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TITLE: AN INSTRUMENT FOR VIRUS IDENTIFICATION BY POLARIZED LIGHT SCATTERING - A PRELIMINARY REPORT

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AN INSTRUMENT FOR VIRUS IDENTIFICATION BY POLARIZED LIGHT SCATTERING

A PRELIMINARY REPORT

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Biological macromolecules are asymmetric structures. As such, they interact differently with left and right circularly polarized light. Circular dichroism (CD), the differential absorption of left and right circularly polarized light, probes changes in the secondary and tertiary structure of molecules in solution. Circular intensity differential scattering (CIDS), which is the differential scattering of left and right circularly polarized light, probes the higher order structure of macromolecular aggregates (1,2). CIDS is given as the amount of light scattered when the incident beam is left circularly polarized minus that scattered when the incident beam is right circularly polarized, divided by the total amount of light scattered by the object, in our case a virus or bacterium.

CIDS is just one element of a 4 X 4 matrix called the Mueller matrix describing the scattering of light from an object at a particular angle and wavelength. All of the information in a beam of light at a given wavelength is contained in a four element vector called the Stokes vector

$$\begin{matrix} I \\ Q \\ U \\ V \end{matrix}$$

I is the intensity of the light in a beam and is usually normalized to one. Q, U, and V describe the polarization properties of the light beam. Q is the tendency toward horizontal linear polarization. It is +1 for horizontal linearly polarized light and -1 for vertical linearly polarized light. U is the tendency toward linearly polarized light tilted at $+45^{\circ}$ to the horizontal scattering plane. It has values ranging from +1 ($+45^{\circ}$) to -1 (-45°). V is the tendency toward right circular polarization. For a right circularly polarized beam V = +1 and for a left circularly polarized beam V = -1. In our instrument the Stokes vectors of the incident and scattered light are manipulated by a series of optical elements represented by 4 X 4 Mueller matrices so that the intensity component of the Stokes vector of the scattered light contains information about the scattering sample.

Fig. 1 shows a schematic of the photopolarimeter. Fig. 1A shows the coordinate system. θ is the polar scattering angle in the horizontal scattering plane. γ is the azimuthal angle indicating the preferred axes for the optical elements. Fig. 1B shows a schematic of the instrument. The argon laser beam passes through a polarizer oriented at γ_1, then through a photoelastic modulator with its fast axis oriented at γ_2 and with sinusoidally varying retardance amplitude δ_2. It is driven at a frequency f_2. The Stokes vector of the incident light beam is now elliptically polarized. The light scatters from a homogeneous suspension of microorganisms in a cylindrical cuvette. The light scattered at an angle θ passes through a second photoelastic modulator with its fast axis at azimuthal angle γ_3 and its retardance amplitude at δ_3 and then through a second polarizer with its passing axis at γ_4. The light finally passes through a laser line filter and impinges on the cathode of a photomultiplier tube. This instrument is conceptually similar to one developed by Thompson et al. (3) based on earlier work by Bickel et al (4) and Hunt and Huffman (5). Modulation frequency f_2 is 50 kHz and f_3 is 47 kHz.

The use of two frequencies produces intensity modulation in the output at a variety of frequencies consisting of linear combinations of the two reference frequencies. Each element of the Mueller matrix for the sample appears at a unique modulation frequency. With $\gamma_1 = \gamma_4 = +45^\circ$ and $\gamma_2 = \gamma_3 = 90^\circ$ the Mueller matrix for the sample is as shown in Fig. 2.

S_{11} is the total scattering intensity and appears as a DC component at zero frequency. S_{14} is the CIDS and appears at 50 kHz. The other polarization sensitive element of particular interest is S_{34} which appears at 44 kHz.

Fig. 3 shows a sample Mueller matrix for the case $\gamma_1 = \gamma_4 = +90^{\circ}$ and $\gamma_2 = \gamma_3 = +45^{\circ}$. In this case, matrix elements in row three and column three are missing and S_{24} appears at 44 kHz instead of S_{34}.

Each matrix element can be calibrated by replacing the sample by optical elements with known Mueller matrices. S_{14} is calibrated by using a quarter wave plate with its fast axis at 0° followed by a polarizer rotated through 180°. The functional dependence should be 0.5 sin 2Θ. Fig. 4 shows this calibration for S_{14}. Fig. 5 shows a similar calibration for S_{34} where the functional dependence should be 0.5 $\sin^2 2\Theta$.

