Cutaneous melanin exhibiting fluorescence emission under near-infrared light excitation

Zhiwei Huang*
Haishan Zeng
British Columbia Cancer Research Centre
Cancer Imaging Department
675 West Tenth Avenue
Vancouver, British Columbia
V5Z 1L3 Canada
E-mail: hzeng@bccrc.ca

Iltefat Hamzavi
Abdulmajeed Alajlan
Eileen Tan
David I. McLean
Harvey Lui
University of British Columbia
Department of Dermatology and Skin Science
Vancouver Coastal Health Research Institute
835 West Tenth Avenue
Vancouver, British Columbia
V5Z 4E8 Canada

Abstract. Under ultraviolet and visible light excitation, melanin is essentially a nonfluorescent substance. This work reports our study on near-infrared (NIR) fluorescence properties of melanins, and explores potential applications of NIR fluorescence techniques for evaluating skin disorders involving melanin. The NIR fluorescence spectrum is obtained using a fiber optic NIR spectrometer under 785-nm laser excitation. *In vitro* measurements are performed on synthetic dihydroxyphenylalanine (DOPA) melanin, melanin extracted from *Sepia* ink sacs, human hair, animal fur, and bird feathers. Paired spectral comparisons of white and black skin appendages show that melanization of hair, fur, or feathers more than doubles the NIR fluorescence. *In vivo* NIR autofluorescence of normal dorsal and volar forearm skin of 52 volunteers is measured. Dorsal forearm skin, which is darker than volar skin, exhibits significantly greater NIR fluorescence. Patients with vitiligo ($n=4$), compound nevus ($n=3$), nevus of Ota ($n=1$), superficial spreading melanoma ($n=3$), and postinflammatory hyperpigmentation ($n=1$) are also evaluated. NIR fluorescence is greater within the lesion than the surrounding normal skin for all these conditions except vitiligo, where the converse was true. The observed melanin NIR fluorescence provides a new approach to *in vitro* and *in vivo* melanin detection and quantification that may be particularly useful for evaluating pigmented skin lesions. [DOI: 10.1117/1.2204007]

Keywords: melanin; near-infrared fluorescence; tissue spectroscopy; *in vivo* diagnosis; skin.

Paper 05224RR received Aug. 5, 2005; revised manuscript received Jan. 13, 2006; accepted for publication Jan. 16, 2006; published online May 23, 2006.

1 Introduction

Over the last decade, optical spectroscopy has rapidly evolved as an *in vivo* tool for diagnosis and characterization of human skin.[1–6] Like all biological tissues, the skin is composed of biomolecules with specific biophysical properties that are determined by their chemical structures and microscopic environments. The biophysical properties of cutaneous chromophores such as melanin, hemoglobin, collagen, keratin, and lipids can be studied noninvasively and *in vivo* using a variety of optical spectroscopic techniques such as diffuse reflectance, fluorescence, and Raman scattering.[2,4,7–13]

Melanins are ubiquitous pigments that largely determine skin, hair, and eye color. Functionally, melanins appear to play somewhat conflicting roles in terms of serving as natural sunscreens while potentially also initiating skin cancers.[14–16] The unique photochemical and photobiological properties of melanins in the skin have been analyzed by optical spectroscopy.[8,17–21] Among cutaneous chromophores, melanin is unusual in that its absorption decreases monotonically with increasing wavelengths from 300 to 1100 nm with an absence of discrete spectral bands or peaks for identifying its structural subunits.[17,20,22] Nevertheless, some structural information about melanin can be derived from visible or near-infrared (NIR) absorption spectroscopy.[13,17] Spectroscopy that assesses the apparent absorption of tissue derived from reflectance measurements has been used as the basis for skin cancer diagnosis. In this instance, diagnosis is based on analyzing the absorbance of melanin, oxyhemoglobin (HbO_2), deoxyhemoglobin (Hb), and water (H_2O) from 400 to 1100 nm within suspicious skin lesions.[1,23,24]

Fluorescence spectra of biologic tissues may often be more informative than optical absorption in terms of molecular structure and functional groups. Moreover, the fluorescence of specific tissue components is strongly influenced by the microenvironment.[18,19,25] Under ultraviolet (UV) or short visible (VIS) light excitation melanin is a relatively low quantum yield fluorophore, and any fluorescence that it does emit is presumably attenuated by melanin's broad spectral absorption.[19] Because of this optical behavior under UV-VIS light excitation, melanin has previously been regarded as a nonfluorescent pigment that is distinct from the autofluorescent lipopigment family.[25,26] However, we have observed that the wavelength of excitation is important in obtaining fluores-

*Current affiliation: National University of Singapore, Singapore 11576.

Address all correspondence to Haishan Zeng, Cancer Imaging Dept., British Columbia Canger Research Centre, 675 West 10th Avenue, Vancouver, BC V5Z 1L3 Canada; Tel: 604–675–8083; Fax: 604–675–8099; E-mail: hzeng@bccrc.ca

1083-3668/2006/11(3)/034010/6/$22.00 © 2006 SPIE

cence from melanin, which may explain why others have regarded melanin as a nonfluorescent pigment.

