Mueller Matrix Microscopy

Author: Marta Baldrís Calmet
Facultat de Física, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain.[*]

Abstract: We describe a microscope that can measure simultaneously all the Mueller matrix elements of a sample with high spatial resolution. This measure is possible thanks to the dual rotating compensator method, which analyses the variation in time of the intensity at every pixel of the camera detector. This work reports the measurement principle, the instrumental details, the calibration, and some applications of the microscope.

I. INTRODUCTION

The Mueller matrix is a useful mathematical tool to characterize the optical properties of a medium at certain wavelength. A wide range of optical properties of the material can be calculated from the Mueller matrix. The optical characterization of materials and media with Mueller matrices is used in many fields of science, for example in the study biological tissues [1], for remote sensing in the ocean [2], for polarimetry of anisotropic chiral media [3], or in liquid crystals studies [4].

The objective of this work is to build up a microscope that can measure the Mueller matrix of a sample with high spatial resolution. The antecedent of this Mueller matrix microscope (MMM) is the Polarized Light Microscope (PLM). The PLM is typically used to study the linear birefringence of optically anisotropic samples and, for example, it has a lot of applications in textile industries (as an indicator of the stretching degree) or in geology (for mineral identification). The Mueller Matrix Microscope represents a generalization of the PLM and it can be also used for all these applications. The main difference between both microscopes is that in PLM the results of linear birefringence are based on the visual analysis of the polarization colors, while in a MMM all the optical properties (linear birefringence, lineal dichroism, circular birefringence, etc) are measured quantitatively and simultaneously.

There are several experimental approaches to measure a complete Mueller matrix, for example, the four photoelastic modulators technique [5] or the dual rotating compensators approach [6]. This last technique is compatible with the uses of a camera as a detector, because the camera detectors (CCD or CMOS) are relatively slow and cannot be used with photoelastic modulators (which work in the KHz range). Camera detectors are suitable to work with much lower frequencies than those allowed by the dual rotating compensators approach, so this technique is suitable for imaging.

In our MMM we apply this last technique, so that polarization of light is modulated by two compensators that rotate at different frequencies. The sample is placed between these two compensators, and a camera is continuously collecting images while the compensators rotate. From the analysis of the variation of this intensity with time, the elements of the Mueller Matrix of this sample can be recovered.

In this article we will show the measurement principle of the Mueller Matrix Microscope, we will explain the calibration process, and finally we will present some examples of different measurements.

II. THEORY AND INSTRUMENTATION

A. Mathematical development

There are two ways of describing a completely polarized light: with Jones vectors and Jones matrices, which are related with amplitudes of electric fields, and with Stokes vectors and Mueller matrices, which are associated with intensities. In our case, we have considered this second method. The Stokes vector of light is given by:

$$\mathbf{S} = \begin{pmatrix} I \\ Q \\ U \\ V \end{pmatrix} = \begin{pmatrix} \langle |E_x|^2 + |E_y|^2 \rangle \\ \langle |E_x|^2 - |E_y|^2 \rangle \\ \langle E_x^* E_y + E_x E_y^* \rangle \\ i\langle E_x^* E_y + E_x E_y^* \rangle \end{pmatrix}, \tag{1}$$

where I, Q, U, V are the Stokes parameters, and E_x, E_y are the amplitudes of electric field.

The changes of a Stokes vector by an optical system or a medium can be given as:

$$\mathbf{S}_{out} = \mathbf{M}\mathbf{S}_{in} \tag{2}$$

where $\mathbf{M}$ is a Mueller matrix of a medium and it expresses how the polarization of light is modified. The Mueller matrix is a 4x4 matrix which contains 16 real parameters and it is written as:

$$\mathbf{M} = \begin{pmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{pmatrix} \tag{3}$$

To measure the Mueller matrix of a sample with the dual rotating compensators technique, we have to build an optical train that includes two polarizers and two rotating compensators as proposed by Azzam [7]. The intensity measured at the detector can be found from the multiplication of the Mueller matrices of each element of the optical system.

$$\mathbf{I} = (1000) \cdot \mathbf{PL}_{90} \cdot \mathbf{WP}_2 \cdot \mathbf{M} \cdot \mathbf{WP}_1 \cdot \mathbf{PL}_0 \cdot (1000)^T \tag{4}$$

[*]Electronic address: mbaldrca8@ub.edu

PL corresponds to the Mueller matrix of the polarizers, where the subscript is the angle of orientation. WP indicates the Mueller matrix of the compensators, and the subscripts indicate whether it is the first or the second.

