Optical delineation of human malignant melanoma using second harmonic imaging of collagen

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Abstract: Skin cancer incidence has increased exponentially over the last three decades. In 2008 skin cancer caused 2280 deaths in the UK, with 2067 due to malignant melanoma. Early diagnosis can prevent mortality, however, conventional treatment requires multiple procedures and increasing treatment times. Second harmonic generation (SHG) imaging could offer diagnosis and demarcation of melanoma borders non-invasively at presentation thereby short-cutting the excision biopsy stage. To test the efficacy and accuracy of SHG imaging of collagen in skin and to delineate the borders of skin cancers, unstained human melanoma biopsy sections were imaged using SHG microscopy. Comparisons with sister sections, stained with H&E or Melan-A were made for correlation of invasion borders. Fresh ex vivo normal human and rat skin was imaged through its whole thickness using SHG to demonstrate this technique is transferable to in vivo tissues. SHG imaging demonstrated detailed collagen distribution in normal skin, with total absence of SHG signal (fibrillar collagen) within the melanoma-invaded tissue. The presence or absence of signal changes dramatically at the borders of the melanoma, accurately demarcating the edges that strongly correlated with H&E and Melan-A defined borders (p<0.002). SHG imaging of ex vivo human and rat skin demonstrated collagen architecture could be imaged through the full thickness of the skin. We propose that SHG imaging could be used for diagnosis and accurate demarcation of melanoma borders on presentation and therefore potentially reduce mortality rates.

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1. Introduction

Skin cancer is one of the most common forms of cancer in the UK totaling around 84,500 registered cases in 2007 with 10,672 case of these being malignant melanoma (MM) [1–5]. The total cost of skin cancer in the UK in 2002 was estimated to be in excess of £190 million [6]. In that year, 63% of the total costs of skin cancer were attributable to MM alone, amounting to 1770 cases [6] and the number continues to rise (1852 in 2006), resulting in a doubling of reported cases since the early 1970’s (Quinn et al., 2001).

Globally, there were more than 161,000 reports of MM alone in 2002, with Australia/New Zealand having the highest incidence followed by Northern America, Northern Europe and Western Europe (67, 27.1, 18.5 and 17.6 cases/100,000, respectively) [7]. These numbers are predicted to continue rise over the next few decades. Although MM becomes more common with increasing age, these trends have changed more recently with an abnormally high incidence in the young; with MM becoming the most common form of cancer in people aged 15-34, a 300% increase between 1975 and 2006 [1]. The greatest increase was found in the 60-79 year old group, particularly in males, which has seen an increase of over 600% in the same period [1].

Early diagnosis and treatment can prevent death for the majority of these patients. The normal route for diagnosis is a Post-Hoc method that builds-in an inherent delay in the diagnostic procedure. This involves histological processing of the tissue followed by analysis/diagnosis by an expert pathologist to assess the severity of the MM and the margins of the invading cancer cells based on Breslow thickness measurements. This may result in the patient having to undergo two or more operations to surgically remove the entire tumour, if some of the affected tissue was not removed at the first biopsy stage. For irregular shaped cancers, simply applying Breslow depth measurements to calculate the margins of the cancer to excise the lesion is inadequate and results in further excision biopsies being necessary to remove all the diseased tissue. This delay can lead to the cancer metastasizing to other tissues and significantly worsen the prognosis for the patient.

During their invasion of surrounding tissues, both Melanoma and Non-Melanoma Skin Cancer (NMSC) utilize the same biological events [8–13]. They are characterized by active invasion and destruction of surrounding connective tissue. Penetration of the cancer through the basement membrane (BM) is the initial step in the complex process of invasion and metastasis. This process when simplified consists of three distinct events: 1. attachment to the BM, via various cell surface receptors. 2. degradation of the BM and extracellular matrix by a series of matrix metalloproteinases (MMPs). 3. cancer cell locomotion into the surrounding tissue induced by chemotaxis. Therefore, a key features of all skin cancers is the degrading and remodeling of the extracellular matrix along the borders of the invading cancer, principally collagen type I, by matrix MMPs [8–13], but little is known about the differential disruption of collagen density and morphology between MM and NMSC. We propose that this degradation of collagen along the borders of the invading cancer can be used to, a) accurately define the margins of the skin cancer, and b) aide in the diagnosis of a malignant condition.

