Invited Paper

Rotating Optical Tweezers

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ABSTRACT

Several methods to rotate and align microscopic particles controllably have been developed. Control of the orientation of a trapped particle allows full three dimensional manipulation, whereas rotating particles are tools for the development of optically-driven micromachines. It has been shown that the orientation of an object in the laser trap depends on its birefringence as well as on its shape. The effect of shape is often referred to as form-birefringence. We report on the trapping, rotation, and in-situ growth of birefringent tetragonal lysozyme crystals in optical tweezers operating at a wavelength of 1064 nm. Variation of the temperature, pH and lysozyme concentration of the solution during growth was used to alter the size, as well as the length to width ratio of the crystals, and hence their orientation in the tweezers. Thus this system serves as a model to study the relative importance of birefringence versus form-birefringence for particle orientation. Crystals with the optical axis skewed or perpendicular to the trapping-beam axis could be rotated by changing the orientation of linearly polarized light. We observed spontaneous spinning of some asymmetric crystals in the presence of linearly polarized light, due to radiation pressure effects. Addition of protein to the solution in the tweezers permitted real-time observation of crystal growth.

1. INTRODUCTION

1.1. ROTATION IN OPTICAL TWEEZERS

Optical tweezers have been used to manipulate and investigate microscopic particles for many years and a wide variety of applications have been explored[1-3]. The optical forces acting to trap microscopic particles result from the transfer of linear momentum from the trapping beam to the trapped object. Various trapping geometries to achieve stable three dimensional trapping have been developed[4, 5]. The most common trap, the single beam gradient optical trap, often referred to as optical tweezers, consists of a tightly focused laser beam, in which the same objective is usually used for focusing the laser beam as well as for observing the trapped object[6]. If the index of refraction of the particle is higher than that of the surrounding liquid the gradient of the electric field intensity creates a net force towards the focal point of the beam in three dimensions. This allows manipulation and relocation of particles with nanometer precision and application of calibrated forces in the piconewton regime[7]. To date, many experiments with biological specimens, such as living cells and viruses, have been performed. Examples are the study of the interaction forces between different motor molecules[8], the use of optical tweezers to study the dynamic of fusion pores[9] and the determination of the elastic properties of single DNA strands[10]. Proper choice of the wavelength of the trapping beam (in a region of high transparency) minimizes damage of biological specimens.

However, light can also carry angular momentum which introduces the ability to controllably rotate or orient optically trapped particles, thereby achieving full three dimensional control over the trapped object. In recent years a variety of methods both to rotate and orient optically trapped microscopic particles have been proposed and successfully tested[11-13].

The optical torque acting to induce rotation of a trapped particle is always a result of the alteration of orbital and/or spin angular momentum of the incident beam by the trapped particle. This can be achieved either by absorption of energy from a beam carrying angular momentum, a change of spin angular momentum by birefringent particles, or a change of orbital angular momentum by the use of asymmetric particles[14-16]. In the case of a birefringent material, circularly

polarized light can be used to make a spherical particle spin within the beam. Special light fields of tailored laser beams and specially shaped particles have also been used for rotation[17].

The external or internal anisotropy of the particles can also be used to control the orientation of the objects in the trap. Orientation effects due to the shape of the particle (external anisotropy) have been reported, and are often referred to as form birefringence. Elongated particles tend, due to the gradient force, to align with their long axis along the axis of the trapping beam. Disc-shaped objects have two long axes and, after aligning one with the beam axis, have an asymmetric shape perpendicular to the beam axis, and the remaining long axis will align with the plane of polarization of the trapping beam. It has been shown that the torque acting to align the particle with the beam axis is typically one order of magnitude larger than the torque which aligns the remaining asymmetry with the plane of polarization[18]. Using this principle the particle can either be aligned by changing the plane of polarization or spun using a rapidly rotating linearly polarized beam. Such an alignment has the advantages of being easy to implement in an existing tweezers setup and provides the maximum of trapping efficiency. Additionally, polarization can be used to control the orientation of symmetrically shaped birefringent particles (internal anisotropy). Positive birefringent spheres align their optical axis along the electric field of linearly polarized light, whereas negative birefringent objects align their optical axis normal to the electric field that can be either parallel or perpendicular to the beam axis.

