Dynamics of Interaction of RBC with optical tweezers

Samarendra K. Mohanty[a], Khyati S. Mohanty [b] and Pradeep K. Gupta[a,*]

[a]*Biomedical Applications Section, Centre for Advanced Technology, Indore, INDIA 452013;*
[b] *Deartment of Electrical Engineering, Faculty of Technology and Engineering, Maharaja Sayajirao University, Baroda, INDIA 390001.*
*pkgupta@cat.ernet.in

Abstract: It has recently been shown that a red blood cell (RBC) can be used as optically driven motor. The mechanism for rotation is however not fully understood. While the dependence on osmolarity of the buffer led us to conclude that the osmolarity dependent changes in shape of the cell are responsible for the observed rotation, role of ion gradients and folding of RBC to a rod shape has been invoked by Dharmadhikari *et al* to explain their observations. In this paper we report results of studies undertaken to understand the dynamics of a RBC when it is optically tweezed. The results obtained support our earlier conjecture that osmolarity dependent changes in shape of the cell are responsible for the observed rotation.

OCIS Codes: (000.0000) General; (140.7010) Trapping; (180.0180) Microscopy.

References and links

1. H. He, M. E. J. Friese, N. R. Heckenberg, H. Rubinsztein-Dunlop, "Direct Observation of Transfer of Angular Momentum to Absorptive Particles from a Laser Beam with a Phase Singularity," Phys. Rev. Lett. **75**, 826 (1995).
2. M. E. J. Friese, T. A. Nieminen, N. R. Hewckenberg, H. Rubinsztein-Dunlop, "Alignment or spinning of laser-trapped microscopic waveplates," Nature **394,** 348-350 (1998).
3. P. Galajda and P. Ormos, "Complex micromachines produced and driven by light," Appl. Phys. Lett. **78**, 249-251 (2001).
4. A. I. Bishop, T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, "Optical application and measurement of torque on microparticles of isotropic nonabsorbing material," Phys. Rev. A **68**, 033802-1 (2003).
5. L. Paterson, M. P. MacDonald, J. Arit, W. Sibbett, P. E. Bryant, K. Dholakia, "Controlled Rotation of Optically Trapped Microscopic Particles," Science **292**, 912-914 (2001).
6. R. Dasgupta, S.K. Mohanty, P.K. Gupta, "Rotation of transparent, nonbirefringent objects by transfer of the spin angular momentum of light," Biotechnol. Lett. **25**, 1625-1628 (2003); see also Opt. & Photon. News **14** (12), 16 (2003).
7. R. Dasgupta, P.K. Gupta, "Rotation of transparent, nonbirefringent objects by transfer of the spin angular momentum of light," Opt. Lett. **30**, 394-396 (2005).
8. S. K. Mohanty, A. Uppal, and P.K. Gupta, "Self-rotation of red blood cells in optical tweezers: prospects for high throughput malaria diagnosis," Biotechnol. Lett. **26**, 971-974 (2004); see also Opt. & Photon. News **15** (12), 19 (2004).
9. J.A. Dharmadhikari, S. Roy, A.K. Dharmadhikari, S. Sharma and D. Mathur, "Torque-generating malaria-infected red blood cells in an optical trap," Opt. Express **12**, 1179-1184 (2004). http://www.opticsexpress.org/abstract.cfm?URI=OPEX-12-6-1179.
10. J.A. Dharmadhikari, S. Roy, A.K. Dharmadhikari, S. Sharma and D. Mathur, "Naturally occurring, optically driven, cellular rotor," Appl. Phys. Lett. **85**, 6048-6050 (2004).
11. H. Rouse, *Elementary Mechanics of Fluids*, Ch. VIII, Wiley Eastern Pvt. Ltd, NewDelhi, India (1970).
12. R. C. Gauthier, "Theoretical investigation of the optical trapping force and torque on cylindrical micro-objects," J. Opt. Soc. Am. B. **14**, 3323-3333 (1997).
13. R. C. Gauthier, M. Ashman and C. P. Grover "Experimental confirmation of optical-trapping properties of cylindrical objects," Appl. Opt. **38**, 4861-4868 (1999).
14. S. C. Grover, R. C. Gauthier and A. G. Skirtach, "Analysis of the behavior of erythrocytes in an optical trapping system," Opt. Express **7**, 533-539 (2000). http://www.opticsexpress.org/abstract.cfm?URI=OPEX-7-13-533.
15. Z. Cheng, P. M. Chaikin and T. G. Mason, "Light Streak Tracking of Optically Trapped Thin Microdisks," Phys. Rev. Lett. **89**, 108303-1(2002).

