ANALYSIS OF THE LONGITUDINAL POINT SPREAD FUNCTION OF A CONFOCAL MICROSCOPY BASED ON THE CHROMATIC ABERRATION PRINCIPLES

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RESUMEN

En este trabajo se evalúa la Longitudinal Point Spread Function (LPSF) de microscopio confocal cromático, el cual funciona bajo un multiplexado focal generado por una lente difractiva de fresnel. El sistema utiliza como fuente de iluminación supercontínuos generados mediante efectos no lineales en fibra óptica monomodo estándar. El análisis espectral de la luz detectada produce la codificación de la altura, identificando la más intensa componente espectral que proviene de la muestra. La sensibilidad de altura del sistema está relacionada con la medida de la respuesta impulsional sobre diferentes posiciones axiales con lo cual se obtiene la LPSF de cada fuente de iluminación.

Palabras Calve: Longitudinal Point Spread Functio, Análisis spectral.

ABSTRACT

In this work the Longitudinal Point Spread Function (LPSF) of a chromatic confocal microscopy is evaluated. Its working is based in the focus multiplexing generated by a diffractive Fresnel lens. The system uses as illumination sources supercontinuous generates by a non-lineal effects in a standard single mode optical fiber. The spectral analysis of the detected light gives the height decoding, by identifying the most intense spectral component coming from the sample. The height sensitivity of the system is related with the measurement of the point spread function on different axial positions, whereby the LPSF for every illumination source is obtained.

Keywords: Longitudinal Point Spread Functio, Spectral analysis.

INTRODUCTION

The principle of chromatic depth scan was introduced by Molesini *et al*., and was adapted confocal microscopy by Akinyemi *et al*[1]. Several setups that use this technique have already been reported but these are robust. Our system is miniaturized (12 cm) and its advantage is to perform a wavelength encoding of the optical axis by using a Fresnel lens in order to eliminate the z-mechanical displacement. In this setup, the illumination sources offers a broader and stability spectrum from 400 nm to 1100 nm. The spectrums were generates by means of a non linear effects in standard single mode optical fiber using pulsed laser radiation. The spectral analysis

of the detected light gives the height decoding, by identifying the most intense spectral component coming from the sample. So, the Full Width Half Maximal Value (FWHM) of the LPSF on different axial positions were measured for obtaining the spectral resolution functions.

CHROMATIC DISPERSION AND CONFOCAL MICROSCOPY

Figure 1 shows a chromatic confocal configuration. Here, a Fresnel lens (diameter: 8 mm, focal: 25 mm for a wavelength of 633 nm) as dispersive element is used. In this way, each individual wavelength λ_0 is focused at one particular point on the optical axis by mean of an achromatic lenses (focal: 20 mm). Each of these points (for its specific wavelength) is confocal with the pointlike detector(Spectrometer). At the stage when an optical spectrum analyzer is used as a pointlike detector, each spectral maximum in the recorded spectrum $I(\lambda_0)$ corresponds to the image of a point situated at a depth z_λ. In this way, each individual slide of the object is located at a depth confocal with the pointlike detector for a unique wavelength. Every individual spectral spike reveals the presence of a confocal reflecting point in the object. Additionally, by mean of a *x-y* scanning apparatus the 3D mapping of an object can be obtained.

