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# Master/slave optical coherence tomography imaging of eyelid basal cell carcinoma

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**1** Optical coherence tomography (OCT) is fast emerging as an additional non-interventional modality for skin tumor detection and diagnosis. A master/slave flying spot OCT configuration was assembled to detect periocular basal cell carcinomas (BCC). A swept source at 1300 nm and sweeping speed of 50 kHz were used. A three-step process was involved. First, 384 channeled spectra using a mirror were stored for 384 optical path differences at the master stage. Then, the stored channeled spectra (masks) were correlated with the channeled spectrum from the BCC tissue to produce 384 en face OCT images (200 × 200 pixels) for the optical path difference values used to acquire the masks. Finally, these en face slices were stacked to form a volume to cross-reference BCC tumor margins in the orthogonal plane. Per each eyelid sample, several en face images of 200 × 200 lateral pixels are produced in the time to scan laterally a complete raster of 1.6 s. Combination of the en face views with the cross-sectioning views allow for better discrimination of BCCs comparable to using cross-sectional imaging alone, as previously reported using the conventional fast-Fourier-transform-based OCT techniques. © 2016 Optical Society of America

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## 1. INTRODUCTION

**2** Basal cell carcinoma (BCC) is the most common form of skin cancer in Caucasians [1] and accounts for 80%–90% of all eyelid malignancies [2,3]. It is estimated that there are 53,000 new BCC cases diagnosed in the UK yearly [4]. The gold standard for tumor diagnosis involves surgical biopsy for histological analysis [5]. To reduce patient morbidity from surgery, non-invasive methods of diagnosis and determining tumor margins have been investigated in recent years. They include reflectance confocal microscopy (RCM) [6], high frequency ultrasound (HFUS) [7], multispectral imaging [8], multiphoton microscopy [9], the fluorescent technique [10], confocal scanning laser microscopy (CSLM) [11], and optical coherence tomography (OCT) [12,13]. Although these technologies provide valuable information, they are not yet widely adopted by mainstream health care professionals to provide the high level of certainty essential for accurate treatment. None of these emerging techniques have been used solely on their own or proved to be superior to the gold standard. These new techniques are often used to bridge the gap of limitation on established technologies, demanding new imaging systems with better penetration depth

and sensitivity. Nevertheless, recent literature has suggested that OCT in *en face* imaging mode presents the potential for improved diagnostic specificity of BCC morphology compared with clinical assessment and dermoscopy alone [14,15].

For this reason, we have developed an *en face* optical coherence tomography (OCT) system powered with our enhanced master/slave (MS) technique [16]. Compared to conventional fast Fourier transform (FFT)-based OCT, the MS technique is tolerant to the non-uniformities in the modulation frequency of the spectra acquired, as well as to dispersion in the interferometer [17]. Therefore, the MS technique allows achievement of the theoretical expected parameters for the sensitivity and for the axial resolution as determined by the bandwidth of the system and by the spectral domain principle. The MS technique leads to attainment of such values with no need for resampling of data. In conventional FFT-based OCT, the axial resolution and sensitivity are as good as the resampling/data linearization methods, which generally operate well at shallow depths only. Additionally, the MS technique gives direct access to information from selected depths, allowing the real-time display of one or more *en face* OCT images from such depths [18]. The MS method has been applied to OCT imaging of the eye fundus,

66 and by harnessing the power of graphic cards, real-time produc-  
 67 tion of *en face* images was made possible [19].

68 This is a pilot study aiming to correlate the histological fea-  
 69 tures of periocular BCC to features seen on the OCT scan using  
 70 the MS technique. To the best of our knowledge, this is the first  
 71 pilot study using the master/slave OCT technique for imaging  
 72 periocular BCCs. Prior to this, three other studies to image  
 73 BCC were conducted by our group using dual-wavelength  
 74 (840 and 1300 nm) time domain (TD)-OCT [20], FFT-based  
 75 OCT [21], and dynamic focus (DF)-OCT [22]. Based on the  
 76 experience we gathered from these three tests, we identified that  
 77 it is essential in performing accurate correlations with histology  
 78 data to acquire high-resolution OCT images and correctly  
 79 identify the margins of any skin lesion precisely. On the wave-  
 80 length selection, we identified that light at 1300 nm suffers less  
 81 scattering in tissue, thus providing better signal strength  
 82 compared to 850 nm when used for imaging BCC [19–23].

83 Independent to our group's research, the use of 1300 nm for  
 84 OCT imaging was further documented by the study of BCC  
 85 that employs a combined RCM/OCT system for *ex vivo*  
 86 imaging [24]. The data processing of OCT signals is based  
 87 on the conventional FFT-based technique. In contrast to the  
 88 MS technique, dispersion compensation and interpolation  
 89 are employed. The resulting set of 512 OCT raster images,  
 90 using their method, could be acquired in 13 s. While the RCM  
 91 channel delivers *en face* images, the OCT is performed in cross  
 92 section. Single *en face* images are inferred by software cut of  
 93 volumes of A-scans and are placed in comparison with the  
 94 RCM images.

95 Another study employed a commercial 20 kHz tunable laser  
 96 to produce 120 stack OCT images of size 6 mm × 6 mm in  
 97 between 10–20 s [25].