#7273 - $15.00 USD
Received 26 April 2005; revised 6 June 2005; accepted 7 June 2005

16. P. Galajda and P. Ormos, "Orientation of flat particles in optical tweezers by linearly polarized light," Opt. Express **11**, 446-451 (2003). http://www.opticsexpress.org/abstract.cfm?URI=OPEX-11-5-446.

1. Introduction

There exists considerable interest in optically controlled orientation or rotation of microscopic objects for applications in microfluidics and nano-technology. Optically driven micromotors can be used for transport of fluids, for mixing fluids in microfluidic chambers or to probe viscosity of microscopic environments. Several optical tweezers based techniques have been used for rotation of absorbing [1], birefringent [2], specially fabricated structures [3] and even non-absorbing and non-birefringent objects [4-7]. It has recently also been shown that RBC can be used as optically driven motor [8-10]. The mechanism for rotation is however not fully understood. While the dependence on osmolarity of the buffer led us to conclude that the osmolarity dependent changes in shape of the cell are responsible for the observed rotation [8], role of ion gradients and folding of RBC has been invoked by Dharmadhikari *et al* [9, 10] to explain their observations. In this paper we report detailed studies on the dynamics of a RBC when it is optically tweezed. Our results do not support the suggestion [9, 10] that RBC folds into rod shape when optically tweezed. A ray optic analysis of the propagation of trap beam through the RBC is presented to explain the observed rotation.

2. Experimental methods

The unpolarized output of a 1064 nm cw Nd: YAG laser (Solid State Laser Division, CAT) operating in fundamental Gaussian mode was expanded using 6X beam expander. This was coupled to the 100X Plan Neofluor oil immersion phase objective of an inverted microscope (Carl Zeiss) through its base port. A spherical lens was used in the path of the near infrared beam to generate conventional point optical tweezers. For generating additional tweezers, the infrared laser beam was split into two beams, which were combined, to result in a small angle between them and coupled to the microscope. A dichroic mirror was also placed at the base port of the microscope to transmit the 1064 nm laser beam and reflect the visible transmitted radiation to a CCD camera. Infrared cut off filter were placed before the CCD to reject the back-scattered laser light. The focal plane of the trapping spots were adjusted by changing the divergence of the beam as it enters the objective back aperture. The trapping laser beam power at the back aperture of the objective was monitored with a power meter (Coherent Inc., USA). The transmission factor of the objective was estimated by use of the dual objective method, which is able to correct for the total internal reflection losses at the objective-oil-glass-water interfaces. The CW Nd: YAG laser trapping beam powers were adjusted to obtain powers of ~ 10 to 200 mW at the focal plane of the objective.

Fresh blood from healthy volunteers was obtained. The RBCs were separated by centrifugation and suspended in Phosphate Buffered Saline (PBS). The shape of RBC is known to depend on the osmolarity of the buffer in which it is suspended. In isotonic buffer (290 mOsm/Kg) it is bi-concave in shape; in a hypotonic buffer (155 mOsm/Kg) it gets swollen and becomes spherical and in hypertonic buffer (> 600 mOsm/Kg) RBC takes a meniscus shape. The dynamics of interaction of red blood cell (RBC) with optical tweezers was investigated at varying osmolarity to examine the influence of the change in shape of RBC on the dynamics of interaction.

3. Model for calculation of viscous torque

In order to get oriented or rotated, the torque generated by the laser beam has to exceed the viscous torque due to the surrounding medium. Following reference [11], viscous drag (F_D) on disk-like RBC can be estimated at low Reynolds number, using $F_D = C_D A \rho u^2 / 2$, where C_D is the drag coefficient, A the projected area of the body on a plane normal to the flow, ρ the density of the fluid, and u the velocity of the moving body. The dependence of the drag force on the shape and size of the object is incorporated through the coefficient C_D.