In this study, we extend the previous work of our group and others on UV/visible autofluorescence[2,3,27] to the NIR domain by studying melanin samples *in vitro* as well as normal, sun-exposed, and diseased human skin *in vivo* under 785-nm laser excitation. Melanins within skin appendages such as hair and feathers were also evaluated. We have observed that synthetic and natural melanins exhibit significant fluorescence emission under near-infrared (NIR) light excitation at 785 nm, which is strikingly different from their apparent nonfluorescent behavior under UV-VIS light excitation.

2 Materials and Methods

This study was approved by the Clinical Research Ethics Board of the University of British Columbia. NIR autofluorescence spectra were acquired from *in vitro* melanin as well as from hair, feathers, and *in vivo* human skin.

2.1 *Melanin, Hair, and Feather Samples*

Natural melanin isolated from cuttlefish ink sacs of *Sepia officinalis* (M2649) and synthetic DOPA-melanin (M8631) were obtained from the Sigma-Aldrich Company, Saint Louis, Missousri. Hair samples were obtained from ten human volunteers (black hair=5, white hair=5) and a domestic house cat with black and white fur. Black and white chicken feathers were also measured.

2.2 *Human Volunteers*

In vivo NIR autofluorescence spectroscopy was carried out on 52 healthy volunteers by measuring and comparing spectra from the sun-exposed, dorsal forearm and the less sun-exposed volar forearm skin of each subject. Patients with the following skin disorders involving melanin were also evaluated by comparing NIR spectra from the abnormal skin lesions to that of the immediately surrounding normal skin: vitiligo ($n=4$), benign compound melanocytic nevus ($n=3$), nevus of Ota ($n=1$), malignant melanoma ($n=3$), and postinflammatory hyperpigmentation ($n=1$). Visible blue light autofluorescence spectra were also acquired in parallel from these skin lesions.

2.3 *Instrumentation*

The spectrometer employed for NIR autofluorescence measurement has previously been described in detail.[4] This system comprises a diode laser at 785 nm (maximum output 300 mW, SDL Incorporated, San Jose, California); a transmissive imaging spectrograph (HoloSpec f/2.2-NIR, Kaiser Optical Systems Incorporated, Ann Arbor, Michigan) with a volume phase technology (VPT) holographic grating (HSG-785-LF, Kaiser Optical Systems Incorporated, Ann Arbor, Michigan); a NIR-optimized back-illuminated, deep-depletion charge-coupled device (CCD) detector (LN/CCD-EEV 1024 $\times$ 256, QE $\geq$ 75% at 900 nm, Princeton Instruments, Trenton, New Jersey); and a specially designed skin fiber-optic probe.[4] This skin probe comprising two optical arms was designed to maximize the collection of tissue NIR fluorescence signals while reducing the interference of Rayleigh scattered light and fiber fluorescence. One optical arm of the probe integrating a collimating lens, a bandpass filter (785 $\pm$ 2.5 nm), and a focusing lens delivers the laser light onto the skin surface with a spot size of 3.5 mm. The other optical arm with collimating and refocusing lenses and a holographic notch plus filter (OD $>$ 6.0 at 785 nm, Kaiser Optical Systems Incorporated, Ann Arbor, Michigan) is used for collecting tissue NIR autofluorescence emission. The holographic notch filter was placed between the two lenses to block the Rayleigh scattered excitation laser light while allowing the NIR autofluorescence signals to pass. The refocusing lens then focused the filtered beam onto the circular end of the fiber bundle (58 $\times$ 100-μm core diameter fibers, NA =0.22). The 785-nm laser is coupled to a 200-μm core diameter fiber (NA=0.22), which in turn is connected to the skin probe via an SMA connector. The NIR autofluorescence that is collected by the fiber bundle probe passes through an integrated holographic notch plus filter (OD $>$ 6.0 at 785 nm, Kaiser Optical Systems Incorporated) and is then fed into the transmissive spectrograph. The holographic grating disperses the incoming light onto the liquid nitrogen-cooled, PC-controlled CCD detector. NIR autofluorescence spectra are displayed on the computer screen in real time and can be saved for further analysis. The system acquires spectra over the range 820 to 920 nm, each within 1 s at an incident irradiance of 1.56 W/cm^2. For visible autofluorescence measurements,[28] a separate system was used consisting of a portable spectrometer equipped with a 2048-element CCD array detector (S2000-FL spectrometer, Ocean Optics Incorporated, Dunedin, Florida), a high-pressure mercury light source (M60100, Oriel Instruments, Stratford, Connecticut), and a quartz Y-type fiber optic skin probe (R200-7, Ocean Optics Incorporated, Dunedin, Florida). The mercury light source was filtered to generate blue light (437 $\pm$ 10 nm band; power=9.5 mW) for fluorescence excitation, and the handheld fiber optic probe was used to conduct both the excitation light to the skin and the emitted fluorescence to the spectrometer. Visible autofluorescence emission spectra were measured from 460 to 750 nm. All spectral measurements were performed in a dark room, and all spectra (including both NIR and visible) were corrected for the wavelength dependence of their respective spectrometer systems using a standard lamp (RS-10, EG&G Gamma Scientific, San Diego, California).

