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Multi-Reflection Polarimetry in Microfluidics

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Abstract— The field of microfluidics promises new portable, low-cost sensing systems, as well as the capabilities to measure the physical or chemical properties of precious samples, for which only small volumes are available. However, when using microfluidic channels with millimeter to micron scale dimmensions, together with optical sensing methods, these configurations result in short path lengths over which the signal can be acquired. Whilst polarimetry would greatly benefit from using small volumes, providing important information on the structure of chiral biomarkers in life sciences, the small interrogation volumes associated with the use of minute samples decreases the numbers of molecules in the light path that cause an optical rotation, and so reduces the sensitivity of the technique. Here we show that when an optical beam, passing through a chiral sample, undergoes multiple reflections from suitably aligned and configured external micromirrors, the usual cancelling out of the optical rotation that occurs when the rotated polarized beam is passed back through a solution following reflection at a single mirror can be negated. This enables the chirality of molecular species present in a microfluidic sample to be measured with increased sensitivity. This approach was validated experimentally using solutions of D-(+)-glucose as a model system, by investigating the effect of multiple reflections of a linearly polarized He-Ne laser beam and a 403 nm diode laser beam across the microfluidic channel. It was found that there was a 30 fold enhancement in the limit of detection with as few as 11 reflections through the sample.

Index Terms—Microfluidics, Polarization, Polarimetry, Reflections.

I. INTRODUCTION

Polarimetry provides useful insights into the molecular structure of optically active media such as chiral molecules in solution, enabling the measurement of the rotation of polarized light, while also determining the concentration of the analyte present [1]. Examples of the use of polarimetry in research has included insights into the dynamics of protein folding, investigations into the denaturation of DNA and RNA as well as the study of the structure of commonly used medicines, [2], [3]. The relatively high value of such biological samples [4], [5] can be used to justify new approaches to miniaturize the device so that smaller amounts of samples that can be measured using this sensing technique.

Different methods of performing polarimetry have previously been implemented in microfluidic systems, allowing the measurement of microscale volumes (e.g. as reviewed in [6]) although in many cases, the short optical pathlengths decrease the optical rotation sensitivity, according to Biot's law [7]. Some microfluidic polarimetry devices have overcome this challenge, improving the sensitivity to within the mM range without increasing the pathlength, either by using back-reflected fringe patterns from the microfluidic channels [8], or by measuring the difference between the refraction angle between two circularly polarized beams with an array of prisms [9], [10]. However, the resulting increase in sensitivity has been limited whilst the requirement for the implementation of long microfluidic channels to increase the effective sampling volume, has proved challenging.

Within the context of non-invasive glucose measurement, other techniques have been described which have achieved increased sensitivities, including, for example by implementing dual-wavelength methods in polarimetry to minimize birefringence errors [11]. Using a real time closed loop feedback system, authors used these methods to achieve sub-millidegree sensitivity [12],[13],[14]. Similar sensitivities were also obtained using an heterodyne signal [15], although this required a 1 cm path length with millilitres sample volume (which may not be suitable for high value samples).

Cavity-ring spectroscopy, classically using a two-mirror cavity without intra-cavity optics, has also been used to increase the optical path length [16] and, although this latter strategy can be applied to polarimetry, it requires additional intra-cavity optics to avoid the light rotation of the forward and backward reflections through the cavity cancelling each other out. To overcome this, Muller et al. [17], used an intra-cavity nonreciprocal signal reversing methodology (i.e. using retarders), enabling an increase of the optical path length of up to 1 km for a 1.63 m optical path length cavity. This method required costly and large experimental equipment including pulsed lasers.

Sensitive detection in polarimetry has also been achieved by using the reflection of the probing light off the wall of the chamber containing the sample [18]. Although this work provided the theoretical foundation for the concept of using multiple reflections, the practical limitations in its implementation enabled only one single reflection, again limiting the overall sensitivity of the method.

In this work, we now overcome the problems associated with short

optical path lengths, inherent to microfluidic systems, by using micromirrors to create multiple reflections across a capillary channel. By arranging the external mirrors so as to obtain *two* reflections before passing through the sample, we overcome the 180° flipping of the polarization that normally occurs following single reflections from a metallic mirror. This simple configuration (shown in Fig. 1) enables us to sum polarization rotation during multiple passes through the solution, whilst avoiding losses or distortions of the optical beam.

To realize this scheme, manufactured corner cube mirrors with aluminium coatings (as used in commercial light fittings) were oriented around a microfluidic channel in order to reflect the light beams so that they made multiple passes through the capillary, placed between two sets of these mirrors (Fig. 1b). Results of theoretical analysis of the technique were confirmed experimentally by investigating the optical rotation of solutions of D-glucose, as a well-known model analyte, that had previously been characterized extensively.