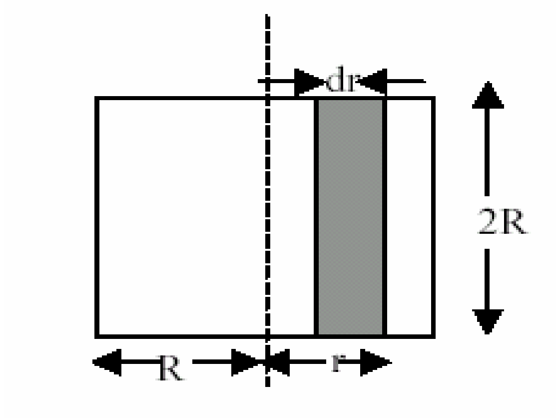

Fig. 1. Schematic of the cross-section of the disk

For simplicity we consider a RBC as a rectangular sheet. For a rectangular sheet, drag torque acting on an element of width dr at distance r from the axis (shown by shaded area in Fig. 1) can be expressed as

$$dT_D = F_D \times r = (C_D.\ 2Rdr\ \rho\omega^2 r^2 / 2).r \qquad \ldots\ldots \quad \ldots.\ (1)$$

Here, ω is the angular velocity of the disk and R is its radius. Hence, drag torque on the discotic object (assumed rectangular) an be written as,

$$T_D = (C_D R.\ \rho\omega^2)[\int r^3\,dr]_0^R = C_D\,\rho\omega^2 R^5/4 \qquad \ldots\ldots \quad \ldots.\ (2)$$

For micrometer dimension disks rotating with angular velocities of a few Hz the Reynolds number (Re) is much less than 1. At these values of Re, C_D can be approximated as C_D = 24/Re.

$$\text{Where } Re = \rho.u.R/\ \eta \sim \rho.\ \omega.R^2/\ \eta. \qquad \ldots\ldots\ldots\ldots \quad \ldots.\ (3)$$

Hence, $T_D = (\rho\omega^2 R^5/4)\ 24/\ (\rho.\ \omega.R^2/\ \eta) = 6\ \omega\ \eta\ R^3.$ ……… ……. (4)

In our case, R ~ 3 x 10^{-6} m, η ~ 1.005 x 10^{-3} N.s/ m^2.

4. Results

RBCs, when suspended in isotonic buffer look discotic. When optically trapped the RBCs get oriented with their symmetry axes perpendicular to the beam axis. We show in Fig. 2 the time-lapse digitized video images of change in orientation of RBC when subjected to optical tweezing.

The time required for a RBC to switch from the initial horizontal position to the vertical orientation was estimated from these time lapse images and was found to depend on both the trapping power and osmolarity of the buffer in which the RBCs were suspended. In Fig. 3, we show the change in the angle of orientation of RBC (suspended in a buffer with an osmolarity of 300mOsm/Kg) as a function of time after it is optically tweezed at two power levels. At the same trap power levels the time required for a RBC to switch from initial horizontal position to the vertical orientation was found to decrease with increasing osmolarity.

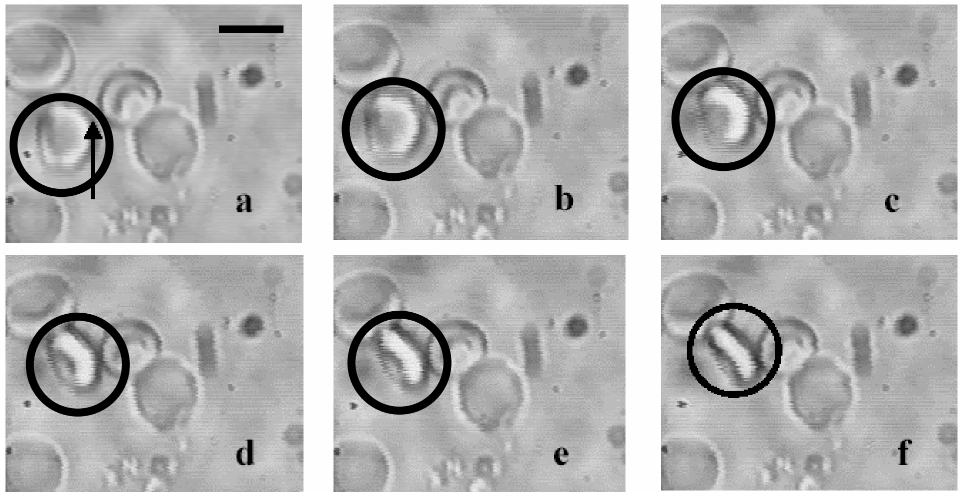

Fig. 2. Time-lapse digitized video images of RBC subjected to optical tweezing with trap beam power of 85 mW. The RBC is shown encircled for clarity and the location of the trap beam is shown by arrow. Time interval between consequent frames was 40 ms. In panel (a) the RBC is in horizontal plane. On being subjected to optical tweezing it gradually orients with its plane along the trap laser beam axis (panels b to f). Scale bar: 5µm