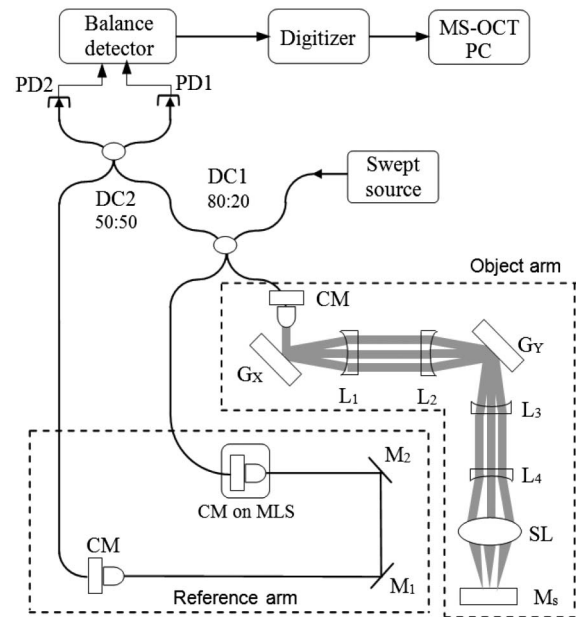
98 None of the OCT systems referenced above are capable of  
 99 producing *en face* images in real time.

100 The use of high-definition OCT as an auxiliary diagnostic  
 101 tool to histology has received an overwhelming positive re-  
 102 sponse by clinicians, as evidenced by a study that looked  
 103 into the effectiveness of locating BCC with OCT. This was  
 104 compared to histology analysis involving both experienced  
 105 and inexperienced dermatologists [26]. We drew inspiration  
 106 from these state-of-the-art research studies in developing our  
 107 master/slave OCT system, with an aim to offer simplified, more  
 108 robust image display in a shorter time.

## 109 2. METHODS AND MATERIALS

### 110 A. Master/Slave OCT

111 The schematic diagram of the MS-OCT system assembled is  
 112 shown in Fig. 1. Light is emitted from a swept source (SS) op-  
 113 erating at 1310 nm central wavelength with a 100 nm sweeping  
 114 range, 50 kHz sweeping speed, and 15 mW output power  
 115 (model SSOCT-1310, Axsun Technology, Massachusetts,  
 116 USA). An interferometer, using two directional couplers DC1  
 117 and DC2, was assembled. The light from the source enters  
 118 the first directional coupler (DC1, model FOBC-2-1310-20,  
 119 AFW Technology, Australia) with a splitting ratio of 80:20.  
 120 One output delivers 80% of light intensity into the reference  
 121 arm, and 20% of light into the object arm.



**Fig. 1.** OCT system configuration ( $G_X$ ,  $G_Y$ , galvo scanners;  $L_1$ ,  $L_2$ ,  $L_3$ ,  $L_4$ , lenses;  $SL$ , scan lens;  $M_1$ ,  $M_2$ , mirrors;  $CM$ , free-space to fiber coupler;  $MLS$ , motorized linear stage;  $DC$ , directional coupler;  $PD_1$ ,  $PD_2$ , InGaAs photodiodes;  $M_s$ , mirror used at the master stage, to be replaced with the BCC specimen at the Slave stage).

F1:1  
 F1:2  
 F1:3  
 F1:4  
 F1:5

122 In the object arm, the interface optics includes an achro-  
 123 matic lens ( $CM$ ) to collimate the output beam of the fiber,  
 124 four lenses for beam expansion ( $L_1$ - $L_4$ ,  $f = 75$  mm, AC-  
 125 127-030-C, Thorlabs), and a scan lens ( $SL$ ). The  $SL$  is an  
 126 OCT-optimized microscope objective (LSM03, 5 ×,  
 127 Thorlabs). For a 4 mm beam diameter incident on the  $SL$ ,  
 128 transversal resolution of the spot on the sample is 15  $\mu$ m.  
 129 The transversal resolution obtained was verified by imaging  
 130 a USAF 1951 resolution target. Two galvo-scanners are in-  
 131 stalled in the object arm, denoted as  $G_X$  (frame) and  $G_Y$  (line)  
 132 in Fig. 1.

133 Both  $G_X$  and  $G_Y$  are driven with triangular ramps. Since  $SS$   
 134 has a tuning frequency of 50 kHz, each of the 200 lateral pixels  
 135 are acquired within 20  $\mu$ s.  $G_X$  is driven with a triangular signal,  
 136 where each ramp duration is 20  $\mu$ s/pixel × 200 pixels = 4 ms,  
 137 with every other ramp discarded, so at a frequency of 125 Hz.  
 138 The frame scanner  $G_Y$  is driven with a triangular signal,  
 139 which for 200 lines requires 1.6 s. With a measured transversal  
 140 resolution of 15  $\mu$ m, the lateral image size is limited to  
 141 3 mm × 3 mm. A single ramp of the frame signal is used for  
 142 data acquisition, while the other ramp is used for data transfer  
 143 to the GPU and data processing. As a result, the time needed  
 144 to acquire each 2D *en face* dataset of 200 × 200 = 40,000  
 145 channelled spectra is 1.6 s.

146 The light into the reference arm is reflected off a set of mir-  
 147 rors ( $M_1$ ,  $M_2$ ), then into the second directional coupler ( $DC_2$ ,  
 148 model FOBC-2-1310-50, AFW Technology, Australia), where  
 149 it is combined with the signal from the object arm to produce  
 150 interference patterns. The outputs of the  $DC_2$  are connected to  
 151 the photodetectors  $PD_1$  and  $PD_2$  of a balance photodetector  
 152 module (model BPD-200, Santec, Japan, 200 MHz). This

153 signal is sent to a digitizer (Alazartech, Canada, model  
 154 ATS9350, 500 MB/s). This is embedded into a PC with an  
 155 Intel Xeon processor (model E5646, 2.4 GHz, 6 cores) and  
 156 a GPU card (GeForce GTX 780 Ti), loaded with a custom  
 157 64-bit LabVIEW 2014 program to display simultaneously  
 158 *en face* images from 40 different depths [28,29].

