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Optical trap loading of dielectric microparticles in air

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Abstract:	We demonstrate a method to trap a selected dielectric microparticle in air using radiation pressure from a single-beam gradient optical trap. Randomly scattered dielectric microparticles adhered to a glass substrate are momentarily detached using ultrasonic vibrations generated by a piezoelectric transducer (PZT). Then, the optical beam focused on a selected particle lifts it up to the optical trap while the vibrationally excited microparticles fall back to the substrate. A particle may be trapped at the nominal focus of the trapping beam or at a position above the focus (which we refer to as the levitation position) where gravity provides the restoring force. After the measurement, the trapped particle can be placed at a desired position on the substrate in a controlled manner. In this protocol, an experimental procedure of selective optical trap loading in air is outlined. First, the experimental setup is briefly introduced. Second, the design and fabrication of a PZT holder and a sample enclosure are illustrated in detail. The optical trap loading of a selected microparticle is then demonstrated with step-by-step instructions including sample preparation, launching into the trap, and use of electrostatic force to excite particle motion in the trap and measure charge. Finally, we present recorded particle trajectories of Brownian and ballistic motions of a trapped microparticle in air. These trajectories can be used to measure stiffness or to verify optical alignment through time domain and frequency domain analysis. Selective trap loading enables optical tweezers to track a particle and its changes over repeated trap loadings in a reversible manner, thereby enabling studies of particle surface interaction.
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TITLE:
Optical trap loading of dielectric microparticles in air

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SHORT ABSTRACT:
A protocol for launching and stably trapping selected dielectric microparticles in air is presented.

LONG ABSTRACT:
We demonstrate a method to trap a selected dielectric microparticle in air using radiation pressure from a single-beam gradient optical trap. Randomly scattered dielectric microparticles adhered to a glass substrate are momentarily detached using ultrasonic vibrations generated by a piezoelectric transducer (PZT). Then, the optical beam focused on a selected particle lifts it up to the optical trap while the vibrationally excited microparticles fall back to the substrate. A particle may be trapped at the nominal focus of the trapping beam or at a position above the focus (referred to here as the levitation position) where gravity provides the restoring force. After the measurement, the trapped particle can be placed at a desired position on the substrate in a controlled manner.

In this protocol, an experimental procedure for selective optical trap loading in air is outlined. First, the experimental setup is briefly introduced. Second, the design and fabrication of a PZT holder and a sample enclosure are illustrated in detail. The optical trap loading of a selected microparticle is then demonstrated with step-by-step instructions including sample preparation, launching into the trap, and use of electrostatic force to excite particle motion in the trap and measure charge. Finally, we present recorded particle trajectories of Brownian and ballistic motions of a trapped microparticle in air. These trajectories can be used to measure stiffness or to verify optical alignment through time domain and frequency domain analysis. Selective trap

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INTRODUCTION:

Ashkin reported the acceleration and trapping of microparticles by radiation pressure in 1970.[1] His novel achievement promoted the development of optical trapping techniques as a primary tool for fundamental studies of physics and biophysics.[2–5] To date, the application of optical trapping has focused mainly on liquid environments, and been used to study a very wide range of systems, from the behavior of colloids to the mechanical properties of single biomolecules.[6–8] Application of optical trapping to gaseous media, however, requires resolving several new technical issues.

Recently, optical trapping in air/vacuum has been increasingly applied in fundamental research. Since optical levitation potentially provides nearly-complete isolation of a system from the surrounding environment, the optically levitated particle becomes an ideal laboratory for studying quantum ground states in small objects,[4] measuring high-frequency gravitational waves,[9] and searching for fractional charge.[10] Moreover, the low viscosity of air/vacuum allows one to use inertia to measure the instantaneous velocity of a Brownian particle[11] and to create ballistic motion over a wide range of motion beyond the linear spring-like regime.[12] Therefore, detailed technical information and practices for optical traps in gaseous media have become more valuable to the broader research community.

