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Determination of pitch rotation in a spherical birefringent microparticle

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Abstract

Rotational motion of a three dimensional spherical microscopic object can happen either in pitch, yaw or roll fashion. Among these, the yaw motion has been conventionally studied using the intensity of scattered light from birefringent microspheres through crossed polarizers. Up until now, however, there is no way to study the pitch motion in spherical microspheres. Here, we suggest a new method to study the pitch motion of birefringent microspheres under crossed polarizers by measuring the 2-fold asymmetry in the scattered signal either using video microscopy or with optical tweezers. We show a couple of simple examples of pitch rotation determination using video microscopy for a microsphere attached with a kinesin molecule while moving along a microtubule and of a particle diffusing freely in water.

Keywords: optical tweezers, pitch rotation, birefringent microspheres, micromanipulation

(Some figures may appear in colour only in the online journal)

A particle can have translational and rotational degrees of freedom. In the mesoscopic world, all three translational degrees of freedom have been recorded either directly with video imaging or under optical tweezers. For an elongated particle, the yaw, which is the rotation about the direction of propagation of the incident light in an optical microscope, and the pitch, which is the rotation about one of the axes orthogonal to the propagation direction, can both be measured relying upon the projection on the image plane [1]. However, for a spherical particle, only the yaw degree of freedom has been measured by making the particle birefringent and measuring the total intensity of light scattered under crossed polarizers [2]. It is relevant for problems where the shape of the particle assumes significance. It is also relevant for optical tweezers where micro-manipulation along all degrees of freedom and subsequent detection is of paramount importance [3–5]. Some systems where the yaw has been studied were in rheology [6], study of hydrodynamic interactions [7], motion of molecular motors like kinesin [8], determination of asymmetry of an RBC [9, 10], measurement of rotational and translational diffusion coefficients of elongated objects in a bath [11] and so on. There have been some attempts to perform micro-manipulation in the pitch degree of freedom but have only been partially successful [12–15]. Generation of such motion using optical-tweezers and subsequent detection is still a matter of research [16].

In this paper, we have studied the effect of the pitch degree of freedom on the conventional method of detection of yaw rotation and subsequently devised another technique to isolate the pitch rotation. We show the application of the technique on the motion of a kinesin motor carrying a birefringent microsphere on a microtubule and find that there is minimal pitch rotation while the kinesin is in motion. We also show how pitch and yaw motion can be independently isolated for a birefringent particle diffusing freely in water.

We have used the Lumerical software to study the scattering properties of the birefringent microspheres. The liquid crystal birefringent microspheres typically assume a bipolar configuration as shown in figure 1(a) [17]. The liquid crystal molecules are oriented along ellipsoidal surfaces. To model this, we have considered a sphere with an ellipsoidal cavity of different refractive indices to simulate a birefringent microsphere. This ellipsoid can be considered to have the equivalent birefringence as the net effect of all the ellipsoidals surfaces inside the actual liquid crystal droplet. The axis of the ellipsoid gives the axis of birefringence of the particle. Initially, as shown in the figure 1, a x-polarized laser beam at 1064 nm has been made incident along the z-axis on a

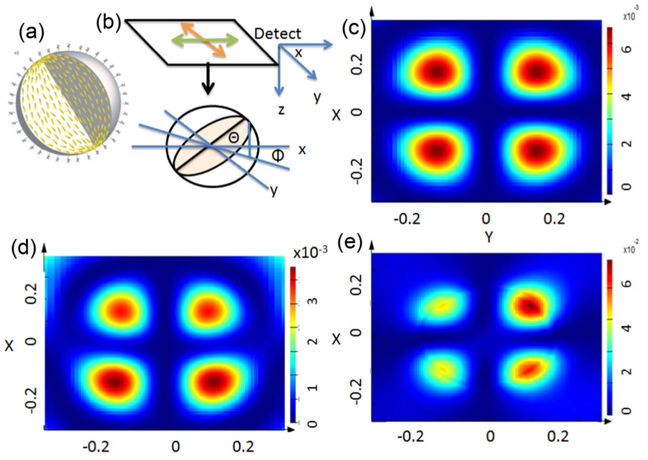

Figure 1. This figure illustrates the simulation. (a) The liquid crystal directors for RM257 given in [17]. (b) A birefringent microsphere has been simulated as a superposition of a sphere and an ellipsoid, the axes of which define the axis of birefringence. The refractive indices of the ellipsoid and the sphere are different. The input polarization is along the x-axis (marked green double arrow) while the detection is done with polarization along the y-axis (marked orange double arrow). (c) The square of the absolute value of x component of the electric field incident on a plane in the backscattered direction when ϕ and θ are both 0. The incident light is oriented along the y-axis. (d) The same configuration as (c) but with $\theta = -45°$. (e) Same configuration as (c) but with $\theta = 45°$ and $\phi = 45°$.