159 The imaging process using the master/slave (MS) method is  
 160 a two-step process. First, at the master stage, a mirror ( $M_S$ ) is  
 161 placed in lieu of the BCC specimen in the focal plane of SL. A  
 162 set of 384 channeled spectra (masks), are collected and stored.  
 163 The masks are acquired for MLS positions separated by  $10\ \mu\text{m}$   
 164 steps using a motorized translation stage, MLS (model MFA-  
 165 CC, repeatability  $0.3\ \mu\text{m}$ , speed  $2.5\ \text{mm/s}$ , Newport) driven by  
 166 a motion controller (ESP301, Newport). These masks represent  
 167 the channeled spectra at the OCT interferometer output for  
 168 384 values obtained by equivalently displacing the mirror  
 169  $M_S$  by  $5\ \mu\text{m}$ . Thus, with 384 masks, an axial depth  
 170 range of  $1.8\ \text{mm}$  in air is achieved.

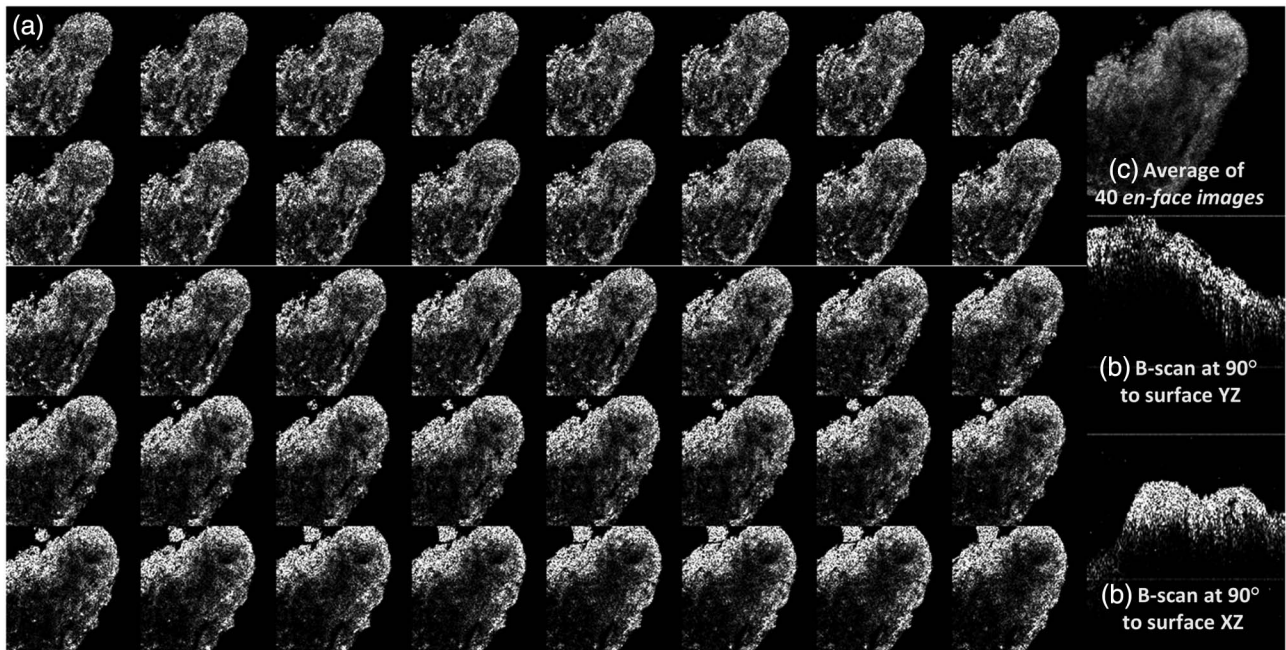
171 A special program was created to allow quick acquisition of  
 172 masks by shifting the MLS to a new incremented position by  
 173 the chosen differential interval ( $10\ \mu\text{m}$ ), followed by time to  
 174 wait for mechanical vibrations to settle and then followed by  
 175 the storage of mask for that position, then moved to the next  
 176 position, and so on. The whole process takes less than 5 min  
 177 and once masks are collected, the process does not need to be  
 178 repeated. Collection of masks is performed with no voltage  
 179 applied to the two galvo-scanners, i.e., using the optical beam  
 180 on-axis.

181 The second stage (slave) involves imaging the BCC specimen  
 182 that replaces the mirror  $M_S$ . Each BCC specimen is position-  
 183 ed in front of the SL. 384 *en face* images are obtained by

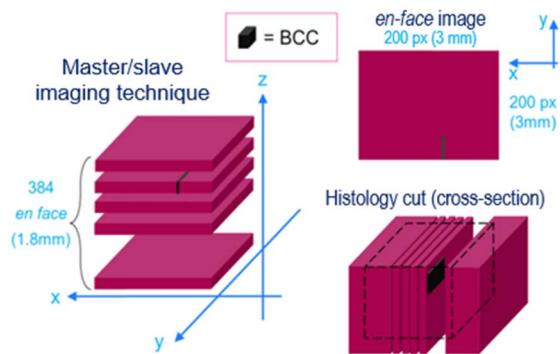
184 correlating the 384 masks with the acquired channeled spectra  
 185 of the BCC tissue, but a reduced number of such images is  
 186 displayed, in this case 40. Figure 2 shows a typical screen of  
 187 the raster displayed by the MS technique. Three categories  
 188 of images are simultaneously presented and they are refreshed  
 189 simultaneously: (a) *en face* OCT images at 40 simultaneous  
 190 depths, (b) two cross-sectional images, and (c) a compound  
 191 image to help with guidance, made from the average of the 40  
 192 *en face* images displayed. The *en face* OCT images and the com-  
 193 pound image have  $200\ \text{pixels} \times 200\ \text{pixels}$  while the cross-sectional  
 194 OCT images have  $200\ \text{pixels}$  lateral and  $384\ \text{pixels}$  in  
 195 depth (along the vertical axis, equal to the number of masks).  
 196 The differential distance between the numbers of *en face* images  
 197 displayed, as well as the depth of the first *en face* images in the  
 198 left corner, can be adjusted with cursors on-screen (not shown,  
 199 but similar to the procedure detailed in [28]).