New experimental techniques are required to load nano/microparticles into optical traps in gaseous media. A piezoelectric transducer (PZT), a device that converts electric energy into mechano-acoustic energy, has been used to deliver small particles into optical traps in air/vacuum[5,12] since the first demonstration of optical levitation.[1] Since then, several loading techniques have been proposed to load smaller particles using volatile aerosols generated by a commercial nebulizer[13] or an acoustic wave generator[14]. The floating aerosols with solid inclusions (particles) randomly pass near the focus and are trapped by chance. Once the aerosol is trapped, the solvent evaporates out and the particle remains in the optical trap. However, these methods are not well suited to identify desired particles from within a sample, load a selected particle and to track its changes if released from the trap. This protocol is intended to provide details to new practitioners on selective optical trap loading in air, including the experimental setup, fabrication of a PZT holder and sample enclosure, trap loading, and data acquisition associated with the analysis of particle motion in both the frequency and time domains. Protocols for trapping in liquid media have also been published.[15,16]

The overall experimental setup is developed on a commercial inverted optical microscope. **Figure 1** shows a schematic diagram of the setup used to demonstrate steps of the selective optical trap loading: freeing the resting microparticles, lifting the chosen particle with the focused beam, measuring its motion, and placing it onto the substrate again. First, translational stages (transverse and vertical) are used to bring a selected microparticle on the substrate to the focus of a trapping laser (wavelength 1064 nm) focused by an objective lens (near-infrared corrected long-working distance objective: NA 0.4, magnification 20X, working distance 20 mm) through the transparent substrate. Then, a piezoelectric launcher (a mechanically pre-loaded ring-type PZT) generates ultrasonic vibrations to break the adhesion between microparticles and a substrate. Thus, any freed particle can be lifted by the single-beam gradient laser trap focused on the selected particle. Once the particle is trapped, it is translated to the center of the sample enclosure containing two parallel conducting plates for electrostatic excitation. Finally, a data acquisition (DAQ) system simultaneously records the particle motion, captured by a quadrant-cell photodetector (QPD), and the applied electric field. After finishing the measurement, the particle is controllably placed onto the substrate so that it can be trapped again in a reversible manner. This overall process can be repeated hundreds of times without particle loss to measure changes such as contact electrification occurring over several trapping cycles. Please refer to our recent article for details.[12]

PROTOCOL:

Caution: Please consult all relevant safety programs before the experiment. All the experimental procedures described in this protocol are performed in accordance with the NIST LASER safety program as well as other applicable regulations. Please be sure to select and wear proper personal protective equipment (PPE) such as laser protection glasses designed for the specific wavelength and power. Handling dry nano/microparticles may require additional respiratory protection.

1. **Design and fabrication of a PZT holder and a sample enclosure**

1.1. **Design a PZT holder and a sample enclosure**

Note: Particular design values vary depending on the selection of a PZT.

1.1.1. Open the computer-aided design (CAD) software package. Draw a two-dimensional (2D) sketch of a holder for a given PZT dimension. Develop the 2D sketch to volumetric features using combinations of Extrude/Extrude-cut.

1.1.2. Click Sketch, draw a rectangle and extrude it to make a rectangular cube.

1.1.3. Sketch a disk on the top surface of the cube to define a circularly recessed feature to cover and hold the ring-type PZT.

1.1.4. Define a central hole to have an optical access for both real-time imaging and trapping.

1.1.5. Define a circular guide along the rim of the central hole to insert a flat metallic (copper) ring to concentrate the ultrasonic power toward the center area as shown in **Figure 2** (a).

1.1.6. Create two bore holes for M6 screws on the PZT holder to be assembled with a bottom plate (purchased, 4 mm thick bottom aluminum plate with a hole in the center), as shown in Figure 2 (c) and (d).

1.1.7. In a similar manner, design a rectangular frame of the sample enclosure. Click Sketch, and draw a rectangle, extrude the rectangle to make it a rectangular box.

1.1.8. Draw a smaller rectangle on the top surface of the rectangular box and extrude-cut the rectangle to make it as a rectangular tube.

1.1.9. Draw a smaller rectangle on the side wall of the tube and Extrude-cut to transform it into the frame of sample enclosure box.

1.1.10. Convert these three-dimensional (3D) models into a stereolithography (STL) file format for a 3D printing process (Figure 2 (b)).

1.2. **3D printing of the designed objects**

1.2.1. Open the design file ("-.STL") from the 3D printer operating software. Lay the object flat 0/.and center the object on (0, 0, 0) by clicking the object to select it and using the alignment functions: "Move", "On Platform", and "Center". Orient the PZT holder to face the delicate features upward. The recessed surface will be faced upward.

1.2.2. In the menu go to the "Settings" and the "Quality" tab. Set the printing values as following, Infill: 100%, Number of shells: 2, and Layer height: 0.2 mm.

1.2.3. Preview the objects to check the total print time and make sure the layered objects will be printed as desired. Export the 3D print file in a ".x3g" format and save it to use in the 3D printer.

1.2.4. Turn on the 3D printer and warm it up until the temperature of the extrusion nozzle reaches an operating temperature, 230 °C. Load the design file from a memory card or network drive.

1.2.5. During the warm up, place the Build platform with blue painter's tape to help objects adhere securely. As a thermoplastic material for the printing job, use a polylactic acid (PLA) filament for both objects.