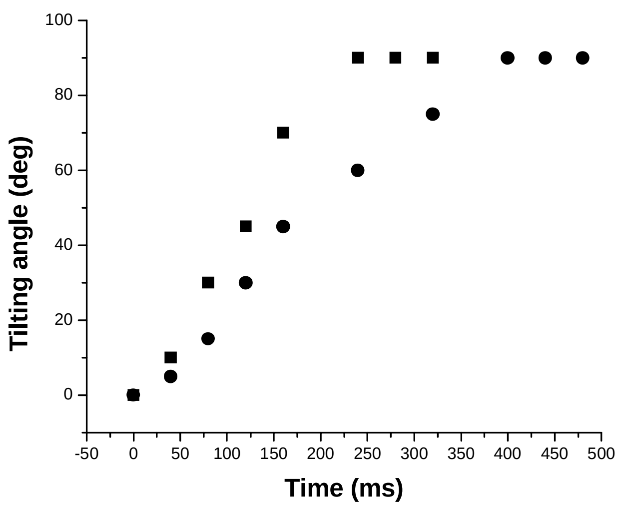

Fig. 3. Angle of orientation (with respect to horizontal) as a function of tweezing time. Square symbols are for 85 mW trapping power and circles are for 60 mW laser power at the trapping plane.

From the measured rate of change of the orientation angle (Fig. 3), the angular velocity and thus the viscous orientational torque T_D can be calculated as a function of time using eq^n (4). The orientational torque was found to increase up to 120 ms and then decrease as the plane of the discotic RBC is aligned with the laser beam axis. The results are shown in Fig. 4. The peak value of viscous orientational torque T_D was estimated to be ~ 1.0 pN.µm at 60 mW and ~ 1.5 pN.µm at 85 mW.

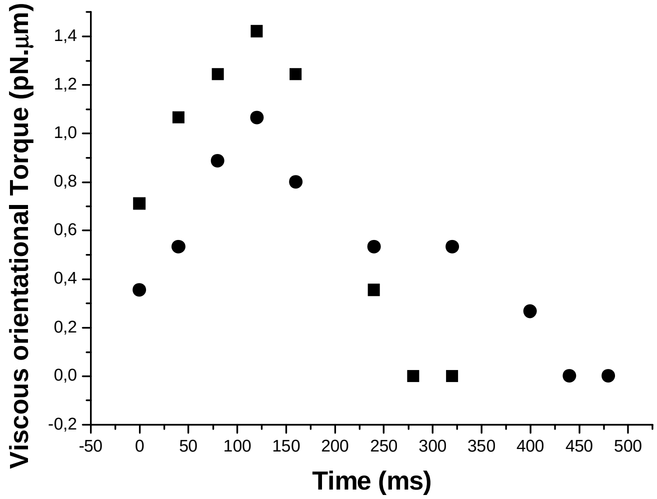

Fig. 4. The estimated viscous orientational torque as a function of tweezing time. Square symbols are for 85 mW trapping power and circles are for 60 mW laser power at the trapping plane.

When the trap beam is switched off, RBC returns to the original horizontal orientation, in approximately 3 to 6 s. Even with the trap on, the vertically oriented RBC could be reoriented to the horizontal plane by subjecting the RBC to a viscous drag on its surface closer to the cover slip by translation of the microscope stage. The results for the case when the stage was moved with a velocity of ~ 80 μm/ sec are shown in Fig. 5. During its reorientation to the horizontal plane the shape of the RBC remains discotic and there is no evidence for any folding to a rod shape. A rod shape object is known to orient with its axis along the trap beam and should show up in the image as a circular spot having the same diameter as that of the rod [12, 13]. From results presented in Fig. 5 switching time was estimated to be ~ 0.2 s. The reorientation of RBC at such fast time scale also does not support folding of RBC in a rod shape.

