Determination of the Refractive Index and Thickness in Tissues by Chromatic Confocal Microscopy

J. Garzón¹, T. Gharbi², J. Meneses³

¹Grupo de Óptica y Espectroscopía. Centro de Ciencia Básica Univ. Pontificia Bolivariana. AA 56006. Medellín, Colombia.
²Département d’Optique PM Duffieux, Institut FEMTO-ST, UMR CNRS 6603, Université de Franche-Comté, 25030 Besançon Cedex, France.
³Laboratorio de Óptica y Tratamiento de Señales, Escuela de Física, Universidad Industrial de Santander. Bucaramanga, Colombia

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Abstract

In this work, a chromatic confocal method to measure the refractive index and thickness of tissues is developed. The model experimental of the method is based on the longitudinal chromatic aberration produced by a diffractive element. The setup is composed by a point polychromatic illumination system, a wavelength-height codification system of confocal microscopy and a spectral detection system. The first two systems form a wavelengths segment on the device output. The spectral analysis of the detected light gives the thickness decoding, by identifying the most intense spectral components coming from the interphase of the tissues.

Key Words: Chromatic aberration, Confocal microscopy, reconstruction 3D.

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1. Introduction

The chromatic confocal microscopy (CCM) to measure the refractive index and thickness of membranes is attractive for various applications, such as optical metrology, spectroscopy, biomedical optics. The CCM has been the object of research in the last years. The first works were conducted in bulk architectures. In those experiments the miniaturization was not present. The point sources of illumination difficulty were obtained. In this paper, we show a simple method of measuring the refractive index and thickness of membranes with a miniaturized device. First, we explain the illumination systems. Then, the CCM described. Finally, the experimental results using this method are presented. The experimental results and a briefly perspectives of the work are provided.
The zero dispersion wavelength of the fundamental mode is $\lambda_0 = 870 \text{ nm}$. Stimulated Raman scattering, self-frequency doubling, and every spectral component is focused on different positions. Thus, the segment of wavelengths $\Delta \lambda$ is created (Fig. 1b) and it is defined by $\Delta \lambda = f(\lambda) - f(\lambda_0)$. Then, there are two lenses, one achromatic lens $AL_2$ with a focal distance of 2 cm and the other lens is a microscopy objective of 50× ($O_2$) with numerical aperture of 0.45 and a working distance of 13.8 mm. Together the lens $AL_3$ and $O_2$ imagined the wavelengths segment $\Delta \lambda$ produced by the Fresnel lens at the system output, forming $\Delta \lambda'$. Thus, the segment of wavelengths image $\Delta \lambda'$ can be adjusted to the desired measurement range. In order to realize a longitudinal chromatic codification of $z$, every focal position $f''(\lambda)$ can be converted to $z$ relative positions. If $f''(\lambda_0)$ is a reference focal position on $\Delta \lambda'$, the $z$ relative positions are given by $z_{\lambda} = f''(\lambda) - f''(\lambda_0)$. On the other hand, if a reflective sample (S) is placed inside the wavelengths segment image $\Delta \lambda'$, a corresponding wavelength is reflected through the system. Afterward, the beam splitter BS will divert the beam toward the microscope objective $O_3$ of 40× with numerical aperture of 0.4. The objective $O_3$ focused the beam over a optical fiber connector which guide the signal to a spectrometer (SC) of bandwidth from 341.73 to 1001.58 nm and 0.34 nm resolution (PC2000-ISA spectrometer card from Ocean Optics), which has an optical fibre input ($FO_2$) of 50/125 μm. Before the fibre input $FO_2$ a pinhole $H_1$ of 10 μm is placed for obtaining a better optical sectioning. This way, the reflected wavelength is detected. Whether the sample S along the optical axis is moved, different wavelengths are reflected and detected by mean of pointlike detector. Thus, the position $z_{\lambda}$ along the optical axis can be coded inside the spectral space defined by the wavelengths segment image $\Delta \lambda'$. Here, we understand the advantage of using a chromatic dispersion confocal system because only placing a membrane on the chromatic dispersion interval its thickness or refractive index can be measured. Using the chromatic codification, the point spread function $PSF^4 I(x,y,z- z_{\lambda})$ is centered on the $z$ position for a
given wavelength. Placing a mirror perpendicularly to the optical axes at \(z_0\) position in the segment of wavelengths, the spectral components of the reflected signal can be calculated by the superposition:

\[
I(\lambda) = \int I(x, y, z_0 - z) \, dx \, dy
\]  

(2)

Experimentally, the measured spectrum of reflected signal \(I(\lambda)\) can be defined as spectral response of the system.

3. Experimental Results

Experimentally a human cornea was taken as sample. It is placed such way that its whole thickness is contained on the wavelength segment \(\Delta\lambda\). So a corresponding wavelength is reflected through the system from every interphase. If a step – step axial scanning is done and the spectrum is taken for every step, a thickness matrix is obtained (Fig. 2). A profile of the thickness matrix shows the optical thickness of the human cornea which is about 250 \(\mu\text{m}\). (Fig. 3).

4. Conclusions

A chromatic confocal method for determination of the topography, refractive index and thickness of tissues has been presented. The CCM permit to do a spectral coding of the depth of an object without using a scanning system in \(z\)-direction. The depth \(z\) corresponding to a wavelength \(\lambda\) in the spectrum depends on the dispersion properties of the diffractive element. The system offers a manner possible of knowing the variation of the refractive index with wavelength. So, the optical properties of the tissues can be known. This method is extremely useful in a number of applications, such as spectroscopy, optical coherence tomography, and microscopy.

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