Consequently, elongated birefringent particles orient themselves according to their shape and birefringence with respect to the beam axis and plane of polarization. Demonstrating the different contributions of external and internal birefringence on the orientation of a particle in the trap was one of the aims of this work.

The driving force for rotation can also originate from a different principle where the trapping light itself does not carry any angular momentum. In this case the torque needed to drive the rotation originates from asymmetric light scattering from the object itself, and the particles rotate in the presence of a linearly polarized trapping beam[19, 20]. Spontaneous rotation of asymmetric objects has also been reported in optical tweezers of an unspecified polarization state[21, 22]. We also observed spontaneous spinning of some asymmetric crystals in the presence of linearly polarized light, due to these radiation pressure effects.

1.2. LYSOZYME CRYSTALS

Whenever atoms or molecules in a liquid state come together to form a solid, they can arrange in one of two ways. They can come together randomly to form an amorphous structure, or they can associate into highly-ordered, three dimensional structures called crystals. Crystals are formed when a chemical compound solidifies in such a way that its atoms or molecules are arranged in a symmetrical, three dimensional pattern which is composed of regular, repeating geometrical units. Ideal crystals form perfect arrays, however perfect crystals are a rarity, because they generally require very slow crystal development and the absence of contaminating substances and outside forces which could disrupt crystallization. Under the proper conditions, crystals can be formed from chemical elements (such as diamonds from carbon), chemical compounds (quartz from silicon dioxide), salts (a solution of table salt left to evaporate), or even proteins.

The crystallization of proteins is an important technique in biochemistry. Because protein crystals are highly-ordered arrays of a protein, the analysis of these crystals can provide important information about the three-dimensional structure of the protein. Protein crystals can be analyzed using X-ray diffraction, which takes advantage of a crystal's ability to scatter X-rays into a regular pattern. Analysis of a protein crystal's diffraction pattern helps determine the three dimensional structure of the folded protein. In addition to giving insights about the structure and function of proteins, structural biology also allows the development of drugs which can interact with specific regions of the protein.

Lysozyme crystals are a widely used model to study nucleation and growth of protein crystals to establish the fundamental understanding that can be applied to the crystallization of other proteins. Lysozyme was one of the first proteins to be crystallized and used in X-ray diffraction experiments. Lysozyme is an enzyme which degrades bacterial cell walls, and is found in abundance in human tears or chicken egg whites. In its most common (tetragonal) form, chicken egg white lysozyme forms a positive uniaxial birefringent crystal, with a well-characterized morphology. Images of lysozyme crystals we have grown in our lab can be seen in Fig. 1
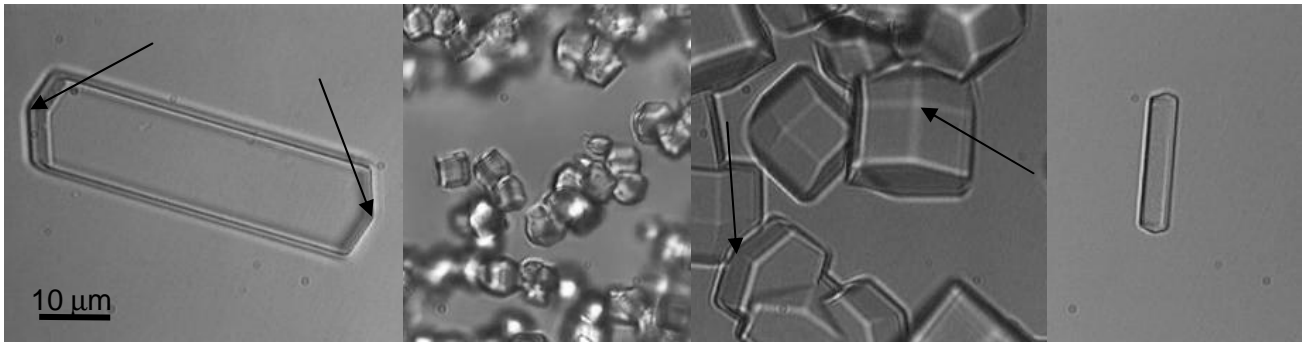
Figure 1: The size and the aspect ratio (length of the *c*-axis to the (110) face width) in these crystals is affected by the pH, temperature and the supersaturation ratio of the host liquid during growth. Arrows indicate the position of the tips of some crystals.

