HORIBA Scientific

Fast & Non-invasive Determination of Skin State FLSS-41

ELEMENTAL ANALYSIS FLUORESCENCE GRATINGS & OEM SPECTROMETERS OPTICAL COMPONENTS FORENSICS PARTICLE CHARACTERIZATION R A M AN SPECTROSCOPIC ELLIPSOMETRY SPE IMAGING



HORIBA's Aqualog[®] is uniquely equipped with a patented simultaneous Absorbance-Transmittance fluorescence Excitation-Emission Matrix (A-TEEM) technology

Introduction

Biological tissues contain chromophores that absorb light, as well as fluorophores that absorb and reemit light (fluorescence effect). Light absorption depends on the chromophores' content and their distribution within the organic matter **[1]**.

The endogeneous fluorescence of skin is due to the presence of specific fluorophores, i.e. aromatic amino acids such as tryptophan and tyrosine, collagen and elastin, porphyrins and flavins [2]. Some physiological and pathological processes such as aging, photoaging [3], psoriasis [4] and skin cancers [5] have shown characteristic changes in skin fluorescence [6].

We have previously reported **[7]** on fluorescence spectroscopy as a tool to characterize and quantify skin aging and photoaging. In the current work we show the application of fluorescence Excitation-Emission Matrix (EEM) analysis for the purpose of rapid identification of skin endogenous markers.

Experimental method

HORIBA's Aqualog is uniquely equipped with a patented simultaneous Absorbance and fluorescence Excitation-Emission Matrix (A-TEEM) technology which provides rapid access to a wide range of parameters. The Aqualog acquires a complete UV-VIS spectrum and reports the Transmission spectrum which can be used to determine the CIE LAB Tri-Coordinate Color Descriptions. Aqualog with subtractive double monochromator for excitation and TE-cooled back-illuminated CCD detector for emission is particularly suited for in-vivo monitoring of endogenous skin fluorescence from a highly scattering background such as human skin, with high sensitivity and precision. The system is equipped with a remote bifurcated fiber bundle (6 mm diameter) accessory which transmits light to the skin and returns the fluorescence emission to the detector. The tip of the fiber bundle is placed in contact with the volunteer skin with minimal pressure.

Results and discussion

Fluorescence Excitation Emission Matrices were measured on at least four different spots on the volar forearm site (Fig. 1).

The Aqualog EEM conditions included an excitation range from 280-400 nm with a 1 nm increment and an emission range of 250-600 nm with a 3 nm increment at medium CCD gain, and with a 1 s integration. These parameters correspond to a very quick 45 second acquisition time.



 $\ensuremath{\textit{Fig}}$ 1. Normalized 2D contour plot of the in-vivo EEM spectra of human skin.



HORIBA

As shown in Fig. 1, the major skin fluorophores, i.e. tryptophan (295 nm/345 nm - marker of cellular proliferation), pepsin digestible collagen cross-links (335 nm/390 nm - structural marker), collagenase digestible collagen cross-links (380 nm/460 nm) and NADH (350 nm /460 nm) peaks, could be easily detected. The decrease of the fluorescence of epidermal tryptophan is particularly important for monitoring non-invasively the premature aging of skin in diseases like diabetes or for UV absorbing Sun Protection products evaluation, as it is in agreement with two effects of aging on epidermal activity: a decrease of proliferation and a thinning of the epidermis **[8]**.

New Synchronous Profile Tool

From the in-vivo 2D contour data a single spectrum can be extracted by using the Profile Tool (Fig. 2).

Conclusions

We conclude that in-vivo fluorescence EEM represents a useful tool for quantitative and qualitative evaluation of skin markers and could also be used for the rapid and noninvasive assessment of cosmetics/pharmaceutical agents.



Fig. 2 2D contour plot of the in-vivo EEM spectra of human skin. Tryptophan emission spectrum extraction is the red spectrum to the right of the contour plot.

References

[1] I. Bliznakova, E. Borisova and L. Avramov, Acta Physica Polonica A, 112 (5), 1131-1136 (2007)

[2] R. Gilles, G. Zonios, R. R. Anderson and N. Kollias, J. Invest. Dermatol 115:704-707 (2000)

[3] N. Kollias, R. Gilles, M. Moran, I. E. Kochevar, and R. R. Anderson, J. Invest. Dermatol. 111, 776-780 (1998)

[4] A. G. Doukas, N. S. Soukos, S. Babusis, Y. Appa, and N. Kollias, Photochem. Phobiol. 74, 96-102 (2001)

[5] L. Brancaleon, A. J. Durkin, J. H. Tu, G. Menaker, J. D. Fallon, and N. Kollias, Photochem. Phobiol. 73, 178-183 (2000)

[6] J. Zhao, F. Feng, M. Tsai, H. Zeng, D. I. McLean, E. Ruvolo, N. Kollias and H. Lui, 217th ECS Meeting, Abstract #1037, The Electrochemical Society

[7] Horiba Application Note FL-12, "Endogenous Skin Fluorescence in-vivo on Human skin"

[8] W. D. Tian, R. Gillies, L. Brancaleon and N. Kollias, J Invest Dermatol. 116(6), 840-5 (2001)



info.sci@horiba.com

USA: +1 732 494 8660 **UK:** +44 (0)20 8204 8142 **China:**+86 (0)21 6289 6060

France: +33 (0)1 69 74 72 00 Italy: +39 2 5760 3050 Brazil: + 55 11 2923 5400 Germany: +49 (0) 6251 8475-0 Japan: +81 (75) 313-81231 Other: +1 732 494 8660

www.aqualog.com



HORIBA