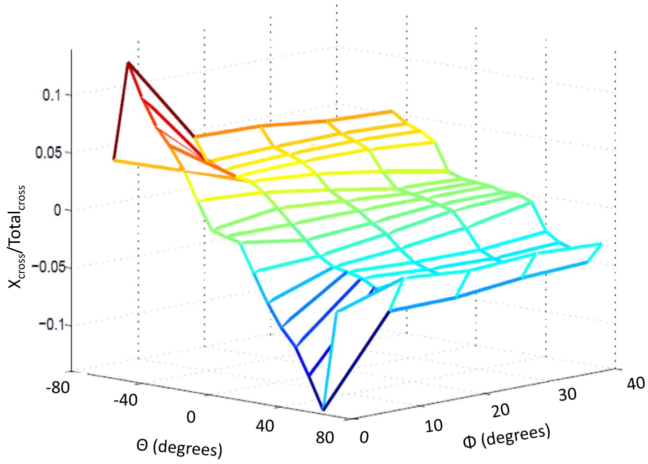

Figure 2. The ratio of the difference in x-halves with total backscattered intensity was plotted as function of θ and ϕ. The ratio depends linearly upon θ from $-60°$ to $+60°$ for all values of ϕ. This can be used for measuring the pitch rotation. The birefringent microsphere is simulated as a superposition of a sphere and an ellipsoid, with refractive indices 1.4 and 1.7, respectively. The ratio with the total intensity ensures that the results are independent of the intensity of incident light.

microsphere of radius 300 nm superimposed with an ellipsoid of semi-major axis 300 nm and semi-minor axes 100 nm.

The axis of the ellipsoid is initially in the x-direction and has been subsequently rotated. We find that as the θ is made non-zero, an asymmetry tends to develop between the halves of the backscatter pattern, which we can use to infer θ as shown in figures 1(d) and (e). We go ahead and calculate this difference-in-halves signal as a function of θ to better understand the behavior.

At first, we make the $\phi = 0$ and rotate θ to find its effect on the asymmetry signal. We find that a good measure of the asymmetry is the ratio of the difference in the halves signal of the backscattered pattern under crossed polarizers to the total intensity of the same pattern. It exhibits a linear region from about $\theta = -60°$ to $60°$, as shown in figure 2. Then, we vary ϕ and find that the system retains the linear regime as a function of ϕ. This ratio is independent of the incident electric field.

We also calculate the backscattered total intensity through crossed polarizers as a function of the θ and ϕ and find that, while it is dependent upon ϕ in the usual $\sin^2(\phi)$ fashion, to 10% accuracy the dependence on θ is minimal, as indicated in figure 3. This can have profound implications in determination of the yaw angle without bothering about the pitch and the roll even when the birefringent particle is freely moving in a bath, and recording the crossed polarizer signal with a video camera.

This is also relevant to the case of the optical tweezers where the incident light is linearly polarized. The particle can be assumed to be aligned in the direction of polarization and exhibiting Brownian motion in all the degrees of freedom.

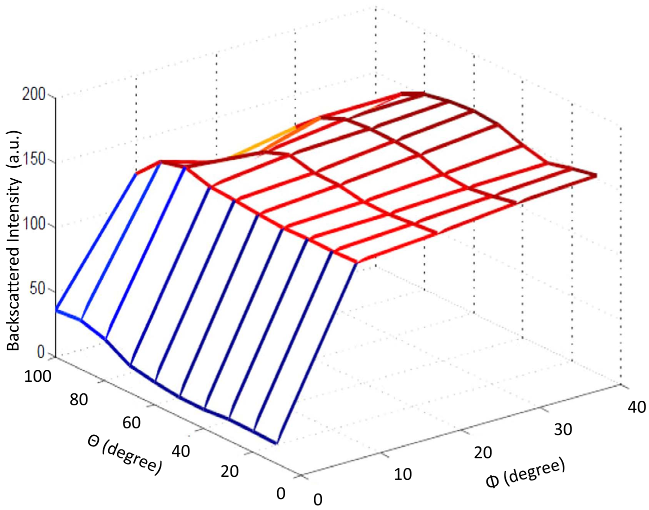

Figure 3. The total backscattered intensity under crossed polarizers is plotted as function of θ and ϕ. We find that the dependence of backscattered intensity on θ is constant within 10%.