2.4 *Statistical Analysis*

To evaluate the mean difference in visible and NIR autofluorescence between the dorsal and volar forearm measurements, paired student t-tests were applied at each recorded wavelength. A two-sided alpha of <0.05 was considered significant.

3 Results

Solid-state *in vitro* melanins (*Sepia* and synthetic DOPA melanins) exhibit prominent fluorescence in the NIR range under 785 nm excitation [Fig. 1(a)], with the spectrum being slightly higher in intensity at longer emission wavelengths. The two broadbands with peaks near 880 and 890 nm represent Raman scattering by melanin.[29] NIR-excited autofluorescence from black human hair, feline fur, and chicken feathers, which are replete with melanin [Figs. 1(b)–1(d)], is similar to that of *Sepia* and synthetic melanin. White hair and white feathers, which presumably differ from their black counter-

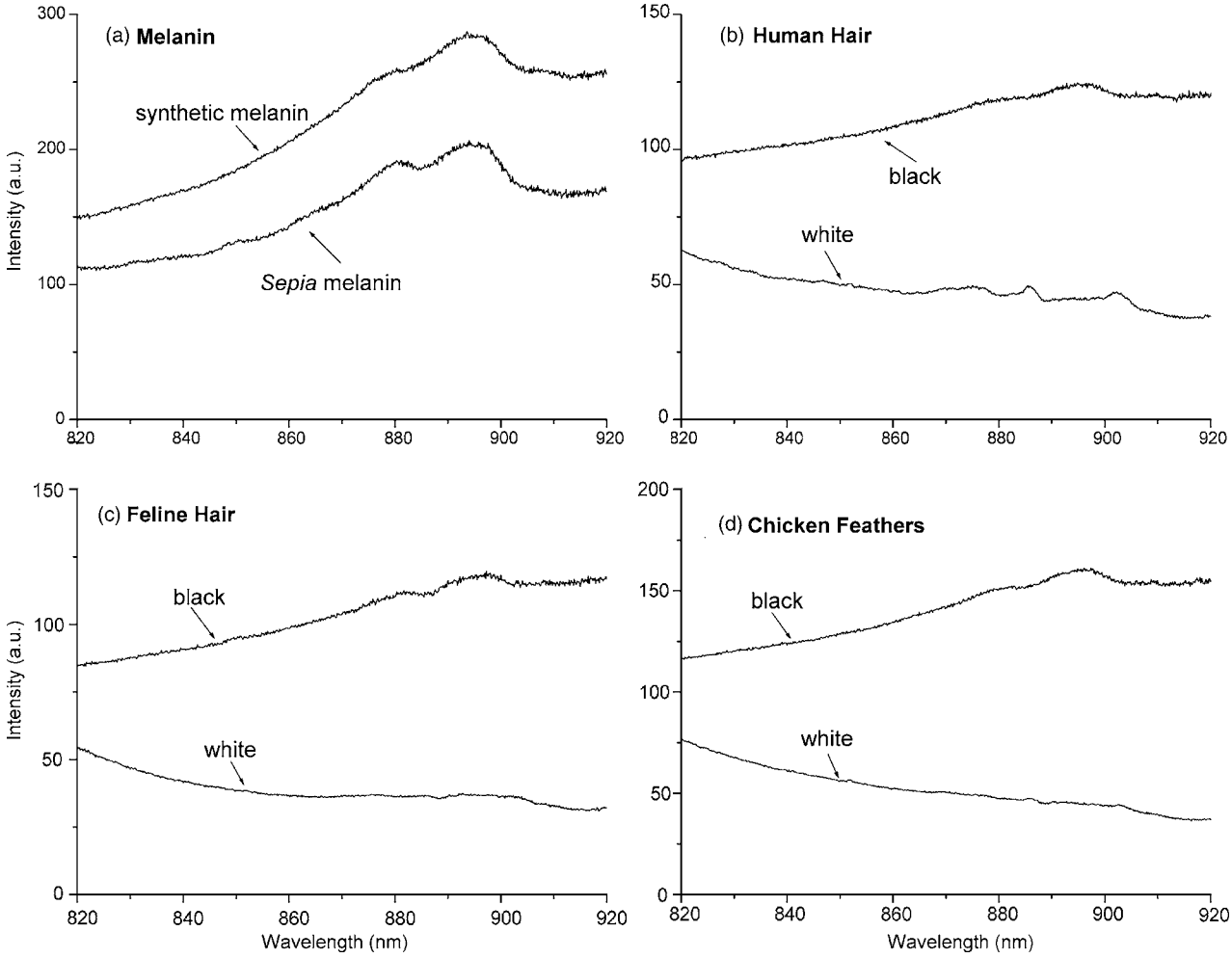

Fig. 1 NIR autofluorescence spectra (820 to 920 nm) obtained from: (a) *Sepia* and synthetic DOPA melanin; (b) human hair; (c) feline fur; and (d) chicken feathers. Excitation wavelength 785 nm; light intensity 170 mW; and integration time 1 s.

parts only in terms of their relative absence of eumelanin, exhibit a markedly lower NIR autofluorescence signal.