200 The compound display was found useful in adjusting  
 201 the relative position of the specimens in front of the scan lens  
 202 SL. Here this uses the 40 images displayed; however, this  
 203 can be modified to use any number in any order of the  
 204 398 *en face* images generated by the end of each scanning  
 205 frame.

206 Each category of images is produced using a special program  
 207 developed in CUDA. The simultaneous display of the three  
 208 categories of images takes advantage of methods developed  
 209 in previous reports [22,29], to speed up the processing of com-  
 210 parison operation at the core of the MS technique to the level  
 211 where it can compete with the FFT-based OCT method, while  
 212 maintaining the other MS advantages (tolerance to dispersion  
 213 and tuning nonlinearities with implications in achieving high  
 214 axial resolution and better sensitivity).



F2:1 **Fig. 2.** Illustration of master/slave raster made from 3 categories of images. (a) 40 *en face* OCT images separated axially by  $5\ \mu\text{m}$  (measured in air),  
 F2:2 (b) two cross-sectional OCT images acquired from two orthogonal orientations, and (c) an average of the *en face* images displayed for guidance.  
 F2:3 The horizontal size of all images and the vertical size of *en face* OCT images:  $3\ \text{mm} \times 3\ \text{mm}$ . The vertical size of the two cross-sectional images is  
 F2:4  $3\ \text{mm} \times 1.5\ \text{mm}$  (measured in air). The *en face* images have  $200\ \text{pixels} \times 200\ \text{pixels}$  while the cross-sectional images  $200\ \text{pixels} \times 300\ \text{pixels}$ .



F3:1 **Fig. 3.** Illustration of assembling cross-sectional scans from *en face*  
 F3:2 images. *En face* images are delivered by the MS-OCT technique.

215 The axial resolution of the system is determined by the  
 216 tuning bandwidth of the optical source. With a SS of  
 217  $\Delta\lambda = 100$  nm bandwidth, we can expect an axial resolution  
 218 of around  $7.5 \mu\text{m}$ .

219 Histology data are collected in the cross-section plane; there-  
 220 fore, to compare and correlate suspected BCC features side by  
 221 side, we need to prepare such cuts from the *en face* images  
 222 produced by the MS technique. Therefore, for subsequent  
 223 analysis, the *en face* slices acquired are stacked to form a 3D  
 224 volume. A slice was cut orthogonally to the *en face* surface  
 225 to produce a cross-sectional OCT image, as shown in Fig. 3.

## 226 B. Tissue Imaging

227 Three consecutive patients over the age of 18 with a biopsy-  
 228 proven periocular nodular BCC, without previous eyelid sur-  
 229 gery, were enrolled into the study. The study protocol was  
 230 approved by the Research and Development Department of  
 231 the Maidstone and Tunbridge Wells NHS Trust and also  
 232 by the Ethics Committee at the School of Physical Sciences,  
 233 University of Kent. Informed consent was obtained from the  
 234 participants.

235 All BCC lesions were surgically excised with predetermined  
 236 2 mm margins of normal skin. The orientation of each speci-  
 237 men was specified by using 6/0 silk sutures of various lengths to  
 238 mark 3 of the margins of the lesion. The excised specimens  
 239 were placed in 10% neutral buffered formalin and transported  
 240 from Maidstone Hospital to the University of Kent within 3 h

241 of excision for OCT imaging. For purpose of image acquisition,  
 242 the BCC tissue was placed on a transparent microscope glass  
 243 slice, with its superior margin aligned to the horizontal direc-  
 244 tion of the optical beam axis at the microscope focus of the  
 245 OCT machine. After OCT imaging was completed, the speci-  
 246 mens were placed back into 10% neutral buffered formalin  
 247 and transported to the Maidstone Hospital Histology  
 248 Department the same day.

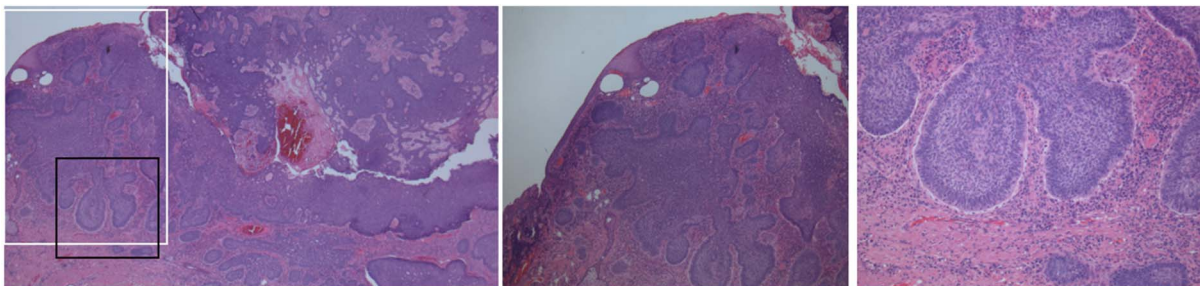
## 249 C. Histology Analysis

250 The specimens were allowed to fix in 10% neutral buffered  
 251 formalin for 24 h and then “bread sliced” at 2 mm intervals  
 252 at 90 deg to the skin surface, retaining orientation as  
 253 denoted by the marking sutures. Each slice was processed  
 254 and paraffin-embedded with serial sections cut at  $2 \mu\text{m}$   
 255 intervals, then mounted on glass slides stained with haematoxylin  
 256 and eosin, and viewed in a conventional light microscope.  
 257 Photomicrographs were captured for comparison with OCT  
 258 images and correlation undertaken by identification of the  
 259 matching features of the OCT and histology images under  
 260 supervision of two of the authors (JS and CC).

