Revista Colombiana de Física, vol. 40, No. 1, Abril 2008

Revista Colombiana de Física RCF

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

J. Garzón[1], T. Gharbi[2], J. Meneses[3]

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

Recibido 22 de Oct. 2007; Aceptado 3 de Mar. 2008; Publicado en línea 15 de Abr. 2008

Resumen

En este trabajo se desarrolla un método para medir el índice de refracción y el espesor de tejidos. El modelo experimental se basa en la aberración cromática longitudinal producida por un elemento difractivo. El dispositivo está compuesto por un sistema de iluminación policromático puntual, un sistema de codificiaciónn longitud de onda – altura de microscopía confocal y un sistema de detección espectral. Los dos primeros sistemas forman un segmento de longitudes de onda a la salida del dispositivo. El análisis espectral de la radiación detectada da la codificación del espesor, identificando las componentes espectrales más intensa que vienen desde la interfase de los tejidos.

Palabras claves: Aberración cromática, Microcopía confocal, Reconstrucción 3D.

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.

© 2008 Revista Colombiana de Física. Todos los derechos reservados.

1. Introduction

The chromatic confocal microscopy (CCM) to measure the refractive index and thickness of membranes is attractive for various applications, such a optical metrology, spectroscopy, biomedical optics. The CCM has been the object of research in the last years.[1] The first works were conducted in bulk architectures[2]. In those experiments the miniaturizing no was present. The point sources of illumination difficultly 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.

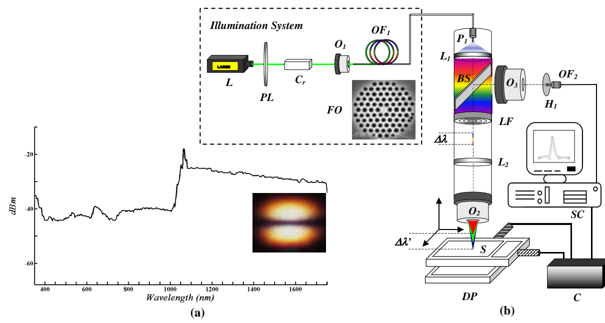


Fig.1 (a) Visible and infrared supercontinuum spectra measured at fibre output[3]. (b) Chromatic confocal microscopy.

2. Illumination System

Fig.1a shows the spectrum of the illumination source and Fig. 1b shows a probe of chromatic confocal microscopy with a illumination system. The white illumination source is constituted by the combination of a high energy pulsed laser (L) and a nonlinear microstructured optical fibre (OF1), as depicted in Fig. 1b. The pump source is a Q-switched Nd:YAG microchip laser (L) delivering 600 ps pulses at $\lambda = 1064$ nm. It is frequency doubled in a KTP crystal (Cr), yielding 420 ps pulses at $\lambda = 532$ nm. The green and infrared radiations are coupled into a 4-m piece of air-silica microstructured fibre (OF1) by mean of microscope objective (O1) . This fibre has been fabricated at XLIM laboratory[7] by the conventional stack and draw process. The hole-to-hole spacing Λ is 2.2 µm, the hole diameter 1.5 µm (FO) and the core diameter equal to 2.8 µm.
The zero dispersion wavelength of the fundamental mode is located at $\lambda \sim 870$ nm. Stimulated Raman scattering, self-phase modulation, cross-phase modulation and four-wave mixing are the main nonlinear effects occurring along the propagation in the fibre. This result in the generation of a wide spectral supercontinuum both in visible and infrared ranges are presented in Fig. 1a. The output beam shows a transversally singlemode distribution[3].

2. Cromatic Confocal Microcopy

Fig. 1b shows a configuration of CCM, where the polychromatic illumination point source was put in the point P1. The polychromatic spectrum coming from P_1 is collimated through an achromatic lens AL_1 with a focal distance of 2 cm, which transforms the luminous signal to plane wave. The luminous wave is then directed toward a beam splitter *BS*. After, a diffractive Fresnel's lens *FL* of 8 mm diameter with focal distance of 25 mm for a wavelength of 633 nm and numerical aperture of 0.6 is placed. The lens *FL* produces the longitudinal chromatic aberration phenomenon and every spectral component is focused on different position along the optical axis, forming a wavelengths segment $\Delta\lambda$. The chromatic dispersion properties of *FL* can be characterized for order +1 by[5]:

$$f(\lambda) \approx f(\lambda_d) - 2f(\lambda_d)\left(\frac{\lambda}{\lambda_d}\right) + 6f(\lambda_d)\left(\frac{\lambda}{\lambda_d}\right)^2 - 3f(\lambda_d)\left(\frac{\lambda}{\lambda_d}\right)^3 + f(\lambda_d)\left(\frac{\lambda}{\lambda_d}\right)^4 \quad (1)$$

