Diffractive elements performance in chromatic confocal microscopy

J. Garzón¹, D. Duque¹, A. Alean¹, M. Toledo¹, J. Meneses² and T. Gharbi³

¹ Grupo de Óptica y Espectroscopía, Centro de Ciencia Básica, Universidad Pontificia Bolivariana. Medellín, Colombia.
² Laboratorio de Óptica y Tratamiento de Señales, Instituto de Física, Universidad Industrial de Santander. Bucaramanga, Colombia.
³ Laboratoire d’Optique P. M. Duffiez, UMRD6603 CNR/Université de Franche-Comté. 16 route de Gray, 25030 Besançon Cedex, France.

E-mail: jgarzonr10@une.net.co

Abstract. The Confocal Laser Scanning Microscopy (CLSM) has been widely used in the semiconductor industry and biomedicine because of its depth discrimination capability. Subsequent to this technique has been developed in recent years Chromatic Confocal Microscopy. This method retains the same principle of confocal and offers the added advantage of removing the axial movement of the moving system. This advantage is usually accomplished with an optical element that generates a longitudinal chromatic aberration and a coding system that relates the axial position of each point of the sample with the wavelength that is focused on each. The present paper shows the performance of compact chromatic confocal microscope when some different diffractive elements are used for generation of longitudinal chromatic aberration. Diffractive elements, according to the process and manufacturing parameters, may have different diffraction efficiency and focus a specific wavelength in a specific focal position. The performance assessment is carried out with various light sources which exhibit an incoherent behaviour and a broad spectral width.

Key words: Diffractive elements, confocal microscopy.

1. Introduction

The chromatic confocal method (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 years¹ ². The first works were conducted in bulk architectures³⁷. In those experiments the miniaturizing no was present and the point sources of illumination difficulty were obtained. In this paper, we show three different chromatic confocal systems with three different Fresnel lens, where the Fresnel lens is the diffractive element. Each system was characterized and its response was contrasted in order to choose a system with the best characteristics and at the same time consider them in the design the future chromatic confocal system.

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2. Principal parts of a chromatic confocal microscope

Our chromatic confocal microscope has three principal systems: illumination system, wavelength-high codification system and detection system (figure 1).

![Figure 1. Main systems of a chromatic confocal microscope.](image)

In a confocal chromatic microscope the light source must be carefully chosen based on criteria such as: spectral width, output power, stability, flatness of the spectrum, etc. The sources used most often are halogen and LEDs sources, but also in recent years supercontinuum. Our illumination system is composed by: a 100× objective, that permit us focusing the light that arrives from the fiber; and a system of spatial filter compose by a 50 µm pinhole and a 10× objective was used for obtaining a polychromatic collimated beam. On the other hand, wavelength-high codification system is formed by: a Fresnel lens which generates the longitudinal aberration, a compression system where the segment of wavelengths is adjusting to the desired measurement range and a nanometric motorized stage for scanning 2D-displacement. Finally, the detection system is composed by a 10× objective which focuses the signal reflected from the specimen in the spectrometer detector.

3. Chromatic confocal probe modelling

The probe has two main elements: a Fresnel lens and a compression system. How was mentioned above, the Fresnel lens generates the longitudinal aberration and the compression system permits adjusting the wavelengths segment to a desired measurement range. If we suppose that a polychromatic source arrives to the input of Fresnel lens, then in the output of it different spectral components are focused on the optical axis at different positions. If $\lambda_1$ and $\lambda_2$ 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. This situation can be carried out by means of a diffractive lens. The chromatic dispersion properties can be characterized for order +1 by

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

(1)
where \( f \) is the focal position, \( \lambda \) is the operating wavelength, \( r_1 \) is the innermost radius of the Fresnel lens, \( \lambda_d \) and \( f(\lambda_d) \) are the design wavelength and the corresponding design focal length, respectively. Expressing the equation (1) as a Taylor expansion around the design wavelength and eliminating the high order terms, the chromatic dispersion can be expressed as:

\[
 f(\lambda) \approx f(\lambda_d) - 2f(\lambda_d) \left( \frac{\lambda}{\lambda_d} \right) + 6f(\lambda_d) \left( \frac{\lambda}{\lambda_d} \right)^2 - 3f(\lambda_d) \left( \frac{\lambda}{\lambda_d} \right)^3 + f(\lambda_d) \left( \frac{\lambda}{\lambda_d} \right)^4
\]

