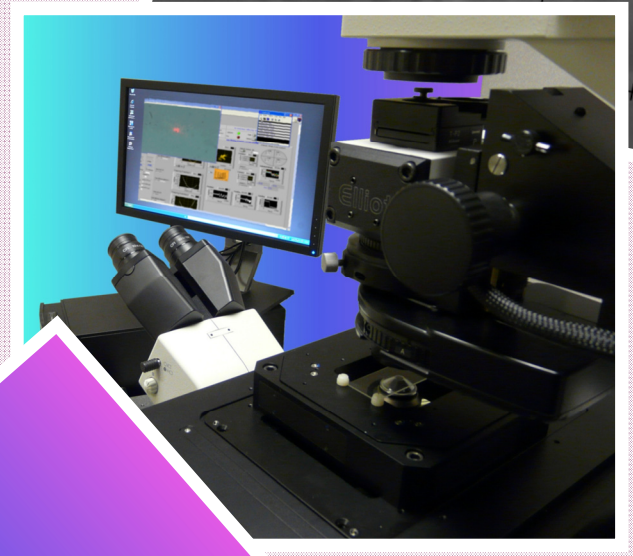
Optical Tweezer Application Notes

4. Photoporation and Transfection



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ELLIOT SCIENTIFIC OPTICAL TWEEZERS APPLICATION NOTES

4. PHOTOPORATION AND TRANSFECTION OF CELLS WITH PULSED LASER LIGHT

Numerous laser sources, such as UV laser beams, pulsed infrared Nd:YAG lasers, or ultra-short pulsed femtosecond beams, can be employed to function as “optical scissors”. Studies have shown cutting of the mitotic spindle and intracellular organelles including chromosomes. In combination with optical tweezers, the ability to selectively cut or dissect sub-cellular components is of immense interest. In this note we concentrate on simultaneous trapping and poration of a single Human Leukaemia (HL-60) cell. The work shown is based on the Elliot AOD tweezers system but we have additionally co-aligned an ultra-short femtosecond pulsed laser into the microscope body to act as a poration laser (Coherent MIRA Optima 900-F, 800 nm, 76 MHz repetition rate, 110 mW average power at objective back aperture).

When we consider poration, we start with the recognition that the cell membrane represents the outer extremity of all eukaryotic cells. In mammals this is a thin (5 nm) bi-layer film of lipids, embedded with various protein molecules at interspersed locations. The membrane encloses the cell, defines its boundaries, and maintains the essential physio-chemical differences between the cytoplasm and the extracellular environment. It is to be noted that the lipid nature of the cell membrane acts as an impermeable barrier to the passage of most water-soluble molecules. Thus the selective introduction of biologically important compounds to the inside of cells remains a key challenge.

Over many years a range of differing techniques have been developed for transfection including direct methods, such as microinjection and gene guns (using gold particle ballistics), through to electroporation, lipid/chemical agents, and viral vectors. Optical methods have now emerged that offer an alternative [1-3], and here we demonstrate the power of combining the Elliot system with a CW infra-red light to trap a cell and simultaneously use a femtosecond laser to porate it.

To prepare samples, HL-60 cells were grown under standard culture conditions (37 °C, 5% CO_2), trypsinised and suspended in culture medium (RPMI supplemented with 10% Foetal Calf Serum and antibiotics (2 mM L-glutamine, 100 units penicillin and 0.0 2mg streptomycin per mL)). The trapping chamber was coated in Poly-HEMA (in ethanol, 20 mg/ml) to prevent the cells from sticking to the surfaces of the glass trapping chamber. The mode-locked femtosecond beam was co-aligned with the trapping laser, its optical pathway including an electronic shutter to control dosage (shutter open time) and provide a trigger mechanism to allow user-controlled firing of the poration beam. The poration beam power was 110 mW at the objective back aperture for all experiments.

Cavitation bubbles and cell movement were observed upon firing, confirming that the poration beam produced additional force on the cell, localised heating, and membrane compromise. Dosage times of 40-120 ms produced clear cavitation bubbles. After poration, the cells were seen to degrade rapidly, changing in morphology and also in their behaviour in relation to the optical trap (not trapping as well as before).

Although the cells were intentionally destroyed by the forceful puncturing of the cell membrane in these demonstrations, reducing the poration beam power and dosage time would allow self-healing pores to form instead. This is the principle behind photo-transfection, for the controlled introduction of exogenous compounds and DNA into selected cells [2]. Overall, this shows the potential of the Elliot AOD system not only to trap arrays of cells, but to perform nanosurgery upon them. Other studies to extend the work could include cell ablation or fusing.

