Spectral degree of linear polarization of light from healthy skin and melanoma

R. Ceolato, M. Golzio, C. Riou, X. Orlik, N. Riviere

Spectral degree of linear polarization of light from healthy skin and melanoma

Romain Ceolato,[1*] Muriel Golzio,[2,3] Cécile Riou,[1] Xavier Orlik,[1] and Nicolas Riviere[1]

[1]*ONERA, The French Aerospace Lab, FR31000 Toulouse, France*
[2]*Centre National de la Recherche Scientifique, Institut de Pharmacologie et de Biologie Structurale, BP64182, 205 route de Narbonne, F-31077 Toulouse, France*
[3]*Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France*
[*]*romain.ceolato@onera.fr*

Abstract: A non-invasive optical technique, based on a supercontinuum laser source and hyperspectral sensors, is established to measure the spectral degree of linear polarization (DOLP) in a broad spectral range from 525 nm to 1000 nm. Several biomaterials of interest, such as healthy and cancerous skins, are considered. The spectral DOLP of melanoma, from 5 mm to 9 mm diameter, are measured and analyzed. An increase of the spectral DOLP is reported for 100% of the melanoma samples compared to healthy skin samples. The spectral DOLP of a given melanoma appears to be correlated to the stage of its development: the larger the melanoma, the higher the DOLP. Such trend could be explained by a decrease of the surface roughness along the evolution of the disease. In addition, a significant spectral dependence of the DOLP is reported for melanoma samples as it exhibits a decrease in the near infrared from 750 nm to 1000 nm.

OCIS codes: (170.0170) Medical optics and biotechnology; (160.1435) Biomaterials; (120.5410) Polarimetry; (290.5855) Scattering, polarization; (310.6188) Spectral properties.

References and links

<jrn>1. A. Breslow, "Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma," Ann. Surg. **172**(5), 902–908 (1970).</jrn>
<jrn>2. B. P. Hazen, A. C. Bhatia, T. Zaim, and R. T. Brodell, "The clinical diagnosis of early malignant melanoma: expansion of the ABCD criteria to improve diagnostic sensitivity," Dermatol. Online J. **5**(2), 3 (1999).</jrn>
<jrn>3. W. K. Andersen and D. N. Silvers, "'Melanoma? It can't be melanoma!' A subset of melanomas that defies clinical recognition," JAMA, J. Am. Med. Assoc. **266**(24), 3463–3465 (1991).</jrn>
<jrn>4. L. Li, Q. Zhang, Y. Ding, H. Jiang, H. B. H. Thier, and J. Z. Wang, "Automatic diagnosis of melanoma using machine learning methods on a spectroscopic system," BMC Med. Imaging **14**(36) 101186 (2014).</jrn>
<jrn>5. D. S. Gareau, J. Lagowski, V. M. Rossi, J. A. Viator, G. Merlino, M. Kulesz-Martin, and S. L. Jacques, "Imaging Melanoma in a Murine Model Using Reflectance-Mode Confocal Scanning Laser Microscopy and Polarized Light Imaging," J. Investig. Dermatol. Symp. Proc. **10**(2), 164–169 (2005).</jrn>
<jrn>6. J. C. Ramella-Roman, K. Lee, S. A. Prahl, and S. L. Jacques, "Design, testing, and clinical studies of a handheld polarized light camera," J. Biomed. Opt. **9**(6), 1305–1310 (2004).</jrn>
<jrn>7. S. Y. Lu and R. A. Chipman, "Interpretation of Mueller matrices based on polar decomposition," J. Opt. Soc. Am. A **13**(5), 1106–1113 (1996).</jrn>
<jrn>8. R. Ossikovski, A. De Martino, and S. Guyot, "Forward and reverse product decompositions of depolarizing Mueller matrices," Opt. Lett. **32**(6), 689–691 (2007).</jrn>
<jrn>9. H. D. Noble, S. C. McClain, and R. A. Chipman, "Mueller matrix roots depolarization parameters," Appl. Opt. **51**(6), 735–744 (2012).</jrn>
<jrn>10. A. Pierangelo, A. Benali, M.-R. Antonelli, T. Novikova, P. Validire, B. Gayet, and A. De Martino, "Ex-vivo characterization of human colon cancer by Mueller polarimetric imaging," Opt. Express **19**(2), 1582–1593 (2011).</jrn>
<jrn>11. B. Laude-Boulesteix, A. De Martino, B. Drévillon, and L. Schwartz, "Mueller Polarimetric Imaging System with Liquid Crystals," Appl. Opt. **43**(14), 2824–2832 (2004).</jrn>
<jrn>12. J. Chung, W. Jung, M. J. Hammer-Wilson, P. Wilder-Smith, and Z. Chen, "Use of polar decomposition for the diagnosis of oral precancer," Appl. Opt. **46**(15), 3038–3045 (2007).</jrn>