Experimental data obtained from this instrument is presented in the paper by Gregg and Salzman in these proceedings. The authors would like to thank Mr. W. Kevin Grace, Mr. Richard D. Hiebert, and Ms. Dorothy M. McGregor for their assistance. This work was performed under the auspices of the US Department of Energy and was suported in part by National Institutes of Health Grant GM 26857 and by the US Army Chemical Research and Development Center.

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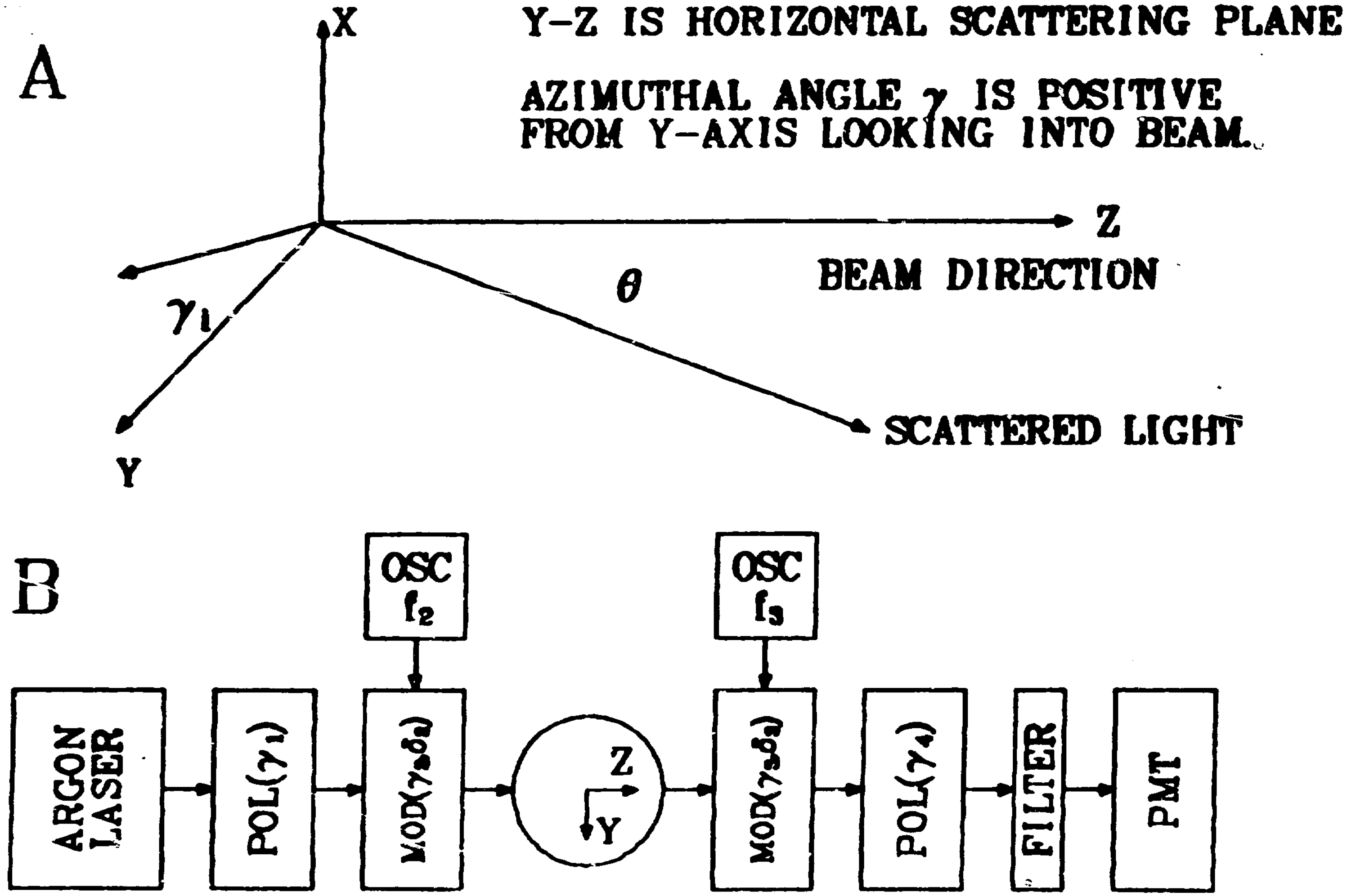
STATIC MUELLER MATRIX PHOTOPOLARIMETER