The optical axis of the crystals points from one to the other tip of the crystal (see Fig. 1 and 2). The difference in the indices along the [001] and [110] axes in the visible is 2.4 x10^{-3}. The tensor elements of the optical activity are two orders of magnitude smaller[23], so chiral effects are minimal, especially at long wavelengths. The difference between the crystal and host liquid indices of refraction is also small (8 x10^{-3})[24, 25]. The ratio of the length *c* to the (110) face width *w* in these crystals is affected by the pH and the supersaturation ratio of the host liquid during growth. Also the size of the crystals can be controlled by varying those parameters (see Fig. 1) Therefore we were able to choose the orientation that the crystals will assume in the tweezers due to the shape birefringence of the particle.

Using lysozyme crystals together with optical tweezers has several potential advantages. While a crystal is trapped, it can grow in free space, in contrast to the usual case where the crystal is lying on a surface, which can alter or hinder crystal growth in that direction. Furthermore, immobilizing the crystal with a laser trap, the growing medium and thereby the growing parameters can be changed for a certain crystal. As a result one can use the same crystal seed for several growing experiments; the crystal can be grown under certain conditions, then dissolved and re-grown under changed conditions, allowing comparison of the quality of crystals from a single seed, used multiple times. Experiments could also be devised to test hypothesis about the termination of growth due to contamination effects.
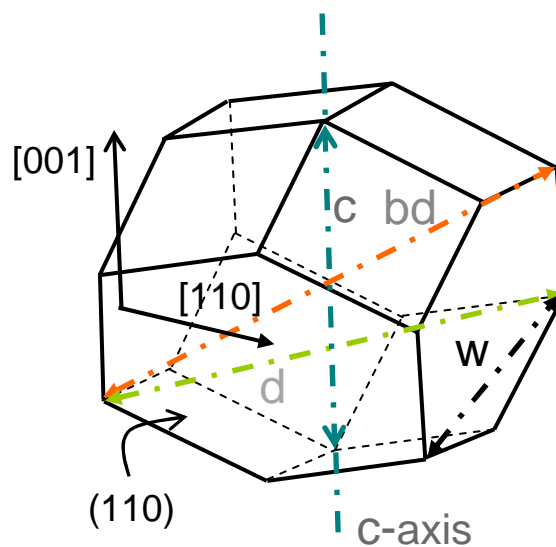

Figure 2: Morphology of tetragonal lysozyme, showing crystallographic axes and faces. Also the nomenclature of the different dimensions used in section 5 is illustrated. The optical axis coincides with the c-axis. The aspect ratio is defined as c:w.

2. EXPERIMENT

2.1. OPTICAL TWEEZERS SETUP

Two separate setups were used in the experiments described here; one for recording video of the rotation and the growth of the particles, and one for measurements of the ellipticity introduced into linearly polarized light passing through the lysozyme crystals. The video recording setup is described in detail by Singer *et al.*[26] Linearly polarized light from an Yb-doped fiber laser operating at 1070 nanometers in the range of 200-500 mW was coupled into a 60x oil-immersion objective of numerical aperture 1.4. The laser light was guided to the objective using a dichroic mirror, allowing viewing and recording of the crystals using the same objective and a white light source. A half-wave or quarter-wave plate in the beam path could be used to either adjust the direction of the plane of polarization or to produce a circularly polarized trapping beam.

