Optical trapping of spermatozoa using Laguerre-Gaussian laser modes

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Abstract. We report results of a study on the use of Laguerre-Gaussian (LG) modes for optical trapping of spermatozoa. The results show that for a given trap beam power the first-order LG mode (LG01) leads to lower photodamage to the cells without compromising the trapping efficiency.

Keywords: laser applications; cells; laser-induced damage; Gaussian beams.

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Optical tweezers are being used for measurements on the motility of spermatozoa and for in vitro fertilization of individual spermatozoon to oocytes. To minimize possible damage to the cells due to exposure to the high light intensity (hundreds of MW cm\(^{-2}\)) at the trap focus, lasers in near-IR region (wavelength \(\sim 1 \mu m\)), where the absorption of the cellular components is minimal, are used. However, even at 1064 nm, negative effects of laser exposure on spermatozoa have been reported, which originate presumably from the nonlinear light absorption.

Studies on the use of Laguerre-Gaussian (LG) laser modes that have a dark spot at the centre (optical vortex), for trapping of microscopic objects, have shown that compared to the TEM\(_{00}\) mode, the LG\(_{01}\) mode leads to an improved axial and transverse trapping efficiency. This has been attributed to the fact that optical trapping force is primarily contributed by the off-axis large conic angle rays. The use of an LG laser beam may therefore enable efficient trapping of the motile spermatozoon while the absence of strong axial intensity and redistribution of power into the doughnut-like region may help minimize the possible photodamage. We have therefore investigated the use of LG modes for manipulation of spermatozoa. The results obtained confirm that as compared to TEM\(_{00}\) Gaussian mode, the use of the LG\(_{01}\) mode leads to significantly lower photodamage for similar trapping efficiency.

The optical tweezers setup consists of a frequency-doubled Nd:YVO\(_4\) laser emitting at 532 nm (Verdi-5, Coherent Inc.). Note significantly lower photodamage for similar trapping efficiency.

Laguerre-Gaussian laser modes can lead to improved axial\(^8\) and transverse\(^9\) trapping efficiency.

The experiments were then kept at 37 \(\circ\)C. The average size of the paddle-shaped head of a goat spermatozoon estimated from brightfield microscope images was \(\sim 8 \times 3.5 \times 1.7 \mu m\) (length \(\times\) width \(\times\) thickness). For vital staining of spermatozoa, propidium iodide (PI) was used. A stock solution of 0.5-mg PI/ml of water was prepared and stored frozen at \(-20 \circ\)C in the dark. The stock solution was added at the time of experiments into the spermatozoa samples so that the final concentration was 5 \(\mu g/ml\). The suspension was incubated for 5 min at room temperature (\(-25 \circ\)C). Laser-exposure-induced DNA damage to the spermatozoa was monitored using acridine orange (AO) staining. AO exhibits green and red fluorescence depending on whether it intercalates into double-stranded nucleic acids (DNA) or single-stranded nucleic acids (damaged DNA and RNA), respectively. For staining the spermatozoa with AO, the spermatozoa samples were mixed with PI and DPH suspension (~4 mM) was prepared by dissolving it in
dimethyl sulphoxide (DMSO). Since DPH has minimum absorption above 400 nm, direct single-photon excitation of the probe molecules from the 532-nm laser beam is unlikely. It has its strongest absorption bands in the region 300 to 400 nm and has weak absorption in the spectral region 240 to 270 nm. The fluorescence emission could be observed using long-pass filters having transmission above 400 nm.

