Holding with Invisible Light: Optical Trapping of Small and Large Colloidal Particles

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Early works on trapping and levitation of small objects by laser beams date back to the 1970s. Optical tweezers are now a widespread tool based on three-dimensional trapping by a single tightly focused laser beam (**Fig. 1a**). In general, the necessary condition for optical trapping of a particle is that the refractive index of the latter is higher than the one of the surrounding media. Due to the shape of the beam and the refraction from the surface of the particle, the bead is pushed towards the zone with higher intensity, i.e. the beam waist of the laser beam. Thus, using light one can manipulate particles without mechanically touching them. Even though they are difficult to work with because of being invisible for the human eye, infrared laser sources are preferred for the lower potential damage on biological samples.

The simplicity of laser tweezers stems from the fact that to construct a trap one just needs a single collimated beam, directed through a microscope objective with a very large aperture. The latter condition implies using short-working-distance objectives, which restrict optical manipulation to the high magnification end of the microscope nosepiece. Certain applications of optical trapping demand long-working distances at moderate magnification. This can be achieved using a two-beam trapping configuration where two counterpropagating laser beams are used (**Fig. 1b**).

Both single- and two-beam trappings have advantages and drawbacks. All of the limitations of the single-beam trap are consequences of the requirement of a very large aperture objective. (i) Such objectives are of immersion type and have extremely short-working distances: one is limited to working at distances not larger than about 10 µm above the chamber bottom. (ii) They are at the high magnification end (100x is standard) of the microscope nosepiece, providing a relatively narrow field of view. (iii) Large aperture means high resolution, which is profitable, but involves, at the same time, tight focusing and very high power density. The latter often causes heating and optical damage to the sample.

The two-beam geometry represents an opposite tradeoff. Beams are weakly focused by low aperture objectives, allowing for long working distances, low magnification and large field of view, and moderate intensities. Drawbacks are (i) a definitely higher complexity of the optical setup, which needs shaping, aligning, and precisely positioning a couple of counterpropagating beams; and (ii) the trapping geometry depends on the particle size.

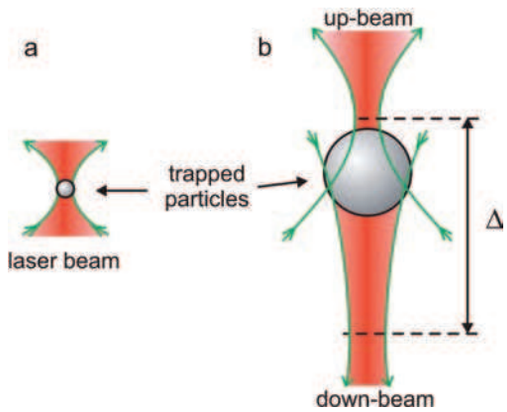

Fig. 1: A schematic illustration of single-beam (a) and double-beam optical trapping (b). In the first case, the laser beam is tightly focused by the objective and the particle is trapped at the beam waist position. In the case of a double-beam trap, two counterpropagating beams are used, up-going and down-going. Their beam waists are located above and below the bead, forming a trapping cage for the particle. The inter-focal distance Δ is set depending on the particle size.

The particle sizes, which one can trap with the two types of traps, also differ. The single-beam tweezers are usually applied to manipulation of particles with diameters between about 0.5 and 5 micrometers. The lower range is set by the limitation from the optical detection of the manipulated particle. Some enhanced detection systems (for example, quadrant photo diodes, which follow the beam deflection from the trapped particles) can reduce this limit. The upper range of particle sizes is set by the diameter of the beam waist, which, in turn is fixed and depends on the objective characteristics. Thus, particles much larger than the beam waist cannot be suitably trapped. With the two-beam trap, one can easily manipulate large particles of tens of microns in size. However, due to the objectives of low magnification, this configuration cannot be applied to particles smaller than about 2 micrometers.