Confocal microscopy has been the subject of intensive investigation in the scientific community in recent years, not least in the field of cancer biology [14,15]. More recently, multiphoton fluorescence microscopy (MPFM) has been used for non-invasive imaging of the epidermis in a number of studies [16,17]. Since MPFM is a non-linear fluorescence phenomenon, only tissues at the focal point of the objective are excited and hence the technique is inherently confocal, i.e., the only two-photon events that can occur in the tissue will be at the focal point of the objective and therefore no confocal pinhole is required to remove out-of-focus blur. Similarly, second harmonic generation (SHG) occurs only at the focal point of the objective in tissues that have a non-centrosymmetric crystalline structure, such as collagen, to produce a strong SHG signal at exactly half the wavelength of pump laser.
wavelength [18–21]. However, with SHG imaging, there is substantially less absorption of energy by the tissue compared to single-photon excitation if the fundamental wavelength is off the resonance wavelength of cellular constituents, and hence, there is negligible thermal or phototoxic damage [15]. As both SHG and MPFM utilize pulsed infra-red light this allows deep imaging into live tissues (400–1000 µm depending tissue turbidity, pigmentation and wavelength) with less scattering of light [15,22,23]. Importantly, the SHG signal is generated without the need to stain the tissue in any way, and so this technique lends itself well to be used as a non-invasive imaging tool [24,25]. Using second harmonic confocal microscopy we have demonstrated that the borders of malignant skin cancers can be easily identified in unstained human biopsy skin sections that correlate well with clinical diagnosis of MM.

We propose that by using SHG imaging the diagnostic process currently used and surgical planning can be dramatically enhanced by accurately delineating the borders of invading cancer cells at the patient’s first presentation. This could significantly reduce the time taken for diagnosis and alleviate the patient of repeated operations and possibly decrease mortality rates.

2. Materials and methods

Sections from human MM biopsies were acquired from the University of Szeged, Hungary with full ethical approval from the host institute and patient consent. Tissue devitalization protocols were standardized for all patients and no samples showed any signs of necrosis prior to excision. Histological preparation of the tissue was identical for all samples. Five samples were diagnosed as superficial spreading MM, one nodular MM and two lentigo maligna MM. Three samples were from female patients and five from males with an age range of 58–75 years. Breslow thickness assessments ranged from 0.304 to 1.672 mm and post-MM excision survival was 30–84 months.

Multiple adjacent fields of unstained human melanoma skin sections (4µm) (n = 8 patients) were imaged using SHG imaging to reveal fibrillar collagen distribution (principally Type I and III), followed by single-photon transmission (bright field) imaging. A detailed description of the experimental setup is described in [19]. Image acquisition was performed on a Leica SP2 AOBs confocal multi-photon laser scanning microscope coupled to a Leica DMRE upright microscope (Leica, Milton Keynes, UK), equipped with a pulsed, mode locked femtosecond (fs) Ti:sapphire Tsunami laser synchronously pumped by a Millenia VII (Spectra-Physics, Mountain View CA), diode pumped solid state frequency doubled laser capable of delivering up to 8.5W pumping power at 532nm.

All SHG image acquisition was performed at 880nm, which has previously been found to be the optimum wavelength for collagen types I and III [19]. The pulse width of the Tsunami was 80 fs with a pulse repetition rate of 80 MHz. Laser power output at the microscope objective was recorded with a coherent power meter and calibrated for all samples to deliver peak power of 0.4 × 10^8 Wcm⁻² and was consistently maintained below the damage threshold of the samples, which for collagen in sections was found to be 1.5 × 10^8 Wcm⁻² [19]. An IST laser spectrum analyzer coupled to a Tektronix TDS 210 oscilloscope was used to tune the laser to the desired wavelength. An electro-optical modulator (EOM) (Linos LIV20) received the laser output before delivery to the confocal microscope through a series of optical mirrors. The EOM allowed the laser intensity at the objective to be controlled and optimized. EOM was set at 90% to circularly polarize the pump laser beam, so as to ensure the incident beam laser remained consistent across all specimens. Thus the potential effects of sample polarization versus fundamental pump beam polarization were minimized.