The visibly discernible differences in the degree of skin melanization between darker dorsal and lighter volar forearm skin of normal volunteers could be demonstrated by *in vivo* NIR autofluorescence and the corresponding autofluorescence difference spectrum [Fig. 2(a)], with the dorsal forearm showing greater autofluorescence than that of the volar aspect in the 820- to 920-nm range ($p < 0.0001$ at all wavelengths). Consistent with this finding was the observation that the depigmented skin of vitiligo lesions had a much lower NIR autofluorescence intensity than that of the normally pigmented surrounding skin [Figs. 2(b)]. Furthermore, the spectra revealed the presence of several embedded Raman signals (e.g., 857, 871, 874, 885, 901, and 909 nm) from both pigment-bearing (normal) and amelanotic (vitiligo) skin. These signals are due to cutaneous proteins and lipids,[4] and their consistent presence in all skin measurements would be expected, since these components are unaffected by vitiligo.

Dark-colored skin lesions due to abnormal hypermelanization (i.e., benign compound nevus, nevus of Ota, melanoma, and postinflammatory hyperpigmentation) all showed higher NIR autofluorescence when compared to the adjacent normal skin [Figs. 2(c)–2(f)]. For heavy pigmented skin lesions, the difference spectra in Figs. 2(c)–2(f) showed an increasing trend of intensity at longer wavelengths, which is in consistent with NIR fluorescence spectra observed from synthetic or extracted melanin samples and melanin-rich black hair or black feathers (Fig. 1). Therefore, melanin is an important fluorophore accounting for *in vivo* skin in the NIR range 820 to 920 nm.

In contrast to NIR-excited skin autofluorescence, visible autofluorescence is lower in the presence of cutaneous melanin, as in the case of a pigmented compound melanocytic nevus versus normal skin under blue light excitation at 437 nm [Fig. 3(a)]. Similarly, normal skin had lower visible autofluorescence than depigmented patches of vitiligo [Fig. 3(b)].

4 Discussion

Although the diagnostic application of cutaneous autofluorescence has been studied intensively over the last decade, most studies have focused on excitation wavelengths ranging between ultraviolet (UV) and shorter wavelength visible light.[2,5,12,28,30–34] A very limited number of autofluorescence