where f is the focal position, λ is the operating wavelength, λ_d and $f(\lambda_d)$ are the design wavelength and the corresponding design focal length, respectively. If $f(\lambda_1)$ and $f(\lambda_2)$ are calculated for λ_1 and λ_2 respectively, then a segment of wavelengths $\Delta\lambda$ is created (Fig. 1b) and it is defined by $\Delta\lambda = f(\lambda_1) - f(\lambda_2)$. Then, there are two lens, one achromatic lens AL_2 with a focal distance of 2 cm and the another 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_2 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 $f''(\lambda_2)$ is a reference focal position on $\Delta\lambda'$, the z relative positions are given by $z_\lambda = f''(\lambda) - f''(\lambda_2)$. 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 band width 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_λ 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_λ position for a

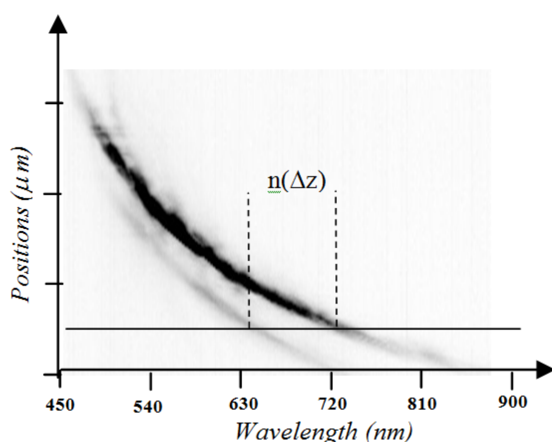
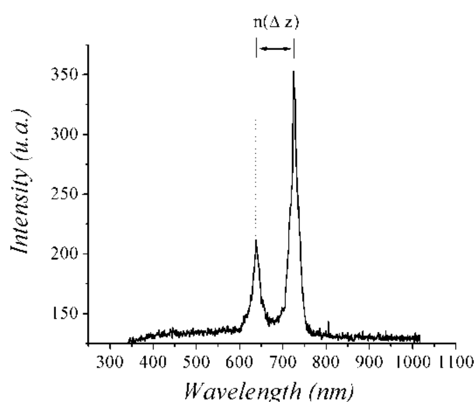


Fig.2 Thickness Matriz for human cornea



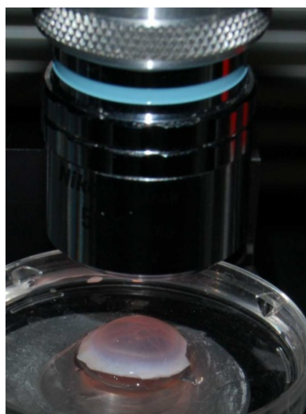


Fig.3 On top optical thickness of the humana cornea. On left side a view of the human cornea used

given wavelength. Placing a mirror perpendicularly to the optical axes at z_o position in the segment of wavelengths, the spectral components of the reflected signal can be calculated by the superposition:

$$I(\lambda) = \int I(x, y, z_o - z_\lambda)dxdy \qquad (2)$$

Experimentally, the measured spectrum of reflected signal I(λ) is affected principally by the impulse response of the detection system. I(λ) can be defined as spectral response of the system.

3. Experimental Results

Experimentalls a human cornea was taked as sample. It is placed such way that its whole thickness is contained on the wavelength segment Δλ'. So a corresponding wavelength is reflected through the system from every interphase. If a step – step axial scanning is done and the spectrum is taked for every step, a thickness matrix is obtained (Fig. 2). A profile of the thickness matrix shows the optical thickness of the humana cornea which is about 250 µm. (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 λ 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.

Acknowledgments

This work was realized in the Département d'Optique PM Duffieux, Institut FEMTO-ST, UMR-6603 CNRS, Université de Franche-Comté. Besançon France. J. Garzón acknowledge the financial support of the Universidad Pontificia Bolivariana and COLCIENCIAS, Colombia.

References

[1] J Garzón R., J. Meneses, G. Tribillon, T. Gharbi and A. Plata. Pure App. Opt. 6 544-8, 2004
[2] G. Q. Xiao, T. R. Corle, G. S. Kino. Appl. Phys. Lett., Vol. 53, Nº 8, p. 716, 1988
[3]V. Tombelaine, C. Lesvigne, P. Leproux, L. Grossard, V. Couderc, J.L. Auguste, and J. M. Blondy. OPTICS EXPRESS / Vol. 13, No. 19 / 2005
[4] T. Wilson. “Confocal microscopy”. Academic Press, London, 1990.
[5] D. Faklis and G. Michael Morris. Appl. Opt. Vol. 34 No. 14 pp 2462-2468. 10 May 1995.