The advantages of using semiconductor light sources for polarimetry have previously been described [18] providing a means of obtaining high optical powers and low flicker noise. Thus, given the inverse squared wavelength dependence of chiral rotation ($1/\lambda^2$), we were also able to show improved enhancements in the optical rotation and further decreases in the limit of detection, when using a higher frequency diode laser (403 nm) as opposed to a He-Ne laser (632.8 nm).

II. Theory

The asymmetric structure of chiral molecules rotates the azimuthal angle of the polarization vector of a propagating linear polarized beam by an amount, α, which is defined as the specific rotation at a given wavelength, λ, and temperature, T. It depends on the concentration, c, of the sample and the optical path length, l [19].

$$[\alpha][\alpha]_\lambda^T = \frac{\alpha}{cl} \qquad (1)$$

In microfluidic samples, the critical dimension of the microscale channels provides a small optical pathlength. In our study, we optimized different spatial arrangements of mirrors to increase the effective optical pathlength within the channel. The coordinate system is depicted in Fig. 1(a), together with an offset zigzag
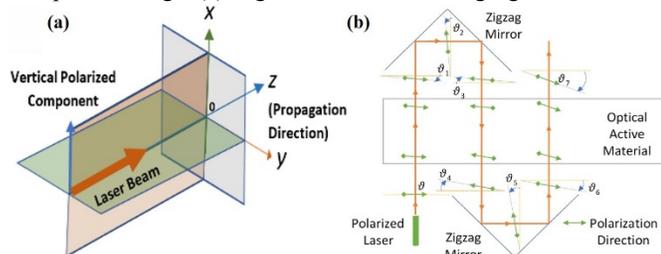
Fig. 1. (a) The co-ordinate system for wave propagation, with the laser beam polarized in the xz plane. (b) Schematic diagram of the operation of the polarization rotation increasing upon multi-reflections through an optically active material in a capillary containing the chiral molecules.

arrangement of metallic mirrors, Fig. 1(b). To understand the change of polarization upon reflection from surfaces, we first implemented a polarization state model for multi-reflections polarimetry, simulated using Matlab.

Fresnel coefficients [21] indicate the phase shift between *s* and *p* of the electric component of the light, which ultimately determines the polarization state after reflection [22]. In most cases, these can be calculated from the angle of incidence and the index of reflection. However, they are not directly applicable to reflections at metals, where more complicated phase shifts can arise, according to Snell's law, with a complex refractive index $N = n + ik$ (where n is the material refractive index, and k is the extinction coefficient) [23].

As stated, the change in polarization was determined by the relative magnitude and phase between the *s*- and *p*-polarized components, r_s and r_p respectively. Each polarization component change was calculated upon reflection, M_r, from the surface as in Equation (2) [24]. The negative sign of r_p refers to the handedness or chirality of the inversion.

$$M_r = \begin{bmatrix} -r_p & 0 \\ 0 & r_s \end{bmatrix} \qquad (2)$$

It is important to note that polarization will be nullified if the light passes forward and backward through a sample containing chiral molecules [21]. Therefore, in our work, the 180° polarization rotation at mirrors, achieved by two successive reflections before interrogating the sample, plays a critical role as it nullifies the 180° flipping of the polarization at each mirror, before interrogating the sample through the subsequent pass, as shown in Fig. 1(b). The polarized laser beam, with polarization angle θ passes through the solution of chiral molecules, which rotates the beam by an arbitrary angle, α, which is flipped at the first tilted mirror ($-\alpha$), and then flipped again by the second mirror (α), passing a second time through the sample ($\alpha+\alpha$), summing with the next rotation of α with each passage. By using aluminium mirrors (as opposed to e.g. silver mirrors), we were able
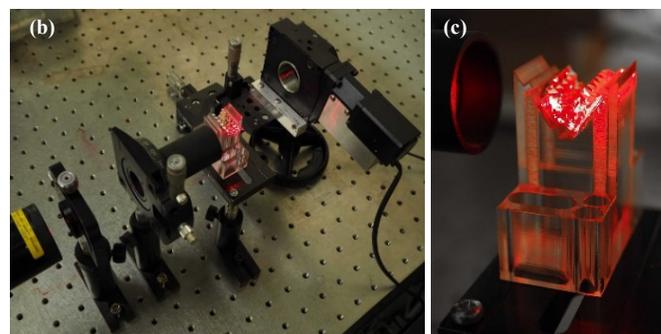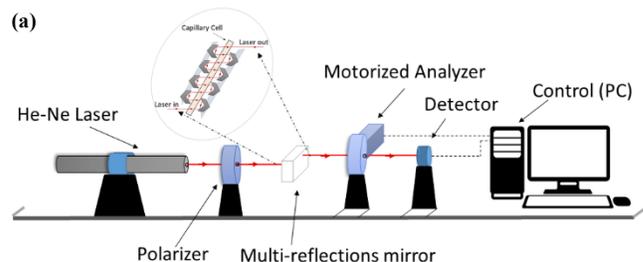
Fig. 2. (a) Schematic diagram of the polarimetry experimental set-up. Light passes through a Glan-Thompson polarizer, then multiple times through the sample, each time summing the optical rotation. The polarization angle of the light was measured using a power meter. The angle of rotation was extracted using curve fitting in Matlab; (b) Photograph of the setup; (c) Photo of mirrors array of the integrated multi-reflections scheme.