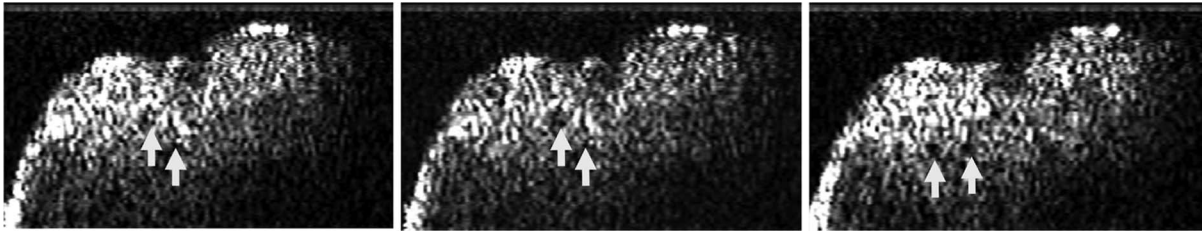
## 261 3. RESULTS

262 The three separate excised BCC specimen biopsy tissues from  
 263 the three patients were imaged using the MS-OCT system.  
 264 Each sample has been marked Case 1, Case 2, and Case 3,  
 265 respectively. To allow comparison to histology data, we as-  
 266 sembled the *en face* images into a 3D volume. A cross-sectional  
 267 OCT image was then sliced orthogonally to the volume surface.  
 268 Using this method, 200 cross-sectional images were recon-  
 269 structed at different lateral positions. We ran our algorithm  
 270 through all these images from the center of the sample to locate  
 271 possible BCC features contained within each of the cross-sec-  
 272 tional images. Diagnosis of BCC via OCT correlation requires  
 273 demarcation of tumor margins to determine BCC invasion,  
 274 and visual interpretation of morphological features as reported  
 275 in pathology-to-OCT correlation studies [30–33].

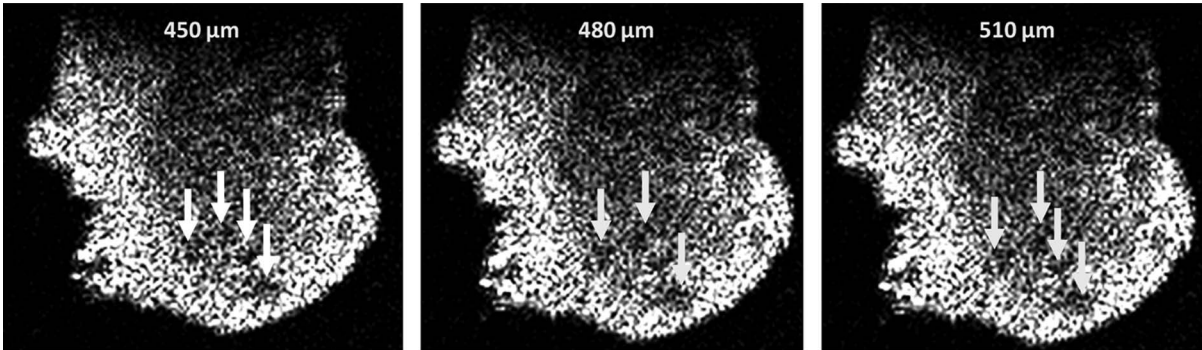
276 Histology images of Case 1 using an Olympus BX50 micro-  
 277 scope are shown in Fig. 4. The haematoxyphilic (purple) areas  
 278 equate to lobules and strands of BCC. The eosinophilic (pink)  
 279 areas are supporting stroma and normal dermis. The corre-  
 280 sponding OCT cross sections are shown in Fig. 5. The areas  
 281 containing BCCs are compared against the marked area in



F4:1 **Fig. 4.** Histology images of the nodular BCC corresponding to tissue sample of Case 1. The images show tumor lobules that correspond to  
 F4:2 honeycomb structures. The lateral size  $\times$  depth size of left image is 5 mm  $\times$  3 mm. The middle image shows an enlarged view of the white boxed  
 F4:3 region in the left image. The right image shows an enlarged view of the black box area in the left image. Magnification: left  $\times 10$ , middle  $\times 20$ , and  
 F4:4 right  $\times 100$ .



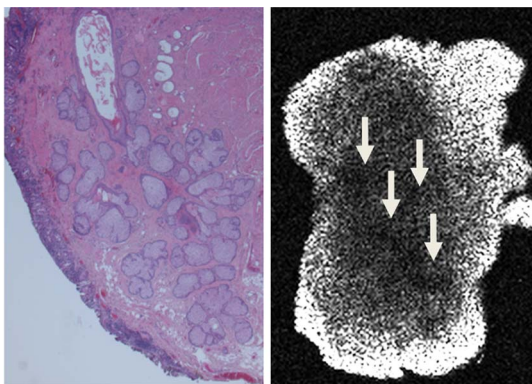
F5:1 **Fig. 5.** Results of Case 1. Cross-sectional OCT images of eyelid BCC taken from three consecutive transverse slices at the center of the sample.  
 F5:2 The left, middle, and right slices are each separated by 15  $\mu\text{m}$ . The size of the image is 1.2 mm  $\times$  0.8 mm. The arrows show the suspected areas of BCC.  
 F5:3



F6:1 **Fig. 6.** Results of Case 1. *En face* OCT images of eyelid BCC taken at different depths. The depth was measured from the top of the sample in  
 F6:2 air. The lateral size of the image is 1.5 mm  $\times$  1.5 mm. The arrows show the areas of BCC correlated with histology data.

282 the histology image. *En face* images taken at 30  $\mu\text{m}$  axial inter-  
 283 val from each other (measured in air), showing features at  
 284 depths of 450, 480, and 510  $\mu\text{m}$  from the top of the sample  
 285 are shown in Fig. 6. The corresponding BCC areas are notice-  
 286 able with several dark spots surrounded by bright regions.