The motion in the yaw degree of freedom can be directly inferred from the total scattered light intensity under crossed polarizers. The ϕ in this case would be small, typically less than 5° [6]. We can perform the detection in the fashion described in [8] with two sets of quadrant photodiodes (QPDs), one placed in the forward scattered direction while the other one in the backscatter direction. A polarizing beam splitter can be placed in the path of the input beam and the light backscattered through the port of of the beam splitter placed onto the QPD. The forward QPD detects the translational motion in the usual fashion [18], while the backscatter QPD can be used to detect pitch. We would have to record the difference in halves signal of the backscatter QPD and then divide by the relevant translation signal. Then, we would have

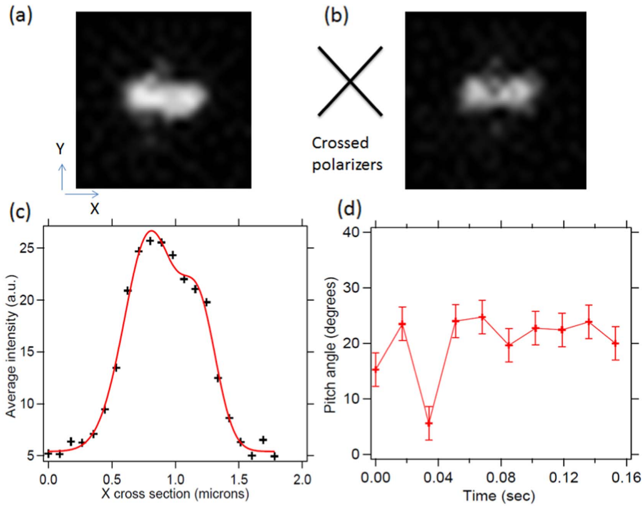

Figure 4. This figure shows the application of this asymmetry technique to determine the pitch rotation of a birefringent microsphere pulled along by a kinesin molecule on a microtubule. (a), (b) Two snapshots of the microsphere during motility. The particle diameter is 800 nm and crossed polarizers oriented along the diagonals. (c) The x average of the 20 pixel region for both these snapshots. (d) The pitch rotation angle as function of time while the kinesin moves on the microtubule.

to divide by the total intensity signal of the backscatter to get the ratio $X_{\mathrm{cross}}/\mathrm{Total}_{\mathrm{cross}}$.

The signal is linear in pitch angle. The equipartition theorem should also hold in this sense too, which can be used to calibrate the pitch signal in the usual fashion [18].

We show an example of determination of the pitch angle for a birefringent microsphere being pulled along by a kinesin molecule on a microtubule in figure 4. The method of preparation of the sample has been mentioned in the [8]. We record the activity using a video camera under crossed polarizers and then zoom into the video image to region 20 pixels by 20 pixels around the center of the particle. Then, we calculate the average X pixel intensity as the direction of orientation of the micro-sphere is in the x-direction. Then, we make a distribution as a function of the pixel position and then fit the intensity distribution to a double Gaussian (figure 4(c)). We use the parameters of the individual Gaussians to estimate the asymmetry in the image. We take the ratio between this difference in the area under the two individual Gaussians to the total area under both Gaussians. The asymmetry was then converted into the pitch rotation angle of the microsphere by using the relation from figure 2. A better estimate of the conversion factor from the relative asymmetry to the pitch rotation angle could be found from the optical tweezers study. However, in this work, we are just relying on the numerically calculated values given in figure 2.

Two sample images of the birefringent microsphere of 800 nm diameter have been shown in figures 4(a) and (b) and a sample average x value indicated in (c). In figure 4(d), we have shown the asymmetry as a function of time as the particle is dragged along by the kinesin molecule. The molecule typically takes 100 steps per second at 1 mM ATP concentration [8], such that in 170 ms, it should take 17 steps. We find that the video is undersampled and hence each individual step could not be resolved. By using the fit of the image cross section to a double Gaussian, we could estimate the amplitude and the width of each half very accurately to infer the asymmetry. It has been found to fit quite well to the cross-section and can be used as an empirical finding. From the values of the pitch rotation angle, we can infer that there is no significant gradual change in the pitch angle as the kinesin pulls the microsphere over the microtubule.