The setup used in the ellipticity measurement is reported in Ref. 27, however a schematic drawing of the principle used is shown in Fig. 3.
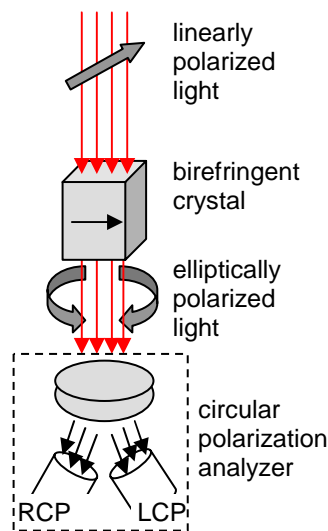

Figure 3. Schematic drawing illustrating the principle to determine the ellipticity that is induced to linearly polarized light after passing through a birefringent particle. Together with the thickness of the crystals, along with the angle of the c-axis relative to the polarization and the measured ellipticity a value for the difference in the indices of refraction along the [001] and [110] directions was determined. The circular polarization analyzer measures the intensities of right (RCP) and left (LCP) circular polarized light that contributes to the elliptically polarized beam.

Briefly, linearly polarized laser beam is incident on the birefringent crystal under study, and after passing through the crystal the light is guided to a circular polarization analyzer. Ellipitcally polarized light, and consequently also linearly polarized light, can be described by a superposition of left (LCP) and right (RCP) circularly polarized light. Using the circular polarization analyzer the intensities of left and right circularly polarized light that contribute to the light after passing through the birefringent crystal can be determined. The thickness of the crystals, along with the angle of the c-axis relative to the polarization was then combined with the ellipticity to yield a value for the difference in the indices of refraction along the [001] and [110] directions.

2.2. LYSOZYME CRYSTAL PREPARATION

The microcrystals of lysozyme were prepared by the batch method. To promote heterogeneous nucleation, a concentrated solution of lysozyme was prepared from the as-purchased powder from Sigma, and allowed to age for 2 weeks at room temperature, prior to crystal growth[28]. The lysozyme powder was dissolved in a 0.6M acetic

acid/deionized water solution to a concentration of 487mg/ml. 20-100 μl of this protein concentrate was placed in 2ml bottles, and then varying amounts of a 5wt% solution of NaCl in water (titrated to a pH of either 7 or 9 using NaOH) were added. The ratio of the volumes of the protein:salt solutions was one to one, two, four, five, ten or twenty. During addition of the salt solution, the micropipette was used to agitate the solution vigorously by pumping the mixture in and out of the tip 10-20 times. This resulted in rapid formation of many nuclei in all but the most dilute solutions. The crystals were formed at, and stable at, room temperature over a period of weeks. Growth experiments were made by adding small amounts of the protein concentrate (approximately 1:4 by volume) to the solution in the optical tweezers. No attempt was made to control the solution pH accurately during these initial experiments.

2.3 SOFTWARE

For the evaluation of the crystals we used a software package developed by Gibson *et al.*[29] The software enabled us to get precise information on the size as well as of the orientation of the trapped particle. This is done by fitting a grid which represents the crystal on the individual images we obtained from the microscope. After the grid fits the crystal best, the size as well as the orientation of the optical axis with respect to the image plane is returned.
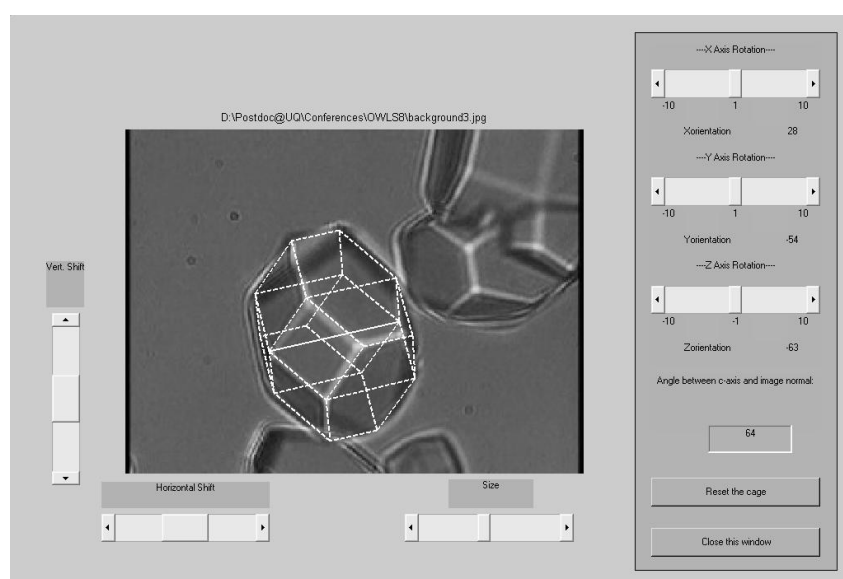

Figure 4: Software that enables a precise determination of the size and the orientation of the individual crystals.

