depth of ~ 6 mm using this laser system has been carried out. Its further applications in welding of Copper, and Aluminium with variation in pulse duration and pulse energy is underway.

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L.8. Long distance axial trapping with Laguerre-Gaussian beams

Optical tweezers require tight focusing of laser light through a high numerical aperture (NA) microscope objective lens to produce the strong gradient forces necessary to overcome axial scattering forces and thus achieve stable three-dimensional trapping. The short working distance associated with a high NA (NA>1) objective limits the axial trapping range to a typical value of ~ 200 μm. Further, because the particles to be trapped are often suspended in an aqueous medium the trap beam suffers significant spherical aberration at the glass-water interface leading to an increased axial spread of the focal volume with increasing distances from the substrate. The resulting reduction in the power density at focus leads to a reduction in the gradient forces and consequently a limited axial trapping range that is significantly shorter (often under 100 μm) than the available working distance of the objective lens. Longer axial trapping range is however required for optical manipulation of objects at free liquid surface.

Studies performed in Laser Biomedical Applications and Instrumentation Division (LBAID), have shown that since the *LG* mode traverses the glass-water interface with a narrower angular range of rays vis a vis the TEM_{00} mode, the spherical aberration gets reduced for the *LG* mode. The resulting smaller axial spread of focal volume for *LG* mode enables longer axial trapping range compared to the TEM_{00} mode.

With ~10 mW of trapping laser power, with LG_{01} trap beam human colon adenocarcinoma (Colo-205) cells could be moved over an axial distance of ~ 240 μm whereas with the same power in the TEM_{00} mode the cells could be moved only up to a distance of ~ 145 μm (fig. L.8. 1). This ability of LG_{01} was used to transport a human colon adenocarcinoma (Colo-205) cell from the bottom layer of the medium to the top surface layer (thickness of the fluid ~200 μm) exposing it to a higher atmospheric oxygen concentration. By tagging the cell with a fluorescent membrane probe (laurdan), the difference in the extent of oxygen mediated fluorescence quenching between the two cases was used to estimate the oxygen diffusion rate in the membrane.

The relative fluorescence intensity for the cells at the

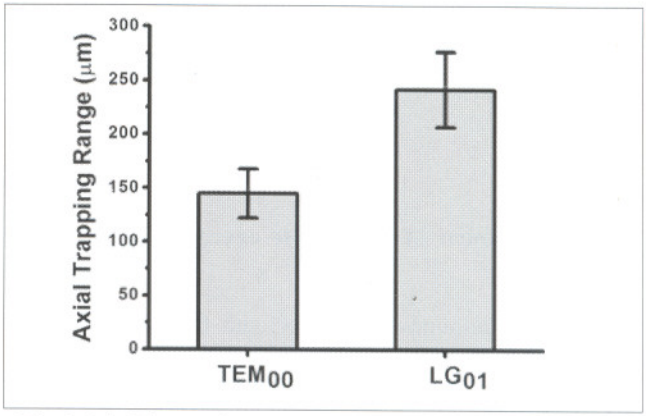

Fig.L.8.1: The axial trapping range observed for colo cells with TEM_{00} and LG_{01} modes. The data shown are the mean over five experiments.

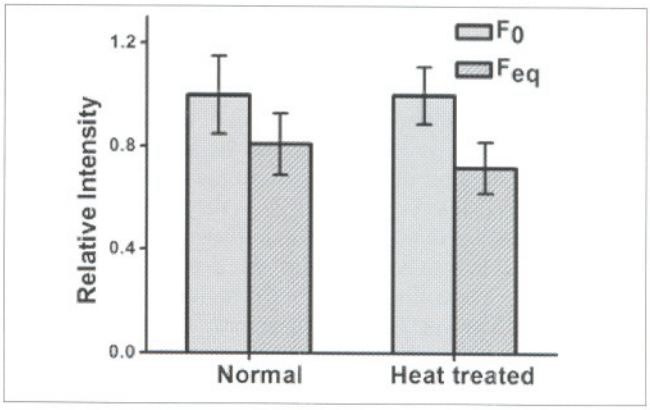

Fig.L.8.2: Observed mean relative fluorescence intensities of cells when trapped at the bottom (F_0) and at the top surface (F_{eq}). The data for cells althrough kept at room temperature (25°C) and cells heat treated for ~1hr at an elevated temperature of ~ 55 °C are shown.

air-liquid interface with respect to the cells trapped deep inside the medium is shown in fig.L.8.2. The data shown is the mean value taken over ten cells. The ratio of mean unquenched fluorescence intensity at bottom (F_0) to reduced fluorescence intensity at top surface (F_{eq}) ratio was measured to be ~ 1.23 yielding an oxygen diffusion rate of ~ 5.2 x10^{-5} cm^2s^{-1}. The value is in reasonable agreement with the value for biological membranes. To further validate these results measurements were also made on cells kept at higher temperature (55°C for 1 hr. The measured values for F_0 and F_{eq} for cells heat treated for ~ 1 hour at a temperature of 55 °C are consistent with the expected increase the membrane permeability of the cells.

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