Revista Bistua
ISSN 01204211
Universidad de Pamplona
Pamplona-Colombia

Measurement Of Optical Thickness In Human Corneas By Mean Of A Chromatic Confocal Probe

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RESUMEN

La técnica de exploración confocal es atractiva en varias aplicaciones de formación de imágenes de microscopía, debido a la capacidad de mejorar la resolución, la recolección de luz dispersada y la discriminación en profundidad. Éste método tiene especial interés en aplicaciones tales como: formación de imágenes de muestras biológicas y materiales semiconductores, donde se requiere una alta definición transversal y longitudinal. En ésta técnica, el seccionamiento óptico o propiedad de discriminación en profundidad ha llegado a ser la principal motivación para el uso de microscopios confocales. En los sistemas confocales clásicos, la altura de cada punto de una superficie es medida por la localización en la dirección z que lleva a la detección del máximo de intensidad. Entre los diversos sistemas existentes se encuentran los dispositivos que exploran paralelamente todos los puntos de la superficie y los sistemas donde la superficie es medida punto por punto. En éste trabajo se presenta el principio de la microscopía confocal donde la superficie es medida punto por punto, con la propiedad de discriminación en profundidad basada en un fenómeno de aberración cromática longitudinal. Como aplicación particular, se presenta una sonda miniaturizada de microscopía confocal cromática para medir el espesor óptico y la topografía de córneas humanas. Una de las principales motivaciones para el desarrollo de este dispositivo de microscopía confocal cromática, se asocia con la necesidad de medir por medio de métodos ópticos rápidos, el espesor central de las córneas humanas extraídas de un donante fallecido. Los resultados presentados en este trabajo corresponden a pruebas preliminares que buscan evaluar las capacidades metrológicas de este sistema confocal cromática. Las córneas humanos utilizadas en esta investigación se obtuvieron del banco de córneas del Hospital Universitario Saint Jacques de Besançon, Francia. Algunos córneas fueron descartadas para la realización de implantes por el número de células vivas por milímetro cuadrado y el porcentaje de variación de su espesor. El modelo experimental se basa en el principio de multiplexación del foco mediante la codificación de longitudes de onda debidas a una lente de Fresnel. El dispositivo se compone de un sistema de iluminación policromático puntual, un sistema de codificación longitud de onda de altura de microscopía confocal y un sistema de detección espectral. El sistema de iluminación cuenta con un espectro continuo y amplio que está constituido por la combinación de una alta energía de pulsos láser sobre una fibra óptica microestructurada de aire sílicia. Los dos primeros sistemas forman un segmento de longitudes de onda sobre la salida del dispositivo. La codificación del espesor óptico y la topografía se basa en la detección de las componentes espectrales más intensas procedentes desde cada interfase orgánica de las membranas. La medición de la sensibilidad está relacionada con el ancho de la respuesta espectral, que depende del poder de resolución del elemento dispersivo y el sistema de detección. Finalmente se presentan los resultados experimentales y una breve discusión de los criterios de resolución junto con las perspectivas de trabajo.

Palabras Clave: Microscopía confocal, aberración cromática, reconstrucción 3D.

ABSTRACT

The confocal scanning technique is attractive in various microscopic imaging applications because of its capabilities for superior resolution, rejection of scattered light and depth discrimination. This method drew special interest in applications such as imaging biological samples and semiconductor materials, in which high definition in both transverse and longitudinal dimensions is required. In this technique, the optical sectioning or depth discrimination property has become the main motivation for using confocal microscopes. In the classic confocal systems, the height of each point of the surface is measured by the z-location of the sample along the optical axis that leads to the maximum detected intensity. Among the various existing systems, we distinguish the systems where all points of the surface in parallel are treated and the system where the surface is measured point by point. In this work, the confocal microscopy principle