3. RESULTS

3.1. BIREFRINGENCE AT 1064nm

The birefringence of our crystals in the visible was confirmed by examining them in the optical tweezers setup using crossed polarizers. The trapping laser was turned off for this experiment.
For measuring the birefringence of the lysozyme crystal for 1064nm linearly polarized light was incident on a crystal, and the induced ellipticity upon passage through the crystal was measured according to the principle illustrated in Figure 3. A change in the ellipticity of a beam is equivalent to a change of the beam's (spin) angular momentum. Figure 5 shows the angular momentum change per photon (normalized by thickness) derived from these ellipticity measurement. The solid curve in Figure 5 corresponds to the angular momentum change per thickness calculated for a crystal with a difference in the indices of refraction along the [001] and [110] directions of $\Delta n = 1.66 \times 10^{-3}$. This value for Δn, which fits the experimentally obtained results best, is somewhat smaller than the value reported in the visible.
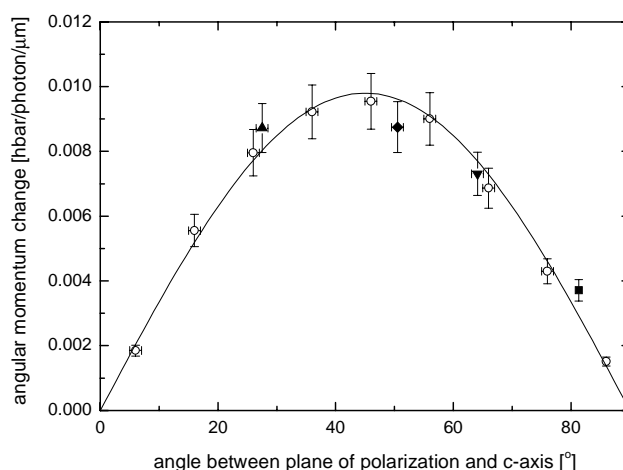
Figure 5.   Measured and calculated values of the angular momentum change vs. angle for several crystals[26] (solid line is calculated for $\Delta n = 1.66 \times 10^{-3}$).

3.2. ROTATION OF LYSOZYME CRYSTALS

Experiments were then performed to verify that the crystal orientation could be controlled using polarization. We expect to see a combination of the effects of shape anisotropy and crystalline birefringence. For crystals with aspect ratios (c:*w*) greater than ~1.3 the c-axis aligned with the beam axis upon capture. In this case, the alignment of the optical axis precluded any polarization effects due to birefringence. The shape anisotropy due to the square cross-section of the crystals was too weak to allow changes in orientation upon rotation of the half-wave plate. However, for smaller aspect ratios, crystals could be trapped with the optical axis perpendicular or skewed to the trap beam. In these cases, the crystals could be rotated using a half-wave plate. The maximum momentum transfer for this case was determined to be on the order of $0.1\hbar$ per photon. Rotation occurs for crystals with shape anisotropy (regular hexagonal faces) less than that of the c-axis aligned ones, indicating that the rotation is due to birefringence. The maximum momentum transfer for circular polarization was calculated to be between one and two orders of magnitude smaller than that for the half-wave plate rotation. Indeed, we were not able to rotate the crystals using circularly polarized light; the torques were not large enough to overcome the viscous forces for particles of this size. Spinning of well-formed crystals, by rotating a half-wave plate could potentially be used to measure the effects of convection on the dissolution of protein crystals.