1.2.6. Print the designed objects. Once the printing job is finished, turn off the printer after it has cooled down.

1.2.7. Detach the printed object from the platform using a chisel. Straighten up the printed objects. If the orientation is appropriately chosen, the PZT holder can be directly used without further post-processing.

1.2.8. For the sample enclosure, prepare one pair of indium tin oxide (ITO) coated coverslips and three glass coverslips to cover the frame. Use a diamond cutter to fit the coverslip to the enclosure.

1.2.9. Wire the two parallel conducting plates using a fast drying silver paint to supply voltage across two plates. Glue these five windows onto the sample enclosure using an instant adhesive glue.

Note: The one pair of ITO coated coverslips are installed on the sample enclosure in parallel (facing each other) to provide uniform electric field and to generate ballistic motion of the naturally charged particle along the electric field. The three conventional coverslip cover the rest of sample enclosure surfaces (top and two other sides) to protect the trapped particle from the external flow of air

2. Optical trap loading of a selected microparticle

2.1. Sample preparation

2.1.1. Store the microparticles in an evacuated desiccator to reduce contact with moisture in the air before the experiment.

2.1.2. Pour out a small portion of microparticles onto a glass slide and immediately put the manufacture's bottle back in the desiccator.

2.1.3. Pick up some of the microparticles with a glass capillary tube. Scatter the particles over the substrate by gently tapping on the capillary while holding the capillary over the coverslip.

2.1.4. Verify the quantity and distribution of deposited particles on the substrate using a dark-field microscope.

Note: In the sample preparation step, the particle is just scattered on a coverslip and imaged with an optical microscope to verify overall arrangement before inserting them (a coverslip with scattered microparticles) between the PZT and PZT holder. Since the surface adhesion is strong enough to hold individual microparticles on the substrate, the adhered particles are firmly fixed unless significant external force is applied.

2.2. Piezoelectric launcher assembly

2.2.1. Obtain all the components of the piezoelectric launcher: the flat bottom plate, insulating film, the PZT, the glass coverslip, a copper ring, the PZT holder, two M6 screws, and the sample enclosure.

2.2.2. Apply a thin film (or tape) on the bottom plate to insulate the PZT. The glass coverslip isolates the top of the stack.

2.2.3. Assemble the stack by centering the PZT on top of the flat plate now insulated with tape, followed by the coverslip, the copper ring, and the PZT holder. Screw the stack together maintaining the centering of the PZT to avoid shorting the PZT to the holder if the holder is conducting as shown in Figure 2 (c) and (d). The copper ring provides an evenly distributed mechanical preload on the stack for plastic PZT holders.

2.2.4. Finally, glue the sample enclosure onto the stack and mount the assembly on an XYZ translational stage in the microscope.

2.3. Configuration of the PZT launcher

Note: Driving the PZT with a high voltage signal has potential electrical hazards. Please consult with safety personnel before the experiment. All the electrical connections should be secured before the experiment. Turn off the amplifier and disconnect PZT leads whenever possible.

2.3.1. Connect the PZT leads to the voltage amplifier and connect the function generator to an input port of the voltage amplifier.

2.3.2. Turn on the function generator and configure it to generate continuous square waves with an output voltage of 1 V. Do not generate the voltage signal until all the connections are verified and secured.

2.3.3. Turn on the voltage amplifier and generate the square wave of output voltage 1 V by enabling the output.

2.3.4. Connect the monitoring output port (output voltage 200 V) of the amplifier to an oscilloscope. Configure the amplifier to have gain of 200 V/V by turning the gain knob on the front panel. Verify that the monitoring output voltage has an amplitude of 1 V as measured by the oscilloscope.

2.3.5. Once the function generator and the amplifier are configured, find the resonant frequency of the PZT launcher by scanning the modulation frequency of the driving signal while the real-time video microscope images adhered particles. Repeat the scanning until the microparticle motion is a maximum. Use this frequency (64 kHz here) to release particles.

Note: The modulation frequency is manually changed (scanned) from zero to 150 kHz to find the resonant frequency.

2.3.6. Configure the function generator to generate a square wave with a specified number of cycles in burst mode. Press the “Burst” button on the front panel and select “N Cycle Burst”.

2.3.7. Choose the burst count by pressing “# Cycles” soft key and set the count to 10 or 20.

2.3.8. Configure the square waveform to generate voltage signals with an amplitude of 600 V (three times the voltage used for continuous excitation) at the resonant frequency of 64 kHz which has found from the previous step. Verify that the pulsing signal releases the target particle in a repeatable manner by ensuring particles move after each pulse.