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015

<jrn>13. M. Cemazar, M. Golzio, G. Sersa, P. Hojman, S. Kranjc, S. Mesojednik, M. P. Rols, and J. Teissie, "Control by pulse parameters of DNA electrotransfer into solid tumors in mice," Gene Ther. **16**(5), 635–644 (2009).</jrn>
<jrn>14. R. R. Alfano and S. L. Shapiro, "Emission in the region 4000 to 7000 A via four-photon coupling in glass," Phys. Rev. Lett. **24**(11), 584–587 (1970).</jrn>
<jrn>15. P.-A. Champert, V. Couderc, P. Leproux, S. Février, V. Tombelaine, L. Labonté, P. Roy, C. Froehly, and P. Nérin, "White-light supercontinuum generation in normally dispersive optical fiber using original multi-wavelength pumping system," Opt. Express **12**(19), 4366–4371 (2004).</jrn>
<jrn>16. J. Dudley, G. Genty, and S. Coen, "Supercontinuum generation in photonic crystal fiber," Rev. Mod. Phys. **78**(4), 1135–1184 (2006).</jrn>
<jrn>17. H. Akbari, L. V. Halig, D. M. Schuster, A. Osunkoya, V. Master, P. T. Nieh, G. Z. Chen, and B. Fei, "Hyperspectral imaging and quantitative analysis for prostate cancer detection," J. Biomed. Opt. **17**(7), 076005 (2012).</jrn>
<jrn>18. G. Lu and B. Fei, "Medical hyperspectral imaging: a review," J. Biomed. Opt. **19**(1), 010901 (2014).</jrn>
<jrn>19. C. Zakian, I. Pretty, and R. Ellwood, "Near-infrared hyperspectral imaging of teeth for dental caries detection," J. Biomed. Opt. **14**(6), 064047 (2009).</jrn>
<jrn>20. R. Ceolato, N. Riviere, and L. Hespel, "Reflectances from a supercontinuum laser-based instrument: hyperspectral, polarimetric and angular measurements," Opt. Express **20**(28), 29413–29425 (2012).</jrn>
<jrn>21. R. Ceolato, N. Riviere, R. Jorand, B. Ducommun, and C. Lorenzo, "Light-scattering by aggregates of tumor cells: Spectral, polarimetric, and angular measurements," J. Quant. Spectrosc. Radiat. Transf. **146**, 207–213 (2014).</jrn>
<jrn>22. A. Al-Qasimi, O. Korotkova, D. James, and E. Wolf, "Definitions of the degree of polarization of a light beam," Opt. Lett. **32**(9), 1015–1016 (2007).</jrn>
<jrn>23. D. A. Haner, B. T. McGuckin, and C. J. Bruegge, "Polarization Characteristics of Spectralon Illuminated by Coherent Light," Appl. Opt. **38**(30), 6350–6356 (1999).</jrn>
<jrn>24. F. Boulvert, B. Boulbry, G. Le Brun, B. L. Jeune, S. Rivet, and J. Cariou, "Analysis of the depolarizing properties of irradiated pig skin," J. Opt. A, Pure Appl. Opt. **7**(1), 21–28 (2005).</jrn>
<jrn>25. S. L. Jacques, "Optical properties of biological tissues: a review," Phys. Med. Biol. **58**(11), R37–R61 (2013).</jrn>

