# Whole-cell manipulation by optical trapping


The contact-free, non-invasive manipulation provided by optical trapping enables us not only to measure physical parameters of individual cells but also to initiate specific responses in a given cell in a defined environment.

Optical trapping is by now a familiar tool for manipulating biological macromolecules, cellular organelles and living cells under the microscope. Among the various techniques that come under the heading 'optical trapping' are optical tweezers, which have become indispensable for nanometer-scale measurements of motor molecules and for analysis of the mechanical properties of DNA and microtubules. Compared with such measurements *in vitro*, however, the use of optical trapping to study whole cells is still preliminary and scattered, although the first reported biological application of optical trapping used living motile bacteria [1]. This article focuses on recent developments in optical manipulation, with emphasis on its technical aspects, and discusses future directions.

### Trapping schemes

Three optical-trapping schemes for whole-cell manipulation are presented in Figure 1. The first scheme (Fig. 1a) shows transport trapping, in which cells are trapped in a parallel laser beam and moved in the beam-propagating direction. The driving force is derived directly from radiation pressure: photon momentum is transferred to cells as a result of the reflection or scattering of light when it hits the cells. This transport scheme is convenient for cell sorting, because cells are easily switched from one transporting beam to another at the intersection point between the two. A robotic cell-sorter capable of classifying individual cells according to their light-scattering intensity was reported by Buican *et al.* [2].

The second scheme (Fig. 1b) is trapping by optical tweezers. In this case, a cell is held stationary at a particular point. The gradient force, which holds the target cell transversely and axially near the focus of a converging beam, is again produced by radiation pressure, but in this case it is generated mainly by refraction of the light that is incident to the target at large angles. For this reason, a lens with a high numerical aperture is needed. A 'tweezers trap' can pick up and manipulate a cell by steering the laser beam such that it holds onto a small organelle inside the cell [1], or an attached bead on the cell surface [3]. The bead facilitates the use of stronger trapping forces with lower optical powers and reduces the optical flux incident on the cell.

Levitation trapping is the third scheme (Fig. 1c). This method is suitable for experimental configurations that require long working distances, in which case it is not possible to use a lens with a high numerical aperture. To compensate for the loss of the trapping force needed for lifting a large cell, a second laser beam is introduced [4]. As shown in Figure 1c, this scheme uses a combination of transport trapping and tweezers trapping. A target cell is positioned axially to balance the pushing forces due to the downward and upward beams, and it is stabilized transversely with a tweezers force generated by the downward beam which is focused more sharply. The cell can be placed in a desired position by moving the translational stage of the microscope.

The trapping force applied to a cell is usually calibrated by measuring the flow velocity at which the cell escapes from the trap. Stokes' drag law relates the velocity and the force at this limit, and forces that are applied to cells in actual biological situations are thus estimated. With a Nd:YAG (neodynium in yttrium aluminium garnet crystal) laser emitting an infrared beam at 1.06 μm — which is the laser most frequently used for optical trapping because of its low cellular absorbance — the actual force generated per 1 mW laser power is typically 0.5 pN. In practice, maximum trapping forces are usually limited not by the laser power but by the extent of the optical hazard resulting from laser irradiation. Assessment of cell viability by subsequent cell division and mitochondrial activity shows that laser powers up to 150 mW can be accommodated without serious damage to the cells, although the long-term effects of laser irradiation are unknown.

### Applications

The freedom that optical techniques provide for the three-dimensional manipulation of single cells, and for selecting between experimental configurations, means that they are widely versatile in their application, despite the limitations described above. As mentioned above, sorting is one promising application for optical cell manipulation; the isolation of yeast cells and their subsequent reculture has been performed using tweezers trapping [5]. The optical method seemed to be effective in culturing pure yeast cells of interest — for example, cells that are all in the same growth phase, or cells with mutant characteristics, as judged by morphological criteria.

Another interesting example is provided by the application of optical methods to cell-density measurement [6]. Sample cells were levitated and then released by extinguishing the trapping laser beam, which made them start falling. The mean density of the cells was evaluated from their falling velocity by applying Stokes' law. The fact that the densities of outer hair cells from the guinea pig