To estimate the efficiency of different laser modes to capture spermatozoa, video data were recorded for about 1 h, and during that period, speeds of the moving spermatozoa that could be captured by the different trapping laser modes were estimated. In our study, only spermatozoa that have fairly straight trajectories were considered. Therefore, the difference between curvilinear velocity (VCL) and straight line velocity (VSL) could be minimized for the cells analyzed in our study. The VSL of a moving spermatozoon could be estimated by noting its initial and final positions. A spermatozoon of interest was observed for 1 to 2 s before trapping. Nearly 50 cells were studied with each type of trap. From these measurements, we estimated for each mode the maximum VSL of the spermatozoa that could just be trapped. For this, we selected five cells with highest VSL from the nearly 50 cells on which measurement was made. The mean and standard deviation of these are plotted in Fig. 1. From Fig. 1 we can see that as compared to a TEM$_{00}$ beam, the LG$_{01}$ beam can trap spermatozoa swimming at a higher speed. However, the third- and fifth-order LG beams fared worse than the TEM$_{00}$ beam. The observation is consistent with earlier reports that while using the LG$_{01}$ mode, the transverse trapping efficiency increases, higher order LG modes lead to a reduction in transverse trapping efficiency. The difference in the VSL of spermatozoa that could be trapped using different laser modes was found to be statistically significant with $p < 0.05$.

When trapped, the motile spermatozoa show strong flagellar and head motion though their position could be held constant by the trap. With increasing trapping duration the flagellar and head motion tends to die out and eventually ceased indicating a paralyzed cell. The time duration for the onset of paralysis of the cells when held continuously under optical trap can be used as an indicator for the detrimental effect of the trap. To measure the photodamage effect, the motions of the trapped cells were recorded at video rate and the time interval ($T_{\text{paralysis}}$) between the capture of the cell and the complete disappearance of any movement was noted. Figure 2(a) shows the data for the TEM$_{00}$ mode and the LG$_{01}$ mode for three trap beam power levels. A total of $\sim$120 cells were studied for the analysis.

The measured $T_{\text{paralysis}}$ shows that cells could remain motile over a longer time as the order (azimuthal index) of the LG mode is increased [Fig. 2(b)]. The viability of the trapped cells when they turned nonmotile was further checked with PI staining. Strong PI fluorescence could be observed for most of the cells within 1 to 2 min after the cell turns nonmotile.

We used AO staining to monitor possible DNA damage in spermatozoa under the trap. The stained cells were irradiated with $\sim$1 mW of TEM$_{00}$ and LG$_{01}$ laser profiles and evolution of AO fluorescence, when excited with 450 to 490-nm excitation, was monitored using a color CCD camera. The temporal evolution of the AO fluorescence with increasing exposure duration for the TEM$_{00}$ and LG$_{01}$ modes is shown in Fig. 3. From the CCD image data (24 bits/pixel, 8 bits for each of the red, green, and blue channels) the intensities of the green (500 to 600 nm) and red (600 to 700 nm) channels (which shows whether AO intercalates into double-stranded DNA or single-stranded DNA/RNA) were estimated. From Fig. 3(a) we can see that for low exposure times ($<15$ s) the red-to-green ratio is small for both the trap beams, showing very little DNA double-strand breaks. However, with increasing exposure duration...
the increase in the intensity ratio occurs more quickly for the TEM00 mode as compared to the LG01 mode. Further, while for the TEM00 mode, the intensity ratio was seen to saturate at \( R=G = 1.2 \) within 60 s, for the LG01 mode, the saturation occurred at \( \sim 75 \) s. These results suggest that the DNA damage rate is faster with the TEM00 mode and implies an increased level of risk to the genetic purity of the spermatozoa. Note here that although the absorption band for AO ranges from 450 to 500 nm, it has small but nonzero absorption (~5%) of the peak value of \( \sim 50,000 \text{ M}^{-1} \text{ cm}^{-1} \) at \( \sim 490 \) nm at the laser wavelength. Considering AO concentration of \( \sim 15 \) \( \mu \text{M} \) used in the samples, the temperature increase at focus resulting from direct absorption of laser light by AO was estimated following the method given in Ref. 15. The estimated temperature rise is \( \sim 0.004 \)°C, which is small to cause any significant DNA damage.