In order to image the complete section we used a motorized stage (Mietzhouser, GmbH), controlled by the Leica software to perform automated montages from multiple fields consisting of up to 42 x 19 fields with up to 10 z-sections of 1µm thickness. Samples were imaged with a 20x, 0.7NA dry objective and 2x zoom producing an image measuring 375 by 375 µm at 512 by 512 pixel resolution. The SHG signal in the backscattered geometry was
captured in the de-scanned detector with the pinhole set to the maximum of 600 µm. The Leica microscope incorporates a programmable, prismatic beam splitter, which is capable of single and multi-chromatic beam splitting. The backscattered photons are subsequently focused through the objective lens with an estimated beam diameter of 0.24 µm (λp = 880 nm) and delivered through the prismatic beam splitter, programmed to collect light at 435-445 nm, before passing it on to the photomultiplier tube (PMT). This calculation is based on the Guoy phase shift and its relationship to the NA of the objective lens, the fundamental wavelength and refractive index of the sample [26]. Thus the beam diameter = 0.32λp / √2NA. The coherent SHG signal formed in the transmission geometry was detected by the transmission detector via the microscope condenser with 445 nm df 30nm band pass filter inserted in the light path (Chroma Inc. UK). In addition, a non-confocal, bright-field image was acquired (sequentially to the SHG image) using single photon excitation at 488nm, which was collected in the transmission PMT. This image served as a reference image to overlay the H&E or Melan-A with the SHG images during the assessment stage. At the end of image acquisition the automatic montage software produced a final compilation image of the all z-projected fields.

Sister-sections were then immunohistochemically stained for the melanoma marker, Melan-A, and imaged using conventional light microscopy, then overlaid on the SHG images for comparison. The extent of invasion and limits of the borders between the two imaging techniques (H&E and or Melan-A) was assessed and marked by two independent histopathologists. The lateral boarders of cancer invasion were marked on the SHG images by an independent researcher, blinded to the histopathologists assessments, then checked for the degree of correlation between the SHG and H&E/Melan-A findings, using Mann-Whitney U-Test and Kendall-Rank Correlation Analysis. The criteria used for the independent evaluation of the MM borders of the SHG images were that one could clearly discriminate undisrupted collagen fiber bundles at the dermal-epidermal junction, adjacent to the MM lesion.

Collagen fiber density was quantified from seven regions using ImageJ image analysis software (Freeware, rsbweb.nih.gov) to calculate the percentage area of collagen fibers for that region. The images were segmented using an adaptive threshold algorithm with a fixed kernel size and offset for all samples. Data was expressed as Area %, calculated using area of collagen fibers/ area of measuring frame x 100. Generally SHG produces very high contrast images with very low or no background and since pump laser beam polarization was circular, segmentation based on threshold values proved to be a robust method. The regions quantified are highlighted in Fig. 2A and were an area of non-lesion skin under the dermal-epidermal junction (labeled A), the lateral melanoma border under dermal-epidermal junction (labeled B), an area under the middle part of the lesion and under the dermal-epidermal junction (labeled C), the areas labeled D, E, F, are all below C but one field vertically deeper to each other, and the area of dermis at the same depth as F but away from the lesion (labeled G in Fig. 2A).

Fresh pieces of whole rat and human skin were cleared of subcutaneous fat and connective tissue, and hair in the case of the rat samples, and mounted onto glass slides in PBS buffer. Human skin samples were collected from The Royal London Hospital Trust, department of surgery with full patient consent and ethical approval, and imaged within four hours. One sample was from 22 year old, breast reduction patient, and the other three samples were breast reconstructions from age rages of 30–45 years. Two samples appeared heavily pigmented whilst the other two appeared very lightly pigmented. SHG image acquisition was performed in both backscattered and forward propagated (transmission) geometries from the epithelial surface and imaged at full depth (deep into the dermis) in order to demonstrate that the technique is transferable to intact live skin. A Leica 20x 0.7 NA, long working distance objective, was used for image acquisition. A single field of 512 x 512 pixels (750 x 750 µm or 430 x 430 µm) at zoom 1.0 or 1.7 at 800 Hz with 2.5µm z-step, an imaging average of 2 frames was acquired for both rat and human ex vivo samples. Typical imaging times for ex
vivo skin samples were 0.7 s per frame and up to 225 z-slices per sample; therefore total-imaging times did not exceed 3 min. 3D-images were generated with Imaris visualization software (Bitplane Scientific Software. Zurich, Switzerland).

3. Results

Three images were obtained for each unstained melanoma section; an SHG image in the forward propagated (Fig. 1A) and backscattered geometries (Fig. 1B), followed by a bright field image (Fig. 1C). Bright field and SHG images superimposed gave an image of collagen distribution within the bright field background for each section (Fig. 1D).