1. Introduction

Among the three types of skin cancer which are the basal cell carcinoma, the squamous cell carcinoma and the melanoma, the latter is the most aggressive due to its strong capacity to metastasize. Thus, early diagnosis is of primary importance [1]. The « ABCDEF method » is usually employed by physicians to determine whether the suspect skin area is cancerous or not. This method, entirely based on subjective observations with conventional optics, consists in characterizing the Asymmetry, the Border irregularity, the Color variation, the Diameter, the Evolutionary and the Funny-looking lesion [2]. The most useful criterion for determining the prognosis is tumor thickness. Thin melanomas, those measuring less than 1 millimeter, have excellent cure rates. The thicker the melanoma, the less optimistic the prognosis. However, a significant subcategory of melanomas escapes clinical diagnosis [3] and the need for a new selective and non-invasive observation method is obvious. Early diagnosis and treatments are essential.

Optical devices have been the subject of several investigations for medical diagnostic of melanoma [4–6]. On the contrary to conventional imaging that uses only the scalar value of the field, polarimetric imaging benefits from additional information carried by the vector nature of light. The Stokes-Mueller formalism describes the entire polarimetric response of a sample including partial depolarization, and the various decompositions of Mueller matrices [7–9] exhibit 16 degrees of freedom that can add discriminant information in terms of skin cancer diagnosis.

These degrees of freedom characterize three main optical properties known as the dichroism, the birefringence, and the ability to polarize or depolarize light. Promising results are provided by polarimetric optical systems for biological tissue characterization. Especially, from the various polarimetric information extracted from a Mueller matrix, the degree of linear polarization (DOLP) of the light scattered from a sample generally gives the highest contrast between slightly different tissue structures and offers in consequence a very discriminant parameter [10]. Noticeable wavelength dependence was also reported, mainly due to different penetration depths [11, 12]. However, the full spectral dependence of the

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015

DOLP of the scattered light from biological tissues as a function of its state (normal, inflammatory, dysplasic, or cancerous) is poorly known at that time.

The spectral and polarimetric measurements reported here are believed to provide valuable insights in the development of non-invasive techniques for melanoma detection at the macroscopic scale. We believe an accurate knowledge of the spectral DOLP is crucial to improve disease diagnosis based on novel hyperspectral or polarimetric techniques.

2. Experimental setup

This section reports the clinical methodology that we use to produce melanoma samples with different diameters. The optical setup used to measure the spectral DOLP of skin samples is briefly presented.

2.1 Clinical methodology

Experimental animal studies were carried out according to the guidelines of the CNRS in compliance with EU directive 86/609/EEC. In the experiments, females C57Bl/6 purchased from Charles Rivers Laboratories (L'arbresle, France) were used. At the beginning of the experiments, the animals were 10–12 weeks old. Mice were kept in a conventional animal facility at a constant room temperature (21°C) and a natural day/night light cycle. Food and water were provided ad libitum. Animals were subjected to an adaptation period of 7–10 days before experiments. As previously described [13], the murine tumor cell line used was B16F10 melanoma (American Type Culture Collection, Manassas, VA) syngeneic to C57B1/6 mice. The doubling time of tumor models was approximately two days. B16F10 cells were routinely maintained in Eagle's minimal essential medium (EMEM; Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal calf serum (FCS; Sigma) and antibiotics in a humidified atmosphere at 37°C, containing 5% CO_2. Two days before tumor-cell injection, the hair was removed using a hair-removal cream (Veet, Reckitt Benckiser, Slough, UK). Animals were kept under isoflurane/air anesthesia during the whole procedure. 1.0×10^6 B16F10 cells in 30 µl of 0.9% NaCl were injected intradermaly with a Hamilton syringe through a 26G needle (Hamilton, Bonaduz, Switzerland). Ten days after, tumors sizes were from 5 to 9 mm in diameter. During this period of time, no necrosis on the skin was observed [Fig. 1].