based on longitudinal chromatic aberration fenomenon is presented. As particular application, a miniaturized probe of chromatic confocal microscopy to measure the optical thickness and topography of human corneas is presented. One of the main motivations for the development of this chromatic confocal microscopy device, is associated with the need to measure by mean of fast optical methods, the central thickness of the human corneas removal from a deceased donor. The results presented in this work correspond to pretesting seeking to assess the metrological capabilities of a chromatic confocal system. The human corneas used in this research were obtained from corneas bank of the Hôpital Universitaire Saint Jacques de Besançon, France. Some corneas were discarded for the realization of implants by the number of living cells per square millimeter and the percentage change in its thickness. The experimental model is based on the principle of focus multiplexing by wavelength encoding due to a phase Fresnel lens. The device is composed of a point polychromatic illumination system, a wavelength-height codification system of confocal microscopy and a spectral detection system. The illumination system has a broad and continuum spectrum and it is constituted by the combination of a high energy pulsed laser and an airsilica microstructured optical fiber. The first two systems form a wavelengths segment on the device output. The coding of the optical thickness and the topography is based in the detection of the most intense spectral components coming from the interphases of the organic membranes. The measurement sensitivity is related with the width of the spectral response, which depends on the resolving power of the dispersive element and the detection system. The experimental results and a briefly discussion of the resolution criterion and the perspectives of the work are provided.

Keywords: Confocal microscopy, chromatic aberration, 3D reconstruction.

1. INTRODUCTION

Chromatic dispersion confocal microscopy (CDCM) draws special interest in applications such as imaging biological samples and semiconductor materials, in which high definition in both the transverse and longitudinal dimensions is required[1]. The CDCM has been the object of research in the last years.[2-9] The first works were conducted in bulk architectures. In those experiments the miniaturizing was not present. A polychromatic illumination point source was difficult to obtain, but with technologies such as the generation of supercontinuum in optical fibres [10] was possible to create configurations of miniaturized confocal scanning systems[2,9]. Chromatic confocal systems requires wide illumination spectra with stable flat homogeneous intensity distribution in order to offer reproducible results over long working times. Therefore, not all the fiber-generated supercontinuum can be used. In this work, a high energy pulsed laser and a nonlinear microstructured optical fiber were used to generate a suitable supercontinuum light source for this system. The resulting broad spectrum, high energy and very high stability allow its application to non-contact metrology in biological materials. One of the main motivations for the development of this miniaturized device is associated with the need to measure by mean of a fast method the central thickness of the human corneas removed from a deceased donor. By which, the supercontinuum was used as a point polychromatic illumination subsystem in a miniaturized device for measuring the optical thickness and the topography of human corneas in real time. The micro-setup is based on the longitudinal chromatic aberration produced by a diffractive element where the height of each point of the surface or interphase is measured by the z-location of the most intense spectral components coming from every biological layers of the sample. The whole experimental setup is composed by the point polychromatic illumination subsystem, wavelength-height codification subsystem of confocal microscopy and the spectral detection subsystem. This paper is presented as follows: in section 2, the chromatic confocal microscopy is explained. Section 3 describes the point polychromatic illumination sources utilized in the chromatic confocal microsocopy. In section 4, chromatic confocal device is described. In section 5, the experimental results and the resolution capabilities of the system are described and analyzed. In section 6, some conclusions and perspectives are given.

2. CHROMATIC CONFOCAL MICROSCOPY

2.1. Chromatic aberration

The chromatic aberration is the deformation of an image due to dispersion phenomenon of non monochromatic light through the lenses of an optical system. In this paper we are interested only in the longitudinal aberration, where the position of the focus image varies with the wavelength. In this case, different spectral components are focused on the optical axis at different positions. If λ_1 and λ_2 are two different spectral components of the illumination source, then their focal distances $f(\lambda_1)$ and $f(\lambda_2)$ are localized on different positions along the optical axis (Figure 1). This situation can be

carried out by means of a diffractive lens. The chromatic dispersion properties can be characterized for order +1 by:1[2, 9]

$$f(\lambda) = \frac{r_1^2}{2\lambda} = f(\lambda_d)\frac{\lambda_d}{\lambda} \tag{1}$$

where f is the focal position, λ is the operating wavelength, r_1 is the innermost radius of the Fresnel lens, λ_d and $f(\lambda_d)$ are the design wavelength and the corresponding design focal length, respectively.