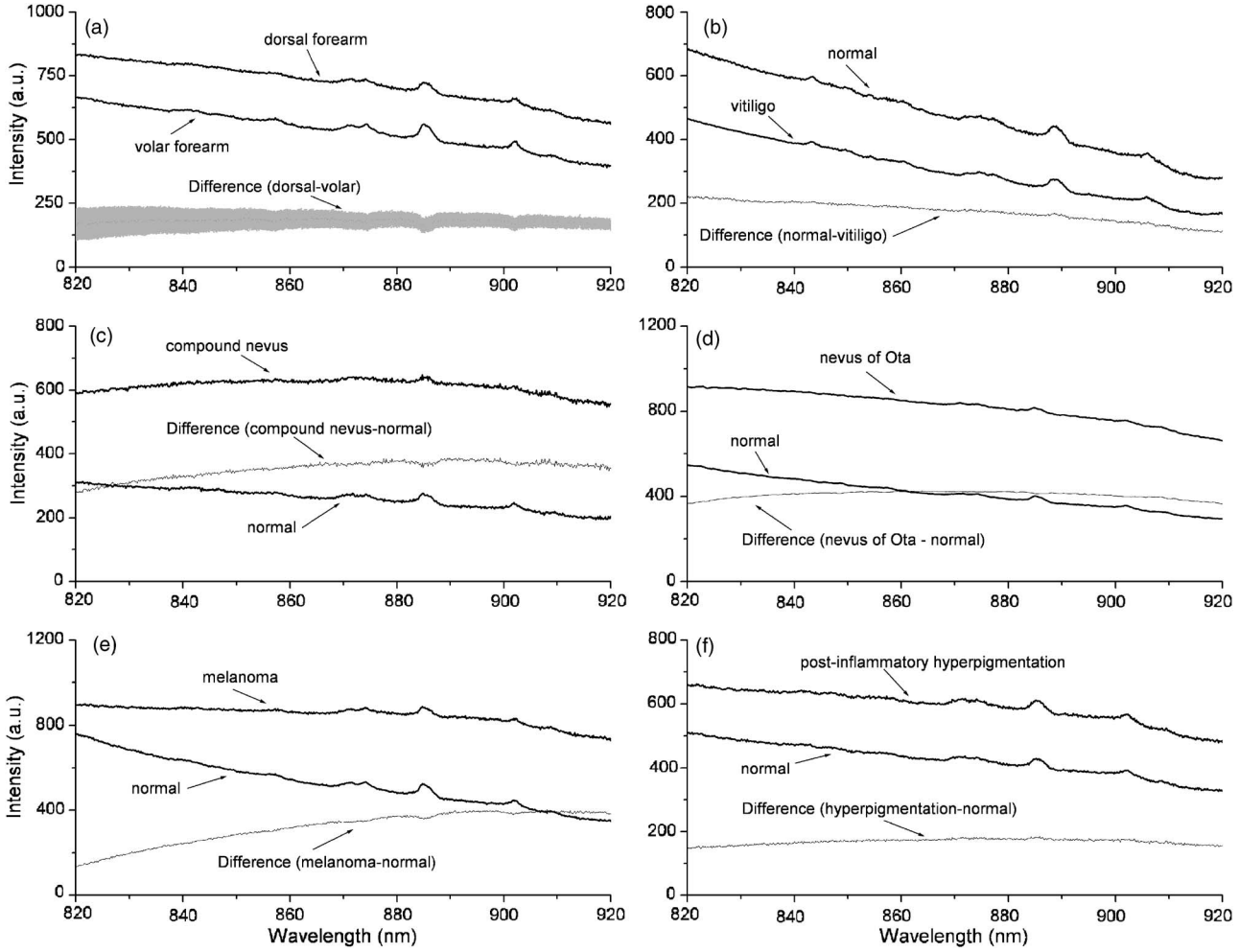

Fig. 2 Paired mean NIR autofluorescence spectra (820 to 920 nm) and the corresponding mean difference spectra obtained from: (a) dorsal and volar sites of the forearm [gray curve is the mean difference spectrum ± SD (n=52)] and skin lesions and their normal surrounding skin sites; (b) vitiligo (n=4); (c) compound melanocytic nevus (n=3); (d) nevus of Ota (n=1); (e) superficial spreading melanoma (n=3); and (f) postinflammatory hyperpigmentation (n=1). Excitation wavelength 785 nm; light intensity 170 mW; and integration time 1 s.

studies have utilized longer red-to-NIR wavelengths, but these were mostly centered on examining tissue fluorescence in relation to porphyrins.[35–37]

We have characterized the NIR autofluorescence properties of melanin obtained by chemical synthesis and tissue extraction, as well as through direct *in vivo* or *in vitro* measurements of skin, melanin-containing appendages (e.g., human hair, feline fur, and chicken feathers), and partially purified melanin. It has been shown by observation and histochemical methods that regardless of ethnicity, epidermal melanin content is significantly greater in chronically sun-exposed skin than it is in corresponding sun-protected skin sites (up to two-fold).[15,21] In this study, sun-exposed skin (dorsal forearm) exhibited significantly more NIR autofluorescence compared to sun-protected skin (volar forearm), confirming that NIR autofluorescence of epidermal melanin parallels the visible degree of pigmentation. The results clearly demonstrate the ability of melanin from a variety of biological sources to fluoresce positively under NIR excitation in a measurable fashion. In contrast, both visible absorption and fluorescence spectroscopy rely on the notion that the relative *absence* of a spectral signal

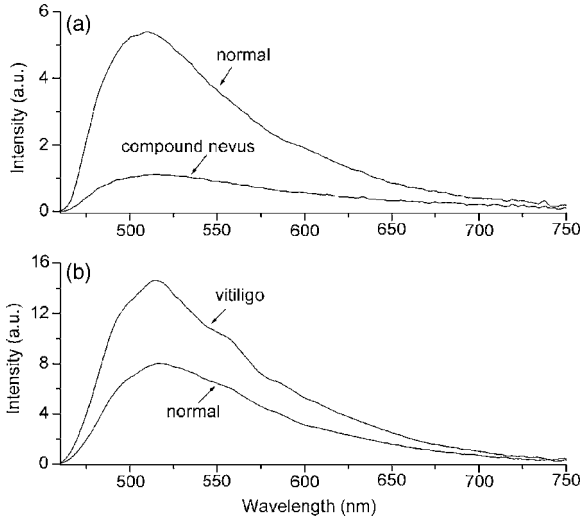

Fig. 3 Paired autofluorescence spectra in the visible range (460 to 750 nm) obtained from skin lesions and their normal surrounding skin site: (a) compound melanocytic nevus, and (b) vitiligo. Excitation wavelength 437 nm±10 nm; light intensity 9.5 mW; and integration time 0.1 s.

implies the *presence* of melanin, and that the degree of suppression of a signal can be used to indirectly estimate the quantity of melanin present.[7,8] The ability to measure a "positive" optical signal in the presence of melanin within the NIR excitation domain thus provides a fundamentally different and more direct approach for *in vitro* and *in vivo* melanin detection.