to ensure that the flipping was as close to 180° as possible and achieve less polarization noise (aluminum has a better ability to flip the polarization at a 45° incident angle compared with silver [25]).

III.   Materials and Methods

The multi-reflection scheme configuration is depicted in Fig. 2 and comprising a 632.8 nm He-Ne laser of 632.8 nm (Melles-Griot) or a 403 nm laser diode (TOPTICA Photonics, ibeam-smart), as linearly polarized sources, together with a Glan-Thompson 100 000:1 polarizer (Thorlabs), oriented at 90° to further improve the polarization state of the beam before passing through the sample. The sample was contained in a 1 mm wide capillary channel sandwiched by two zigzag arrays of mirrors, Figs. 1 and 2, (each element comprising 2.5 mm PMMA Al coated cubes as mirrors). The mirrors arrays were arranged at a 45° angle horizontally but only 35° vertically, requiring a 55° tilted holder to achieve parallel propagation in the horizontal plane.

The 1000 nm thick Al layer was evaporated on the cubes using an electron beam evaporator. The electron beam evaporation was angled and rotated through the process in order to evaporate the aluminum on the different faces of cube mirrors. The reflectance was optimized empirically, achieving 63% [26]. In order to align the laser, the cube mirrors were arranged to be linearly shifted by 2.5 mm (the width of each mirror) with respect to each other, so that the laser could be reflected twice before passing through the sample volume, to nullify the 180° flipping of the polarization at each mirror before interrogating the sample through the subsequent pass. The total number of passes were always odd numbers, up to a maximum of 11 passes.

The output light from the final pass was collected using an analyzer (Edmunds) on a motorized mount (Newport) to rotate the polarizer before being detected by a power meter (Coherent, UK). The data (measured as the power as a function of angle of rotation of the analyser) was exported to a PC to calculate the optical rotation due to the sample. The data was fitted to the Malus law,generating a sine wave, and compared with a water reference as a standard. The optical rotation was then the phase shift between the two sine waves (that of the specific glucose concentration sample measured and that of the reference (water)). The power meter was adjusted for the

wavelengths used and was controlled with a serial cable to a PC using a LABVIEW™ (13.0.1) routine to measure the signal as the analyzer was rotated. All sample solutions were prepared using research grade D-(+)-glucose in distilled water, as a model chiral molecule (Sigma-Aldrich). The glucose solutions were allowed to mutarotate and stored at 4°C. Prior to measurements, they were allowed to reach thermal equilibrium at room temperature (25oC).

IV.   Results and Discussion

A. Multi-Reflections Scheme

Fig. 3(a) shows the optical rotation for different D-(+)-glucose concentrations, for different numbers of passes between 1 and 11. As the concentration of D-(+)-glucose decreases, so does the optical rotation, while adding reflections (and thus increasing the path length), increases the total amount of rotation measured.

The limit of detection (LOD) was defined as three standard deviations above the optical rotation of the water, as background, and was found to decrease with increasing numbers of passes through the sample (following a relationship of ~ 1/ number of passes). Although the phase shift is proportional to the number of passes (as it accumulates linearly), the quantitative experimental relationship varies from a strict proportional relationship due to the noise behaviour.