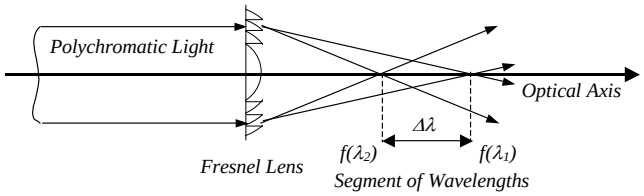


Figure 1. The longitudinal chromatic aberration. λ_2 greater than λ_1 .

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 (Figure 1) and it is defined by:

$$\Delta\lambda = f(\lambda_1) - f(\lambda_2) \tag{2}$$

Experimentally, the segment of wavelengths $\Delta\lambda$ defined by equation (2) can be adjusted to a desired measurement range by mean of a scheme of an optical imaging system useful for reducing the segment of wavelengths $\Delta\lambda$ to $\Delta\lambda$'. If a plane mirror is placed inside the image wavelengths segment $\Delta\lambda$' in a reference focal position $f''(\lambda_2)$, a corresponding wavelength is reflected through the system. Therefore, the z relative positions of the plane mirror are given by[2]:

$$z_\lambda = f''(\lambda) - f''(\lambda_2) \tag{3}$$

2.2. Confocal microscopy

Figure 2 shows a schematic diagram of a conventional confocal microscope. The monochromatic light reflected by the object being tested is spatially filtered near the photodetector. Any light reflected from a point in the object which is not confocal with the pointlike detector is drastically attenuated. This has the effect of selecting a slide in the object. Only the points of the object belonging to the focal point of the objective are detected. The other issue is that such a system improves the lateral resolution of the images. In order to scan the volume of the object, an XYZ scanning device is required.

In confocal systems, if we consider a planar reflector axially scanned through focus, the variation in the detected intensity as a function of its position is given by[1]:

$$I(u) = \left(\frac{\sin(u/2)}{u/2}\right)^2, \qquad u = \frac{8\pi}{\lambda} z \sin^2\left(\frac{1}{2}\alpha\right) \tag{4}$$

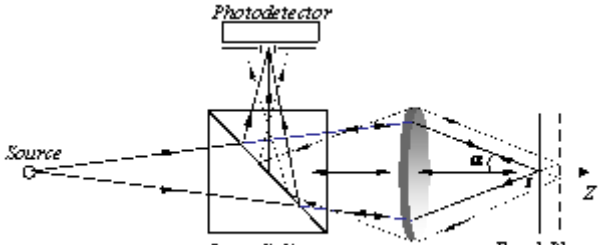


Figure 2. Conventional confocal microscope

where u is a normalized axial coordinate related to the real axial distance z from the planar reflector position to the focal plane, λ is the wavelength and $sin(\alpha)$ is the numerical aperture. That variation of the intensity as a function of z or u constitutes the basis of a confocal microscopy. As the sample S is shifted along the optical axis, the detected intensity reproduces the longitudinal response $I(z)$. The maximum intensity is detected when the sample is exactly at focus ($u = 0$). From a measured point to a neighbor one, the displacement of the sample needs to conserve the detected maximum intensity that corresponds to the height variation between the two points.