That melanin exhibits strong NIR autofluorescence and Raman emission under NIR wavelength excitation is perhaps surprising, given the conventional acceptance that melanin was essentially nonfluorescent when measured in the UV and short visible wavelengths. The biophysical basis for melanin autofluorescence remains largely unknown. It has been suggested that it may be induced by partial oxidative breakdown of melanin, and therefore depends on structurally defective melanin polymers.[19,38] Our data show that prominent NIR autofluorescence emission appears to be a common feature of natural and synthetic melanins, and that melanin is one of the major fluorophores responsible for cutaneous NIR autofluorescence. The observation that the melanin-poor vitiligo lesions still exhibit significant NIR autofluorescence confirms that other fluorophores such as hemoglobin, porphyrin, collagen, etc. in the skin also contribute to cutaneous NIR autofluorescence.[4,29]

The NIR fluorescence line shape of white hair, white feather, and light colored skin tissue observed in this study is similar to that of other tissue types (e.g., nonskin tissues with no melanin content) reported in the literature.[36] The common feature of this fluorescence line shape is that the fluorescence intensity monotonically decreases with wavelength and shows no maxima. In comparison, the NIR fluorescence line shape of melanin (Fig. 1) is quite unique in that the fluorescence intensity increases with wavelength and appears to have a maximum. A complete understanding of these phenomena warrants further investigations.

In Fig. 2, all the difference spectra have positive values, confirming the significant contributions of melanin to *in vivo* skin tissue NIR fluorescence. However, one may note that in Fig. 2, not all the difference spectra follow the line shape of the pure melanin spectrum. The difference spectrum between normal and vitiligo (normal–vitiligo) does not show a maximum and is slightly titled toward the shorter wavelength end. We are building a tissue optics model and planning a Monte Carlo simulation approach to under this phenomenon. Tentatively, we have the following explanation for this phenomenon.

We use the "normal–vitiligo" difference spectrum as an example and assume that the only difference between vitiligo and its surrounding normal skin is melanin. Due to the lack of melanin absorption in vitiligo, excitation light can penetrate deeper down into the tissue than in normal skin; re-emitted fluorescence will also be easier to escape. Thus, when measuring vitiligo we are sampling a larger tissue volume and with higher detection efficiency than measuring normal skin. We are getting more nonmelanin-related fluorescence signal from vitiligo than from normal skin. The proportion of this extra nonmelanin related fluorescence signal should increase with wavelength, since longer wavelength fluorescence photons have higher chances to escape out of the tissue. On the other hand, melanin adds a strong fluorescence signal to the normal skin. Therefore, the line shape of the difference spectrum (normal–vitiligo) will not be exactly the same as the fluorescence spectrum of pure melanins. Instead, it will be determined by the balance between the extra melanin fluorescence signal generated in normal skin and the extra nonmelanin-related fluorescence signal detected from vitiligo. Similar considerations can also be applied to other spectral pairs shown in Fig. 2. Therefore, for heavy pigmented lesions [Figs. 2(c) and 2(e)], the difference spectra have a shape more close to that of the pure melanin, while for more lightly pigmented lesions [such as normal skin versus vitiligo in Fig. 2(b)], the difference spectra deviate significantly from a pure melanin spectral shape and the maximum may disappear. We expect that our Monte Carlo modeling, which accurately accounts for all the tissue optics effects on the difference spectra, will either confirm this consideration or slightly modify it.

We have observed two obvious characteristic Raman bands near 880 and 895 nm from melanin samples, black hair, and black feather (Fig. 1). For *in vivo* spectra of melanin-rich skin lesions shown in Fig. 2, these two Raman bands are not very obvious. They may have been masked, to a certain extent, by other strong Raman signals that originate from other biomolecules, such as proteins, lipids, porphyrin, etc., in skin.[4,29,39] However, if we remove the fluorescence background from these *in vivo* spectra and concentrate on looking at the Raman signals, the melanin's contribution to the total Raman signals becomes more evident (data not shown).

Changes in NIR autofluorescence spectra directly correspond to visible differences arising from melanin in pathological conditions such as vitiligo, malignant melanoma, benign compound melanocytic nevi, nevus of Ota, and inflammatory hyperpigmentation. Thus, NIR autofluorescence spectroscopy can potentially capture differences in melanins for pigmented skin lesions *in vivo*. It appears that the NIR autofluorescence technique may be particularly beneficial to the diagnosis of pigmented lesions, because these dark skin lesions can be lit up under NIR excitation. With UV/VIS excitation, melanin largely behaves as a photon absorber (Fig. 3), making visible fluorescence or reflectance measurements less informative. As previously mentioned, a "positive" NIR autofluorescence signal may be particularly useful for noninvasive analysis of lesions such as malignant melanoma, which can be difficult to differentiate from other pigmented but nonmelanin containing lesions using UV-VIS fluorescence or reflectance (i.e., apparent absorption) spectroscopic techniques.[24]

5 Conclusions

In aggregate, our results demonstrate that under NIR excitation, melanins exhibit prominent autofluorescence within the skin and its pigmented appendages, whereas with UV-visible excitation, any potential melanin fluorescence is essentially undetectable. Practical applications for melanin quantification and diagnostic evaluation of pigmented skin lesions are supported by this observation. Furthermore, it appears clear that the fluorescence properties of melanins are highly wavelength dependent, which in turn merits further detailed exploration.

