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# Deconvolution Method for Multiphoton Microscopy: An Application to Thick Ocular Tissues

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## ABSTRACT

Multiphoton (MP) microscopy of thick biological tissues is limited by the presence of aberrations and scattering. In addition, living samples impose an additional limit due to uncontrolled movements. To overcome these constraints, deconvolution methods are proposed as an alternative to well-established adaptive optics procedures. A marginal blind deconvolution approach for the reconstruction of MP images of both ex-vivo and living ocular tissues is shown and discussed. The quality of the images reconstructed by this method is compared to that of the original ones in terms of the achieved resolution and inherent artefacts, among other criteria.

**Keywords:** Second harmonic generation, deconvolution, ocular tissues.

## 1. INTRODUCTION

MP microscopy suffers from poor optical image quality at deeper layers within static thick samples [1,2]. The reasons are based on the inherent specimen-induced aberrations and scattering. In addition, if dynamic samples are intended to be imaged, the uncontrolled movements will also affect the final image [3]. The result is a “blurred” image where the required visualization of details, especially in biological tissues, presents serious constraints.

During the last decades, adaptive optics has been established as a useful procedure to enhance MP imaging microscopy [1-7]. The use of either deformable mirrors or spatial-light modulators has been reported in the literature. Although some approaches used a wavefront sensor combined with the adaptive element in closed-loop [8,9], others used wavefront sensorless schemas [5,7,10,11]. Adaptive optics MP microscopy was firstly used to analyze static samples, but it has recently been oriented to living tissue imaging, in particular neuroscience applications [12].

As an alternative, deconvolution techniques that use the point spread function (PSF) have been proposed in microscopy, including confocal, structured illumination and more recently two-photon [13-17]. Deconvolution is an image processing method able to deblur images, which often requires PSF information. Although at shallow planes the PSF can be easily characterized by simulation, for deeper planes within the sample this is more difficult, since it is distorted. To overpass this, here we propose an approach based on the so-called *marginal blind deconvolution*. This image restoration method is based on a sound statistical framework that avoids the usual degeneracies encountered in blind deconvolution and makes it entirely unsupervised [18]. The method has been applied to MP images of ocular tissues providing both second harmonic generation (SHG) and two-photon excitation fluorescence (TPEF) signals.

## 2. METHODS

### 2.1 Experimental system

Images were acquired with a home-made MP microscope developed at the Laboratorio de Óptica of the Universidad de Murcia [6]. A mode-locked laser system, set to 800 nm and a repetition rate of 76 MHz, was used as illumination source. The laser beam was scanned across the sample under study and the MP signal was recorded in the backward direction by the detector (photomultiplier). Two spectral filters were used to isolate the corresponding TPEF and SHG signals. A step-motor attached to the long working-distance objective (20x, 0.5 NA) was used to record MP images along the Z direction (i.e. for different depth locations). A schematic diagram is depicted in Fig. 1.

Different thick ex-vivo ocular tissues were involved in the experiment. These included retinas, scleras and corneas of both human donors and a number of animal models. Whereas retinal samples provide TPEF signal, the corneal stroma and the sclera give a fairly strong SHG signal. For the MP images of the living human eye, an additional compact clinically-oriented microscope also developed in our lab was used (details on this instrument can be found in [19]). The exposure time was 1 s for ex-vivo samples and 0.45 s for the in-vivo ones. The entire system was controlled via custom LabView™ software. For image processing the software was developed in Matlab™.

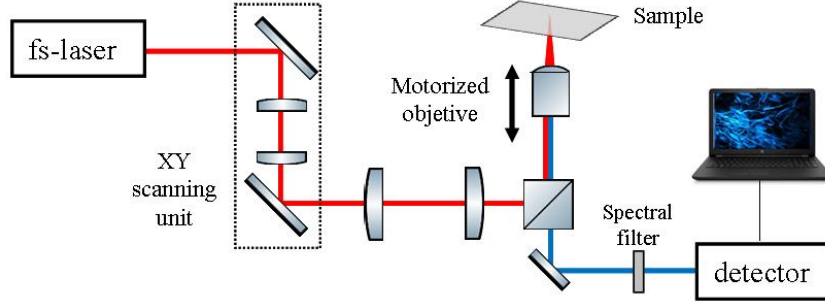


Figure 1. Schematic diagram of the MP microscope used for the purpose of this work.