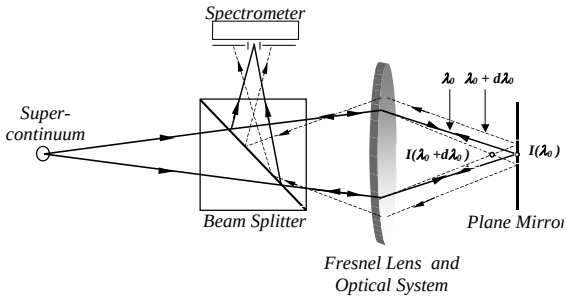


Fig. 1. Polychromatic confocal microscope

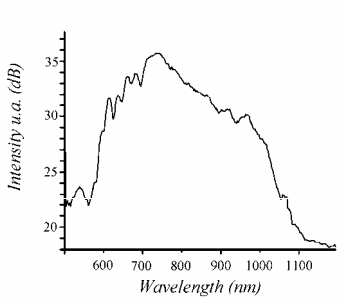


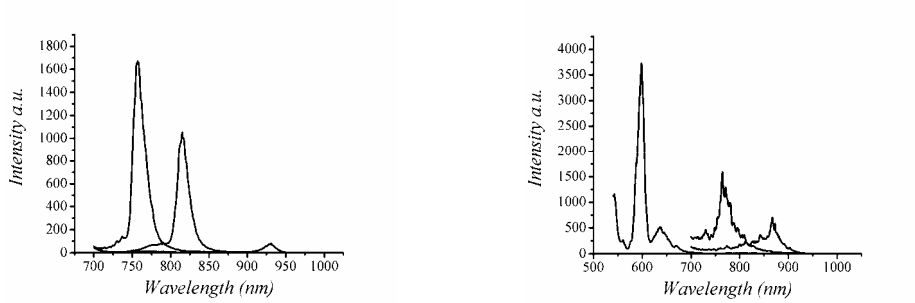
Fig. 2. Supercontinuum

The illumination system consisting in launching pulsed radiation laser in a dispersion shifted fiber (length: 600 m, NA: 0.15, core diameter: 8 μm) by means of an objective, to produce a supercontinuum, figure 2. Supercontinuum generation[2] is mainly based on a double Raman cascade initiated by a multimode four-wave mixing process around the pump laser wavelength at 532 nm (10 mW, pulse width of 0.6 ns, repetition rate of 8 kHz) and 815 nm (22 mW, pulse width of 0.6 ns, repetition rate of 7 kHz). On the other hand, in a classical confocal systems the 3D point spread function (3PSF) $I_p(\lambda)$ is an important parameter for the characterization of confocal imaging systems[3-4]. Placing a mirror perpendicularly to the optical axes at z_o position, the reflected signal $I(\lambda)$ can be calculated by the superposition of the spectral components on the detector of radius r_{det}:

$$I_p(\lambda) = 2\pi \int_0^{r_{\det.}} I(r,\lambda) r dr \qquad (1)$$

EXPERIMENT AND RESULTS

Axially scanning a plane mirror, the spectral response $I(\lambda)$ for different z positions on the optical axis was measured for two illumination sources, figure 3. Every position of the mirror establishes the focal plane for a determined wavelength. The first illumination source (supercontinuum) was generated by the laser of 532 nm, and the second illumination source was generated by the laser of 815 nm.



(a) (b)

Fig. 3. Spectrum detected for three different positions on the optical axis: **(a)** First source. **(b)** Second source

The most intense spectral components are extracted from digitalized spectra, with a resolution of 0.34 nm given by the spectrometer card. By means of a biunivocal relation between the intensity maximum of the spectral response and the position z of the mirror, a calibration curve $Z(\lambda)$ was obtained. That curve represents the wavelength-height conversión. Figure 4 shows the calibration curve for this experiment.

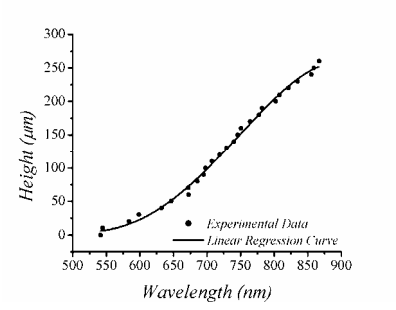


Fig. 4. Calibratio $10^{-2}\lambda^2 + 13.2\lambda - 1536)\mu m$

The axial resolution is related with the measurement of the FWHM of the spectral response $I(\lambda)$. Figure 5 shows the FWHM as a function of the most intense spectral component of $I(\lambda)$. By mean of a linear regression, a spectral resolution function $FWHM(\lambda)$ was obtained for both sources:

$$FWHM_1(\lambda) = (-0.289\lambda + 31.01)\,\mu m \quad (2)$$

$$FWHM_2(\lambda) = (0.037\lambda - 19.202)\,\mu m \quad (3)$$

On the other hand, the lateral resolution (LR=1.22λ/2NA), according to the classic Rayleigh equation is an amount proportional to the wavelength. The LR values are an estimation of the size of the illumination spot on a determined surface. Our system requires a *x-y* lateral displacement stage to inspect two-dimensional regions. If the scanning pitch of the *x-y* lateral displacements stage is greater than LR values, the lateral resolution of the chromatic confocal microscopy is defined by one.

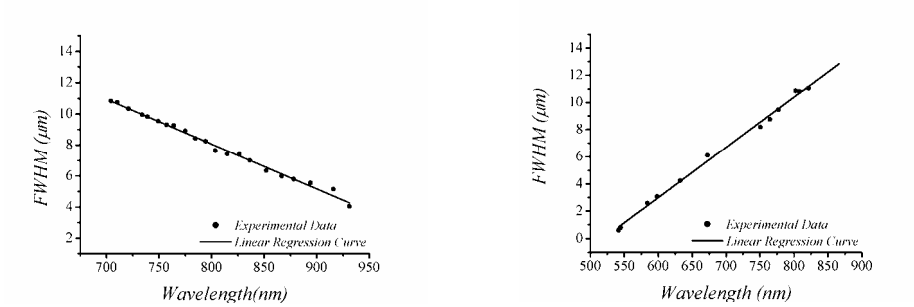


Fig. 5. (a) *$FWHM_1$* as a function of the detected wavelength, first source. **(b)** $FWHM_2$ as a function of the detected wavelength, second source.

CONCLUSIONS

The longitudinal point spread function of a confocal microscopy based on the chromatic aberration and its influence in the axial and lateral resolutions has been presented. If the power of the source increase, the spectral responses I(λ) are most intense and the FWHM values increase proportionally with the wavelength. Instead, if the power of the source decrease, the spectral responses I(λ) has minor energy and the FWHM values decrease proportionally with the wavelength. In general, if the power or wavelength of the pulsed laser launched on the fiber are changed, a new spectral resolution function must be calculated. The utility of one or another source depends of the applications. For example the first source can be used for reconstruction 3D of surface. The second source can be used for measuring thickness on multi-layer films because it has more energy.

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