Imaging the Distribution of Melanin in Human Skin Lesions with Pump-Probe Microscopy

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Melanoma diagnosis poses tremendous challenges in dermatopathology; recent studies show discordance rates in pathology as high as one in seven[1], and the severe consequences of missing a melanoma diagnosis has increased the number of biopsies taken and lowered the threshold for diagnosis of early melanoma[2]. This leads to unknown but likely significant societal costs and morbidity from associated unnecessary treatments. Clinicians need better technology to increase the specificity of current diagnostic techniques.

We have previously reported a technique that resolves the two kinds of melanin in human skin using two-color pump-probe microscopy[3]. Performing principle component analysis on a set of images reveals that 99% of the variance can be attributed to two components. These components map cleanly to eumelanin and pheomelanin, which gives a quantitative way to image their distribution in tissue. This technique, which is compatible with standard pathology procedure, has been used to image the microscopic morphology of the distribution of eumelanin and pheomelanin in human tissue slides. It has revealed that melanomas tend to have an inhomogeneous distribution of melanins and higher fractional eumelanin content compared to other lesions.

Here we report on a variety of extensions of that work. Studies on the dependence of laser parameters reveal the effects of the increased ground state depletion signal in pheomelanin relative to eumelanin. The contrast depends on the difference between the pump and probe wavelengths and has been demonstrated at a variety of near-IR wavelength pairs. Contrast depends on the relative polarizations of the beams and is most prominent when they are parallel. Utilizing the wavelength flexibility of the system, we have begun using a 750nm pump beam and 850nm probe beam rather than our previous 720nm/810nm combination in order to increase depth resolution when transitioning from 5µm thin slices to thick slices around 100-200µm. The contrast at this wavelength combination is shown in figure 1.

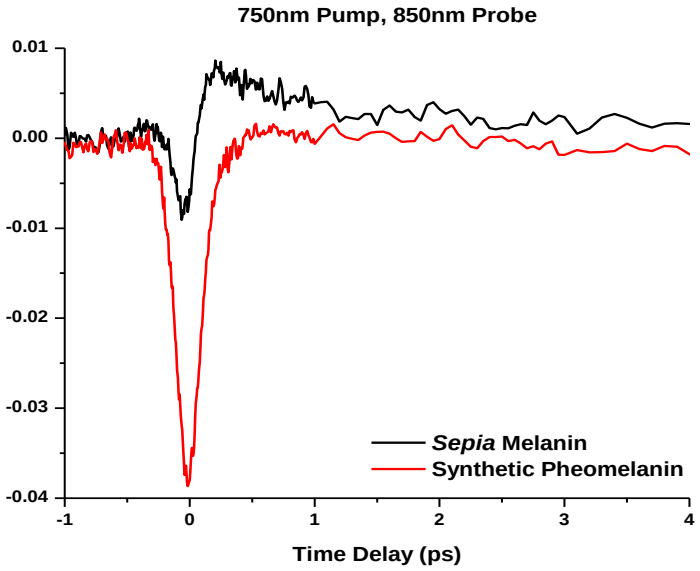

Figure 1: Delay scan of cuvette melanins using 2.6mW of 750nm pump beam and 3.5mW of 850nm probe beam.

High resolution imaging has identified microstructure with variable eumelanin/pheomelanin ratios in individual melanosomes. We have demonstrated epi-mode detection and imaging *in vivo* of melanoma induced in human skin grafted to nude mice. We have also demonstrated three-dimensional sectioning capabilities[4]. As a result, it can be used for imaging thick slices of tissue (to a depth of around 120μm), allowing a clinician to examine a large section of a lesion all at once. This has been shown on a fresh, excised human mole, on which the rete ridges can be seen using melanin as a source of contrast.

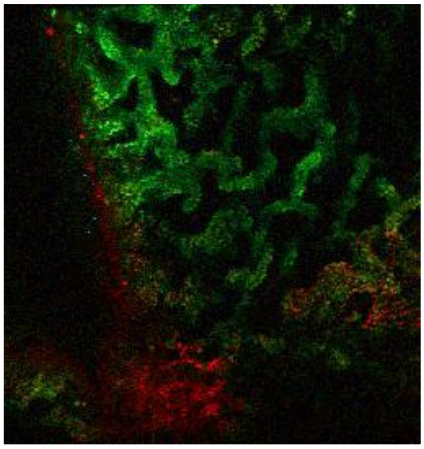

Figure 2: Epi-mode image of an excised human mole. Rete ridges are visible in this image, which is taken 45μm deep with 720nm pump and 810nm probe. Color scale is based on princple component analysis. Pheomelanin appears green, and eumelanin appears red.

This work has led to observations of the damage threshold levels of ultrafast laser power for human skin. Thermal damage has been observed with 30mW of power for a highly pigmented *ex vivo* mole and greater than 60mW for lightly pigmented human skin xenographed onto a mouse model. Thermal damage is immediately obvious, but other damage mechanisms such as DNA damage and formation of reactive oxygen species that may occur at lower power levels have long-term effects.

We have quantified eumelanin and pheomelanin morphology using wavelet analysis and other image processing methods to further improve the specificity of the observed melanoma characteristics. This distills information about the scale of the features and the degree of spatial heterogeneity. One of the main characteristics revealed by wavelet analysis is that benign nevi have more intensity at fine scale size. Preliminary data show that wavelet transformation gives greater than 87% accuracy in classification. Adding to our previously published results [3], additional slides have been included to determine threshold eumelanin level for malignant lesions, an optimal 38% eumelanin yields 91% sensitivity and 77% specificity. The combination of these imaging processing methods can potentially improve certainty in melanoma diagnosis.

In summary, we have found that melanin contrast depends on the difference between the pump and probe wavelengths. With this flexibility, we have begun imaging deeper in the NIR. We have imaged thicker slices of tissue and, in the process, gained an idea of the amount of ultrafast laser power that pigmented tissue can withstand. We have started using more sophisticated data processing techniques and found the optimal eumelanin threshold level. Taken together, these results provide new insights on the chemical morphology and biochemical heterogeneity of normal and diseased tissue, and provide valuable diagnostic information.

Acknowledgements

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