The SHG collagen signal showed a rapid transition at the two lateral melanoma borders, from very low or no SHG signal to that of normal collagen distribution found in non-lesion areas of skin. Medial to the melanoma borders there was a dramatic reduction in collagen SHG signal making the identification of the lateral boarders of cancer invasion unambiguous (Fig. 1D, 2A, 3C and 4B).

![Fig. 1. Montage of SHG images in the forward propagated (A), backscattered geometry (B), and bright field image (C). Superimposed images of bright-field and SHG images indicate collagen distribution within each section (D). Scale bar = 1mm](image)

Quantification of collagen fiber density from different regions around the MM lesion showed varying degrees of fiber density, expressed as Area %, ranging from 0.8% in the region labeled (C) (Fig. 2A) in the middle of the MM lesion, to 38% per unit area, in non-lesion area of skin labeled (A and G) (Figs. 2A and B). In regions labeled (D, E and F) there was a gradual increase of collagen fiber density from 9.1% to 19.88% to 33.2%, respectively.
(Figs. 2A and B). This increase correlated with increasing distance from the MM lesion towards a more ‘normal’ collagen fiber density. Each microscopic field (one tile in the montage) is equivalent to 375μm; therefore the collagen fiber density goes from almost zero in region C to 33.2% in region F, a distance of 1500μm (Figs. 2A-B). In the non-diseased areas of all skin sections we found a normal distribution of collagen up to the dermo-epidermal junction. Linear regression of collagen fiber density from region C (minimum SHG signal) to region G (maximum SHG signal in non-diseased area) showed an $R^2$ value of 0.9849 suggesting that collagen fiber density is a good biomarker for malignancy (Fig. 2B).

![Collagen fibre density graph](image)

Fig. 2. (A) Enlarged view of Fig. 1D showing regions from which collagen fiber density was quantified. $A =$ Area of normal skin under dermal-epidermal junction, $B =$ Lateral melanoma border under dermal-epidermal junction, $C =$ Area under the middle part of the lesion and under dermal-epidermal junction, areas $D, E, F,$ are all below $C$ but one field vertically deeper to each other, area $G$ is at the same depth as $F$ but away from the lesion ($A$). (B) Graph of collagen fiber density (Area %, ± SE) of the individual regions of A ($n = 5-8$). On some samples not all regions could be quantified because the biopsies were cut to shallow, i.e., not enough dermis depth present, therefore the deeper regions were absent and hence there is no data and the n-number vary. Note regression line illustrating a linear correlation of collagen fiber density ($R^2 = 0.9849$) from region $C$ (mid-point of MM lesion) to region $G$ (deep area of non-lesion skin). Scale bar = 1 mm
Assessment of the lateral melanoma borders derived from SHG images versus H&E images (Fig. 3A) by the histopathologist were found, on direct observation, to co-localize in almost exactly the same positions (Fig. 3C). Cross-reference control comparison showed that melanoma borders obtained from H&E and Melan-A stained sister sections were also well co-localized (Figs. 3A and B, Figs. 4A and B).

Non-parametric statistical analysis using Mann-Whitney U-Test revealed no significant difference between the lateral melanoma borders as determined by an independent histopathologist using H&E sections and those assessed by an independent scientist using the SHG images of the corresponding unstained sister section (Fig. 5A). p-values for the right lateral border comparison was $p = 0.9491$ representing a mean difference of 0.85 mm ± 0.18 mm (SE) ($n = 7$) and for the left border $p = 0.9164$ representing a mean difference of 0.55 mm ± 0.13 mm (SE) ($n = 8$), respectively (Fig. 5B). The total p-value for both sides was $p = 0.9339$. Kendall-Rank Correlation Analysis revealed a highly significant correlation between the lateral melanoma borders derived from histopathologists assessment and those from SHG images. p-value for the right lateral border comparison was $p = 0.0069$ ($n = 7$) and for the left...
Fig. 4. High magnification images of Fig. 3. Melan-A staining (A), SHG/brightfield image (B), corresponding to left hand border (red boxes) in Figs. 3B and 3C, respectively. Note that the SHG signal starts to reappear at the dermo-epidermal junction at the point where MM lesion is absent. Scale bar = 1mm

side $p = 0.0260$. ($n = 8$) The tied $p$-values for the right and left sides were $p = 0.0056$ and $p = 0.0260$, respectively. The total $p$-value for both sides correlation analysis was $p = 0.0012$ (Fig. 5B).