Acknowledgments

The authors wish to thank Dr. Jerry Shapiro and Ms. Michelle Zeng for their assistance with the experiments. This work was

supported by the National Cancer Institute of Canada with funds from the Canadian Cancer Society, the Canadian Dermatology Foundation, and the VGH & UBC Hospital Foundation In It for Life Fund.

References

1. R. Marchesini, M. Brambilla, C. Clemente, M. Maniezzo, A. E. Sichirollo, A. Testori, D. R. Venturoli, and N. Cascinelli, "*In vivo* spectrophotometric evaluation of neoplastic and non-neoplastic skin pigmented lesions - I. Reflectance measurements," *Photochem. Photobiol.* **53**, 77–84 (1991).
2. H. Zeng, C. MacAulay, D. I. McLean, and B. Palcic, "Spectroscopic and microscopic characteristics of human skin autofluorescence emission," *Photochem. Photobiol.* **61**, 639–645 (1995).
3. L. Brancaleon, A. J. Durkin, J. H. Tu, G. Menaker, J. D. Fallon, and N. Kollias, "*In vivo* fluorescence spectroscopy of nonmelanoma skin cancer," *Photochem. Photobiol.* **73**, 178–183 (2001).
4. Z. Huang, H. Zeng, I. Hamzavi, D. I. McLean, and H. Lui, "Rapid near-infrared Raman spectroscopy system for real-time *in vivo* skin measurements," *Opt. Lett.* **26**, 1782–1784 (2001).
5. N. Kollias and G. N. Stamatas, "Optical non-invasive approaches to diagnosis of skin diseases," *J. Investig. Dermatol. Symp. Proc.* **7**, 64–75 (2002).
6. P. J. Caspers, G. W. Lucassen, and G. J. Puppels, "Combined *in vivo* confocal Raman spectroscopy and confocal microscopy of human skin," *Biophys. J.* **85**, 572–580 (2003).
7. N. Kollias and A. Baqer, "Spectroscopic characteristics of human melanin *in vivo*," *J. Invest. Dermatol.* **85**, 38–42 (1985).
8. N. Kollias and A. Baqer, "On the assessment of melanin in human skin *in vivo*," *Photochem. Photobiol.* **43**, 49–54 (1986).
9. R. Gillies, G. Zonios, R. R. Anderson, and N. Kollias, "Fluorescence excitation spectroscopy provides information about human skin *in vivo*," *J. Invest. Dermatol.* **115**, 704–707 (2000).
10. G. Zonios, J. Bykowski, and N. Kollias, "Skin melanin, hemoglobin, and light scattering properties can be quantitatively assessed *in vivo* using diffuse reflectance spectroscopy," *J. Invest. Dermatol.* **117**, 1452–1457 (2001).
11. K. J. Jeon, S. J. Kim, K. K. Park, J. W. Kim, and G. Yoon, "Noninvasive total hemoglobin measurement," *J. Biomed. Opt.* **7**(1), 45–50 (2002).
12. N. Kollias, G. Zonios, and G. N. Stamatas, "Fluorescence spectroscopy of skin," *Vib. Spectrosc.* **28**, 17–23 (2002).
13. A. Matas, M. G. Sowa, G. Taylor, and H. H. Mantsch, "Melanin as a confounding factor in near infrared spectroscopy of skin," *Vib. Spectrosc.* **28**, 45–52 (2002).
14. M. R. Chedekel, "Photochemistry and photobiology of epidermal melanins," *Photochem. Photobiol.* **35**, 881–885 (1982).
15. S. Alaluf, D. Atkins, K. Barrett, M. Blount, N. Carter, and A. Heath, "Ethnic variation in melanin content and composition in photoexposed and photoprotected human skin," *Pigment Cell Res.* **15**, 112–118 (2002).
16. J. P. Ortonne, "Photoprotective properties of skin melanin," *Br. J. Dermatol.* **146**, 7–10 (2002).
17. I. A. Menon, S. Persad, H. F. Haberman, and C. J. Kurian, "A comparative study of the physical and chemical properties of melanins isolated from human black and red hair," *J. Invest. Dermatol.* **80**, 202–206 (1983).
18. S. D. Kozikowski, L. J. Wolfram, and R. R. Alfano, "Fluorescence spectroscopy of eumelanins," *IEEE J. Quantum Electron.* **QE-20**, 1379–1382 (1984).
19. J. M. Gallas and M. Eisner, "Fluorescence of melanin-dependence upon excitation wavelength and concentration," *Photochem. Photobiol.* **45**, 595–600 (1987).
20. N. Kollias and A. H. Baqer, "Absorption mechanisms of human melanin in the visible, 400-720 nm," *J. Invest. Dermatol.* **89**, 384–388 (1987).
21. S. Alaluf, D. Atkins, K. Barrett, M. Blount, N. Carter, and A. Heath, "The impact of epidermal melanin on objective measurements of human skin color," *Pigment Cell Res.* **15**, 119–126 (2002).