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015

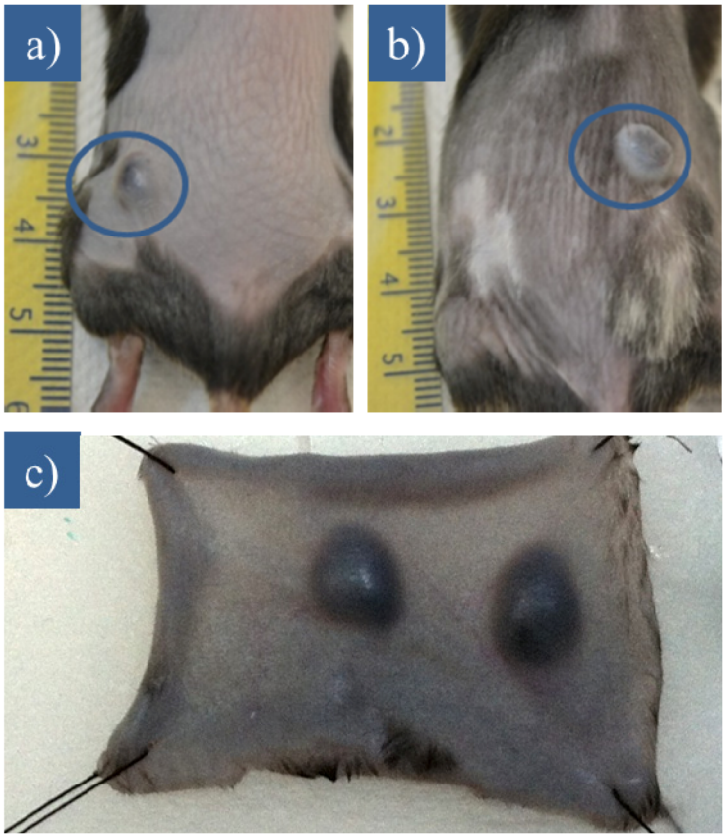

Fig. 1. Photographs of B16F10 melanoma with 5 to 9 mm diameter from female C57Bl/6 mice. Photographs a) and b) display in-vivo melanoma before sacrifice and photograph c) displays ex-vivo photograph after sacrifice.

Ten days after, tumors sizes were from 5 to 9 mm in diameter. Mice were sacrificed and the skin on the back of the mouse was carefully removed and put on a gaze humidified with saline buffer (NaCl 9‰). Sutures are used to maintain the skin on a planar position. Then the skin sample was imaged in the next 3 hours. See Fig. 1.

2.2 Optical setup

Supercontinuum laser sources are the result of nanostructured fiber optics combined with compact pulsed lasers [14, 15]. The primary laser pulses propagate through a non-linear medium and produce a directional white-light continuum, or supercontinuum, by spectral broadening [16]. Recently, multispectral and hyperspectral sensors have been used intensively in the optics community [17]. Recent advances in sensor instrumentation have facilitated the use of hyperspectral sensors for numerous scientific fields, including hyperspectral imaging for biomedical applications [18]. The combination of supercontinuum laser sources with hyperspectral sensors are of rising interest for numerous applications [19].

Let us report a novel technique to measure spectral and polarimetric backscattered radiance. Measurements are conducted using a supercontinuum laser-based instrument developed by ONERA, the French Aerospace Lab [20]. The laser source is fully unpolarized at full power and is coupled to wide-band polarizers to select incident linear polarization states. All measurements are fully interfaced using homemade software. A proper measurement procedure based on relative calibration extended to hyperspectral and polarimetric measurements was reported elsewhere [21].

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015

Only backscattering measurements are addressed here in order to compute the backscattered spectral $DOLP(\lambda)$ defined as [22]:

$$DOLP(\lambda) = \frac{\left| I_p(\lambda) - I_s(\lambda) \right|}{I_p(\lambda) + I_s(\lambda)}$$

where $I_p(\lambda)$ and $I_s(\lambda)$ are respectively the backscattered spectral *p*-polarized and *s*-polarized intensities measured by the optical setup. The wavelength is denoted by λ that ranges from 525 nm to 1000 nm. A schematic of the instrument is shown on Fig. 2.