SHG imaging of whole, $ex$ $vivo$ rat (Fig. 6A) and human skin (Fig. 6 B) samples yielded detailed images of collagen morphology through the entire thickness when imaged from the epidermis, in both backscattered and transmission geometries. In the case of rat skin, SHG
transmission images were detected through the whole thickness of the skin, although only the upper 150 µm is shown in (Fig. 6A). The blue channel is the two-photon auto fluorescence image of the upper epidermis, whilst the red and green channels are the transmitted and back-scattered SHG signals, respectively. In the lightly pigmented human skin samples, we were able to image through the whole thickness of epidermis and dermis to depths of approximately 300µm in the back-scattered geometry (green signal) and over 1000µm in the transmission geometry (red signal) (Fig. 6B). The heavily pigmented human samples could not be imaged from the epidermal surface, presumably because of the absorption of the 880nm wavelength light by melanin. However, if the samples were imaged from the dermal surface, clear SHG images of collagen were detected in both forward and back-scattered geometries (images not shown). Scale bars = 50 µm for each orthogonal plane.

These data support our hypothesis in that there are no significant differences between the two methods of identifying the borders of the MM lesions. Furthermore, it also emphasizes the fact that the extent of MM lesion can be asymmetrical and hence using Breslow thickness alone to empirically define a radius from the center of the MM lesion can in many cases be insufficient. This effect can be compounded in cases where there is very irregular shaped, MM lesions and would lead to more than one attempt to surgically remove the tumor.
Fig. 6. SHG imaging of normal, ex vivo, live rat skin (A) and human skin sample (B) showing collagen morphology throughout the entire thickness when imaged from the epidermis, in the transmission (red) and back-scattered (green) geometry. Rat skin, SHG transmission images (red) and back-scattered SHG signal (green) were detected through the whole thickness of the skin (A). The blue channel is the two-photon auto fluorescence image of the upper epidermis, whilst the yellow represents the colocalization of the red and green channels. In lightly pigmented human skin samples, we were able to image through the whole thickness of epidermis and dermis to depths of approximately 300µm in the back-scattered geometry (green signal) and over 1000µm in the transmission geometry (red signal) (B). Scale bars = 50 µm for each orthogonal plane.

4. Discussion

We have demonstrated that SHG microscopy is a rapid and reliable method for the clinical management of MM by defining the borders of the lesions and the extent of dermal invasion
(Breslow thickness) based on collagen morphology and density. Our results show that we can accurately define the lateral borders in histological sections of MM biopsies, which correlate significantly with the current gold standard assessments (H&E and Melan A). This study provides initial evidence that collagen density in and around the suspected MM lesion may possibly be used as a biomarker for skin cancer diagnostics; further studies are in progress to confirm if collagen density and architecture can differentiate a MM from a squamous cell or basal cell carcinoma as well as other skin tumors. Therefore, the in vivo applicability and identification of skin tumors using SHG microscopy is at present independent of lesion type so clear identification/confirmation is still required. The scant data in the literature about collagen fiber density around MM and NMSC lesions warrants more detailed studies but until then current diagnostic practices will still require immunophenotyping. In the diseased areas we observed a gradual decline in collagen density. This was most dramatic in the areas directly under the melanoma, which showed the weakest SHG signal; in most cases there was a complete absence of SHG signal due to the destruction of collagen by MMP’s produced by the malignant melanocytes. In the MM lesion areas the collagen morphology was greatly affected and was present in very short, thin ‘wispy’ fibers. The SHG signal then intensified with increasing depth below the lesion, until the collagen took on a more normal appearance. Furthermore, we have demonstrated that SHG imaging can detect collagen in freshly excised human and rat skin samples in both the back-scattered and transmission geometries; up to 300 µm and 1 mm, respectively.

SHG imaging has been used to detect collagen non-invasively in many tissues including human skin [27,28]. It is a non-absorptive, non-linear, two-photon optical effect that can detect non-centrosymmetric crystalline structures such as collagen type I and III in unstained, live and fixed tissues [27,28]. It typically employs infrared or longer wavelength pulsed lasers, enabling good depth penetration and very little or no phototoxicity to tissues. Furthermore, the non-absorptive nature of the technique results in conservation of energy and therefore little or no heat is dissipated into non-pigmented tissue from the laser source, which would otherwise damage or even destroy live tissues.