22. M. Shimada, Y. Yamada, M. Itoh, and T. Yatagai, "Melanin and blood concentration in human skin studied by multiple regression analysis: experiments," *Phys. Med. Biol.* **46**, 2385–2395 (2001).
23. R. Marchesini, N. Cascinelli, M. Brambilla, C. Clemente, L. Mascheroni, E. Pignoli, A. Testori, and D. R. Venturoli, "*In vivo* spectrophotometric evaluation of neoplastic and non-neoplastic skin pigmented lesions - II: Discriminant analysis between nevus and melanoma," *Photochem. Photobiol.* **55**, 515–522 (1992).
24. M. Elbaum, A. W. Kopf, H. S. Rabinovitz, R. G. Langley, H. Kamino, M. C. Mihm Jr, A. J. Sober, G. L. Peck, A. Bogdan, D. Gutkowicz-Krusin, M. Greenebaum, S. Keem, M. Oliviero, and S. Wang, "Automatic differentiation of melanoma from melanocytic nevi with multispectral digital dermoscopy: a feasibility study," *J. Am. Acad. Dermatol.*, **44**, 207–218 (2001).
25. M. Elleder and J. Borovansky, "Autofluorescence of melanins induced by ultraviolet radiation and near ultraviolet light. A histochemical and biochemical study," *Histochem. J.* **33**, 273–281 (2001).
26. M. Senba, "Staining properties of melanin and lipofuscin pigments," *Am. J. Clin. Pathol.* **86**, 556–557 (1986).
27. L. Brancaleon, G. Lin, and N. Kollias, "The *in vivo* fluorescence of tryptophan moieties in human skin increases with UV exposure and is a marker for epidermal proliferation," *J. Invest. Dermatol.* **113**, 977–982 (1999).
28. H. Zeng, C. MacAulay, D. I. McLean, H. Lui, and B. Palcic, "Miniature spectrometer and multi-spectral imager as a potential diagnostic aid for dermatology," *Proc. SPIE* **2387**, 57–61 (1995).
29. Z. Huang, H. Lui, X. K. Chen, D. I. McLean, and H. Zeng, "Raman spectroscopy of *in vivo* cutaneous melanin," *J. Biomed. Opt.* **9**(6), 1198–1205 (2004).
30. K. T. Schomacker, J. K. Frisoli, C. C. Compton, T. J. Flotte, J. M. Richter, N. S. Nishioka, and T. F. Deutsch, "Ultraviolet laser-induced fluorescence of colonic tissue: basic biology and diagnostic potential," *Lasers Surg. Med.* **12**, 63–78 (1992).
31. H. Zeng, C. MacAulay, B. Palcic, and D. I. McLean, "A computerized autofluorescence and diffuse reflectance spectroanalyser system for *in vivo* skin studies," *Phys. Med. Biol.* **38**, 231–240 (1993).
32. H. Zeng, C. MacAulay, D. I. McLean, and B. Palcic, "Reconstruction of *in vivo* skin autofluorescence spectrum from microscopic properties by Monte Carlo simulation," *J. Photochem. Photobiol., B* **38**, 234–240 (1997).
33. H. Zeng, C. MacAulay, D. I. McLean, B. Palcic, and H. Lui, "The dynamics of laser induced autofluorescence decay in human skin-experimental measurements and theoretical modeling," *Photochem. Photobiol.* **68**, 227–236 (1998).
34. S. K. Chang, M. Follen, A. Malpica, U. Utzinger, G. Staerkel, D. Cox, E. N. Atkinson, C. MacAulay, and R. Richards-Kortum, "Optimal excitation wavelengths for discrimination of cervical neoplasia," *IEEE Trans. Biomed. Eng.* **49**, 1102–1111 (2002).
35. E. B. Hanlon, I. Itzkan, R. R. Dasari, M. S. Feld, R. J. Ferrante, A. C. McKee, D. Lathi, and N. W. Kowall, "Near-infrared fluorescence spectroscopy detects Alzheimer's disease *in vitro*," *Photochem. Photobiol.* **70**, 236–242 (1999).
36. G. Zhang, S. G. Demos, and R. R. Alfano, "Far-red and NIR Spectral wing emission from tissues under 532 and 632 nm photo-excitation," *Lasers Life Sci.* **9**, 1–16 (1999).
37. S. G. Demos, M. Staggs, L. Gandour-Edwards, R. Ramsamooj, and R. White, "Tissue imaging for cancer detection using NIR autofluorescence," *Proc. SPIE* **4613**, 31–34 (2002).
38. S. Ito, "Advances in the pigmentary system," in *Physiology and Pathophysiology*, J. J. Nordlund, R. E. Boissy, V. J. Hearing, R. A. King, and J. P. Ortonne, Eds., pp. 439–450, Oxford University Press, New York (1998).
39. Z. Huang, H. Lui, D. I. McLean, M. Korbelik, and H. Zeng, "Raman spectroscopy in combination with background near-infrared autofluorescence enhances the *in vivo* assessment of malignant tissues," *Photochem. Photobiol.* **81**, 1219–1226 (2005).