## 2.2 Deconvolution procedure

In MP, as in many imaging modalities, the image can be modelled as the noisy convolution of the sought object with a PSF that is partially unknown, for the reasons described above. The conventional way of tackling this lack of knowledge is to perform a “blind” or “myopic” deconvolution, consisting of a joint estimation of both the sought object and the PSF, if possible with additional constraints such as PSF band-limitedness and positivity, object positivity and support, etc (see [20] for a review). In biomedical imaging, where the object extends beyond the field of view and the image has a limited contrast, the object support constraint is inapplicable and the positivity is ineffective. In this case the blind deconvolution has been shown to be degenerate [18,21], in the sense that the estimation leads to a wrong couple object-PSF even if the noise level is arbitrarily small.

The problem basically lies on the fact that there are too many unknowns for too little data, and the idea for a solution is to try and estimate “only” the PSF, on average for all possible objects within a given class. This idea is embodied in the so-called *marginal blind deconvolution* [18] used in this communication, where the likelihood is marginalized over the unknown object (of a given Power Spectral Density (PSD)), and maximized as a function of the sole PSF. To further constrain the problem, the PSF is parameterized with sparsity, and here we assume it is the linear combination of half a dozen more or less defocused PSFs, so that our unknowns are the coefficients of this linear combination. Finally, because the PSDs of the object and that of the noise are actually unknown, we must also estimate them. To this purpose, we also use a sparse parameterization for the object’s PSD with only three parameters [22], so that the statistical contrast of our estimation (ratio of number of data over the 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 [18]. Fig. 2 shows a sketch of the procedure here used.

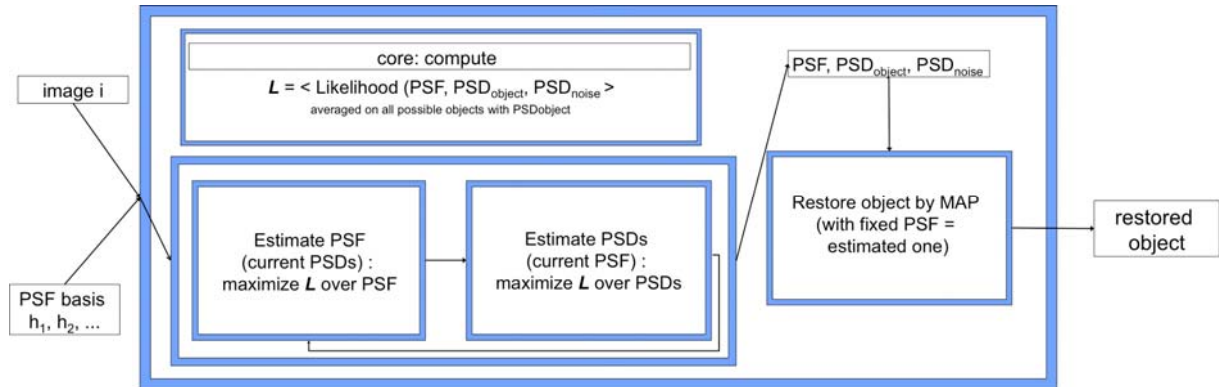


Figure 2. Sketch of the deconvolution procedure used in this work.

## 3. RESULTS

The technique was firstly performed at particular planes within ex-vivo ocular tissues providing different MP signals. SHG images of a porcine cornea (stroma) before and after deconvolution are depicted in the upper panels of Fig. 3. A simple visualization reveals the increase in quality of the deconvolved image when compared to the original one. The imaged plane was randomly chosen. The histogram corresponding to each SHG image is also shown (bottom panels). It can be observed how the gray levels “move” to the right in the deconvolved image. In particular, for this example, the maximum intensity value increases from 111 to 163. In addition it is worth noticing that, as expected, the procedure keeps the total intensity constant for each pair of images.