3.3. AUTOROTATION

Asymmetric and compound (two or more adhered) crystals picked up by the tweezers showed spontaneous rotation in the presence of linearly polarized light. As reported by Higurashi *et al.*[30], particles with parallel faces at different distances from the center of rotation lead to unbalanced torques, and can induce quite rapid rotation, due to refractive effects alone. When crystals displayed spontaneous rotational behavior, after a period of five to ten minutes, the rotation slowed, then stopped. If the crystals were released and re-trapped in the same orientation, there was no resumption of the rotation, but if the re-trapping event resulted in a different orientation of the crystal, rotation was seen to resume in many cases. Close observation of the crystals showed that there was some erosion of the edges of the crystal during rotation; proteins in the crystals are weakly bound, and can be removed by rheological forces. This erosion presumably reduced the asymmetry of the crystals until there was no longer adequate torque to cause rotation.

3.4. IN-SITU GROWTH OF CRYSTALS

There is considerable interest in the protein crystal growth community in observing the habit of crystals in a controlled, but changing environment. Past efforts have been hampered by the difficulty of reliably examining a particular crystal seed as conditions are changed. The use of optical tweezers makes this possible. To establish this, we increased the protein concentration of the solution while observing a trapped crystal. In the presence of small seed crystals, the bulk of the excess lysozyme should induce additional growth of existing crystals, rather than the nucleation of new ones.

Figure 6 shows the increase of the crystal volume of the trapped crystal as a function of time after the protein concentration has been changed described above. Although the increase of volume was extremely fast (time-lapse of graph is 70 seconds) it kept its perfect tetragonal shape. It can be seen that the crystal grows primarily by addition of material on the (110) faces, as would be expected at a high protein concentration [31].
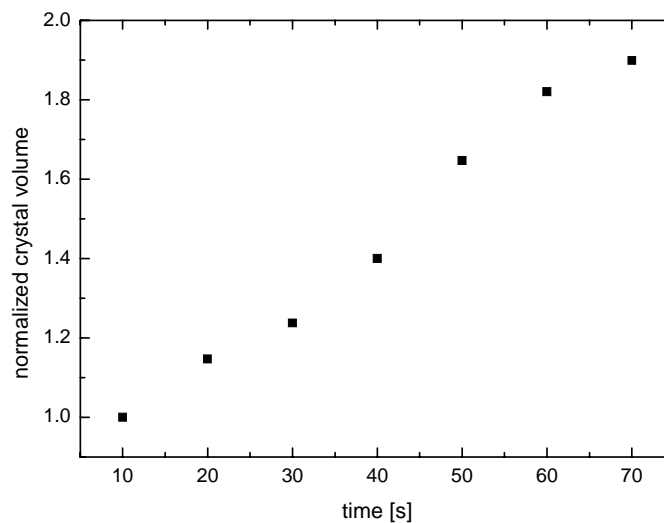

Figure 6: Change of crystal volume after the protein concentration of the surrounding liquid is increased.

3.5. EQUILIBRIUM ORIENTATION

Both external as well as internal anisotropy governs the orientation of a particle in a trap. Positive uni-axial birefringent particle tend to align their optical axis perpendicular to the beam axis. This is utilized when spinning a birefringent sphere using circular polarized light or by rotating the plane of polarization of linearly polarized light[12]. Elongated particles align with their long axis along the beam axis[27].

As mentioned above lysozyme crystals are positive birefringent and their aspect ratio can be changed by changing the growing conditions. As a result one can determine which axis is the crystals long axis with respect to the optical axis. We define the aspect ratio as the ratio of the length of the crystal c to the (110) face width w. Geometrical considerations reveal that for the given tetragonal shaped crystals the body diagonal bd (see Figure 2) is always longer than the cross-sectional diagonal d of the crystal. However bd asymptotically approaches b for small aspect ratios. Consequently, the body diagonal bd, as the crystal's longest dimension lines up with the beam axis. Different aspect ratios lead to different angles between the body diagonal and the optical axis. However, the alignment of the particle in the beam does also depend on the internal birefringence, which tends to align the optical axis perpendicular to the beam axis.