Until social attitudes to skin cancer prevention are changed, early detection and treatment is the key to reducing mortality rates. Therefore the major challenge for skin cancer diagnostics is the early detection of non-metastasized malignant lesions and to reliably differentiate these from the less life threatening malignant cancers such as SCC, BCC and benign nevi BN, at the time the patient presents to the clinician. The most significant risk to life is MM and early detection and excision is crucial if the chances of survival are to be maximized [29,30]. SCC and BCC represent a much lower risk to life expectancy and are highly treatable if the correct diagnosis is made early on in the disease [29,30]. BN present little or no risk to patient survival; however, accurate diagnosis is required, as these can often resemble MM lesions. In addition, an apparently BN may become dysplastic and progress to a MM.

One of the key features of all skin cancers is the remodeling of extracellular matrix by MMP’s, principally collagen type I and III, which is clearly seen to be disrupted along the borders of the invading cancer cells [31] and missing in the diseased tissue itself. However, current diagnostic procedures do not include assessment of collagen density/architecture as a parameter, even though this is a key event in skin cancer progression and can potentially be used as a biomarker. We propose that collagen architecture, and its presence or absence can be used as, 1) a reliable marker of MM invasion through tissues, and 2) that it can be used to accurately define melanoma invasion borders.

A number of non-invasive and experimental imaging techniques are currently being used in dermatology clinics throughout the world including the Dermatoscope [32,33] and single- or two-photon epifluorescence confocal microscope based systems [34]. However, anecdotal reports and clinical interpretations of these techniques have been varied, with a tendency towards over diagnosing non-malignant conditions such as SCC and BCC putting them
incorrectly into the MM category [32,33]. This leads to unnecessary surgery, pain and psychological stress for patients, believing that they have a MM, and ultimately, leads to increased costs to the Health Services. More importantly however, is the reliability and accuracy in misdiagnosis of a MM where current, non-histological methods, have a maximum of 87% specificity rate compared to CLSM of 98% [34].

The data from this study clearly shows that there can be very significant differences in the distance from the center of the tumor to the left and right hand borders of irregular shaped MM lesions; In Fig. 5A, sample numbers 3 and 6, show an approximately 50% differences in left- and right-hand borders, illustrating that the tumors in these samples were asymmetric. If this asymmetry were significant then clinical management of MM based on Breslow thickness measurements would not be sufficient in determining how much tissue to excise from the center of the tumor so as to encompass all the borders. The current practice can lead to multiple excision biopsies being performed, and in some cases, putting the patient at increased risk of tumor metastasis. Therefore, present-day methods for skin cancer diagnostics are complicated by the lack of a reliable non-invasive assessment technique that could eliminate the excision biopsy stage of the assessment if it is found to be non-malignant. A recent study suggests that collagen SHG signature is a promising marker for MM diagnosis [35] and this study supports that notion.

Any future diagnostic instrument will depend on the ability to image through the surface of the skin and thus the back-scattered signal is more important in this respect. Selecting the appropriate wavelength to ensure adequate depth penetration and to overcome absorption of the light by melanin will be crucial to the success of any such instrument. We found that at 880nm we could not detect an SHG signal from heavily pigmented human skin samples from the epidermal surface, but was able to when imaged from the dermal surface. This result emphasizes the need to fully assess the tissue wavelength absorbance characteristics so that SHG imaging can be performed in pigmented as well as non-pigmented samples. Other groups have shown, with custom built microscopes that longer infra-red wavelengths (1200 –1300 nm) can overcome the absorbance of shorter infra-red wavelength (880 nm) we have used in this study [28]. However, in lightly pigmented skin the areas immediately outside of a suspected cancerous lesion would still be penetrable by 880nm light, although longer wavelengths would be more appropriate. Our research is aimed at developing a low resolution, fiber optic, handheld device that can give rapid and reliable assessment of collagen presence/absence outlining the MM lesion, in the first instance, and then a high resolution lens-based system for differentiating between, MM, SCC, BCC and BN based on collagen architecture and density. It is important to note that the low resolution system needs only to penetrate the skin to just below the dermal-epidermal border as this will detect the presence (presumptive normal, non-diseased tissue) or absence (presumptive MM lesion) of the collagen SHG signal. The clear and unequivocal data from this study provide good evidence that SHG imaging can be a viable alternative to the existing diagnostic procedures.

5. Conclusions

In conclusion, this study provides good evidence that SHG imaging has the potential to improve on the current excision biopsy stages of MM diagnosis with a single assessment at first presentation of the patient. This can potentially shorten the diagnostic process and by demarcating the boundaries accurately ensure that the entire MM is removed first time thereby saving lives.

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