We call the polarizer and the compensator placed before the sample (PL_0 and WP_1) polarization state generator (PSG), whereas the compensator and the polarizer that are placed after the sample right before detector (WP_2 and PL_{90}) are called polarization state analyser (PSA).

The orientation of both polarizers is arbitrary as long as it is well known. In our case, the polarizers are crossed to obtain more contrast during the alignment.

The Mueller matrices of our crossed polarizers are:

$$\mathbf{PL}_0 = \begin{pmatrix} 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \quad \mathbf{PL}_{90} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \tag{5}$$

Notice that they are assumed to be ideal and do not have any dependence.

The Mueller matrix of a compensator depends on the retardation that it causes to polarized light, and on the orientation of its optic axis. During the measurement, the compensator is continuously rotating so its orientation is changing with time at a certain angular velocity. The Mueller matrix of the compensator is given by:

$$\mathbf{WP}_i = \mathbf{R}(-\theta_i) \cdot \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & cos(\delta_i) & sin(\delta_i) \\ 0 & 0 & -sin(\delta_i) & cos(\delta_i) \end{pmatrix} \cdot \mathbf{R}(\theta_i) \tag{6}$$

$$\mathbf{R}(\theta_i) = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & cos(2\theta_i) & sin(2\theta_i) & 0 \\ 0 & -sin(2\theta_i) & cos(2\theta_i) & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \tag{7}$$

where δ is the retardation of the compensator, θ is the orientation of the fast axis, and $\mathbf{R}(\theta)$ is the rotation matrix.

The parameter δ depends on the wavelength of light used during the measurement, and its value is determined during the calibration.

In our instrument, θ_i varies with time due to the rotating compensator setup. The value of θ_i with time is given by:

$$\theta_i(t) = \omega_i t + \phi_i \tag{8}$$

where ω is the angular speed, which is different to each compensator, and it can be adjusted before the measurement with the motor controller. ϕ is the phase (the orientation of the compensator at time zero).

If we multiply the matrices in Eq. (2) we will find the intensity expression, that can be parameterized as:

$$I(\theta_1, \theta_2) = \sum_{i,j=1}^{4} c_{ij}(\theta_1, \theta_2) m_{ij} \tag{9}$$

where m_{ij} are the Mueller matrix elements of the sample, c_{ij} are the coefficients that parametrizes the contribution of the device elements, and it includes all the orientation information of the compensator.

From (9) we must determine the value of every coefficient c_{ij}. If we have calculated these coefficents we will find:

$$\begin{aligned}
c_{00} &= 1 \\
c_{01} &= C^2_{2\theta_1} + C_{\delta_1} S^2_{2\theta_1} \\
c_{02} &= C_{2\theta_1} S_{2\theta_1} - C_{\delta_1} C_{2\theta_1} S_{2\theta_1} \\
c_{03} &= S_{\delta_1} S_{2\theta_1} \\
c_{10} &= -(C^2_{2\theta_2} - C_{\delta_2} S^2_{2\theta_2}) \\
c_{11} &= -(C^2_{2\theta_2} + C_{\delta_2} S^2_{2\theta_2})(C^2_{2\theta_1} + C_{\delta_1 1} S^2_{2\theta_1}) \\
c_{12} &= -(C^2_{2\theta_2} + C_{\delta_2} S^2_{2\theta_2})(C_{2\theta_1} S_{2\theta_1} - C_{\delta_1} C_{2\theta_1} S_{2\theta_1}) \\
c_{13} &= -S_{\delta_1} S_{2\theta_1} \left(C^2_{2\theta_2} + C_{\delta_2} S^2_{2\theta_2}\right) \\
c_{20} &= C_{\delta_2} C_{2\theta_2} S_{2\theta_2} - C_{2\theta_2} S_{2\theta_2} \\
c_{21} &= -\left(C^2_{2\theta_1} + C_{\delta_1} S^2_{2\theta_1}\right)\left(C_{2\theta_2} S_{2\theta_2} - C_{\delta_2} C_{2\theta_2} S_{2\theta_2}\right) \\
c_{22} &= -\left(C_{2\theta_2} S_{2\theta_2} - C_{\delta_2} C_{2\theta_2} S_{2\theta_2}\right)\left(C_{2\theta_1} S_{2\theta_1} - C_{\delta_1} C_{2\theta_1} S_{2\theta_1}\right) \\
c_{23} &= -S_{\delta_1} S_{2\theta_1} \left(C_{2\theta_2} S_{2\theta_2} - C_{\delta_2} C_{2\theta_2} S_{2\theta_2}\right) \\
c_{30} &= S_{\delta_2} S_{2\theta_2} \\
c_{31} &= S_{\delta_2} S_{2\theta_2} \left(C^2_{2\theta_1} + C_{\delta_1} S^2_{2\theta_1}\right) \\
c_{32} &= S_{\delta_2} S_{2\theta_2} \left(C_{2\theta_1} S_{2\theta_1} - C_{\delta_1} C_{2\theta_1} S_{2\theta_1}\right) \\
c_{33} &= S_{\delta_2} S_{\delta_1} S_{2\theta_2} S_{2\theta_1}
\end{aligned}$$

We have used a short notation, for example: $cos(2\theta_1) \equiv C_{2\theta_1}$ i $sin(\delta_1) \equiv S_{\delta_1}$

The intensity detected by the camera pixel to pixel over time and the parameters c_{ij} are the known variables of the system, while m_{ij} are the unknown. These equations depend on the retardation of the compensators (δ_1 and δ_2) and on the orientation (θ_1 and θ_2). As it is shown in Eq. 8, θ_1 and θ_2 depend on the angular speed and the phase.

B. Device

In this section we will explain the instrumental details of our microscope, and of the computer that performs the data acquisition.

The light travels from the bottom to the top of the system, as shown in Fig. 1.

The light source of the microscope is a white LED (Metaphase MP-LED-150). The PSG part is composed of a polarizer (high contrast linear polarizing film) mounted on a manual precision rotating stage (Newport UTR80S) and a compensator mounted on a precision rotation motorized stage (Newport SMC100) which rotates with angular velocity of $\omega_1 = 5°/s$.

The sample zone is made up of one bandpass filter (which selects the operative wavelength), the sample holder and the microscope objective (10X Mitutoyo

Plan Apo Infinity-Corrected Long WD, or 50X Mitutoyo Plan Apo HR Infinity-Corrected), which can easily be replaced.

The PSA part is made up of the same elements as PSG and its compensator rotates with angular velocity $\omega_2 = 25°/s$. The top of the microscope is made up of a camera (uEye UI-3370CP) and its telecentric objective . It is important to mention that the optical system has been built on an antivibration table, to avoid external vibrations.

The camera has a resolution of 752×480 (0.36 MP) and is sensitive to all the visible range and a small part of UV. We have chosen the LED light source because it features a high intensity in all the camera range, so we can select the wavelength of study using narrow interference bandpass filters.

The camera and the two motors (controlled with Single-Axis Controller, SMC 100) are connected to a personal computer, using the USB 2.0 bus. With a software made by Labview 2012 we are able to move the motors, collect all data and calculate the Mueller matrix values.

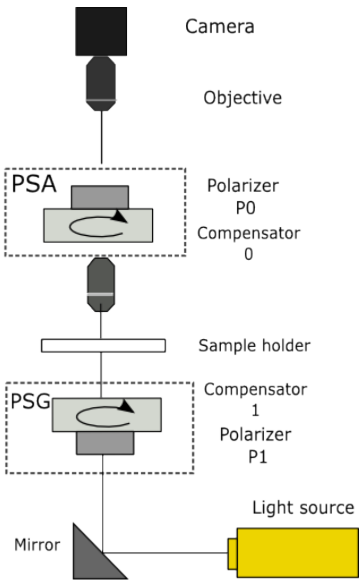

Figure 1: Schematic of the microscope.