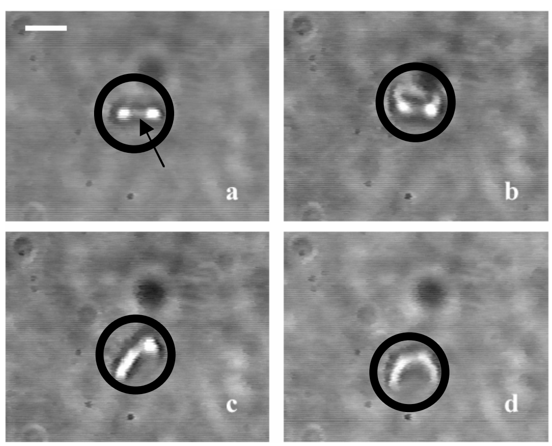

Fig. 5. Time-lapse images of reorientation of an optically trapped RBC back to the horizontal plane by subjecting it to a viscous force. The RBC is shown encircled for clarity and the location of the trap beam is shown by arrow. Time interval between consequent frames was 80 ms. Scale bar: 5μm.

#7273 - $15.00 USD
Received 26 April 2005; revised 6 June 2005; accepted 7 June 2005

In solutions having osmolarity > 1000 mOsm/Kg, RBC was observed to rotate continuously around the axis of the laser beam. The speed of rotation as a function of osmolarity for a trap beam power of 85mW is shown in Fig. 6. The maximum rotational speed was obtained at an osmolarity of 1200 mOsm/Kg. Referring to Fig. 6 and using eq^n (4), the viscous torque on the rotating (120 rpm) RBC was estimated to be ~ 2.0 pN.µm at 85 mW.

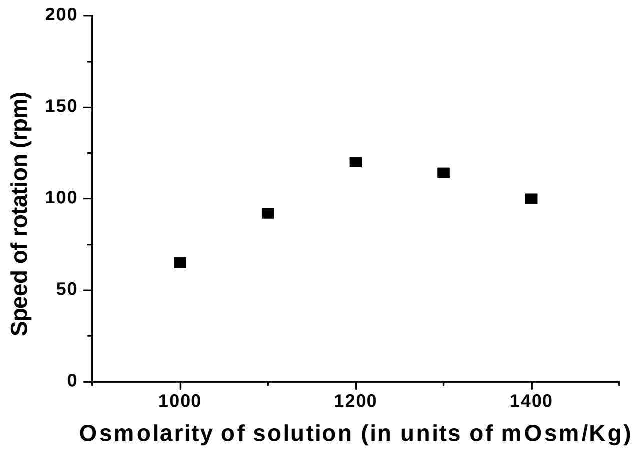

Fig. 6. Dependence of the speed of rotation of RBCs on the osmolarity of the buffer solution. The trap beam power was 85 mW.

5. Discussion and conclusion

The observed edge-on-orientation of discotic RBC when optically tweezed in an isotonic buffer is consistent with earlier reports wherein it has been shown that disk-shaped objects get oriented with their symmetry axes perpendicular to the beam axis, since this maximizes the overlap of disk's volume with the region of highest electric field [12-15]. The thickness of RBC falls in the range of 1.25 to 2.0 µm. From the results presented in references [12, 13] for trapping of cylindrical objects it follows that the RBC should get stably trapped with edge on orientation, i.e. with its central axis transverse to the beam. Here, it is also pertinent to note that when the trapping beam was made linearly polarized using a polariser, the vertically oriented RBC gets aligned with its symmetry axis perpendicular to the electric vector of the trapping beam. Rotation of the trapped RBC could be effected by rotation of the plane of polarization of the trapping beam by use of a half wave plate, much like what has been demonstrated earlier [4, 16].