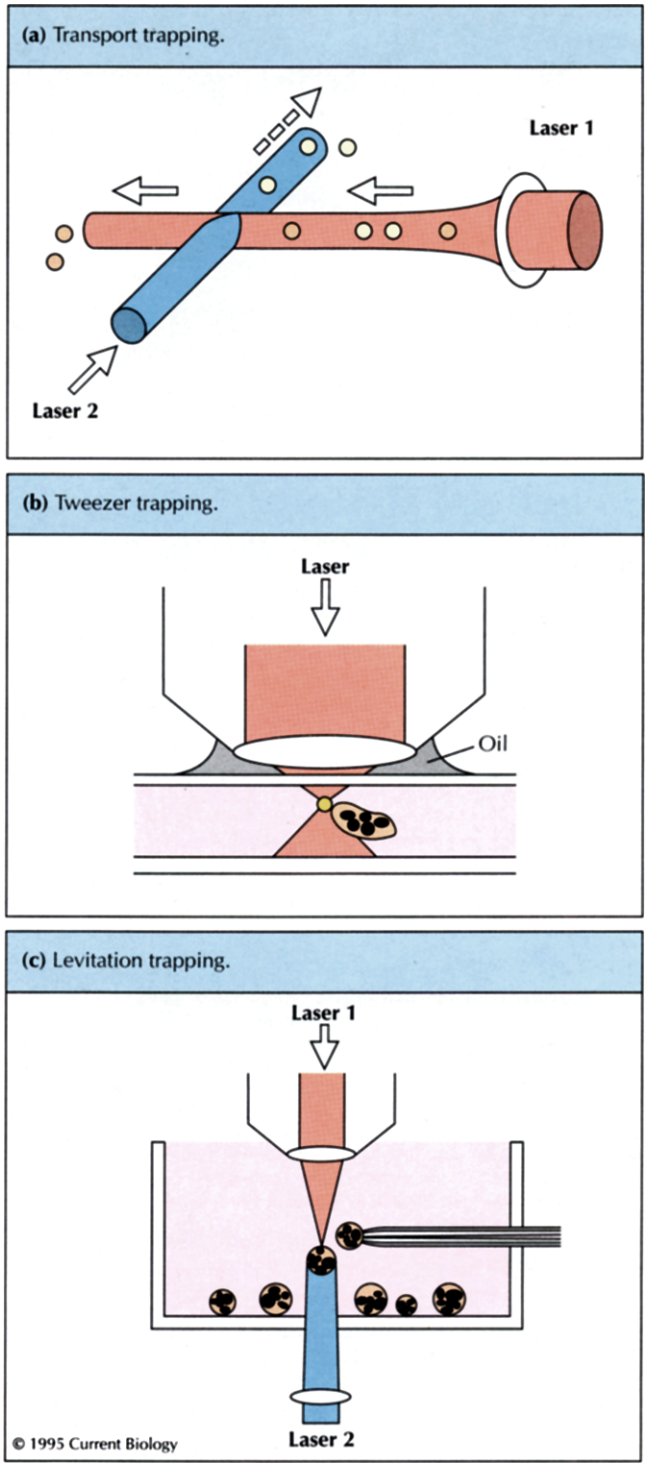


**Fig. 1.** Optical-trapping schemes for whole-cell manipulation. **(a)** Cells floating in medium are pushed along the column of a laser beam. The direction of the transporting beam can be changed by turning on an additional laser beam when cells of interest (yellow) come to the crossing of the beams. **(b)** A lens with a high numerical aperture can produce a gradient force with which to trap a microsphere or a small cell near the focusing point of a single laser beam. Three-dimensional manipulation of a target can be achieved in medium in a sealed-off container made of coverslips. **(c)** A target cell is trapped using two counter-propagating beams. The use of a long-working-distance water-immersed lens, in combination with a micropipette and/or a microelectrode, allows wide-range manipulation of larger cells in three-dimensional space.

cochlea were quasi-constant, despite variations in cell length, indicates that their density may not play a major role in the intrinsic tuning mechanism of the cochlea. The forces generated by bacteria [7] or spermatozoa [8] have also been evaluated using optical methods, by holding individual cells in a tweezers trap and then reducing the optical power until they escaped. Thus, it was clearly demonstrated that human spermatozoa produce three times greater forces upon exposure to the cumulus mass of the oocyte than beforehand.

The enhanced stability of the bead-assisted method of holding a cell against flowing medium has been used effectively to study the membrane skeleton [3]. The rapid replacement of medium by fresh flowing medium is needed in preparations of fresh red blood cells, in order to dissolve the membrane lipid and leave behind a cell ghost. Using bead-assisted trapping, the elasticity of the membrane skeleton of human red blood cells was observed by high-resolution microscopy during and after extraction with non-ionic agents. It was shown that freshly extracted membrane skeletons were highly flexible, but that they underwent salt-dependent and temperature-dependent changes within minutes of extraction. Seeger *et al.* [9] have used the precision provided by optical cell manipulation to initiate specific contact between a human natural killer cell and an erythroleukemia cell. Real-time observations of the cell killing process, especially of the blebbing observed in the attacked cell, could thus be recorded on a time scale of a hundred seconds.