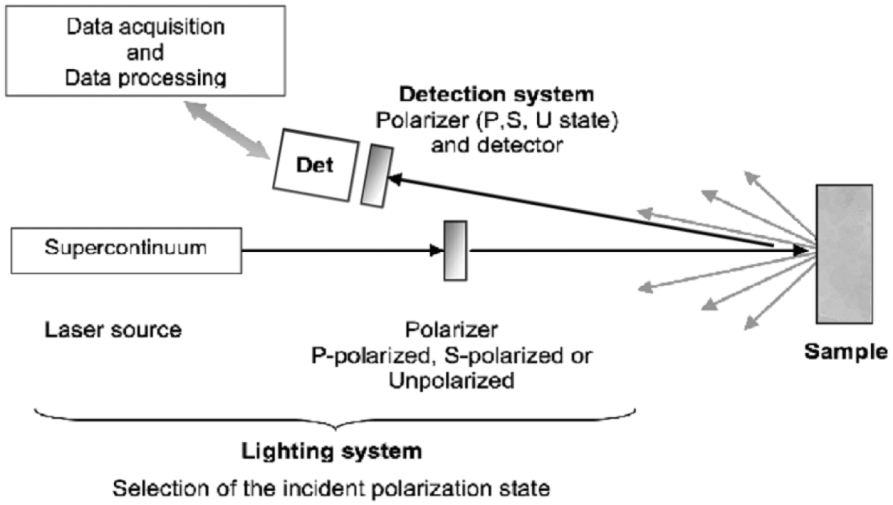

Fig. 2. Schematic of a supercontinuum laser-based instrument to measure spectral degree of linear polarization of samples.

The incidence angle, defined as the angle between the laser and the normal of the sample, remained constant and fixed at 4°. In order to ensure measurements reproducibility and comparison, the solid angle of collection of the detection system remained constant in the following results.

3. Results

This section reports experimental results from our supercontinuum laser-based device for healthy and cancerous skin samples with melanoma. Several melanoma samples are prepared and measured to investigate the dependence of the spectral DOLP upon the diameter of melanoma.

3.1 Reference measurements

Hyperspectral and polarimetric reflectance measurements from our device are validated using a white Lambertian reference surface (LabSphere SRS-99 Spectralon®). Literature reported strong depolarization of polarized coherent light from laser sources by Spectralon®. Similar results are reported to illustrate the validity of our method.

The spectral DOLP for the above white Lambertian sample, computed from our measurements, was found lower than 0.03 for the whole spectral domain. No spectral dependence was found. These results are consistent with other measurements provided by different measurement techniques [23].

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015

3.2 Healthy skin measurements

We first propose to measure the spectral and polarimetric properties of healthy skin samples from three different mice. The mean spectral DOLP of these healthy skin samples were measured and presented in Fig. 3. Healthy skin was found to be a strongly depolarizing medium. Similar spectral DOLP were measured for all the healthy skin sample with no spectral variation from visible to near infrared.