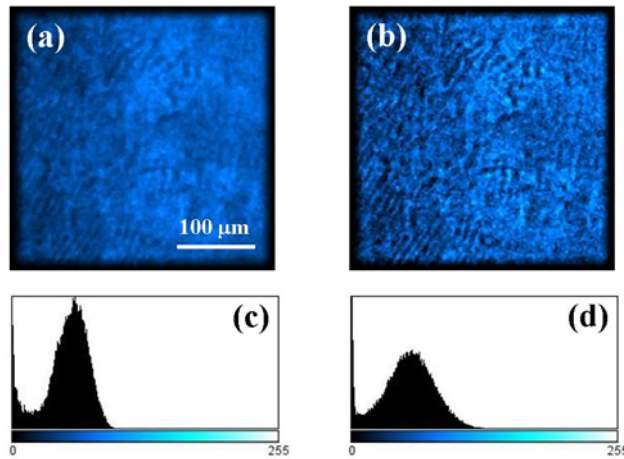


Figure 3. SHG images of an ex-vivo corneal tissue before (a) and after deconvolution (b). The corresponding histograms are also shown (c, d).

Fig. 4 presents the results for a plane within a biological sample providing TPEF signal (non-stained fixed chicken retina). The differences between both images are readily observable.

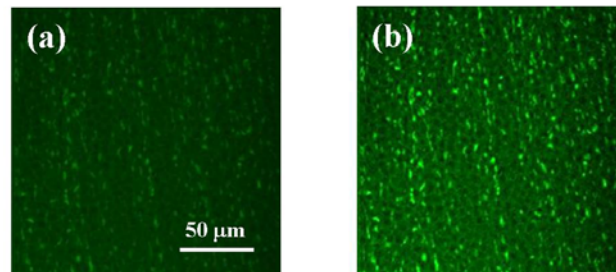


Figure 4. TPEF images of a retinal tissue (ganglion cell layer) before (a) and after deconvolution (b).

For the sense of completeness, the procedure was also applied to MP images of the living human eye. An illustrative example of the image restoration through this deconvolution method is shown in Fig. 5. For this sample the maximum intensity value increased 28%.

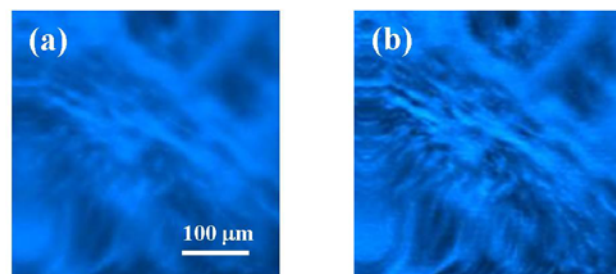


Figure 5. Comparison of SHG images of a living human cornea before (a) and after deconvolution (b).

As it can be seen in the examples here reported the quality of the images was enhanced, independently of having static or dynamic nature (i.e. ex-vivo and in-vivo). Moreover, a better visualization of the biological features was also seen. Different image quality parameters such as signal-to-noise ratio and acutance were used to quantify the improvement.

#### 4. CONCLUSIONS

Along this work, a deconvolution scheme to enhance MP images of ocular tissues has been presented. Images obtained before and after deconvolution were compared. The algorithm was able to improve the quality of the images independently of their location. The increase in contrast and resolution was noticeable in all the images, although the enhancement was more noticeable at deeper regions where the original images were of lower quality. Morphological and structural details that couldn't be observed in the original images were visible after deconvolution. This tool might help not only to improve MP imaging of biological samples, but they could also be used as a tool for the detection and analysis of pathologies producing microscopic changes in the tissue.

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