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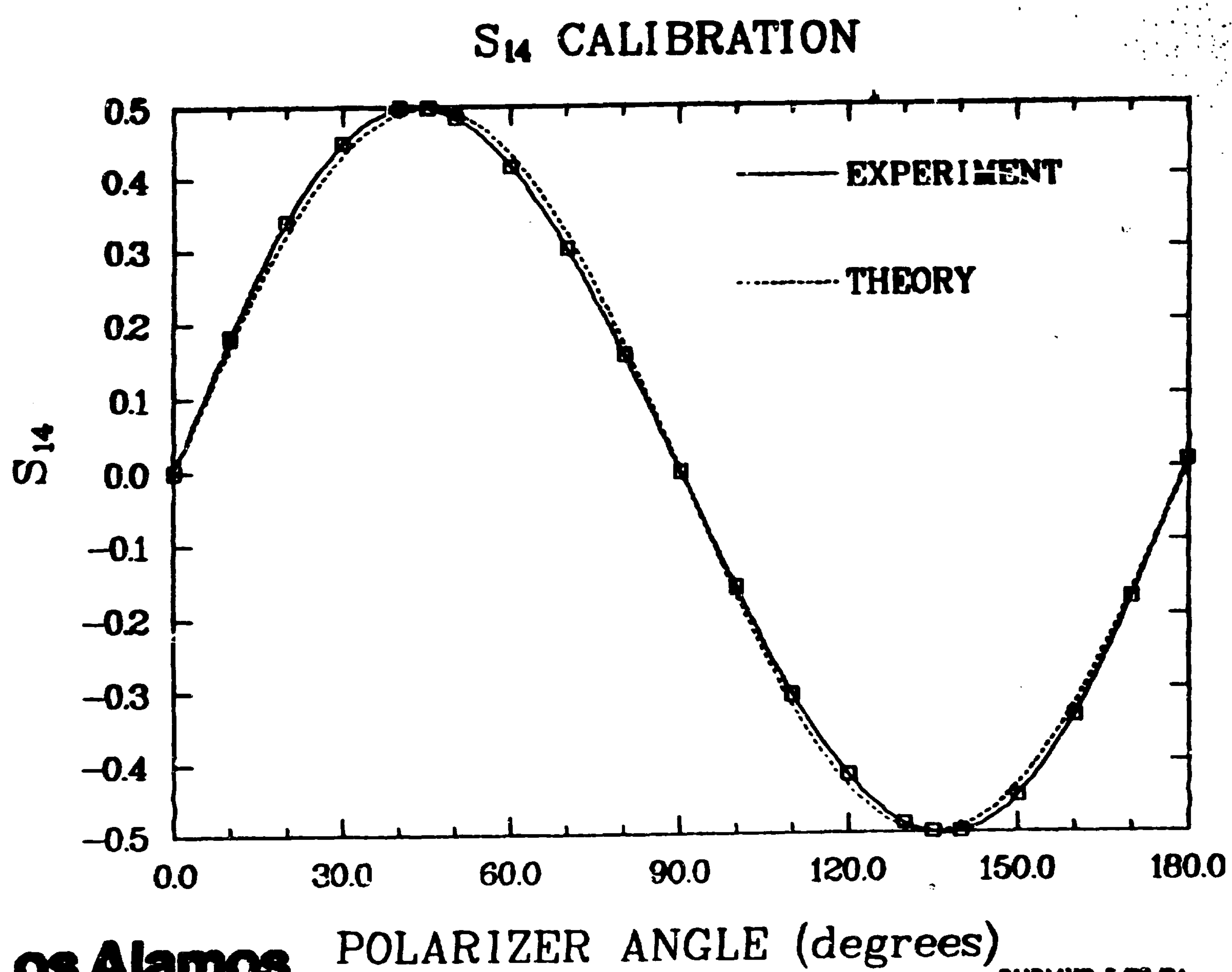
MUELLER MATRIX

S_{11}(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)