It is known that due to very high power density present at the trap beam focus, significant two-photon absorption from the cw trap beam can take place, Refs. 7, 16, and 17, respectively, leading to possible damage to cell DNA having its absorption peak \( \sim 260 \) nm. Therefore, the observed lower degree of DNA damage with the LG modes may be attributed to the fact that peak intensity present in an optical vortex profile is significantly lower than that of a TEM00 Gaussian beam, and this can account for the observation that cells remain motile for a longer period of time with the increasing order of LG modes. To estimate the two-photon absorption for the TEM00 and LG01 modes, their intensity profiles at trapping plane must be determined.

It has been shown that for an optical system consisting of two media separated by a plane interface the diffracted field of the LG mode, at the point \( (r, \theta, \phi) \), can be expressed as the function of conic angle \( \theta \) as\(^{18, 19}\)

\[
E(p) = \int_0^a \int_0^{2\pi} \Psi_{m,l}(\theta) \cos^{1/2} p(\theta, \phi) \exp[i(k_0(r_p + \psi_p))] \times \exp(i\phi) \sin \theta \, d\theta \, d\phi, \tag{1}
\]

where the origin of the coordinate system is assumed at the Gaussian focus in the absence of the aberration caused by the refractive index interface and

\[
\Psi_{m,l}(\theta) = A_0 \exp \left[ -\gamma \frac{\sin^2 \theta}{\sin^2 \alpha} \right] \times \left( \frac{\sqrt{2} \gamma \sin \theta}{\sin \alpha} \right)^{|m|} L^{|m|}_i \left[ 2\gamma \frac{\sin^2 \theta}{\sin^2 \alpha} \right], \tag{2}
\]

and

\[
\gamma = \frac{a}{w_0}, \tag{3}
\]

where \( a \) is the microscope objective aperture radius, \( \alpha \) is the largest conic angle determined by the NA of the objective lens, \( A_0 \) is the amplitude, \( w_0 \) is the beam radius at waist, and \( L^{|m|}_i \) are the Laguerre polynomials. Here \( l \) is the radial mode number, and \( m \) is called the azimuthal index, with \( l = 0 \) and \( m = 0 \) denoting a zeroth-order Gaussian mode (TEM00); \( k_0 \) is the free space wave number of the optical beam; and \( P(\theta, \phi) \) and \( \psi_p \) represent the polarization distribution and aberration effect, respectively.\(^{18}\)

The intensity distribution of the TEM00 and higher order LG modes computed using Eq. (1) are shown in Fig. 4(a). Note that only the left circularly polarized (LCP) state is considered.

**Fig. 3** Time evolution of fluorescence spectra from AO-stained spermatozoa in terms of (a) intensity ratios for the red and green channels. The data are averaged over ~10 cells and presented as mean ± standard deviation. The corresponding light dosages are also shown along the time axis. The intensity ratios are statistically significantly different with \( p < 0.05 \) (Student’s \( t \) test). (b) Time lapse images of AO-stained spermatozoa. (Color online only).

**Fig. 4** (a) Intensity profiles of different laser modes and (b) estimated and observed DPH fluorescence when excited by the TEM00 mode and LG01 mode. The error bars indicate standard deviation of data from the mean value. The values are normalized with respect to the total fluorescence intensity estimated/observed with the TEM00 mode.
for $P(\theta,\varphi)$, as the LCP state produces most symmetric intensity distribution and complete disappearance of axial intensity at the focus in the case of the LG_{01} mode. For identical power, the peak intensities at focus for the LG_{01} beam is $\sim$40% of that produced by a TEM_{00} beam [Fig. 4(a)].