III. MEASUREMENT PRINCIPLE AND CALIBRATION

A. Measurement principle

The two basic parameters that control the acquisition are the frame rate of the camera (that is always kept at 50 fps) and the number of acquired images N. We have to adjust N to a value high enough to allow the slowest compensator to do a complete turn (typically this means that N is at least 3600 in our microscope).

Equation (9) expresses the intensity $I(\theta_1, \theta_2)$ as a summation extended to all the elements of the matrix as a multiplication of the coefficients $c_{ij}(\theta_1, \theta_2)$ with the elements of the Mueller matrix m_{ij}. The variables θ_1 and θ_2 are change over time, so the intensity changes too.

We have defined a new **A** vector, which includes all the 16 elements of the Mueller matrix sample, so we can express this equation as:

$$I(t) = \mathbf{C}^T(t)\mathbf{A} \quad (10)$$

where I is an scalar number, and $\mathbf{C}^T$ is a vector that includes all the system coefficients found in the last section, where the superscript "T" denotes transposition.

If we take N measurements, we can include the intensity analysis in time restating this equation as a multiplication of a matrix by a vector:

$$\mathbf{I} = \mathbf{C}^T\mathbf{A}$$

And now **I** is a vector with N elements, $\mathbf{C}^T$ is N × 16 matrix, and **A** is the vector containing all the Mueller matrix elements.

Using the algebra properties we can isolate vector **A** multiplying **C** on each side of the equation. With the following result:

$$\mathbf{CI} = \mathbf{CC}^T\mathbf{A} \quad (11)$$

we can express **A** like:

$$\mathbf{A} = \mathbf{RI} \quad \text{where} \quad \mathbf{R} = \mathbf{C}^T \cdot (\mathbf{CC}^T)^{-1} \quad (12)$$

To solve this equation we need the determinant of $\mathbf{CC}^T$ to be different from zero, because that warrants that this matrix will be invertible. As the $\mathbf{C}(\theta_1, \theta_2)$ matrix depends on the angular speed of both compensators, we have to find a relation of angular speeds in which the determinant of $\mathbf{CC}^T$ takes maximum values.

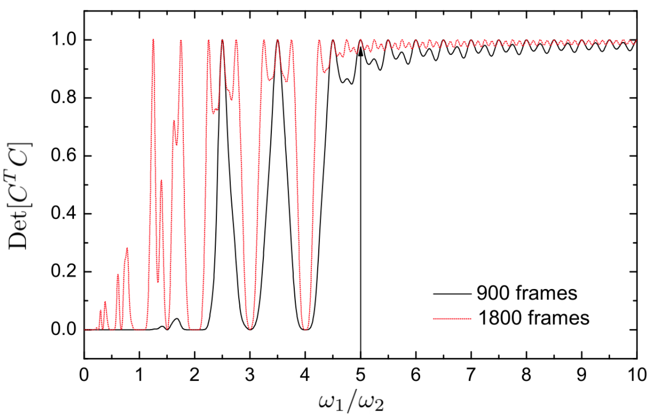

Figure 2: The graphic of evaluation of the determinant of the relation C^TC as a function of ω_1/ω_2.

In Fig. 2 we represent the value of the determinant depending on the different relations of two motors velocities. As we can see, there are several relations of rotations speed that are suitable (when the determinant takes maximum values).

In our microscope system we have chosen the relation of 1:5 for the motors velocity. This is the reason why the PSG motor rotates at $5°/s$ and the PSA motors does it at $25°/s$. Once we have determined suitable values for the angular speeds, the measurement can start. Every pixel will give us a different reading of the intensity so we have to repeat the calculation of Mueller matrix elements for every pixel (in our case 360000 times). We will expose the results as a 4×4 matrix of images. The value of every Mueller matrix element is codified in a color scale.