While single-beam tweezers are commercially available, double-beam traps are found only as home-built setups. Being aware of the advantages of having both configurations, recently in our lab, we developed a complete setup, which combines single- and two-beam trapping **[1]**. Both functions were integrated into a commercial microscope (Zeiss Axiovert 200M), and are compatible with all observation modes of the microscope (phase contrast, differential interference contrast, fluorescent microscopy). The system is fed by a continuous wave

Nd:YAG laser with wavelength 1064 nm. We evaluated the performance of the setup in both trapping modes with latex particles, either fluorescent or not, of different sizes, in the 1–20 µm range. In addition, the trapping ability for manipulating oil droplets and polymer capsules (the latter were provided by the Interface department) was also tested; see **Fig. 2**. Both single-beam and double-beam configuration can be used in the case of capsule manipulation. Because the capsules are much larger than the beam waist, in the single-beam configuration the laser beam is focused on a point located at the shell of the capsule where the force is applied. With the double-beam trap, one can capture the complete capsule in the trapping cage.

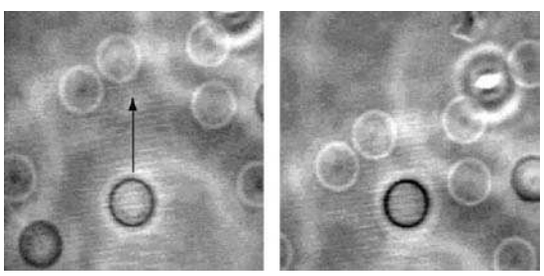

Fig. 2: Demonstration for trapping a polyelectrolyte capsule (phase contrast microscopy). In the setup, the laser beam is immobile and the sample stage is displaced. We trapped a single capsule, levitated it from the bottom of the observation chamber so that the rest of the capsules is out of focus (first snapshot) and displaced the sample stage. In this way, the particle was moved relative to the surrounding solution of capsules (compare with the background in the second snapshot). The direction of the relative displacement is indicated with an arrow in the first snapshot. The capsule diameter is approximately 6 micrometers.

Currently, the setup is used for the manipulation of micron beads with molecular motors attached to them (PhD project of Janina Beeg). The question we attempt to tackle concerns the collective transport of molecular motors. A considerable amount of studies have addressed the transport properties of single motor proteins. But the collective transport performed by several motors, as in the context of transport in cells, has not been studied in detail. As molecular motor we use kinesin, which walks on microtubule tracks. A micron-sized particle with certain kinesin coverage is trapped with the laser tweezers (single-beam mode) and brought to a selected microtubule; see **Fig. 3**. Only a certain fraction of the motors are involved in the bead displacement. The transport properties like walking distance, binding rate and escape force are characterized.

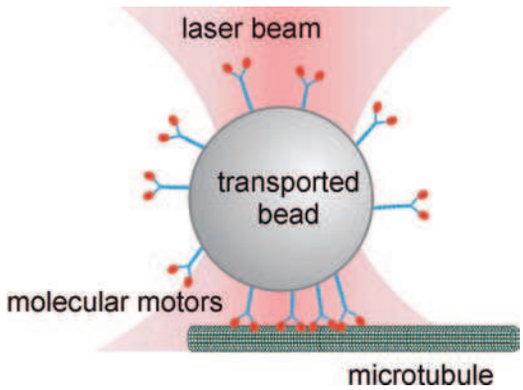

Fig. 3: A schematic illustration of the transport of a bead by several kinesin motors along a microtubule. The particle coverage with motors can be varied depending on the preparation conditions. The bead is trapped by optical tweezers and positioned at a microtubule. If released from the trap, it walks away being pulled by several motors. Switching on the trap again can apply a force in the picoNewton range which is enough to stop the processing bead.

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References:

[1] Kraikivski, P., Pouligny, B. and Dimova, R.: Implementing both short- and long-working-distance optical trapping into a commercial microscope, Rev. Sci. Instrum. **77**, 113703 (2006).
[2] Beeg, J., Klumpp, S., Dimova, R., Gracia, R. S., Unger, E. and Lipowsky, R.: Transport of beads by several kinesin motors, submitted.