Diagnostic Assessment Of Human Skin Cancers Using Second Harmonic Imaging.
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Introduction
Malignant melanoma (MM) incidence has doubled in the last twenty years and it continues to grow faster than any other cancer. It is the second most common cancer in the 15-34 year old age group. One of the key features of all melanomas is the remodelling of collagen (and other extracellular matrix proteins), which is broken down along the invading melanoma border by matrix metalloproteases. Using second harmonic generation (SHG) we can easily image collagen on unstained sections of MM biopsies and in normal ex vivo human skin samples. We propose that SHG imaging can provide a rapid means of delineating skin cancer borders and provide an accurate diagnosis of a malignant condition, allowing for earlier interventional treatment and thereby, save lives.

Materials and Methods
Multiple adjacent fields of unstained human melanoma skin sections (4µm) were imaged using two-photon SHG imaging to reveal collagen distribution (principally Type I), followed by single-photon transmission (bright field) imaging. All the fields were automatically montaged to generate an image of the entire section. “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, limits of the borders and the degree of correlation between the two imaging techniques (H&E and or Malan A versus SHG) was assessed. Fresh pieces of whole human skin were placed in PBS buffer, mounted onto glass slides and imaged directly using SHG to demonstrate that the technique is transferable to whole, live human skin.

Results
SHG microscopy on unstained human melanoma sections produced very detailed images of collagen fibre distribution. Quantitatively, there was a highly significant degree of correlation (p<0.002) between SHG images on unstained sections and Melan-A/H&E stained “sister” sections. The leading edge and total depth of melanoma invasion could easily be identified in all samples. SHG imaging of ex-vivo human skin produced very detailed images of collagen fibres through the full thickness of skin in both the forward and back scattered geometries (>300µm).

Conclusions
We have demonstrated that SHG microscopy is a rapid and reliable method for diagnosis of malignant melanoma borders and the extent of dermal invasion (Breslow thickness). Thus, using SHG imaging, the current excision biopsy stages of MM diagnosis can be replaced with a single assessment at first presentation of the patient, thereby shortening the diagnostic process and possibly save lives.