L.5. Optical Trapping of Spermatozoa using Laguerre Gaussian Laser Modes

Optical tweezers are being increasingly used for assessment of quality of sperm sample and *in-vitro* fertilization. Sperm cells, being very motile in nature, high laser powers (~100 mW) are required to trap them. At such power levels, possible light induced damage to the cells is a major concern. Studies on the use of *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 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 *LG* laser beam may therefore allow efficient trapping of the motile spermatazoa while the absence of strong axial intensity and redistribution of power into the doughnut like region may help minimize the possible photo damage. At LBAID, the use of *LG* modes for manipulation of spermatazoa has therefore been investigated.

The efficiency of different laser modes to trap sperm cells was estimated by measuring the speed of the moving sperm cells that could be captured by these modes having identical powers. In our study only spermatozoa that are having fairly straight trajectories were considered. The straight line velocity (VSL) of a moving spermatozoon could be estimated by noting its initial and final positions. 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 ~50 cells on which measurement was made. The mean and standard deviation of these are plotted in figure 1.

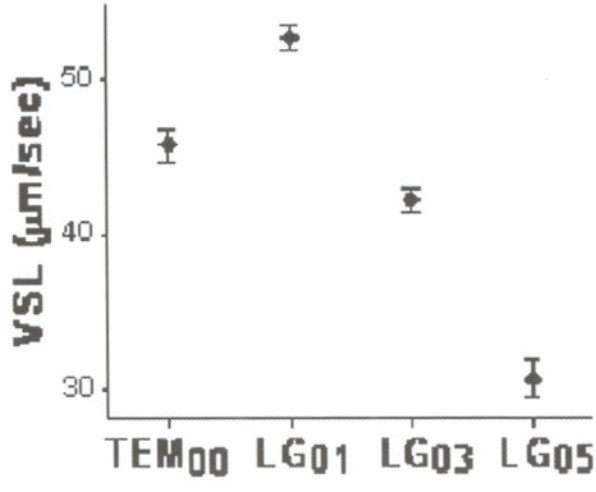

Fig.L.5.1 : Mean VSL of spermatozoa that could be just trapped by different laser modes having identical trapping power of ~ 140 mW at the specimen plane. The data presented are the mean ± standard deviation.

From fig.L.5.1 it can be seen that as compared to TEM_{00} beam LG_{01} beam can trap spermatozoa swimming at a higher speed. However, the 3rd and 5th order *LG* beams fared worse than the TEM_{00} beam. This is due to the fact that in case of higher order *LG* modes whole beam does not interact with the trapped sperm cell which therefore leads to reduction in trapping efficiecy.

When trapped, the motile spermatazoa 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

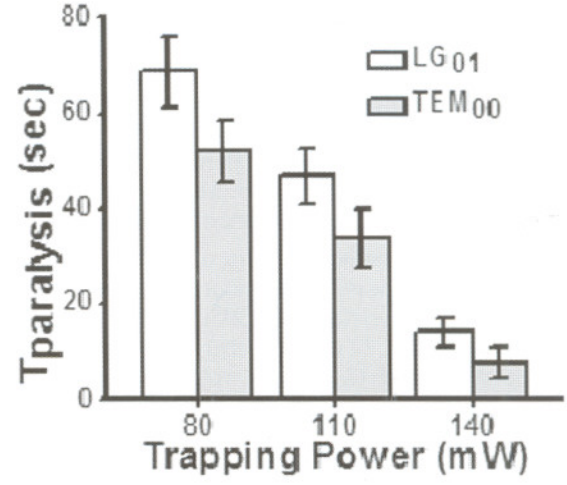

Fig.L.5.2 : $T_{paralysis}$ of the trapped spermatozoa under TEM_{00} and LG_{01} mode. The data presented are the mean ± standard deviation.

the time interval between the capture of the cell and the complete disappearance of any movement ($T_{paralysis}$) was noted. In fig. L.5.2 the data for TEM_{00} mode and LG_{01} mode are shown for three trap beam power levels. A total of ~ 120 cells were studied for the analysis. The viability of the trapped cells when they turned non-motile was further checked with PI staining. Strong PI fluorescence could be observed for most of the cells within 1-2 minutes after the cell turns non-motile. These observations show that use of LG_{01} mode causes reduced photodamage during optical manipulation of sperm cells.

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