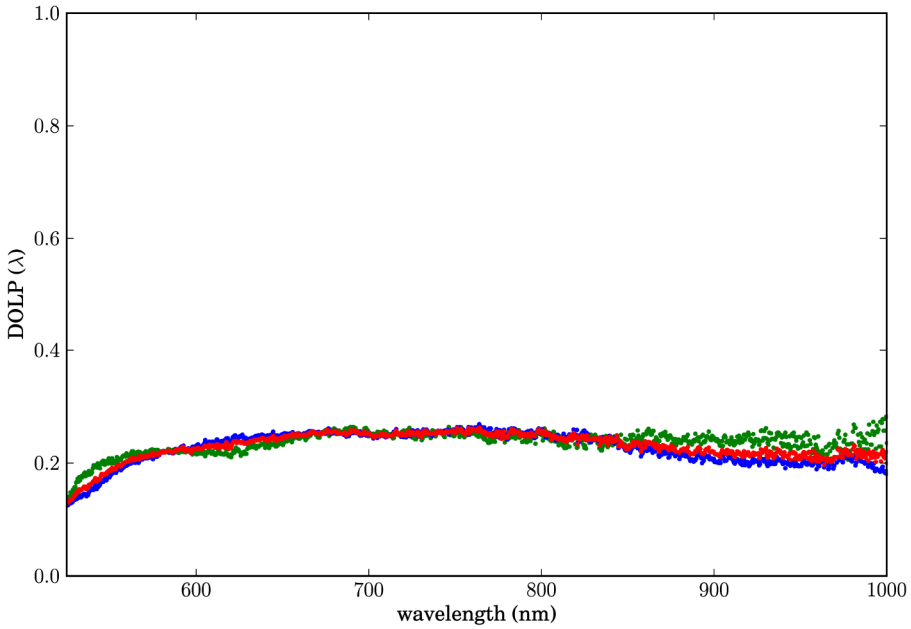

Fig. 3. Spectral DOLP measured from spectral and polarimetric backscattered radiance of healthy skin samples from three different skin samples from three different female C57Bl/6 mice.

The above results are reproducible for different individuals and are also close to previously reported measurements for pig skin samples [24]. For healthy mice skin, the mean spectral DOLP was found to be equal to 0.23. Spectral DOLP measurements are useful information in the development of spectro-polarimetric imaging devices. It accurately quantifies the polarimetric contrast of an object of interest, *i.e* melanoma, compared to the background, i.e. healthy skin.

3.3 Melanoma measurements

Let us investigate the spectral and polarimetric properties of cancerous skin samples. The minimum illuminated area of tissue is conditioned by the experimental setup and by the supercontinuum laser source divergence. Thus, all the presented measurements were performed for melanoma with a minimum size of 23 mm^2. The solid angle of collection of the detection system remained small to only measure the light reflected by the area of interest, i.e. the melanoma.

Figure 4 shows the spectral DOLP from 525 nm to 1000 nm for healthy and cancerous tissues. The studied cancerous samples exhibit melanoma with regular and spherical shapes. Their size ranges from 23 mm^2 to 68 mm^2.

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015

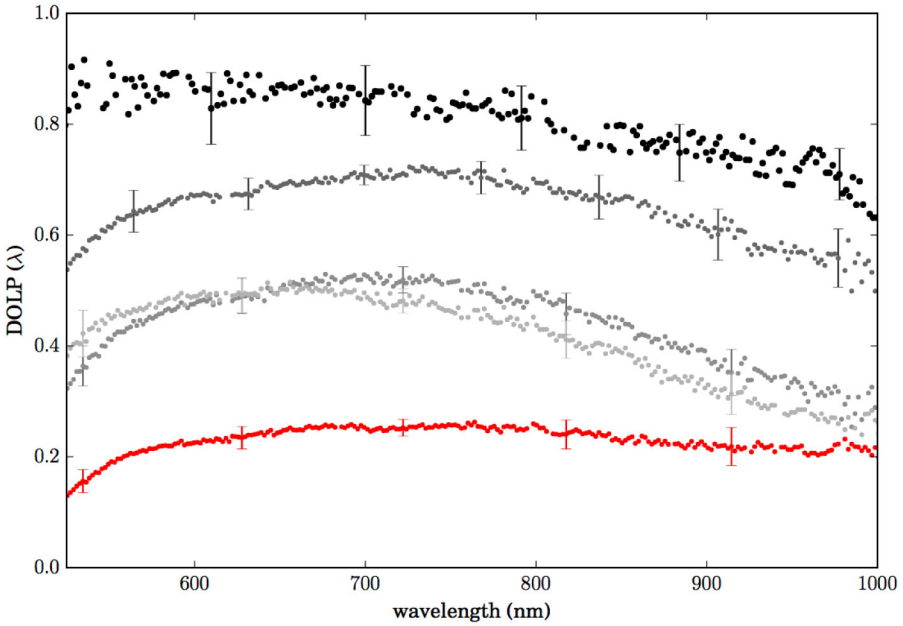

Fig. 4. Spectral DOLP of a) healthy skin (averaged over three different female C57Bl/6 mice) and cancerous skin with B16F10 melanoma areas respectively of b) 23 mm^2, c) 26 mm^2, d) 58 mm^2, and e) 68 mm^2 (from four different female C57Bl/6 mice).