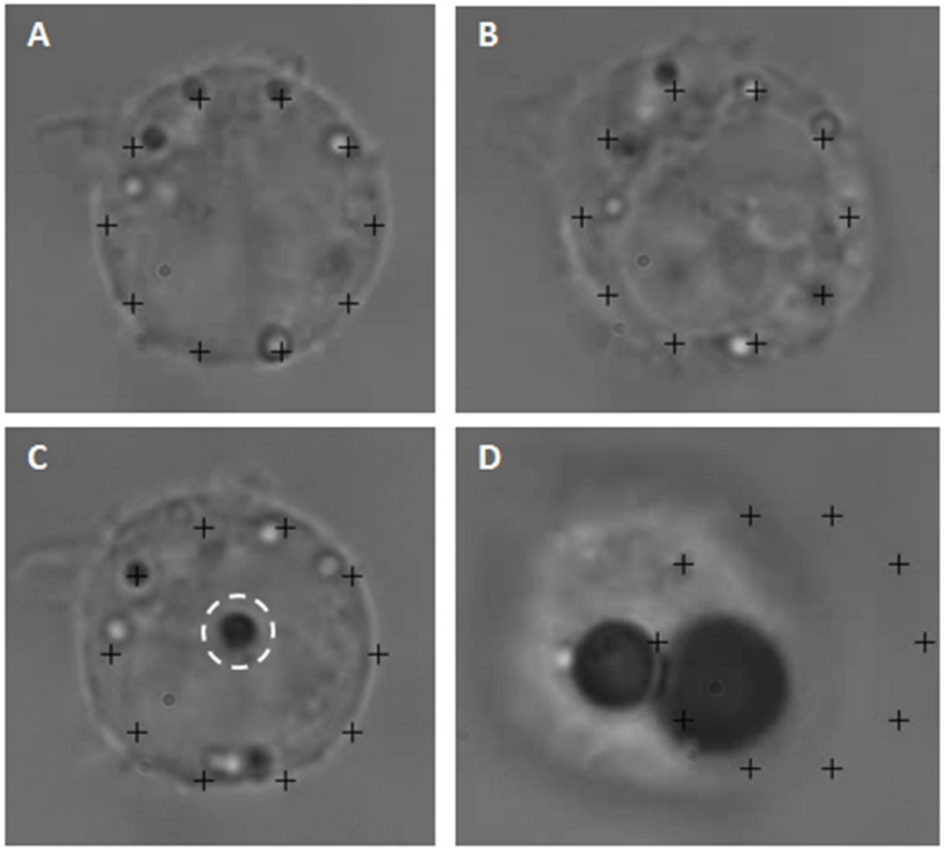

Figure 6: Poration of a trapped human leukaemia (HL-60) cell by a femtosecond laser co-aligned with the trapping beam. The cell is held in a circular array of 10 optical traps (trapping beam power = 52 mW at the objective back aperture). The poration beam (800 nm, 76 MHz, 200 fs pulses, 40 ms shutter speed, and 110 mW at the objective back aperture) is targeted near the centre of the cell. The cell is shown before (A), during (C,D) and after (B) poration. Before poration (A) the cell looks healthy, after poration (B) the membrane has become severely compromised, the cell has increased in volume, and the internal structure has been changed significantly. During poration cavitation bubbles can be observed (C, inside white circle, and D, large black bubbles) and the cell experiences a force which momentarily displaces it relative to the trapping array. The accompanying videos are:

- HL60 Optoinjection - 1
- HL60 Optoinjection - 2

[1] U. Tirlapur and K. Konig, "Targeted transfection by femtosecond laser," *Nature* 418 290 (2002)
[2] D. J. Stevenson, F. J. Gunn-Moore, P. Campbell, and K. Dholakia, "Single cell optical transfection," *Journal Of The Royal Society Interface* 7, 863--871 (2010)
[3] C. T. A. Brown, D. J. Stevenson, X. Tsampoula, C. McDougall, A. A. Lagatsky, W. Sibbett, F. J. Gunn-Moore, and K. Dholakia, "Enhanced operation of femtosecond lasers and applications in cell transfection," *Journal Of Biophotonics* 1, 183--199 (2008)

This case study was performed at the University of St. Andrews using a standard Elliot Scientific E3500 AOD Optical Tweezers system.

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