287 A more extensive type of BCC was spotted from another  
 288 region of Case 1 as shown in Fig. 7. The tumors spread around  
 289 in a non-uniform pattern surrounded by healthy tissue. In  
 290 the *en face* image, noticeable patterns of uneven dark stretches  
 291 indicate the presence of tumor. One definite indicator of  
 292 BCC in OCT images is the reduction of signal intensity.

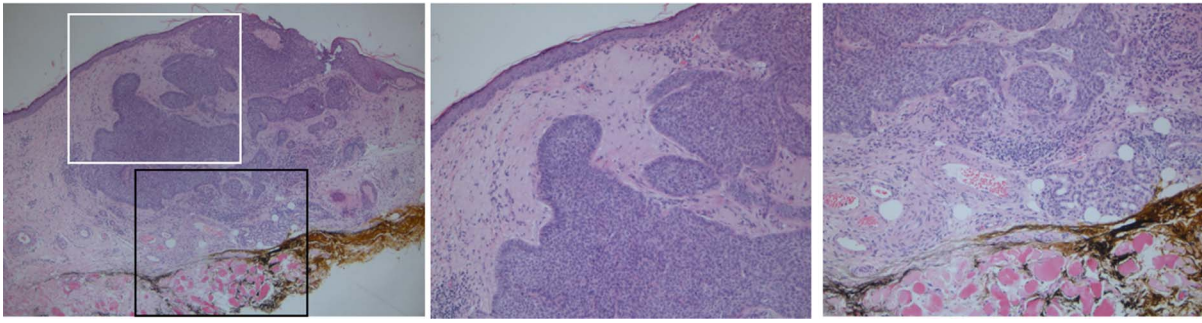


F7:1 **Fig. 7.** Results for another region of Case 1. (Left): histology image  
 F7:2 showing blocks of BCC tumors. (Right): *en face* OCT image of  
 F7:3 corresponding region taken at a depth of 780  $\mu\text{m}$  from top of sample  
 F7:4 (measured in air).

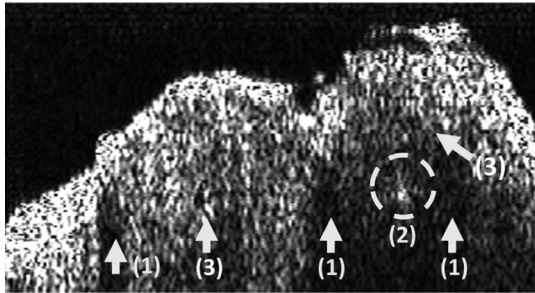
293 The presence of BCC in the *en face* images in Figs. 5, 6,  
 294 and 7 is manifested in clusters of signal-free dark spots,  
 295 rather than in continuous bounded areas, primarily due to  
 296 the position of BCC tumor spreads across multiple axial layers  
 297 of *en face* images.

298 The second experiment was carried out using samples of  
 299 Case 2. Clinical microscopy images are shown in Fig. 8.  
 300 The purple areas are identified as BCC by the histologist.  
 301 Cross-sectional and *en face* images taken from this case with  
 302 features associated with BCC are shown in Figs. 9 and 10.  
 303 Various features are detected in the cross-sectional OCT images  
 304 in Fig. 9: (1) sweat ducts identified by a consistent shallow  
 305 shaft, (2) cross-sectional blood capillary showed up as a highly  
 306 reflective circle, and (3) suspected BCC tumors with irregular  
 307 reflectivity contrasts. Other features suspected to be BCC in  
 308 OCT images (detected by an early report using time-domain  
 309 OCT with dynamic focus [22]) can also be seen in images gen-  
 310 erated using the MS-OCT. These features are noticeable in  
 311 Fig. 10 with scattered high-reflectivity areas that exhibit size  
 312 enlargement as we move deeper into depth. BCC tissues are  
 313 usually highly reflective at 1300 nm, leading to dark scattered  
 314 margins surrounded by low-reflectivity tissue. The tissue areas  
 315 that are lacking these random dark patches are identified as  
 316 healthy tissue.

317 An immediate practical advantage of *en face* imaging is  
 318 showing the direction in which tumors metastasize. With each  
 319 *en face* image displaying the area of BCC in the transverse plane  
 320 in constant intensity, less complicated analysis is required to  
 321 identify the tumors, compared to measuring the reduction



F8:1 **Fig. 8.** Histology images of superficial BCC from tissue sample of Case 2. The image on the left shows a tumor exhibiting nested and budding  
 F8:2 patterns. The lateral size  $\times$  depth size of the left image is 4 mm  $\times$  3 mm. The middle image is an enlarged view of the white boxed region in the left  
 F8:3 image. The right image is an enlarged view of the black box area in the left image. Magnification: left  $\times$ 20, middle  $\times$ 50, and right  $\times$ 50.



F9:1 **Fig. 9.** Results of Case 2. Cross-sectional OCT images of eyelid  
 F9:2 BCC taken from the center of tumor. (1) Sweat ducts. (2) Blood capillary  
 F9:3 under the dermis layer. (3) Suspected areas of BCC. The lateral  
 F9:4 size  $\times$  depth size of image is 1.5 mm  $\times$  0.6 mm.

322 of signal strengths in the cross-sectional OCT images. This feature  
 323 is particularly important to determine the lateral borders of  
 324 a BCC tumor adjacent to healthy tissue.

325 The MS-OCT ability to detect the pigmented type of BCC  
 326 is further evidenced in Case 3. Histology images in Fig. 11  
 327 show tumor lobules concentrated in pigment surrounded by  
 328 healthy tissue. Due to the high reflectivity of BCC, the higher  
 329 concentration of tumor cells blocked light transmission in the  
 330 tissue, producing a signal-free dark spot in the middle of the  
 331 cross-sectional OCT image as evidenced by arrows in Fig. 12.