B. Precision and errors

Before calibration, we are going to determine the precision of the angular phase due to the velocity of the motors. This angular precision can be found by:

$$\Delta\phi = \omega \cdot \Delta t \tag{13}$$

where ω is the angular speed, and Δt is the inverse of the frame rate. We obtain the following precision in the determination of the phase.

$$\Delta\phi_1 = 5 \cdot \frac{1}{50} = 0.1°$$

$$\Delta\phi_2 = 25 \cdot \frac{1}{50} = 0.5°$$

So, the angular precision for the PSG motor that moves $\omega_1 = 5°/s$ is $0.1°$, and for the PSA motor that moves $\omega_2 = 25°/s$ it is $0.5°$.

In Ref.[8] we can see the propagated error associated with each element of the Mueller matrix for small errors in the determination of phases ϕ_1 and ϕ_2 and retardation δ_1 and δ_2 values.

C. Calibration

In the calibration we are going to determine the values of retardation of the compensators, δ_1 and δ_2, and the offset phase ϕ_1 and ϕ_2.

The value of the offset phases has to be constant in all the wavelength range, while retardation values can change slightly depending on the wavelength of measurements.

To calibrate our microscope we have taken into account the fact that in the absence of sample the Mueller matrix that we should measured is the identity matrix. To avoid any distortion made by the objective we have removed it during the calibration. To start the calibration we make a measurement and adjust these four parameters until we obtain the identity matrix. For every wavelength that we want to study we have to repeat the calibration.

Filter (nm)	δ_2 (°)	δ_1 (°)	ϕ_2 offset (°)	ϕ_1 offset (°)
400	71.5	133		
430	88	115		
532	91.7	89	148.9 ±0.5	-103.5 ±0.1
545*	90.9	86.5		
610	84.7	74.5		

* means broadband

Table I: Retardance and phase parameters of the PSA and PSG determined during calibration.

Table I shows the calibration parameters for our microscope at different wavelengths. Once we have adjusted these parameters, if we want to measure the Mueller matrix of any sample we will introduce the objective. The objectives we use are strain free but, they may still introduce some minor changes in the polarization that we have not considered in our calculation. To correct its perturbations we are going to measure a "blank measurement" without sample, and we can extract the objective effects as a matrix $\mathbf{M}_{obj}$. We must always measure this matrix whenever we replace the objective, modify the aligment of the system or change the wavelength of measurement.

To correct the objective effect in our sample measurements we are going to carry out the following calculation:

$$\mathbf{M} = \mathbf{M}_{obj}^{-1} \cdot \mathbf{M}_{sample} \tag{14}$$

and we are going to repeat this operation for every pixel.

IV. MEASURES

The calculus to find the optical properties from a Mueller matrix are complex and it is explain in the Ref.[8]. In this section we will show some of the measures made using the Mueller matrix microscope. We will expose three examples, a measurement of linear birefringence in textile polymer, a measurement of the Mueller matrix of a mosquito wing and a measurement of the Mueller matrix of the benzil polycrystalline.

A. Mosquito wing

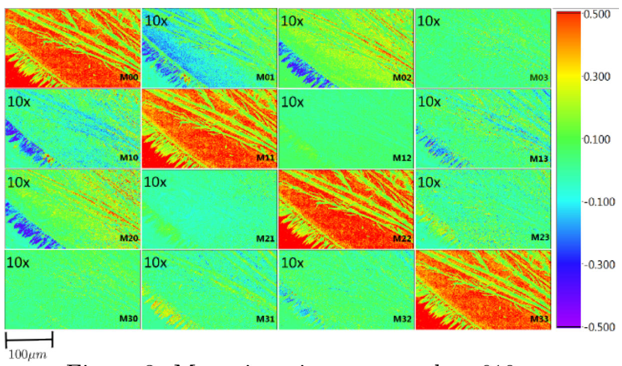

Figure 3: Mosquito wing measured at 610 nm.

In this figure we shows the Mueller matrix of a mosquito wing. We can see that the little feathers of a portion of mosquito wing present high optical anisotropies, as linear birefringence and linear dichroism.

