3D QUANTIFICATION OF MELANIN IN HUMAN SKIN IN VIVO
BASED ON MULTIPHOTON MICROSCOPY AND IMAGE PROCESSING

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Information on the amount and distribution of melanin in the skin is essential for medical and
cosmetic applications. The most common way to estimate these quantities is based on 2D white
light imaging associated with histological staining. This approach is inappropriate for in vivo
applications. Another approach consists in using multiphoton microscopy that offers the
possibility to image the skin in vivo in a non invasive way. For example; the melanin in the basal
layer of the epidermis is often highly concentrated and results in fluorescence intensity stronger
than that of the other endogenous fluorophores. But methods based on fluorescence intensity
levels are not always satisfactory, because this intensity signal may be disrupted by other
fluorophores also having a strong fluorescence intensity (e.g. keratin in the stratum corneum),
and secondly it does not take into account low concentration melanin with a fluorescence
intensity comparable to that of other endogenous fluorophores. A more specific method consists
in taking into account the melanin fluorescence lifetime (Fluorescent Life Time Imaging, FLIM).
However, classical FLIM imaging is too long to obtain 3D data, and is in practice limited to
chosen 2D slices, obtained at a depth chosen by the operator.
In this study, we propose a new quantitative method based on “reduced” FLIM data acquisition
(4 time channels) for each pixel of the 3D-image. The acquisition time of this “Fast-FLIM”
protocol is compatible with in vivo investigations. From these images, using image processing
methods including a 3D automatic segmentation method [2], we were able to extract quantitative
parameters such as the global 3D melanin content in the whole epidermis. In addition, normalized
layers inside the 3D-delimited epidermis were defined in depth and a specific algorithm leads to
the normalized profile of melanin as a function of normalized depth, from the Dermal-Epidermal
Junction (DEJ) to the Stratum Corneum (skin surface).
This new method has been tested on a clinical trial on 15 aged (70-75y) human female volunteers
focusing on the ventral and dorsal sides of the forearm and compared to histological results.
Moreover, results obtained on a clinical study including 45 female volunteers (18-55y) having
ITA grade value on the ventral side of the forearm between I to IV will be shown.
In conclusion, the method based on multiphoton microscopy coupled with Fast-FLIM seems to
be a promising strategy to detect and quantify 3D melanin in the human skin in vivo.

3D segmentation of multiphoton images: a key step for the quantification of human skin", Skin Res.