To understand why meniscus shaped RBC in hypertonic buffer rotates, whereas discotic RBC in isotonic buffer gets oriented along the trap beam, we discuss the propagation of the trap beam through the different shapes of RBC. In an isotonic buffer, the cross-section of a discotic RBC oriented along the trap beam in the plane of the paper is as shown in Fig. 7(a). Consider a trap beam coming out of the plane of paper and having focal point in the plane of the paper. The rays a and b of the trap beam (shown as solid arrows) coming out of the plane of the paper are refracted in the direction shown by dotted arrows. As a result the RBC will experiences forces $\mathbf{F_a}$ and $\mathbf{F_b}$ in the directions shown in figure. Similarly, RBC will experiences forces $\mathbf{F_{a'}}$ and $\mathbf{F_{b'}}$ due to rays a' and b' coming out from diametrically opposite points of the cell. As shown in the figure, due to the symmetry in the shape of the RBC, the resultant force due to rays emerging from diametrically opposite points of the cell are equal and opposite. Therefore, in such a situation the RBC does not experience any rotational torque and remains aligned along the trap beam.

#7273 - $15.00 USD Received 26 April 2005; revised 6 June 2005; accepted 7 June 2005

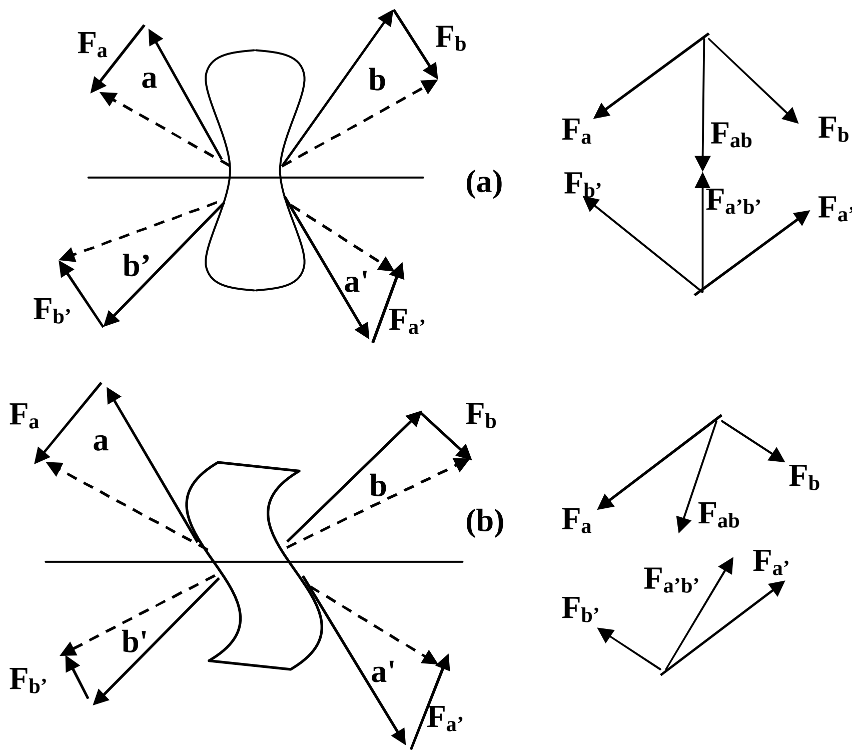

Fig. 7. Force diagram when RBC is trapped in isotonic (a) and hypertonic buffer (b).

In Fig. 7(b) we show a schematic of the cross section of RBC in a hypertonic buffer. The cross-section is distorted and a schematic of the shape is shown). In this case, the ray a of the trap beam (shown as solid arrow) coming out of the plane of the paper near the convex side travels more inside the RBC and thus deviates more form its original path as compared to the ray b coming out near the concave side. Therefore, the forces $\mathbf{F_a}$ and $\mathbf{F_b}$ experienced by the RBC differ in magnitude and direction as shown in figure. Similarly, the RBC experiences unequal forces $\mathbf{F_{a'}}$ and $\mathbf{F_{b'}}$ due to the rays a' and b' emerging from diametrically opposite points of the cell. Therefore, in such a situation the RBC experiences a rotational torque around the trap beam axis as shown in the figure. Thus the above analysis makes it clear that the observed rotation arises due to structural asymmetry of RBC at higher osmolarity and in this respect it is similar to that previously reported for specifically structured objects [3].

To conclude, we have shown that osmolarity dependent changes in the shape of RBC can account for the observation that meniscus shaped RBC in hypertonic buffer rotates, whereas discotic RBC in isotonic buffer gets oriented along the trap beam.

Acknowledgments

Authors would like to thank Shri T.P.S. Nathan, Head, Solid State Laser Division and members of his division for providing the Nd: YAG laser and their help in smooth running of the laser. Authors would also like to thank Dr. A. Uppal for the blood samples.