As a result, if the crystal was initially elongated along the optical axis (optical axis of crystal parallel to beam axis), changing the aspect ratio of the crystal will skew the optical axis by a certain angle with respect to the beam axis due to form-birefringence. Taking into account the geometrical considerations made above, the angle between the optical axis and the beam axis would asymptotically approach 90 degrees. However, since the particle is also birefringent, at a

certain point the torque caused by the birefringence snaps the optical axis of the particle perpendicular to the beam axis (see Figure 7(a)).
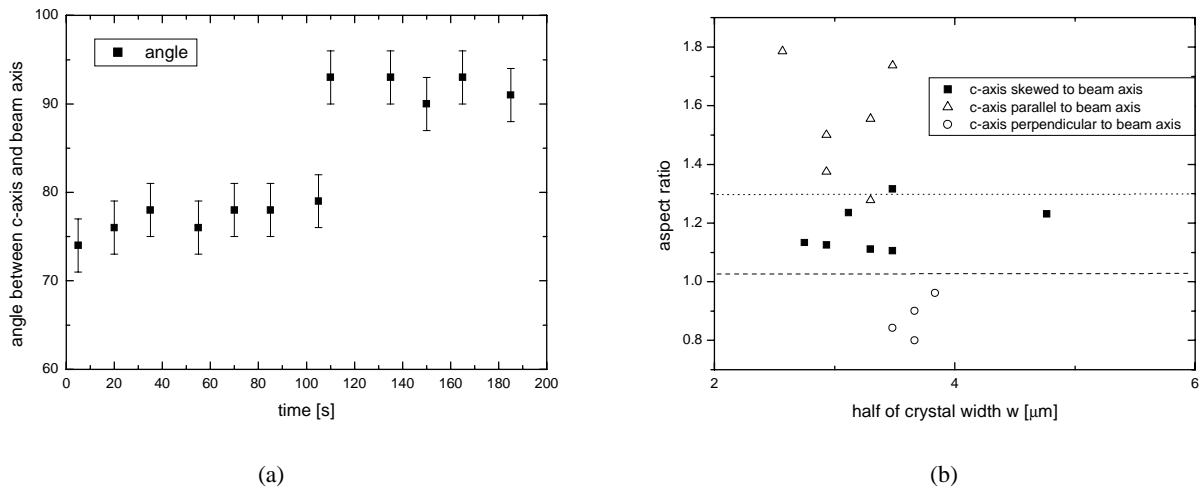

(a)

(b)

Figure 7: (a) Angle between c-axis of crystal and beam axis as a function of time; due to an increase of protein concentration the crystal is growing preferentially in the direction perpendicular to the c-axis. As a result the aspect ratio of the crystal changes with time. (b) Orientation of different trapped lysozyme crystals on aspect ratio. There is a strong correlation between different aspect ratios and particles orienting themselves with the c-axis parallel, perpendicular or skewed with respect to the beam axis.

The graph in Figure 7(a) shows a growth experiment described in Section 3.4 where the angle between the crystal's optical axis and the beam axis is approaching an angle of 90 degrees (t < 105 s), but at a certain angle it snaps to 90 degrees, a position that would be reached only asymptotically due to form birefringence.

The evaluation of a number of particles with different aspect ratios gave the same results, revealing that their orientation is determined by the aspect ratio, as shown in Figure 7(b). As can be seen from this figure crystals with aspect ratios of more than ~1.3 align with the optical axis parallel to the beam axis, whereas crystals with aspect ratios smaller than ~1 are trapped with the optical axis perpendicular to the beam axis. Particles with aspect ratios which lie in between those values arrange themselves with the optical axis skewed to the beam axis.

Also simulations using the T-matrix method[32, 33] to calculate the orientation of the crystal qualitatively agree with our experimental obtained data. However, to be able to compare the torques due to internal birefringence and different particle elongations quantitatively one needs to have reliable values of the relative refractive index (contrast), which are not available so far.

4. CONCLUSIONS AND SUMMARY

Orientation and rotation of microscopic particles can be achieved by using an asymmetric beam or polarized light together with particles having some external or internal anisotropy. Several methods to rotate and align microscopic particles controllably have been developed. It has been shown that the orientation of an object in the laser trap depends on its birefringence as well as on its shape. We have used lysozyme crystals to study the orientation of particles in optical tweezers depending on both the birefringence and the form-birefringence. The ability to immobilize and align the crystals during changes of the host solution (temperature, protein or salt concentration), suggests the use of optical tweezers for studies of protein crystal growth made either by video observation or x-ray diffraction.

5. ACKNOWLEDGEMENTS

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