2.3. Chromatic confocal microscopy

In a different manner, Figure 3 shows a polychromatic confocal configuration. Here, a Fresnel lens is used as dispersive element. In this way, each individual wavelength is focused at one particular point on the optical axis. Indeed, there is a wavelength-dependent distribution of the focal points. Each of these points (for its specific wavelength) is confocal with the pointlike detector. At the stage when an optical spectrum analyzer is used as a pointlike detector, each spectral maximum in the recorded spectrum corresponds to the image of a point situated at a depth z_λ defined by the equation (3). In this way, each individual slide of the object is located at a depth confocal with the pointlike detector for an unique wavelength. This is an easy way of spectrally coding the depth of an object, as each individual spectral spike reveals the presence of a confocal reflecting point in the object. If we suppose the wavelength λ_0 to be exactly focused on the sample surface, that case corresponds to $u(\lambda_0) = 0$. If the value of the wavelength becomes $\lambda_0 + d\lambda_0$, because of the dispersion, the intensity distribution along the optical axis is shifted and the focus position no longer corresponds to the sample one. That case corresponds to $u(\lambda_0 + d\lambda_0) \neq 0$, i.e., the sample is out of focus[2]. In our system the height measurement range is conditioned by the dependency of the focal distance on the wavelength, given by equation (1), and the bandwidth of the light source. Using the chromatic codification, the point spread function PSF $I(x,y,z- z_\lambda)$ is centered on the z_λ position for a 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:

$$I(\lambda) = \int I(x, y, z_o - z_\lambda)dxdy \tag{5}$$

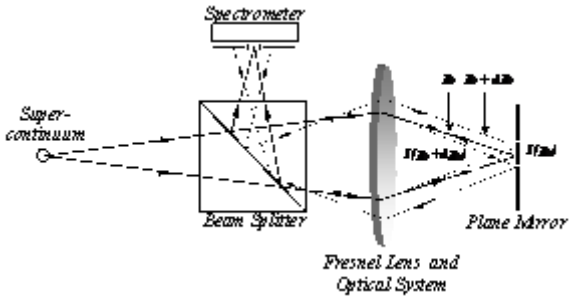


Figure 3. Chromatic confocal microscope.

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

3. ILLUMINATION SOURCES FOR CHROMATIC CONFOCAL MICROSCOPES

In chromatic confocal microscopy is very important the spectral band width of the illumination sources, because it defines the dynamics range of measurement given in the equations (2) and (3). Additionally, its size plays a roll on the optical sectioning in confocal systems. The construction of these sources has been hardly obtained, but with the advent of the technologies of supercontinuums generation in different types of optical fibers was possible to create point polychromatic illuminations sources. However, not all supercontinuum generated by optical fiber can be used in confocal systems, because the spectrums must be wide, with stable energy and easily reproducible for working for long time. Currently, there are several methods for generating supercontinuums in fibers[10,11], but in this work the supercontinuum used is constituted by the combination of a high energy pulsed laser and a nonlinear microstructured optical fibre. Its broad spectrum and high energy, which is very stable, allows its application to non-contact metrology in biological materials. Figure 4 shows the experimental device where a polychromatic point source is obtained. The supercontinuum is built by the combination of a high energy pulsed laser (L) and a nonlinear microstructured optical fibre (OF1). 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 means of microscope objective (O1) . This fibre has been fabricated at XLIM laboratory[11] by the conventional stack and draw process. The hole-to-hole spacing Λ is 2.2 µm and the hole diameter 1.5 µm. This leads to a core diameter equal to 2.8 µm and consequently ensures a nonlinear propagation of the optical wave guided in the core. 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. The results in the generation of a wide spectral supercontinuum both in visible and infrared ranges are presented in the reference 11. In this experiment, the spectrometer only allows to detect until 1100 nm. The output beam or output spot (FO) shows the far field transverse distribution (LP_{11} mode)[11] . This illumination source can be considered as a point source with a wide spectral band that allows us to use it in a chromatic confocal microscopy system.

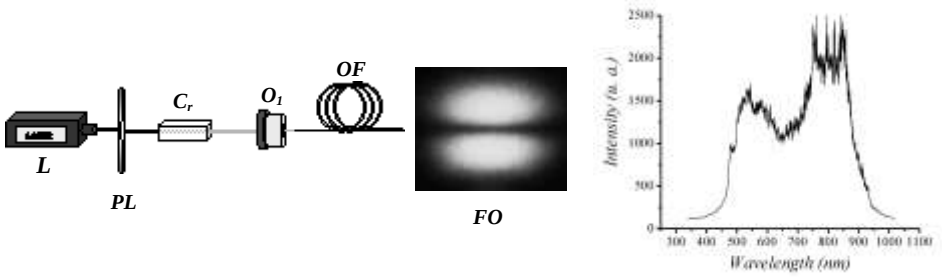


Figure 4. Illumination system and its spectrum.

