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## **(Regular Article)**

## A Novel Image Analysis Method to Evaluate Carbonylated Proteins in the Stratum Corneum

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## Abstract

We established an algorithm for the image analysis of carbonylated proteins (CPs) in the stratum corneum (SC) by eliminating the multiplex SC area using Threshold-based image Processing (TBIP) of image histograms. CPs are produced by various aldehydes yielded by reaction with lipids and reactive oxygen species. The carbonyl modifications are therefore irreversible and CPs are markers of oxidative stress. Recent studies suggested that CPs in the SC are not only markers of oxidative stress but also might be triggers leading to alterations of skin conditions. The content of CPs in the SC (SCCP level) can be visualized by labelling with fluorescent hydrazide derivatives after collecting the SC non-invasively using the adhesive tape-stripping method. However, the strong fluorescence emitted from the multiplex SC area interferes with the comprehensive evaluation of SCCP levels by image analysis. Therefore, a more reliable and accurate method to measure SCCP levels is required. Image histograms of fluorescence-labeled CPs in the SC were obtained. The TBIP method simply separates each image into monolayer and multiplex areas based on the gradation value which is decided by the image histogram shape. SCCP levels were calculated by the image of the selected monolayer area and were compared with simple SCCP levels calculated without image separation of monolayer and multiplex areas of the SC. The comparison result of SCCP levels with and without the TBIP method indicates that the TBIP method reduces not only the value of the SCCP level but also minimizes the deviation in SCCP levels in the fluorescence images. Thus, the TBIP method provides sensitive results to evaluate levels of CPs in the SC.

Key words: carbonylated proteins, stratum corneum, image analysis.