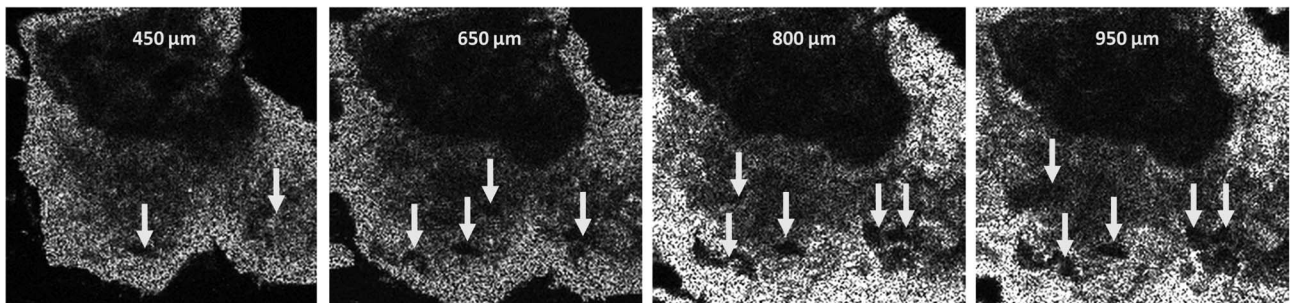
332 This region can be further verified by scanning through dif-  
 333 ferent layers of the *en face* images in Fig. 13. As we move further  
 334 in depth, more common features pointing to potential BCC

335 start to unfold. OCT images are placed side by side with his-  
 336 tology images where correlation of tumor features was per-  
 337 formed. In Fig. 11, the depth data from *en face* images are  
 338 in agreement with the histology data that show structures of  
 339 BCC at approximately 0.5 mm underneath the dermis. *En face*  
 340 images in Figs. 6, 10, and 13 are in their original form, free  
 341 from any quality adjustment. In the slightly pixelated cross-sectional  
 342 OCT images of Figs. 5, 9, and 12, predominant tumor-like  
 343 features remain easily recognizable. Studies have shown  
 344 that image post-processing methods, such as spatial com-  
 345 pounding and de-convolution algorithms, can be used to re-  
 346 duce speckle and enhance signal-to-noise ratio in the  
 347 acquired multilayer images [34,35].

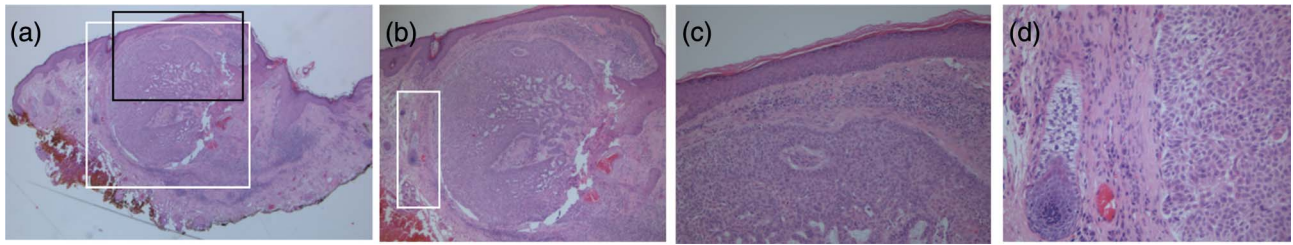
#### 4. COMMENTS ON TECHNOLOGY

348  
 349 Based on previous experiments to characterize BCC employing  
 350 OCT systems at two different wavelengths, 830 and 1300 nm,  
 351 we have concluded that 1300 nm was better suited for imaging  
 352 eyelid skin tissue [20]. MS-OCT is an improvement on the  
 353 technique used in two previous setups, using SS-OCT [21]  
 354 and DF-OCT [22].

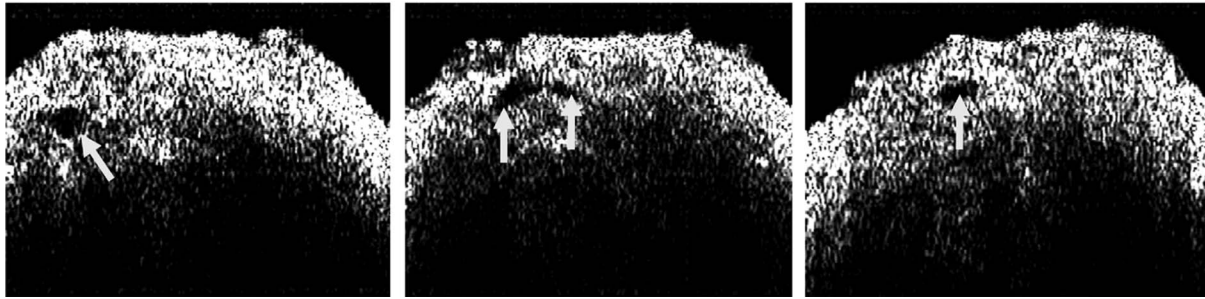
355 The MS-OCT technique proved successful in detecting com-  
 356 plex morphological features of skin in all three specimens im-  
 357 aged. Several skin features that were observed using the DF-  
 358 OCT technique [35] are also present in the MS-OCT *en face*  
 359 images. These are: (1) nodular structure of abnormal tissue,  
 360 (2) superficial structure of scattered tumor margins, and (3) cross  
 361 section of blood vessels, sweat ducts, and sweat glands in the  
 362 upper dermis.