4. CHROMATIC CONFOCAL DEVICE

The whole miniaturized experimental setup is composed by a point polychromatic illumination subsystem, a wavelength-height codification subsystem of confocal microscopy and a spectral detection subsystem. Figure 5 shows a whole schematic diagram of the miniaturized probe of confocal microscope and its photograph. The point polychromatic illumination system was described in the previous section and the output beam was put in the point P_1 on the wavelength-height codification subsystem. This subsystem is formed by two achromatic lenses L_1 and L_2, a Fresnel lens *FL*, and an objective O_2. The polychromatic spectrum coming from P_1 is collimated through the achromatic lens L_1 of focal distance of 2 cm, which transforms the luminous signal to plane wave. After, the 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 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 dispersion properties of *FL* are characterized for order +1 by the

equations (1) and (3). Then, the two lenses, achromatic lens L_2 with a focal distance of 2 cm and the microscopy objective of 50× (O_2) with numerical aperture of 0.45 and a working distance of 13.8 mm, imagined the wavelengths segment Δλ produced by the Fresnel lens at the system output. If the focal distance of L_2 is f_1, f_2 is the focal distance of O_1 and d is the separation distance between f_1 and f_2, then $f_1 + f_2 + d$ is the separation distance between L_2 and O_1. Therefore, the segment of image wavelengths $\Delta\lambda$' can be adjusted to the desired measurement range using f_1, f_2 and d.

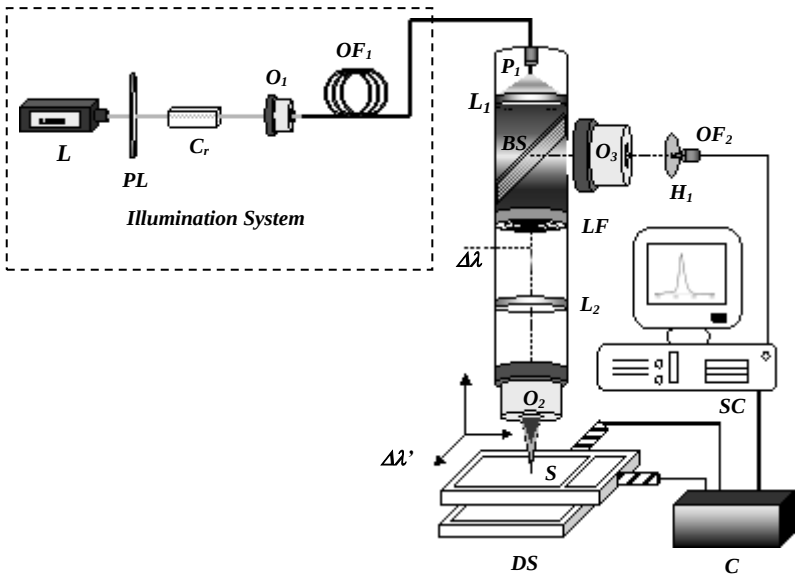


Figure 5. Diagram of the chromatic confocal microscope and its illumination system. On the right side: Photograph of the probe.

In order to obtain 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 the equation (3). On the other hand, if a reflective sample (S) is placed inside the image wavelengths segment Δλ', a corresponding wavelength is reflected through the setup. By means of the beam splitter BS the signals is deviated towards the spectral detection subsystem by a microscope objective O_3 of 40× with numerical aperture of 0.4. The objective O_3 focuses the beam over an optical fiber connector which guides 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 means of pointlike detector. Thus, the position z_λ along the optical axis can be coded inside the spectral space defined by the image wavelengths segment Δλ'. Each of these points (for its specific wavelength) is confocal with the optical spectrum analyzer or pointlike detector. Each spectral maximum defined in equation (5) in the recorded spectrum corresponds to the image of a point situated at a depth z_λ defined by the equation (3). In this way, each individual slide of the object is located at a depth confocal with the pointlike detector for an unique wavelength. This is an easy method of spectrally coding the depth of an object, as each individual spectral spike reveals the presence of a confocal reflecting point in the object. Here, we understand the advantage of using a chromatic dispersion confocal system because only by placing a membrane on the chromatic dispersion interval, its thickness or refractive index can be measured. Additionally, by means of x–y transverse displacement stage (DS), the 3D mapping of an object can be obtained.[2]

