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Low concentration *Phloxine B* staining for high chemical contrast, nonlinear microscope mosaic imaging of skin alterations in pseudoxanthoma elasticum

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Abstract: Pseudoxanthoma elasticum (PXE) is an autosomal recessive metabolic disorder characterized by ectopic mineralization of soft connective tissue. Histopathology findings include fragmented, mineralized elastic fibers and calcium deposits in the mid-dermis. Nonlinear microscopy (NLM) can be used for visualization of these histopathological alterations of the mid-dermis in PXE-affected skin sections. Upon introducing a normalized 3D color vector representation of emission spectra of three of the main tissue components (collagen, elastin and calcification) we found that due to their broad, overlapping emission spectra, spectral separation of emission from elastin and calcification is practically impossible in fresh-frozen or unstained, deparaffinized PXE sections. However, we found that the application of a low concentration *Phloxine B* staining after the deparaffinization process creates an imaging contrast for these two tissue components, which enables spectral decomposition of their fluorescence images. The obtained concentration maps for calcium deposits can be well suited for the determination of illness severity by quantitative analysis.

1. Introduction

Pseudoxanthoma elasticum (PXE, OMIM#264800) is an autosomal recessive metabolic disorder characterized by progressive ectopic mineralization of soft connective tissue [1]. To date, diagnosis of PXE is confirmed by skin biopsy and/or genotyping. Histopathology findings include fragmented, clumped and mineralized elastic fibers and calcium deposits in the mid-dermis, mainly consisting of calcium hydroxyapatite and calcium hydrogen phosphate [2]. According to ultrastructural studies, fine precipitates inside the elastic fibers appear which then build up to form bulky deposits; this process eventually leads to deformation and breakage of the elastic fibers [3]. Coiled, split collagen fibers with irregular diameter and flower-like collagen fibrils are also observed sometimes [4].

Nonlinear microscopy (NLM) is a high-spatial resolution imaging technique that has been increasingly used in dermatological research including non-invasive detection of skin cancer [5] and stain-free examination of dermal connective tissue alterations [6–8]. Among the different NLM modalities, two-photon excitation fluorescence (TPEF) is suitable for the visualization of endogenous fluorophores, such as elastin and keratin, whereas collagen with its chiral structure generates second-harmonic generation (SHG) signal [9].

Recently, we have shown that NLM is able to visualize the histopathological alterations of the mid-dermis in PXE-affected skin using formalin fixed, paraffin embedded, deparaffinized PXE sections. Besides SHG imaging of collagen, we successfully visualized calcification,

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