European Conferences on Biomedical Optics 2021 (ECBO) © OSA-SPIE 2021

Low Concentration *Phloxine B* Staining for High Chemical Contrast, Nonlinear Microscope Mosaic Imaging of Skin Alterations in Pseudoxanthoma Elasticum

L. Fésűs,[1,2] L. Martin,[3] N. Wikonkál,[1,2] M. Medvecz[2] and R. Szipőcs[1,*]
[1] *Wigner RCP, Institute for Solid State Physics and Optics, P.O. Box 49, H-1525 Budapest, Hungary*
[2] *Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, Budapest, Hungary*
[3] *PXE Reference Center (MAGEC Nord), Angers University Hospital, Angers, France*
** Author e-mail address: szipocs.robert@wigner.hu*

Abstract: Low concentration *Phloxine B* staining was applied for nonlinear microscope mosaic imaging of skin alterations in *Pseudoxanthoma Elasticum* sections helping in determination of illness severity by quantitative analysis of calcium deposits in the skin.

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 with 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, two types of calcification were described: fine precipitates inside the elastic fibers in their center and bulky deposits which by surrounding the fibers, lead to deformation and breakage [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 chiral structure collagen 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 (FFPE) and then deparaffinized PXE sections. Besides SHG imaging of collagen, we successfully visualized calcification and detrimental changes to elastic fibers with TPEF [10]. However, in this preliminary study, we captured two-channel NLM images (SHG and TPEF), where TPEF signals originating from elastin and calcification were detected by a single NDD channel. Thus, their optical differentiation was not feasible and solely was based on their morphology.

In our present work we have investigated fluorescent properties of these two tissue components and found that in fresh-frozen PXE sections, they have a broad, overlapping emission spectrum [11]. Introducing a normalized 3D color vector representation of their emission spectra, we have learned that distinction of elastin and calcification for high contrast imaging is rather difficult (no data shown in this paper). However, we realized that by applying some low concentration, (hence low absorption) *Phloxine B* staining during the deparrafinization process of PXE sections, their spectral distribution considerably differs from each other, since *Phloxine B*, as a pink (fluorescent) staining for basic tissue components has a relatively higher concentration in calcification. As a consequence, these two tissue components can be optically distinguished by laser scanning NLM, even without damaging the tissue due to laser light absorption in stained sections.

In this work our aim was to develop an improved imaging and mathematical apparatus (spectral decomposition) to obtain three-color mosaic imaging of deparaffinized PXE sections using NLM, where elastin, calcification and collagen is visualized with distinct colors, to make images suitable for quantitative analysis and further investigation. By applying an artificial red, green, and blue color encoding for enhanced mapping co-localization of mineralization and elastic fibers, high spatial resolution, high contrast histological mosaic images have been recorded for low concentration *Phloxine B* stained, deparrafinized PXE sections.