5. EXPERIMENTAL RESULTS

5.1 Experimental calibration procedure

In this device the wavelength–height conversion is done by means of an experimental calibration procedure and the implementation of equation (3). Using a metallic mirror as the object, the spectral response $I(\lambda)$, equation (5), for different z_λ positions was obtained.

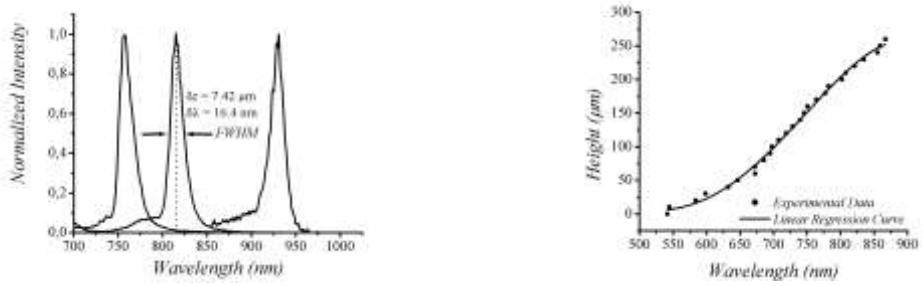


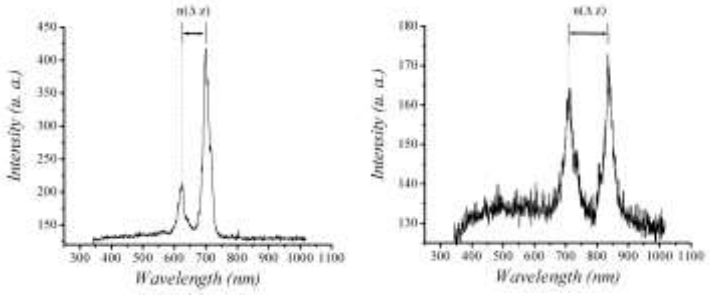
Figure 6. (a) Spectrum detected and normalized for three different positions on the optical axis *z*, (b) Calibration curve

Figure 6(a) shows three normalized spectral responses $I(\lambda)$ for three different z positions. Thus a calibration curve was built by means of a least square fitting of the experimental relation between the most intense spectral components of $I(\lambda)$ and z_λ positions of the mirror. Figure 6(b) shows the calibration curve of the system. In chromatic confocal microcopy, the axial resolution is related with the measurement of the Full Width Half Maximal Value (FWHM) of the spectral response $I(\lambda)$. The FWHM for every spectral response $I(\lambda)$ on the different *z* positions inside the segment of image wavelengths $\Delta\lambda'$, was measured and by means of a linear regression, a spectral resolution function was obtained: $FWHM(\lambda) = -0.289\lambda + 32.01$. Moreover, the lateral resolution LR, according to the classic Rayleigh equation ($LR = 1.22\lambda/2NA$), is an amount proportional to the wavelength and inversely proportional to the numerical aperture of the objective used (O_2 : 50×, numerical aperture of 0.45). From 550 to 900 nm the LR values change from 0.75 to 1.22 μm respectively.

5.2 Applications of the chromatic confocal microscopy

5.2.1 Optical thickness of tissues

One of the main motivations for the development of this device, is associated with the need to measure by mean of a fast method the central thickness of the human corneas removed from a deceased donor. The results presented in this section correspond to pretesting seeking to assess the metrological capabilities of the system. The human corneas used in this research were obtained from corneas bank of the Hôpital Universitaire Saint Jacques de Besançon, France. Some corneas were discarded for the realization of implants by the number of living cells per square millimeter and the percentage change in its thickness.



