Deconvolution Procedure to Improve Second Harmonic Generation Images of the Human Living Eye

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Abstract: An unsupervised deconvolution method has been proposed to enhance the quality and resolution of second harmonic generation images of the living human cornea and sclera acquired with a compact custom multiphoton microscope. © 2022 The Author

1. Introduction

Second Harmonic Generation (SHG) microscopy has become a very useful tool in biomedical research because of its ability to visualize and detect changes in collagen-based tissues without the use of external markers, in particular, those in ocular structures such as the cornea and sclera [1, 2]. Although this technique provides intrinsic sectioning capability and reduced photodamage, SHG images are affected by aberrations at deeper locations within the tissue [3]. In addition, when imaging living samples (i.e., under dynamic conditions) the technique must also deal with non-controlled movements.

To reduce the impact of the aberrations, adaptive optics (AO) approaches have been often used [4,5]. Since AO devices often become complex and costly when living specimens are involved, alternative methods such deconvolution might be also useful for image improvement [6]. This is a post-processing method able to deblur images, which usually requires information about the point spread function (PSF). However, deconvolution of SHG images of the human cornea and the sclera is rather difficult since the PSF is not well-known. Therefore, for these cases both the PSF and the object must be estimated, what is known as blind or myopic deconvolution [7].

In the present work an entirely unsupervised deconvolution method known as marginal blind deconvolution has been used [8] to improve the quality (spatial resolution and contrast) of SHG images of different ocular structures of the living human eye.

2. Methods

2.1 Experimental setup

The setup is a custom compact clinically-oriented SHG microscope adapted for ergonomic imaging of the living human eye. It uses a XYZ chinrest and two additional cameras (LCC) to control for the correct position of the eye, and a fixation target to facilitate stability and minimize ocular movements during measurements. Fig. 1 shows a schematic diagram of device. Further details can be found in [9]. The whole system was controlled through a C++ software via a data acquisition card (DAQ). SHG images (0.45s exposure time) from different ocular structures and locations were acquired in healthy volunteers and then evaluated with image quality metrics such as SDQI [10], contrast and structural dispersion [11].

Fig. 1. Sketch of the home-made instrument used in this work. The illumination source is a Ti:sapphire femtosecond laser (800 nm, 76 MHz). S, electro-mechanical shutter; NDF, variable neutral density filter; BE, beam expanded; DIC, dichroic mirror; OB, long walking-distance microscope objective; PMT, detection unit.
2.2 Deconvolution procedure

In biomedical imaging, the process is three-dimensional and can be modelled (at least locally) as a 3D convolution of the 3D observed object with a 3D PSF, of which only a slice, i.e., a 2D image, is usually recorded. But estimating a 3D object from a 2D image is obviously very difficult because we have too many unknowns for too little information. The idea for the approach presented herein is to estimate the PSF, on average for all possible objects within a given class (the basis of the marginal blind deconvolution [8]). Here the likelihood is marginalized over the unknown object (of a given Power Spectral Density (PSD)), and maximized as a function of the PSF. To reduce the unknowns, we assume the PSF is the linear combination of multiple PSFs with more or less defocus, so that our unknowns are the coefficients of this linear combination. Finally, to estimate the PSDs of the object and noise, we also use a parameterization for the object’s PSD with only three parameters [12], so that the statistical contrast of our estimation (number of data/number of unknowns) remains much greater than unity, and the appealing theoretical properties of maximum likelihood estimation such as consistency are retained in practice, as verified by simulations [8].

3. Results

Figure 2 depicts an example of SHG images of a living human cornea before and after deconvolution. An improvement in image quality is readily observable. In particular, analyzed images provide enhancements in SDQI between x1.75 and x10 with the consequent resolution and contrast increase while maintain the total intensity (i.e., energy conservation). This improvement allows a better detection of biological features and an accurate analysis of the organization.

![Fig. 2. Original (a) and deconvolved (b) SHG images of the living human eye.](image)

4. Conclusions

A marginal blind deconvolution algorithm has been applied to SHG images of the living human cornea and sclera to correct the effects of PSF worsening (i.e., sample-induced aberrations). Certain structures within the tissues that couldn’t be observed in the original images were visible in the deconvolved ones. The successful behavior of this procedure in the in-vivo human eye might serve as a clinical tool for early detection of pathologies associated with microscopic changes in tissue organization.

References