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MULTIPARAMETER LIGHT SCATTERING FOR RAPID VIRUS IDENTIFICATION

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INTRODUCTION

Multiparameter light scattering (MLS) is a term we have used to describe the simultaneous measurement of multiple elements of the Mueller matrix at specific wavelengths and scattering angles. This 4 X 4 matrix describes the polarization sensitive transformation of an incident beam of light into a scattered beam of light by a scattering object such as a virus or suspension of virus particles. The Mueller matrix contains a great deal of information about the internal structure and shape of the virus particle. This information is sufficient in many cases to enable discrimination among a wide variety of different viruses of clinical significance.

The purpose of this paper is to describe a photopolarimeter for making simultaneous angular distribution measurements of up to eight of the Mueller matrix elements from a suspension of virus particles.

PHOTOPOLARIMETER

Fig. 1 shows a schematic of the photopolarimeter. The 488 nm light from the argon laser (50 - 250 mW) passes through a glan-laser prism polarizer (POL1) to ensure that a high fraction of the transmitted light is linearly polarized at a specific angle with respect to the horizontal scattering plane. The beam then passes through a photoelastic modulator [MOD(F1)] operating at 50 kHz (Hinds International, Portland, Oregon). The beam incident on the sample is elliptically polarized with its handedness alternating left and right at 50 kHz. The incident intensity is not modulated. The sample is contained in a cylindrical quartz cuvette 50 mm in diameter with 2 mm thick walls. The entrance window is a 0.17 mm thick piece of coverslip. A Rayleigh horn is used to dump the transmitted beam. The cuvette is cut from a piece of extruded quartz tubing so its optical properties are not optimal.

Fig. 1. Schematic of the multiparameter light scattering photopolarimeter.

MULTIPARAMETER LIGHT SCATTERING (MLS)

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The telescope to detect the scattered light is mounted on a computer controlled rotary stage. The acceptance half angle of the telescope is 0.5 degrees. The scattered light passes through a second photoelastic modulator [MOD(F2)] operating at 47 kHz. Modulation at a second frequency makes it possible to measure separately each of the Mueller matrix elements rather than linear combinations of the elements as some other investigators have done. The scattered light then passes through a second linear polarizer (POL2), a laser line filter and impinges on the photomultiplier tube (PMT). This photopolarimeter is conceptually similar to one developed by Thompson, et al¹ that uses four electrooptic modulators and can measure simultaneously all 16 elements of the Mueller matrix from a suspension sample. This instrument is in turn based on a single modulator instrument developed by Hunt and Huffman² and used by Bickel, et al³ to make measurements on a number of bacterial and viral suspensions.

Fig. 2A shows the coordinate system used in the photopolarimeter. The azimuthal angle convention follows that used by Bohren and Huffman⁴. Fig. 2B shows a schematic top view of the photopolarimeter with the azimuthal angles indicated for each of the optical elements. The retardance, δ , of each modulator is set at 2.4048 radians. At this retardance the total scattered intensity, which is used to normalize the other Mueller matrix elements, is independent of the polarization of the scattered light.

Fig. 2. (A) Coordinate system used to describe the MLS photopolarimeter. (B) Schematic top view of the photopolarimeter showing the parameters for each of the optical elements.

With two modulators eight of the matrix elements can be measured simultaneously. The two modulation frequencies produce intensity modulation at the PMT at a variety of frequencies consisting of linear combinations of the two input frequencies. Each element of the Mueller matrix appears at a unique modulation frequency. With the azimuthal angles of the passing axes of POL1 and POL2 at $+45^\circ$ and the azimuthal angles of the fast axes of MOD1 and MOD2 at $+90^\circ$ (case I) the measurable Mueller matrix elements are as shown in Fig. 3. The numbers in parentheses are the frequencies in kilohertz at which each matrix element is measured. The dashes in some of the parentheses indicate that there is no contribution from that particular matrix element.

Fig. 3. Sample Mueller matrix for case I.

MUELLER MATRIX

$$\begin{array}{cccc}
 S_{11}(\text{DC}) & S_{12}(-) & S_{13}(100) & S_{14}(-50) \\
 S_{21}(-) & S_{22}(-) & S_{23}(-) & S_{24}(-) \\
 S_{31}(94) & S_{32}(-) & S_{33}(6) & S_{34}(44) \\
 S_{41}(47) & S_{42}(-) & S_{43}(53) & S_{44}(-3)
 \end{array}$$

With the polarizer passing axis azimuthal angles at $+90^\circ$ and the modulator fast axes at $+45^\circ$ (case II), the Mueller matrix is as shown in Fig. 4. Note that row two and column two are not present with this configuration.