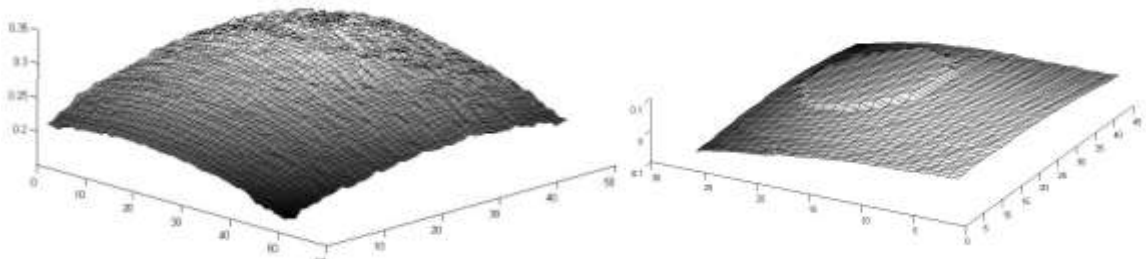
(a) (b)

Figure 7. (a) and (b), corneal optical thickness in different spectral regions.

Experimentally a test human cornea (Figure 7(a)) was placed in such a way that its whole thickness is contained on the wavelengths segment Δλ'. Particularly, the objective O_2 in the device described in the diagram of Figure 5 was changed by an objective of 20× and its calibration parameters stored in the system. So, two wavelengths were detected by the spectrometer from the anterior and posterior corneal surface (Figure 7(b)). By mean of a step – step axial scan on the segment Δλ', fifteen spectrums were stored in the system. Figure 7(b) and Figure 7(c) show the first and fifteenth spectral responses experimentaly detected, respectively. Taking as refractive index value 1.336, the average optical thickness of the test human cornea was 449,47 ± 12 μm. This method is extremely useful on a number of applications in ophthalmology because this result evidences a fast nondestructive method for scanning different regions of human corneas. For example at a later development stage, the device can be adapted to measure the ratio between the radius of the anterior and posterior corneal surface and to study its effect on the corneal effective refractive index. These procedures involve some statistical comparisons with other measuring techniques like optical coherence tomography, ultrasound and interferometric techniques.

5.2.2 Construction of range images and the reconstruction 3D

To obtain the form of a surface was necessary to carry out x–y transverse displacements, which were accomplished by two cross step-step motors of 0.05 μm of resolution and 25 mm of travel range. Figure 5 shows the position where the displacement stages (DS) are located. For every shift of the stage the most intense spectral component coming from the surface was detected. This maximum spectral component was associated with an axial point of the calibration curve, see Figure 6(b). Thus, every sampled point in the surface has a defined axial position. This procedure leads to the construction of a matrix whose size depends on the number of steps chosen by the displacement stage. Additionally, the minimum, maximum and intermediate values found by the calibration curve are mapped to an 8 bits monochrome image. This matrix is called range image. By this procedure the range images of some human corneas were rebuilt. Figure 8 shows the results 3D for two corneas. Again, this results evidence the importance of measuring the central optical thickness of the human cornea by means of a fast nondestructive method.



(a) (b)

Figure 8. Topography of the human corneas: (a) Real time 3D reconstruction of a normal human cornea using the chromatic confocal microscope (b) Topography of an abnormal human cornea.

To conclude this section, we can say that this system offers the possibility of studying the topography, refractive index and thickness in tissues without the need for a high-resolution spectrum analyser, however the system can be optimized by means of the use of optical fibre couples.