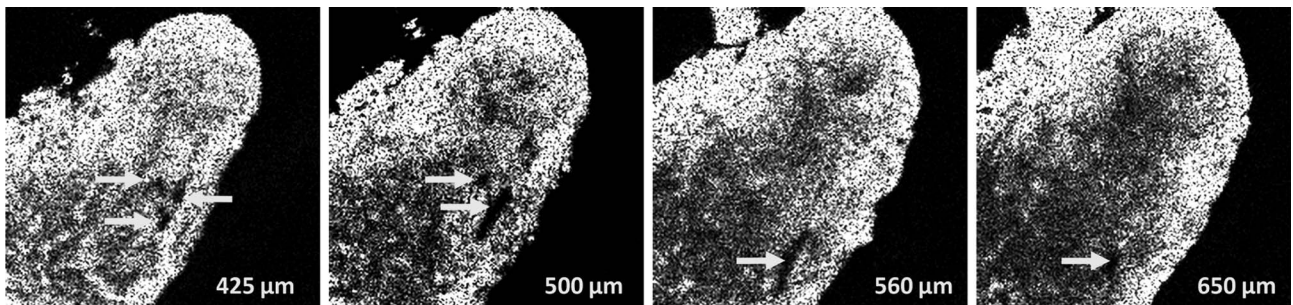
F10:1 **Fig. 10.** Results of Case 2. *En face* OCT images of eyelid BCC taken at different depths of 450–950  $\mu$ m. The depth was measured from the top of  
 F10:2 sample in air. The arrows show the areas of BCC correlated with histology data. The size of the image is 1.5 mm  $\times$  1.5 mm.



F11:1 **Fig. 11.** Histology images of Case 3. Image in (b) shows an enlarged view of the white boxed region in image (a). Image (c) shows an enlarged view of the black box area of image (a). The image (d) shows an enlarged region of image (b) indicating presence of nodular-pigmented-type BCC tumor lobules beneath the epidermis. Magnification: (a)  $\times 20$ , (b)  $\times 50$ , (c)  $\times 100$ , (d)  $\times 200$ .



F12:1 **Fig. 12.** Results of Case 3. Cross-sectional OCT images of suspected BCC taken at three lateral positions from the center of sample. The arrows show the areas with pigment correlated with histology images. The lateral size  $\times$  depth size of the image is  $1.5 \text{ mm} \times 0.6 \text{ mm}$ .



F13:1 **Fig. 13.** Results of Case 3. *En face* OCT images of eyelid BCC taken at different depths in the range  $425\text{--}650 \mu\text{m}$ . The depth was measured from the top of the sample in air. The arrows show the areas of BCC. The size of the image is  $1.5 \text{ mm} \times 1.5 \text{ mm}$ .

363 BCCs in cross-sectional OCT images can be identified by  
 364 three main phenomena: (a) abnormal reflectivity profile as we  
 365 go in depth, (b) obscure regions with clear margins, and  
 366 (c) repetitive inhomogeneous features different from the sur-  
 367 rounding cells. Similar morphological features are also used  
 368 to identify potential BCC candidates in the *en face* images.  
 369 A side-by-side comparison with histology images for all three  
 370 cases further validates the use of these criteria in OCT images  
 371 with high certainty to locate and detect various types of BCC.

372 In the first two BCC structures, nodal and superficial, the  
 373 presence of BCC tissue in *en face* image can be determined  
 374 from the absence of back reflected signal in a bounded area with  
 375 clear edges. The presence of some random, low-reflectivity  
 376 black spots indicate distribution of pigmented BCC structures.  
 377 The lack of signal from BCC structures can be explained by  
 378 higher absorption due to their denser structure compared to

379 normal tissue [20,21]. This feature is predominately visible  
 380 on OCT systems operating at  $1300 \text{ nm}$  than on systems using  
 381 shorter wavelengths.

382 Conventionally, averaging multiple *en face* images usually  
 383 improves the signal to noise, allowing better location of  
 384 BCCs otherwise unnoticeable in a cross-sectional OCT image,  
 385 but this is done on the expense of axial resolution.

386 The master/slave method can equally be applied to broad-  
 387 band sources, by using a spectrometer, in order to achieve better  
 388 axial resolution [37].

## 5. CONCLUSIONS

389 In this pilot study, *en face* images of BCC samples taken from  
 390 three patients were produced using the novel master/slave  
 391 method. In all cases, the suspected BCC regions showed sig-  
 392



nificant reduction in the signal strengths compared to uniform signal reflectivity in neighboring tissues. In some cases, the presence of potential BCC can be identified by irregular scattering of high reflectivity dark spots (Fig. 6) and inconsistent light distribution pattern (Fig. 7) in skin tomograms.

All features associated with BCC demonstrated in our three previous studies [20–22] are also seen with *en face* images obtained using MS-OCT. Three common types of BCC were identified and fitted well with histology criteria. In particular, nodular BCC are noted with their low reflectivity of light at 1310 nm, closely corresponding to BCC on correlation by the histologist following standard laboratory processing. Other subtypes of BCC may also be picked up by their irregular reflectivity profile in *en face* images. High accuracy in distinguishing uncertain tumor lesions from healthy skins was established [15].

A further study has been planned once a portable handheld probe incorporating master/slave technique is manufactured. This will ease the transport of technology for clinical trials at hospitals, allowing imaging to be carried out in real time, in three possible scenarios, involving both *in-vitro* and *in-vivo* experiments: (1) pre-surgery diagnosis, (2) intra-operative assessment of precise tumor margins, (3) post-surgical side-by-side histology assessment. *In-vivo* OCT imaging would be preferred for its enhanced clinical value to dermatologists and pathologists [37–39], as the water content and skin optical properties are preserved. We acknowledge possible signal distortions due to *in-vitro* imaging.

The use of the MS-OCT technique has allowed *en face* images to display noticeable BCC features in real time. With no further image processing required, rapid assessment of BCC extent and surgical margins in excised skin specimens can be carried out, allowing for enhanced management of patients in the clinical setting.

This is a preliminary study to assess the values of the *en face* display in diagnosing BCC. The MS technique is ideally suited to deliver such display as the image is assembled directly while laterally scanning with no need to wait for a whole assembly of A-scans to be subsequently cut.

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