These results are valid for B16F10 melanomas. However, the dysplasic process that occurs in human melanomas is also expected to change the nature of the tissue and in particular its roughness and its reflectance. These properties are both able to modify the spectral degree of linear polarization along the broad spectral range of study.

3.4 Discussions

The constituents of skin tissues (*i.e.* collagen, elastic fibers) strongly absorb and scatter light depending on the wavelength and angle of incidence. In addition, complex processes, such as multiple scattering, also modify the polarization state of reflected light. Figures 2 and 3 reveal significant differences of the spectral DOLP between typical healthy skin samples (in red) and cancerous skin samples with melanoma (in grey and black). Let us highlight several properties extracted from these results:

1. *Melanoma samples tend to maintain polarization states*. A global increase of the spectral DOLP is reported for 100% of melanoma samples compared to healthy skin samples. Light-scattering by skin tissues is mainly due to volume scattering. In the case of polarized light such as lasers, it generally results in a loss of polarization due to multiple scattering. The presence of melanoma on skin samples reduce such loss of polarization.

2. *The larger the melanoma, the higher the degree of polarization*. With the evolution of the disease, the melanoma evolves toward a glossy aspect. Such glossy aspect is the direct result of a decrease of roughness of the melanoma surface. Thus, the surface scattering contribution is enhanced and results in higher DOLP values. This latter interpretation is consistent with the fact that we observe an increase of the spectral DOLP as a function of the melanoma size that is correlated with the stage of evolution of the disease.

3. *Melanoma exhibits a significant spectral dependence of the DOLP, contrary to healthy skin.* We report from measurements a reproducible spectral dependence of the DOLP

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015

for cancerous skin with melanoma. No spectral dependence is reported for healthy skin samples from visible to near infrared. However, all the measurements carried out on melanoma reveal a decrease of the spectral DOLP from visible to near infrared. Melanin pigments can be considered as the main absorber for the cancerous skin samples due to the high concentration of melanin in the melanoma compared to hemoglobin for instance. However, it was reported that absorption of melanin is greatly reduced for near infrared wavelengths [25]. As a consequence, the decrease of the spectral DOLP in the infrared is explained by the decrease of absorbance of the melanin in this spectral domain. In other words, as the absorption decreases, light propagates deeper and is depolarized by multiple scattering.

4. Conclusion

Among the skin constituents, the concentration of melanin pigments varies from healthy skin samples to cancerous skin samples with melanoma. This change in melanin concentration influences greatly the spectral and polarimetric properties of healthy and cancerous skin tissues. Moreover, the absorption of melanin pigments strongly depends on the wavelength. Using a supercontinuum laser-based device, hyperspectral sensors and wavelength independent polarization optics, we report measurements of the spectral DOLP of healthy and cancerous skin samples.

The spectral DOLP values for murine B16F10 melanoma samples were found significantly higher than healthy skin in the entire spectral domain 525 nm – 1000 nm. The observed correlation between the state of development of the B16F10 melanoma and the corresponding value of the spectral DOLP is explained by the reduction of surface roughness of the melanoma along its evolution that tends toward a glossy aspect. While healthy skin exhibits a near flat spectral DOLP with a value around 0.2, all the cancerous samples show higher values with a decrease of their DOLP in the infrared region. This tendency has been explained by the decrease of absorption of melanin pigments in the near infrared domain.

We believe that in addition to detect cancerous lesions, this technique can be able to evaluate the stage of evolution of the disease. These results are crucial in the design of future spectro-polarimetric imaging system to perform earlier and more efficient diagnosis for skin cancer.

Acknowledgments

This research work was supported by ONERA, The French Aerospace Lab and Midi-Pyrenees Region funding. The authors wish to acknowledge the two anonymous reviewers of this research article.

#232054 - $15.00 USD Received 12 Feb 2015; revised 3 Apr 2015; accepted 13 Apr 2015; published 15 May 2015