6. CONCLUSIONS

A chromatic confocal method to determine the optical thickness and topography of tissues has been presented. The miniaturized chromatic confocal probe allows to perform a spectral coding of the depth of an object without using a scanning system on z- direction. The depth *z* corresponding to a wavelength λ in the spectrum depends on the dispersion properties of the diffractive element. The wavelength gap between the intensity peaks indicates the measurement of the optical thickness in mono-layer tissues or multi-layer membranes. The experimental results in 3D reconstruction of human corneas evidence the importance of measuring their central optical thickness by means of a fast nondestructive

method. The step – step axial motorized scanning increases the work dynamic range of the system. Thus, the work dynamic range can depend upon the travel range of the step – step motor. This work has several perspectives. First, the illumination source has a wide and stable spectrum which can be used for exploring a surface using radiation in the infrared region, for example for application on the skin, comparable with the optical coherence tomography technique. Second, the step – step axial scanning with this illumination source enables us to build an optical thickness matrix whether every spectrum in each step is stored in an empty matrix. Therefore, the optical properties of a membrane on wide spectrum can be analysed. This method is extremely useful on a number of applications in ophthalmology. Third, the axial resolution can be increased taking the illumination range in the far infrared or increasing the magnification of the microscope objective at the system output.

ACKNOWLEDGMENTS

J. Garzón acknowledges the financial support of the Universidad Pontificia Bolivariana de Medellín Colombia, the Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología "Francisco José de Caldas", COLCIENCIAS and the Servicio Nacional de Aprendizaje SENA, by means of the research, code: 1210-405-20354.

REFERENCES

1. T. Wilson. *Confocal microscopy*. Academic Press, London, 1990
2. J Garzón, T. Gharbi, J. Meneses, *Determination in Real Time of Optical Thickness and Topography of Tissues by Chromatic Confocal Microscopy*, J. Opt. A: Pure Appl. Opt. 10 (2008) 104028 (8pp).
3. J. Garzón R., T. Gharbi and J. Meneses, *Measurement of the topography, refractive index and thickness in tissues by mean of a chromatic confocal method*, American Institute of Physics. CP **992**. pp. 846-851, May. (2008)
4. J Garzón, T. Gharbi, J. Meneses, *Determination of the Refractive Index and Thickness in Tissues by Chromatic Confocal Microscopy*, Rev. Col. Fís. **40**, No. 1, pp. 140 - 142. Abril. (2008).
5. J Garzón, J. Meneses, A. Plata, T. Gharbi, G. Tribillon, *Analysis of the Longitudinal Point Spread Function of a Confocal Microscopy Based on the Chromatic Aberration Principles*, Rev. Col. Fís. **38**, No. 2, pp. 561-564. (2006).
6. J. Garzón R, J. Meneses, A. Plata, G. Tribillon and T. Gharbi, *Chromatic confocal method for determination of the refractive index and thickness*, Proc. SPIE. **5622**, 805-810 (2004)
7. J. Garzón R, J. Meneses, A. Plata, G. Tribillon and T. Gharbi. *Axial resolution of a chromatic dispersion confocal microscopy*, Proc. SPIE. **5622**, 766-771, (2004).
8. J. Garzón R, J. Meneses, G. Tribillon, T. Gharbi and A. Plata. *Chromatic confocal microscopy by means of a continuum light generated through a standard single mode fiber*, Pure and Applied Optics. **6**, issue 6, pp 544-548, (2004).
9. C. Courvoisier, A. Mussot, R. Bendoula, T. Sylvestre, J. Garzón Reyes, G. Tribilion,B. Wacogne, T. Gharbi, and H. Maillotte. *Broadband Supercontinuum in a Microchip-Laser-Pumped Conventional Fiber: Toward Biomedical Applications*, Laser Physics. **14**. No. 4, pp 507-514, (2004).
10. J. Garzón R, J. Meneses A. Plata, T. Gharbi, G. Tribillon. *Chromatic Confocal Profilometer*, Rev. Col. Fís. **35**, No. 2, pp. 376-379. (2003).
11. Tombelaine V., Lesvigne C., Leproux P., Grossard L., Couderc V., Auguste J. L. and Blondy J. M. *Ultra wide band supercontinuum generation in air-silica holey fibers by SHG-induced modulation instabilities*. *Opt. Exp*. Vol. 13, **19**. pp. 7399-404. (2005).