In Vivo Quantification of Melanin Mass Density in Human by Using Third Harmonic Generation Microscopy

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Poster Session

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Abstract

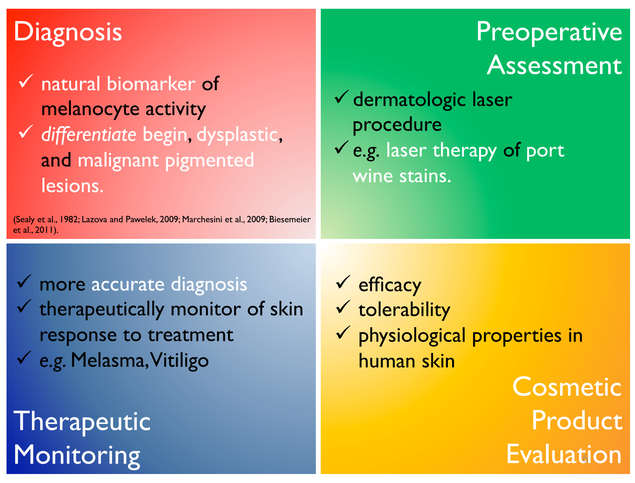
Melanin is the primary determinant of human skin color and can be accessed noninvasively by optical means. The melanosome is a unique membrane-bound organelle where melanin biosynthesis takes place. Melanosomes can be divided into eumelanosomes and pheomelanosomes depending on the type of melanin synthesized: either eumelanin or pheomelanin. Eumelanin is the dominant component of human epidermal melanin, representing more than 90% of total epidermal melanin. Melanin deficiency has been connected with various genetic abnormalities and disease states. Melanin can also serve as an endogenous marker to assist the diagnosis of pigmented skin diseases, including melanoma. Melanoma is less common than other skin cancers, but causes the majority of deaths related to skin cancer. In this work, by calibrating the third-harmonic generation (THG) enhancement ratio versus eumelanin mass density in live melanocytes, we realized, for the first time, in vivo quantification imaging of melanin mass density in human by using the noninvasive THG microscopy, together with a high penetration capability and a submicron 3D spatial resolution. Calibration process was performed by using live melanoma cells. Clinical trials, in vivo imaging on human skin including different skin types and melanin-deficient skin, were conducted. Comparison of our in vivo results with published ex vivo data is performed and high consistency is obtained. This work is sponsored by National Health Research Institute.

Introduction

Human Melanin

- Primary determinant of human skin color, physical barrier to protect from UVR
- Assist the diagnosis of pigmented skin diseases, including melanoma, hyper/hypomelanosis
- Early diagnosis reduce mortality and morbidity
- Divided into eumelanin and pheomelanin depending on the type of melanosome producing melanin
- Eumelanin is the dominant component of human epidermal melanin, representing more than 90% of total epidermal melanin (even in the lightest skin)

Quantification



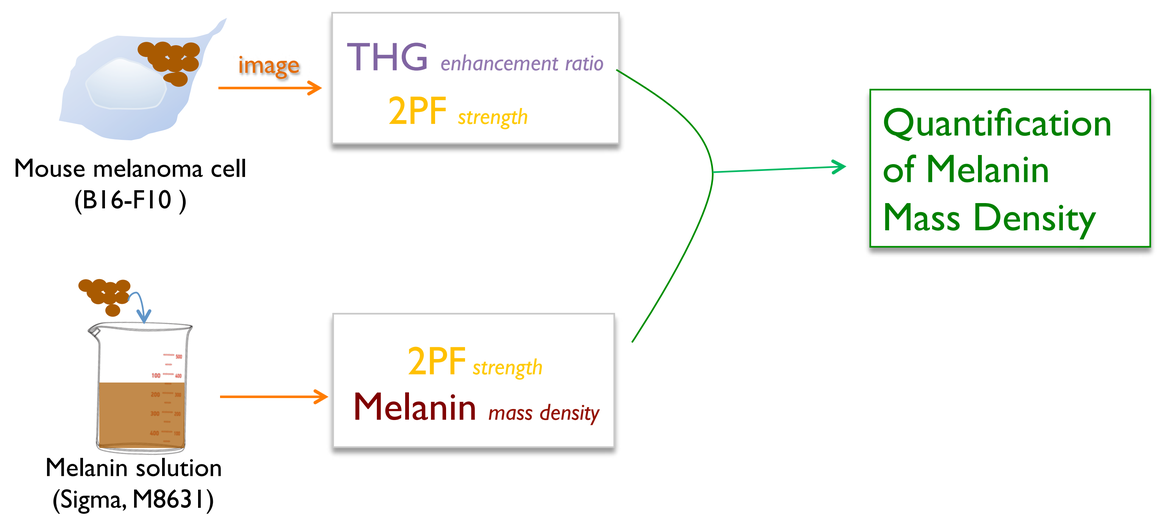
Previous Study

- *In vivo* imaging - TPEFM / PPM / RCM / PAM / HGM
- *In vivo* differntiation of eu/ pheomelanin - HPLC / AS / RS / TPEFM / PPM
- But no one can provide the information of melanin content with absolute estimation values with depth information

