Second Harmonic Generation: An Attractive Tool for Collagen Visualization in 3D Skin Model

N. Berthelemy, D. Gauché, G. Fargier, S. Cadau, C. Jeanmaire, D. Rival, S. Pain, V. André-Frei
BASF Beauty Care Solutions France, 3 Rue de Seichamps, Pulnoy
E-mail: nicolas.berthelemy@basf.com

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Collagens play a pivotal role for the maintenance of structural integrity and in the determination of tissue function [1]. In skin, the dermis contains predominantly type I collagen and with lesser amounts type III collagen and type V collagen [2]. Type I Collagen is a fibrillar collagen that forms fines, isolated and oriented fibers in the papillary dermis and, thicker, crisscrossed fibers in the reticular dermis. With ageing, the collagen content decreases and its organization and functionality are progressively deteriorated [3]. Second-harmonic generation (SHG) is a nonlinear second order optical process combining the advantages of a non-linear microscopy approach with a coherent modality able to probe molecular organization of collagens in normal human skin and 3D reconstructed models.

Different 3D skin equivalent models developed using bioengineering techniques are available today. Among them, our model, based on a collagen sponge scaffold, induce extracellular matrix synthesis, and present major interest for the visualization and the evaluation of active ingredients boosting collagen synthesis. In order to observe and control the quality of the fibrillar collagen organization in this model, we decide to use SHG technique that provides structural information without exogenous staining. We also evaluate the effects of an *Origanum majorana* extract, applied on this dermis model to reinforce the synthesis and spatial organization of collagen.

In normal human skin, collagen fibers generate a strong SHG signal and form a very dense network throughout the dermis. In the reconstructed dermis model, the SHG signal allows the observation of thin fibers of collagen, with unfulfilled extracellular spaces. The treatment with *Origanum majorana* produces larger and denser type I collagen fibers filling extracellular spaces with a fully organized three-dimensional collagen network. This study demonstrates that SHG method is a suitable and informative method to evidence organized collagenic structures in human skin but also in 3D skin equivalent model for pharmacodynamics studies and evaluation of ingredient improving skin density and firmness.

Figure 1: SHG signal observed in human skin and reconstructed dermis treated or not with *Origanum majorana* extract

[1] L. Rich and P. Whittaker, "Collagen and picrosirius red staining: a polarized light assessment of fibrillary hue and spatial distribution," *Braz. J. morphol. Sci*, **22**, 97-104 (2005).
[2] JH Chung, JY Seo, HR Choi, MK Lee, CS Youn, G Rhie, KH Cho, KH Kim, KC Park, and HC Eun, "Modulation of skin collagen metabolism in aged and photoaged human skin in vivo", *J Invest Dermatol* **117**, 1218-1224 (2001).
[3] ML. Koehler, K. König , P.Elsner, R. Bückle, and M. Kaatz, **"**In vivo assessment of human skin aging by multiphoton laser scanning tomography," *Opt Lett*., **31**, 2879-2881 (2006).