The fluorescence yield resulted from two-photon absorption can be expressed as,

$$\phi_{FL,2-ph} = Q \sigma_{2-ph} \left( \frac{I}{h\omega} \right)^2 N,$$

where $\sigma_{2-ph}$ is the mean molecular two-photon absorption cross section, $h\omega$ is the photon energy, $Q$ is the quantum yield for the dye, and $N$ is the number of fluorophore molecules present in the excitation volume. Therefore, the ratio between total fluorescence yield with TEM_{00} and LG_{01} modes can be given as

$$\left( \frac{\phi_{FL,2-ph}}{\phi_{FL,2-ph}} \right)_{TEM_{00}} = \frac{\int_{x,y} I^2_{TEM_{00}}(x, y) \cdot dx \cdot dy}{\int_{x,y} I^2_{LG_{01}}(x, y) \cdot dx \cdot dy}$$

To verify these estimates we measured the two-photon fluorescence yield for DPH when excited with the TEM_{00} and LG_{01} modes. The yield with the LG_{01} profile was measured to be about 52% of that with the TEM_{00} profile, which is in good agreement with the estimate of 56%. Since the probe has a very weak absorption band above 400 nm, direct excitation of the probe molecules by the laser beam due to the single-photon process is unlikely. Notably, a similar observation of reduced two-photon fluorescence from trapped dye-doped polystyrene beads was obtained by Jeffries et al., with LG modes at 1064 nm wavelength. But with small (diameter 100 nm to 1 µm) dye-doped trapped beads, the ratio of total two-photon excited fluorescence observed for LG modes and TEM_{00} mode were much smaller ($\sim$10%) than observed in our studies ($\sim$50%). The difference likely resulted from the incomplete overlap of the annular intensity pattern of LG modes with smaller trapped beads.

Although the detailed mechanisms of cell damage due to light irradiation are not fully understood, photochemical and photothermal effects are believed to be responsible. In the UV region, light absorption by nucleic acids and proteins can result in photodamage. In the visible region, the generation of reactive oxygen species (ROS) and free radicals produced subsequent to photooxidation of cellular components may damage the lipid membrane, proteins, and nucleic acids through oxidative reactions. In particular, cytochromes can absorb strongly near a 532-nm laser wavelength. Even in the near-IR wavelengths where cellular components do not have significant absorption, photoinduced damage has been observed. While for near-IR wavelengths below 800 nm, multiphoton absorption has been shown to contribute to photodamage, the origin of photodamage for longer wavelengths is still poorly understood. Studies performed on spermatozoa using both cw and pulsed trap beams at 1064 nm showed much pronounced damage with short pulses for the same average power, suggesting that transient heating at the trap focus or photochemical effects resulting from multiphoton absorption may be responsible.

With cw trap beams, the rise in temperature should be much smaller ($\sim$1°C/100 mW of trap power). Therefore, damage was much reduced, but for 300-mW cw power, noticeable damage was present for exposure durations exceeding 2 min. The comet assay technique, which has higher sensitivity, revealed a significant level of DNA damage to cells even when trapped for few tens of seconds using $\sim$120 mW of cw laser power at 1064 nm the origin of which is not fully understood. Studies carried out on ROS generation in cells exposed to pulsed and cw 1064 nm trap beams has also provided qualitatively similar results. While significant ROS generation in trapped cells took place with pulsed 1064-nm trap beams, with cw trap beam detectable ROS generation occurred at longer exposure times.

To conclude, the use of an optical vortex for manipulation of spermatozoa offers significant advantages in terms of reduced photodamage to the cells without compromising the trapping efficiency with the LG_{01} mode. Note that in this study, we could use trapping power of up to $\sim$140 mW, which was primarily limited by the diffraction efficiency and damage threshold of the SLM used for the generation of the LG modes. This power level is capable of manipulating spermatozoa having modest swimming speed ($\sim$50 µm/s). For cells with higher motility, a trapping power of $\sim$500 mW or more may become necessary. This can be achieved by use of diffractive optical elements offering high diffraction efficiency or methods suitable for direct generation of high-power vortex modes in the laser cavity.

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References