Harmonic Generation Microscopy (HGM)

- Harmonic generation microscopy provides imaging capability in human skin
- Melanin Enhanced THG in Cytoplasm
- Melanin content is highest in the basal layer of human skin
  Lower melanin content in the spinous and granular layers

Materials and Methods

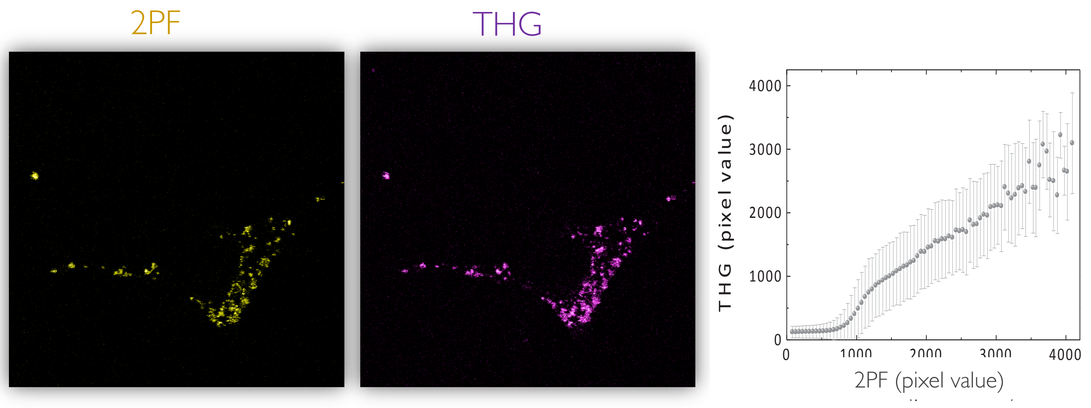


- Commercial available Leica Microsystems (LEICA TCS SP5) integrating a multi-photon microscope and a harmonic generation microscope were utilized to take images
- The excitation laser source was tuned to 1230 nm and focused to the sample using a high numerical aperture (NA) water-immersion objective

Result

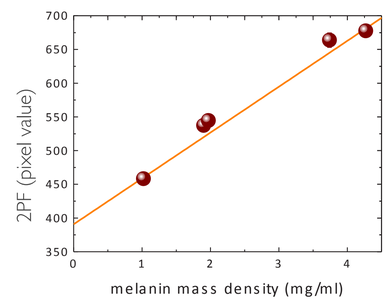
Simultaneous THG and TPEF Images of Living B16 Melanoma Cells

Endogenous epi-TPEF and epi-THG images are recorded simultaneously and the roughly positive correlation between THG and TPEF can be observed as shown in Figure.



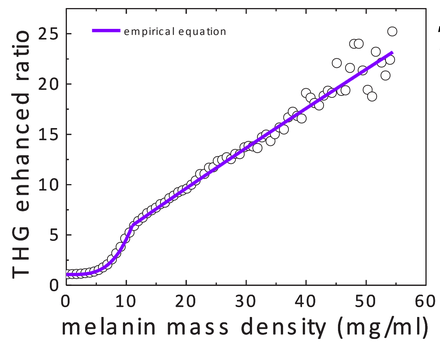
Melanin Mass Density (MMD) Calibration

- Melanin dispersed in a solvent with difference mass densities up to 4.27 mg/ml was utilized to calibrate the quantity of melanin content.
- Connecting TPEF signal to MMD by taking TPEF images of artificial melanin samples.
- A strong linear relation ($R^2 = 0.99$) between measured TPEF intensity and melanin mass density is observed.
- $TPEF = 68.00948 \times MMD + 390.75773$



Empirical correlation between MMD and THG enhancement

- MMD = 0 -> Background THG
- Calculate THG enchantment ratios



$$THG\ enhancement\ ratio = \begin{cases} 1.19\times10^{-3}\ MMD^{3.47} + 1.06 & for\ MMD \le 11.00\ mg/ml \\ 5.04\times10^{-1} \times MMD^{0.95} + 1.06 & for\ MMD > 11.00\ mg/ml \end{cases}$$

Conclusion

In this study, the relation between the THG enhanced ratio and MMD was constructed to quantify the melanin in human skin by using HGM. This is the first time to obtain *in vivo* human imaging and quantitative information of melanin at the same time. HGM with high penetration depth, high resolution, noninvasive nature and melanin quantification ability therefore becomes a promising technique for application such as disease prognosis, therapeutically monitoring, follow-up and cosmetic product evaluation.

Reference

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