B. Textile polyester

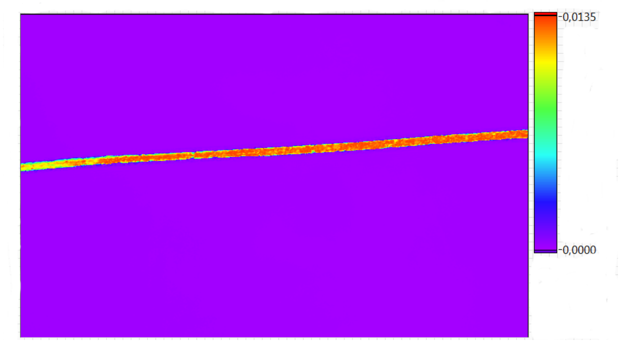

Figure 4: Birefringence values of textile polyester fiber PET650, measured at 532 nm.

Figure 4 is an example of birefringence measurement on textile polyester fiber. In this case the fiber has a value of linear birefringence of 0.0135 radians. In the textile industries the optical property of linear birefringence is useful to determine the stretching of thread.

C. Benzil polycrystalline

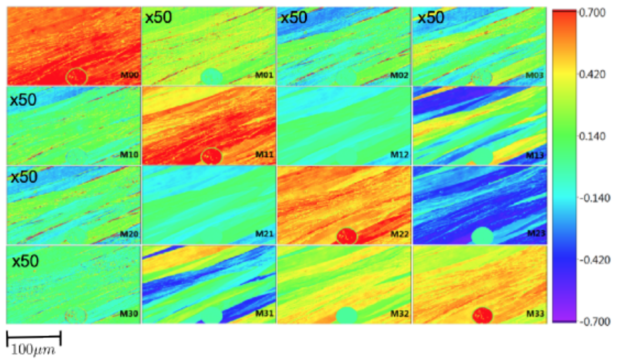

Figure 5: Benzil polycrystalline measured at 440 nm.

Benzil is a organic compound. This measurement corresponds to a thin polycrystalline sample grown from the melt. We have made this measure at 440 nm because at this wavelength there is a edge of an absorption band. From this results we obtained a small contribution of circular dichroism.

VI. CONCLUSIONS

1. We have built a microscope which can study any sample that show some transparency in the 400nm-650nm range.
2. We can measure simultaneously all the optical properties of a sample with good spatial resolution of the microscope objective.
3. Compared to a polarized light microscope, this microscope offers more accurate quantitative results and keeps the same applications.
4. All the elements of the Mueller matrix can be determined with a precision better than 0.5 %.

Acknowledgments

I would like to thank all the help and all the support that Oriol Arteaga and Joan Antó have given me during this project, this work wouldn't have been possible without their help. I also want to thank Adolf Canillas, for all the support that gave me in the lab and to Enric Beltran for the opportunity that he gave me when he asked me to take part in this project.

[1] A. Nezhuvingal, Y. Li *" Mueller matrix optical imaging with application to tissue diagnostics"* Proc. SPIE 4961, Laser-Tissue Interaction XIV (2003).
[2] KJ. Voss, E.S. Fry *" Measurement of the Mueller matrix for ocean water"*, Applied Optics **23**, 4427-4439 (1984).
[3] O. Arteaga, *" Mueller matrix polarimetry of anisotropic chiral media"*, Ph.D. thesis, University of Barcelona (2010).
[4] J.M. Bueno *" Polarimetry using liquid-crystal variable retarders"* Pure Applied Optics **2**, 216-222 (2000).
[5] O. Arteaga, J. Freudenthal, B. Wang and B. Kahr, *" Mueller matrix polarimetry with four photoelastic modulators: theory and calibration"*, Applied Optics **51**, 6805-6817 (2012).
[6] K. Ichimoto, K. Shinoda, *" Photopolarimetric Measurement System of Mueller Matrix with Dual Rotating Waveplates"*, Publications of National Astronomical Observatory of Japan **9**, 11-19 (2006).
[7] R.M. Azzam, *" Photopolarimetric measurement of the Mueller matrix by Fourier analysis of a single detected signal"* Optics Letters **2**, 148-150 (1998).
[8] O. Arteaga, M. Baldrís, J. Antó, A. Canillas, E. Pascual, E. Bertran *" A Mueller matrix microscope with a dual continuous rotating compensator setup and digital demodulation"* Submitted to Applied Optics (2014).
[9] O. Artega, A. Canillas *" Analytic inversion of the Mueller-Jones polarization matrices"* Optics Letters **35**, 559-561 (2010).