MUELLER MATRIX

S_{11}(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)

Fig 3


S_{14} CALIBRATION
EXPERIMENT
THEORY
S_{14}
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
−0.5
0.0
30.0
60.0
90.0
120.0
150.0
180.0
POLARIZER ANGLE (degrees)

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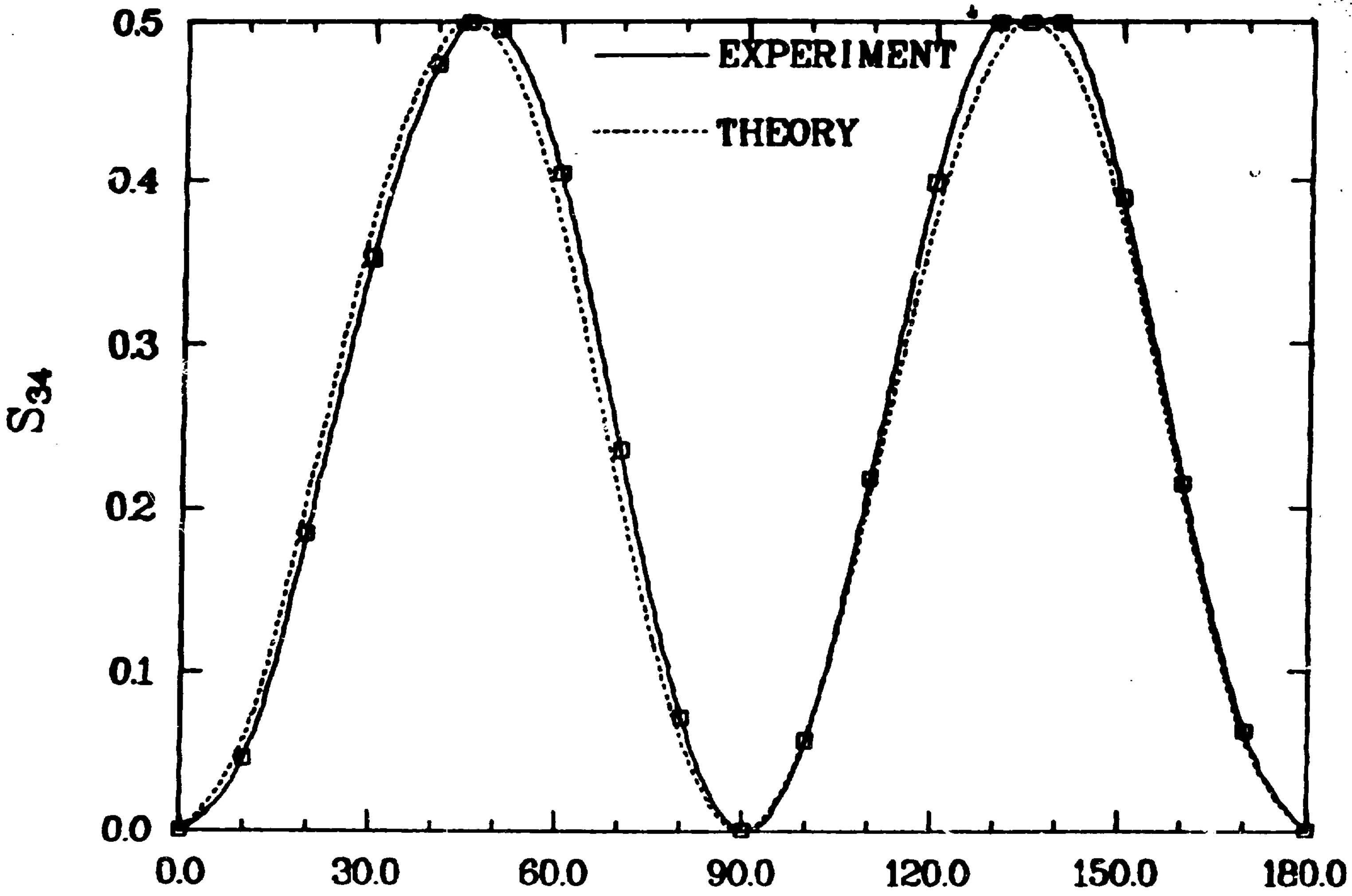
S_{34} CALIBRATION



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