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Fluorescence lifetime imaging of pH in the stratum corneum

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Fluorescence lifetime imaging of pH in the stratum corneum.

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Abstract

Fluorescence lifetime imaging microscopy (FLIM) is being developed to explore the pH gradient in the uppermost layer of the skin's epidermis, the stratum corneum (SC). The SC provides the primary barrier between the environment and the body's interior. It is also considered to play an essential role in normal skin renewal each month (cornification). Over the extremely thin stratum corneum (~20 µm) the pH has been reported to gradually change from 4-5.6 at the skin's surface to pH 7.2 at the stratum corneum-granulosum junction. This corresponds to an increase of up to 1000-fold in the proton concentration over the 20-40 SC cellular layers. Tape-stripping measurements have been employed to study pH changes in the SC; however, such measurements are invasive and provide little information on the location and shape of the proposed SC pH gradient. Consequently, we are developing FLIM experiments to explore non-invasively pH changes within the heterogeneous tissue. For the initial investigations, swine biopsies are labeled with a lifetime-resolved pH-sensitive fluorescein-derivative dye. Upon two-photon excitation at 780 nm, the fluorescence lifetime of the label is measured as a function of SC area and depth. The applicability of fluorescence lifetime imaging to the study of pH measurements in spatially heterogeneous systems like skin is discussed. This work is supported by NIH grant RRO3155.