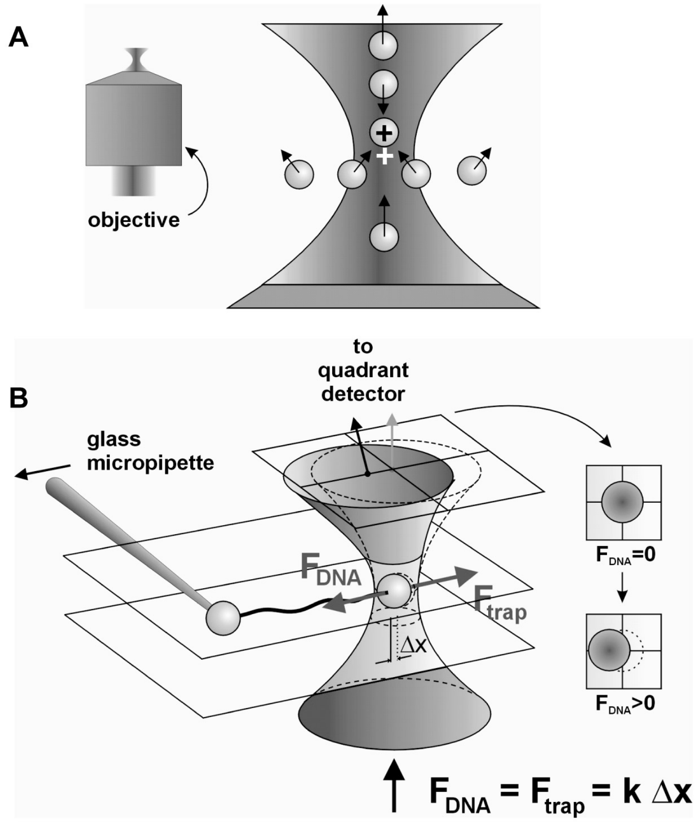

FIGURE 1. Schematic of operation principles of optical tweezers (OT). (*A*) Using a high-numerical-aperture objective lens, a parallel beam of light can be focused to a small spot with a minimum waist diameter of ~500 µm. This minimum is determined by the diffraction limit. Within the tightly focused beam configuration, transparent micron-sized polystyrene beads experience forces that differ in magnitude and direction, depending on their location with respect to the focal point (*white cross*). Just below the focal point (with respect to the direction of the beam) is a position where the net force exerted on the bead by the light is zero. This is the optical trap position (*black cross*). Displacement of the bead from this position will result in a net restoring force pointing to the trap position, effectively establishing a stable trap for the bead. (*B*) For measuring forces on an individual DNA molecule, its ends are attached to two 2.6-µm beads using biotin–streptavidin coupling. The displacement (Δx) of the bead within the trap, caused by applying a stretching force on the DNA tether, is proportional to this force, and the proportionality factor is referred to as the spring constant, *k*, or "stiffness" of the optical trap, indicating its "strength." Projecting the beam that transmits through the bead onto a quadrant detector allows accurate measurement of the bead displacement (Δx), and therefore the force exerted on the bead by the light (F_{trap}). The force acting on the DNA molecule (F_{DNA}) is identical to the force exerted by the optical trap (F_{trap}) but pointed into the opposite direction, such that the net force exerted on the bead is zero.