(2)

If \( f(\lambda_1) \) and \( f(\lambda_2) \) are calculated for \( \lambda_1 \) and \( \lambda_2 \) respectively, then a segment of wavelengths \( \Delta \lambda \) is created and it is defined by:

\[
\Delta \lambda = f(\lambda_1) - f(\lambda_2)
\]

(3)

Experimentally, the segment of wavelengths \( \Delta \lambda \) defined by equation (3) can be adjusted to a desired measurement range. Therefore we can use an optical imaging system for reducing the segment of wavelengths \( \Delta \lambda \). The system (compression system) is composed of achromatic lenses \( AL_2 \) and a microscopy objective \( O_1 \). If the focal distance of \( AL_2 \) is \( f_1 \) and \( f_2 \) is the focal distance of \( O_1 \), then \( f_1 + f_2 + d \) is the separation distance between \( AL_2 \) and \( O_1 \). On the other hand, if \( f'(\lambda_2) \) is the image point of \( f(\lambda_2) \) by \( AL_2 \) and \( a \) is the separation distance between \( f_1 \) and \( f'(\lambda_2) \), it is possible to demonstrate by the Newton’s equation\(^{12}\) that imaging system obeys the following expression:\(^1^8\):

\[
\Delta \lambda = f_1^2 \quad \text{and} \quad \Delta \lambda'(a - d) = f_2^2
\]

(4)

Therefore the final equation to obtain \( \Delta \lambda' \) is:

\[
\Delta \lambda' = \frac{f_2^2}{f_1^2 - d(\Delta \lambda)} (\Delta \lambda)
\]

(5)

Thus, the segment of image wavelengths \( \Delta \lambda' \) can be adjusted to the desired measurement range using \( f_1, f_2 \) and \( d \). In order to get a longitudinal chromatic codification of \( z \), every focal position \( f''(\lambda) \) can be converted to \( z \) relative positions. 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:

\[
z = f''(\lambda) - f''(\lambda_2)
\]

(6)

Equation 6 shows how is possible to build a calibration curve between the \( z \) relative positions and the wavelengths.

Figure 2. Optical imaging system used to reduce the segment of wavelengths \( \Delta \lambda \).
4. Experimental results

In this section we portray the main characteristics of the three systems tested such as: calibration curves, resolution curves and some responses of the systems (spectrums) for certain points of interest. We also do an analysis of these characteristics in order to have criterions to decide which the system with the best performance is.

The differences between the systems are in the diffractive elements or Fresnel lenses. All the diffractive elements have 8 mm of diameter, focus of 2 cm for the wavelength design of 632 nm. But the diffraction efficiency of the Fresnel lens of systems 1 is 85%, for system 2 is 80% and 95% for system 3. We chose the first system how the basic system, in this way we are going to compare system 1 with the others systems (2 and 3). The reason for this election is because the first system was the first system that we implemented, in other words it is our oldest system.

![Figure 3. Calibration curves of the three systems (a) System 1 (b) System 2 and (c) System 3](image)

Figure 3 shows the calibration curves of the three systems. Each curve was obtained by mean of a mirror movement through focal axes of the system under study. Thus for each axial movement of the
mirror, the spectral component focalized in the surface of the mirror was detected by the spectrometer. All curves are almost equal i.e. the distribution of spectral through focal axes of each system was very similar each other. The segment of wavelengths that we used was from 510nm to 690nm with a measurement range of 0.4mm. It is coherent with the theoretical calculus of the compression system because we made the calculus to achieve the same compression of the dynamic range of the wavelengths.