We have used a levitation trapping scheme to study cell adhesion [4,10]. By making a pair of cells contact each other at the tip of a micropipette, as shown in Figure 2, we quantitatively evaluated the characteristics of adhesion — the adhesion probability and the adhesion strength — for different cell types. One cell was picked up from a dissociated population placed on the bottom of a container, and was lifted to the tip of a micropipette. A second cell was then manipulated to make a binary contact with the first, which was held by suction on the micropipette. Homotypic and heterotypic pairs of endodermal and ectodermal epithelial cells of *Hydra* were made in this way, and we found that homotypic cell pairs could adhere to each other within a certain period, whereas the heterotypic pairs could not.

The adhesion probability of the homotypic endodermal cell pairs was higher than that of the homotypic ectodermal cell pairs: the former could contact each other firmly in less than 30 seconds, whereas 60 % of the latter remained non-adherent even after 6 minutes of forced contact. The adhesive strength, measured as a separation force, was estimated to be as large as 30 pN for an ectodermal pair, but the cell pulled by optical trapping broke before separation in the endodermal pairs. The tissue-specific adhesivity difference that we measured could explain the microscopic cell–cell sorting process that occurs in random cell aggregates from *Hydra*; such aggregates are capable of regenerating into a complete organism.

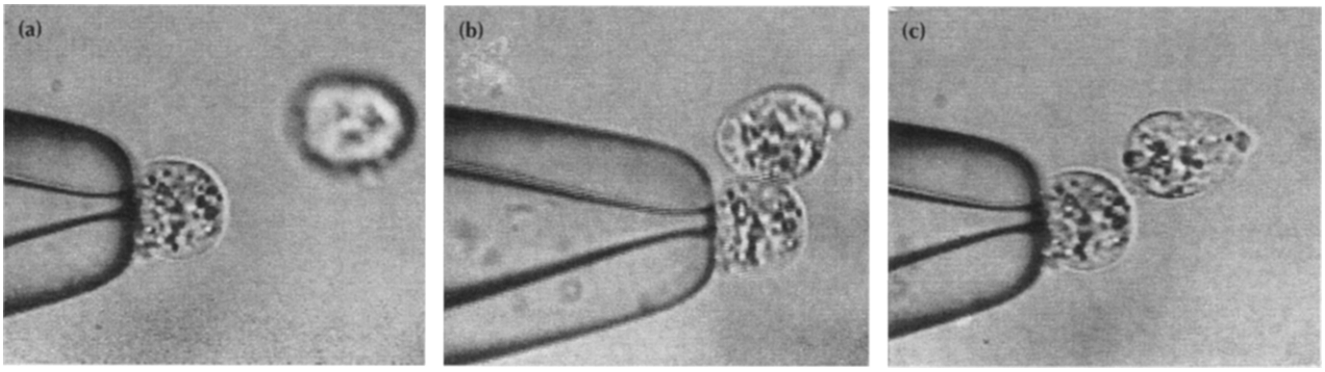


**Fig. 2.** Formation and separation of a cell pair at the tip of a micropipette by means of optical trapping. **(a)** A first cell has been transported onto the tip of the micropipette. A second cell to be trapped is seen slightly out of focus. **(b)** The second manipulated cell has adhered to the first, so it does not fall when the laser beam is extinguished. **(c)** The second adhered cell is pulled away from the first and so is slightly deformed.

What are the future goals for optical cell manipulation? Cell-cell interaction and cell–cell communication will certainly be important fields for the application of optical methods. The characterization and measurement of the adhesive forces between cells, or within a cell sheet, will help directly in understanding the micro-processes that occur during development and cell sorting. Unfortunately, however, the physical parameters obtained using these techniques usually do not directly reflect cellular signalling process, so additional information will be required in order to evaluate their physiological significance.

A micropipette, which can serve as a microelectrode or an injector of sensitive dyes as well as a way to hold cells, seems to be an attractive device for use in such combined analyses; differential interference contrast or laser confocal microscopy will also be useful. The fusion of contacting cells, triggered by irradiation with an ultraviolet laser [11], is another promising field of study. The use of optical trapping techniques for such purposes as the selection and transport of cells will be helpful in the collection of fused cells for culture. The accumulation of successful applications of these optical techniques will doubtless lead us further to novel applications in cell manipulation, as well as to new developments in the technique itself.

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