We have also tried to use the pitch rotation angle determination technique to another problem, that of free diffusion of a birefringent particle in water. Here, the problems of pitch angle determination in the presence of a changing yaw angle becomes challenging. However, as we discussed in this manuscript, the yaw angle determination is unaffected by the change in the other angle. The total backscattered intensity through a set of crossed polarizers still provides a good estimate of the rotation angle. The total backscattered intensity is proportional to $\sin^2(\phi)$. Further, once the axis of yaw orientation is determined, we simply have to find the asymmetry along that axis to estimate the pitch angle. It may be noted that even in this case, the asymmetry signal is normalized by the total backscattered intensity. We then proceed to take the asymmetry value at various instants of time and find the pitch mean square displacement (MSD) as a function of time. Since the process is purely diffusive, we should find that this MSD goes as $\mathrm{MSD}_\theta(t) = 2D_\theta t$, where D_θ is the rotational diffusion coefficient in water and t is the time. However, we know that the $D_\theta = 8\pi\eta a^3$, where η is the viscosity of water and the a is the radius of the particle. Once such sample dataset has been shown in figure 5. The figure 5(a) indicates a typical cross-section for a 1.6 μm diameter particle while the (b) indicates the total backscattered intensity under crossed polarizers, which determines the yaw angle. The figure 5(c) indicates the pitch angle which has been calibrated by estimating the diffusion coefficient from (d). In figure 5(d) we have plotted the MSD as a function of time and find that the relation is linear indicating that the aspects of rotational diffusion motion has been well captured by this technique.

In conclusion, we have described a new technique to determine the pitch rotation in spherical birefringent microspheres. A method of rotating the microsphere controllably in the pitch degree of freedom has not yet been demonstrated, but when available, this technique can be used to study such motion. We also calculate that there is only a maximum cross talk to the extent of 10% between pitch rotation and yaw rotation as inferred from the total backscattered intensity under crossed polarizers. We have studied the intensity of such microspheres in a bath while tracking them at high resolution with video recording and directly inferred the yaw rotation while simultaneously estimating the pitch angle. We have also determined the pitch motion for microsphere being pulled along by a kinesin molecule. This technique is simpler than distinguishing a shift and a modulation of the backscattered intensity suggested with nanorods [16].

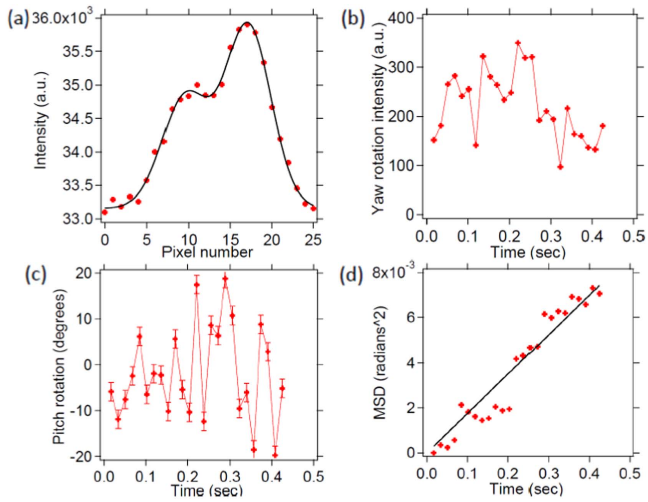

Figure 5. This figure shows the application of this asymmetry technique to determine the pitch rotation of a birefringent microsphere diffusing freely in water. We find the asymmetry along the two axes and then find the axis in which the particle is oriented. In (a), we show a typical cross section for a particle diffusing in water along the axis of yaw orientation. In (b), we show the total intensity scattered through the crossed polarizers that indicates the yaw rotation angle. The total intensity scattered goes as $\sin^2(\phi)$ (c) shows the pitch rotation angle estimated from the cross sectional asymmetry for the particle diffusing in water. It may be noted that this asymmetry has been normalized by the total scattered intensity. The calibration factor has been estimated using the diffusion coefficient mentioned in (d). (d) We estimate the MSD of the pitch angle in radians with time. Since the particle is diffusing freely, it is expected that it shall follow the law $\mathrm{MSD}_\theta(t) = 2D_\theta t$, where D_θ is the rotational diffusion coefficient in water and t is the time. We find that MSD indeed increases linearly with time and the slope has been used to find the pitch orientation in (c). The particle diameter is 1600 nm.

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