Figure 4 shows the axial resolution curve for every system. In order to obtain each curve we scanned with a mirror the optical axes of each system, in this way the spectrum related with each point of optical axes was detected and then the FWHM (Full Width Half Maximum) of each spectral pulse was measured. After the prior process the following results was obtained. The Systems 1, 2 and 3 were analyzed for the same segment of wavelengths and they exhibited an axial resolution in the range from 25um to 48um, from 29um to 57um and 7 um to 13.5um respectively. An important improvement in the system 3 was observed because the value of axial resolution decreased dramatically, so it set from 7 um to 13.5um (compared with the system 1). The other hand, axial resolution in the system 2 made worse because its value set from 29um to 57um. Other important issue is that in the three systems the axial resolution seems to make better if the wavelength increases.

![Resolution Curve of System 1](image1.png)

![Resolution Curve of System 2](image2.png)

![Resolution Curve of System 3](image3.png)

**Figure 4.** Axial resolution curves of the three systems (a) System 1 (b) System 2 and (c) System 3
The following figures portray some relations about the energy or energy efficiency of the systems. In order to obtain these graphics we chose several points (specific wavelengths) along of focal axes of the system and we moved the mirror to these positions for then catching the signal by mean of a spectrometer. This process was applied to each system in the same wavelengths. The figure 5 shows the spectral responses of the three systems in particular points of the focal axes using an integration time of 100ms in the spectrometer detector. We can see the pulse with more energy corresponds to the system 2, then the pulse with second major energy belong to the system 3 and finally the pulse related with the system 1. We observe a proportional relation that conserve along of the wavelength segment that we used.

![Figure 5](image)

**Figure 5.** Responses of the three systems using integration time of 100ms in the detector (a), (b), (c) and (d).

In the figure 6 appear the spectral responses of the three systems but now with an integration time of 200ms. The figure 6 (a) shows that at wavelength of 559nm the sensor of the spectrometer is saturated because of the high energy of the system2, but it does not occur with the others two systems. A similar thing occur in the figure 6 (b), but now the energy of the pulse associate with the system 3 begin to saturate the sensor. Other thing passes with the first system, which has not reached to saturate the sensor yet.

The process to obtain the graphics of the figure 7 was interesting. First we fixed the integration time of the sensor in 200ms and then we worked with the system 1 (probe 1). So, we did a scanning in the axial direction in order to obtain the signal in different positions. Then we did a similar thing with the other systems (system2 or probe 2, system 3 or probe 3), but this time we varied the integration time until obtain the same energy or intensity in the detector. This process permit us to compare the
reduction in the integration time that we can achieve using the system 2 and system 3. For example, the figure 7 (a) shows that if we used the system 2 we can obtain the same response of the system 1 but now with only a integration time of 105ms instead of 200ms. A similar thing pass with the system 3 (figure 7 (b)), we can obtain the same result using an integration time of 133ms. This analysis was made for a wavelength of 584nm.

Figure 6. Responses of the three systems using integration time of 200ms in the detector (a), (b) and (c).

Figure 7. Responses of the three systems using different integration times in the detector (a), (b), (c) and (d).
The figure 7 (c) and (d) shows a similar behavior of the systems as those of figure 7 (a) and (b), but in this case the wavelength was 644nm and the integration time of the system 2 and system 3 decreased a little bit more compare with the previous case. Here we achieved that the systems 1, system 2 and system 3 had the same intensity using the integration times of 200ms, 92ms and 121ms respectively.

5. Conclusions
Three chromatic confocal systems have been studied. The second system presented an important improvement in energy compared with the first system, but both systems showed a similar axial resolution. When the third system was compared with the first one, it showed an improvement in energy and axial resolution as well. The previous ideas let us to say that it is possible obtain a better performance of one chromatic confocal system if we consider and analyze the characteristics of the wavelength-height system, for example: compression system and in a special way the characteristics of Fresnel lens. The study of different systems permitted us finding other systems with a significant reduction of the integration time, which is something basic and important in order to obtain a system that can wok in real time. Also we found that these improvements are together with an increase (system 3) or decrease (system 2) in the axial resolution. It would be interesting doing the theoretical modeling of all systems in order to understand the effect of each optical element in the resolution and efficiency of energy, in special the study of the Fresnel lens.

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