The LOD was four times lower for three passes at 10.0 mM D-(+)-glucose (compared with 40.0 mM for a single pass). After 11 passes, the LOD was 1.5mM, a 25 times enhancement in sensitivity. These results show an improvement of ~5 times compared to the LOD for measurements previously made in urine samples, whilst using a volume 1000 times smaller [27]. Thus, for a given D-(+)-glucose concentration, e.g. 100.0 mM in Fig. 3(b), the optical rotation increased seven times using 11 passes compared with one pass. The decrease in power with the number of passes was linear with the number of passes, Fig. 4 (a), which confirms the theory, described in the preceding section.
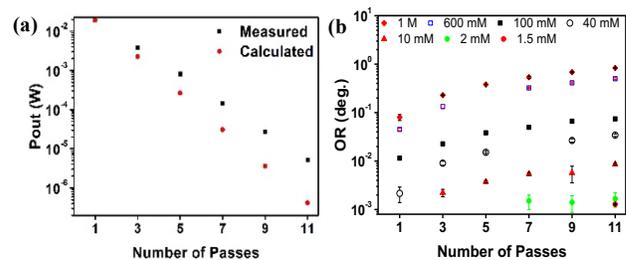
Fig. 4. (a) Calculated (in red) and measured (in black) output power (Pout) from the cube mirrors. The reflectivity of the mirrors depends upon the measurement of input and output power of the coated Al layer which was ~63%. The input power of the He-Ne laser was 19.6 mW; (b) Increment ratio of the optical rotation between each set of measurements at each number of passes in Fig. 3(a) with the one pass (without reflections) for 1.5 mM D-(+)-glucose (red squares), 2.0 mM (green circles), 10.0 mM (red triangles), 40.0 mM (white squares), 100.0 mM (black squares), 600.0 mM (blue squares), 1 M (red circles), The error bars show the standard deviation (n=3) were always <4%.
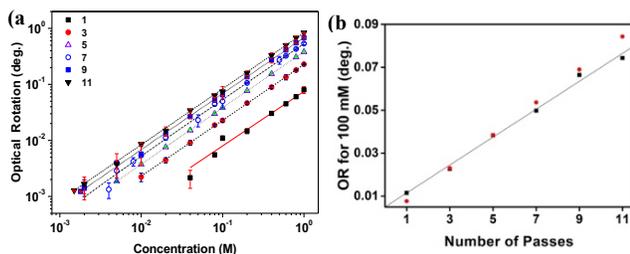

Fig. 3. (a) Optical rotation for different glucose concentrations using the multi-reflections setup and the He-Ne laser (632 nm) for increments of the number of passes (n=1 to11). The error bars show the standard deviation (n=3), which was always <4%; (b) Optical rotation of D-(+)-glucose (100.0 mM) for different numbers of passes), both experimental (in black, with a linear fit added) and theoretical (in red). The linear fits were always at least R^2>0.997.
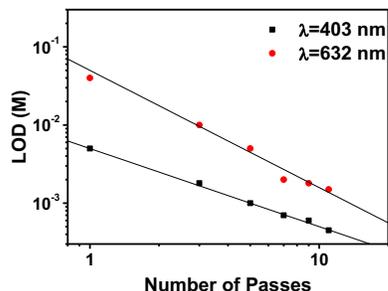
Fig. 5 Limits of detection for the He-Ne laser (red) and the diode laser (black) for different numbers of passes (up to n=11 passes) of the optical beam through the microfluidic cell. The black solid lines are added for ease of visualization.

B. Using Shorter Wavelength along with the Multi-Reflections Scheme

A second strategy to increase the rotation of the polarization for a chiral molecule at constant pH and temperature is to decrease the wavelength of the light source. As stated, there is an inverse squared wavelength dependence of chiral rotation $(1/\lambda^2)$, which results in the increased optical rotation and decreased limits of detection, when using a higher frequency laser. Semiconductor light sources can also provide high optical powers and low flicker noise. In order to validate that our multi-reflection system can accommodate both of these optimization strategies, we used a 403 nm diode laser, giving a further increase in the optical rotation by a factor three (Fig. 5). When applying the multi reflections scheme with the same number of passes (11), the increment ratio for each number of passes was similar to that for the He-Ne laser (Fig. 5), whilst the limit of detection $(4.5 \times 10^{-4}\,M)$ decreased by more than three fold.

V. CONCLUSION

In this work, we have overcome the problems associated with short optical path lengths, inherent to microfluidic systems, by using micromirrors to create multiple reflections across a capillary channel. By using an external configuration of mirrors that gave *two* reflections before passing through the sample, we negated the 180⁰ flipping of the polarization that normally occurs following single reflections. We demonstrate that for a He-Ne laser, we can enhance the detection limit of D-(+)-glucose by ~30 times after only 11 passes. We also demonstrate that using shorter wavelength sources we can further decrease the limit of detection. Additional studies using an intense, collimated beam need now to be realized before further advantages of using multiple reflected light for the measurement of optical activity can be identified.

ACKNOWLEDGMENTS

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