Fig. 4. Sample Mueller matrix for case II.

MUELLER MATRIX

$$\begin{array}{cccc}
 S_{11}(\text{DC}) & S_{12}(100) & S_{13}(-) & S_{14}(50) \\
 S_{21}(94) & S_{22}(6) & S_{23}(-) & S_{24}(44) \\
 S_{31}(-) & S_{32}(-) & S_{33}(-) & S_{34}(-) \\
 S_{41}(47) & S_{42}(53) & S_{43}(-) & S_{44}(3)
 \end{array}$$

Fig. 5 shows a block diagram of the electronic circuitry needed to measure two of the matrix elements. As the scattering angle changes the anode current is kept constant by controlling the high voltage bias on the photomultiplier tube (PMT). This effectively normalizes all the matrix elements to the total scattered intensity. The preamplified signal is fed to several lockin amplifiers (EGG-PAR 5301). The PDP-11/44 computer controls the rotary stage (Aerotech) and the lockin amplifiers over an IEEE-488 bus. The 44 kHz frequency needed for the S_{34} matrix element is generated from the two input oscillator frequencies with the multipliers and filters shown in Fig. 5.

Fig. 5. Block diagram of the electronic circuitry used for measuring the S_{14} and S_{34} Mueller matrix elements.

CALIBRATION

Each of the Mueller matrix elements is calibrated by rotating the detector arm to 0° and replacing the sample cuvette with various combinations of optical elements whose Mueller matrices are known. For example S_{14} and S_{34} are calibrated by inserting a 488 nm quarter wave plate with its fast axis at 0° followed by a polarizer. The polarizer is rotated in steps through azimuthal angles ranging from 0° to 180° . S_{14} behaves as the sine of twice the azimuthal angle and S_{34} behaves as the sine squared of twice the azimuthal angle. These calibrations are shown in Figs. 6 and 7. These calibrations also enable the determination of the correct phase offsets between the reference and signal waveforms.

Fig. 6. S_{14} calibration

Fig. 7. S_{34} calibration

CUVETTE BIREFRINGENCE

Ideally the cuvette should exhibit no strain birefringence at either the input or output windows. A strain of less than 10 nm of retardance at a specified wavelength per centimeter of window material is considered strain free in the optics industry. S_{14} is affected only by strain in the input window while S_{34} is affected equally by strain in the input and output windows. Strain in the input window causes S_{12} and S_{13} to have contributions at the same frequency as that for S_{14} . Since S_{12} can be as large as -1 (at 90°) this presents a potentially severe problem. Modelling studies, however, indicate that for input window strains as large as 24.4 nm/cm and an S_{12} value of -0.5 and S_{14} value of 0.001 the contribution at 50 kHz from S_{14} is twice as large as that from S_{12} and the S_{12} contribution does not change as the S_{14} value varies over a wide range. This occurs for the configuration in Fig. 3 when the fast axis for the strain is a $+45^\circ$ with respect to the horizontal scattering plane. Our modelling effort also indicates that a set of measurements in both the configurations in Figs. 3 and 4 will enable the degree of strain and the orientation of its fast axis in each of the input and output windows to be determined. Once these four numbers are known each matrix element can be corrected appropriately.

RESULTS

We have presented data elsewhere^{5,6} showing discrimination with S_{14} among the following viruses: influenza (3 types), dengue fever (4 types), encephalitis (4 types), and hepatitis. These data were obtained with the cuvette mentioned above and were not corrected for strain induced contributions from S_{12} or S_{13} . As a result we will not present these data here.

CONCLUSIONS

An instrument for making multiparameter light scattering (MLS) measurements from viral suspensions has been described. Calibration methods and a technique for the correction of artifacts was also presented. This measurement technique may have broad applications in cell biology in general and in virology in particular. It may be possible to use MLS to identify viruses directly from clinical specimens.

Bustamante, et al⁷ and Zeitz, et al⁸ have developed theories that may explain the behavior of the S_{14} matrix element as a function of scattering angle for virus specimens. S_{14} appears from the theories to be sensitive to long range order such as supercoiling in the DNA or RNA in a virus.

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