2.4. Selective optical trap loading

Note: The PZT launcher assembly is installed on a manual linear translation xy stage. The particles can be translated relative to the fixed beam focus by moving the translational stage.

2.4.1. Remove the laser line filter to identify the focus of the trapping beam by rotating the microscope turret (**Figure 3** (a)). Move the motorized focusing block back and forth vertically around the best focus of the visible image to optimize focus.

2.4.2. Once the focus position is verified, put the filter back to give a clear real-time video without interference from the trapping beam.

2.4.3. Translate the sample to place a selected particle at the focus position of the trapping laser. Focus on the particle to image the center of a selected particle, which places the nominal trapping position below the particle center by about one half radius while leaving the levitation position above the particle.

2.4.4. Adjust the power supply connected to the electro-optic modulator (EOM) driver to set the optical trapping power. The optimal power depends on particle size and material. The optical power was found through repeated trials to determine the power sufficient to levitate the particle without ejecting it from the beam. Here, use an optical power of 140 mW at the back focal plane of the objective to trap the 20 µm diameter polystyrene (PS) particles.

2.4.5. After the center of the selected particle is aligned, actuate the piezoelectric launcher with several pulses. The change of the particle image from a static focused image to a moving blurred image indicates successful loading to the levitation position.

2.4.6. Translate the levitated particle vertically about a millimeter above the substrate by moving the objective lens up to prevent possible surface interactions. Then reduce the optical power to transition the levitated particle (Figure 3 (b)) into the nominal trapping position (Figure 3 (c)) which is more stable.

Note: The optical power of trapping laser can be modulated by an electro-optic modulator (EOM). The EOM regulates the output power with a bias voltage supplied through a digital power supply. One can observe the transition from the levitation to trapping position through the CCD while slowly reduces the optical power.

2.4.7. For the position measurement, as depicted in Figure 3 (c) to (d), carefully move the center of the PZT holder to the optical axis and then move the objective lens up (vertically) to translate the particle into the middle of sample enclosure (9 mm above the substrate) where the fringe electric field is minimized.

2.4.8. After performing the measurement as described below, place the particle on the substrate by moving the objective down until the particle touches the substrate. Since most of the particles are applied near the corners, the trapped particle can be easily recognized and re-trapped when it is placed in the central area. This enables reversible trap loading to measure changes occurring beyond a single trapping event such as contact interactions of the particle and substrate.

3. Data acquisition

3.1. Align the condenser and the focusing lens to maximize the QPD “SUM” signal with a particle in the trap.

3.2. Align the focusing lens to nominally zero the X and Y channels of the QPD, as shown in Figure 4 (c).

3.3. Repeat the adjustment of the condenser and the focusing lens until the Fourier transformed position signals (or power spectrum density (PSD) plots) of the X and Y channels superimpose to show balanced sensitivity. Properly aligned QPD signals (X and Y) show almost identical behavior, as shown in Figure 4 (b).

3.4. Once the QPD alignment is verified, connect the voltage amplifier to the two ITO plates. Connect the voltage monitoring output signal of the amplifier to the DAQ system to record the step excitation signal and the induced particle trajectory synchronously.

3.5. Supply a continuous square wave of 400 V to generate an electric field (Figure 4 (d)) that moves the particle transversely to the optical axis by about 500 nm (Figure 4 (e)). Measure the step response of the trapped particle using the QPD.

3.6. Average multiple periods as necessary to reduce the effects of Brownian motion. The induced motion can be used to measure the optical force over a wider range of motion than that of thermal fluctuations.[12,17] Figure 4 (d) and (e) shows averaged signals of applied voltage and the induced particle trajectory over 50 iterations of step excitation.

REPRESENTATIVE RESULTS:

The PZT launcher is designed using a CAD software package. Here, we use a simple sandwich structure for the preloading (a PZT clamped with two plates), as shown in Figure 2. The PZT holder and the sample enclosure can be fabricated from a variety of materials and methods. For a quick demonstration, we choose 3D printing with thermoplastic as illustrated in Figure 2 (d). Based on the fabricated components, optical trap loading is shown in Figure 3. For selective loading, the reflected trapping laser is blocked during the experiment by a filter installed on a microscope turret to protect the CCD camera while the visible light passes the filter for imaging in reflection as illustrated in Figure 1. A calibrated CCD camera also facilitates quantitative measurement by allowing measurement of the particle diameter and additional position detection. The diameter of a target particle can be used to calculate the mass which yields trap stiffness from the natural frequency, as discussed below. The trajectories measured using the CCD camera are also used to calibrate the QPD voltage signal for measuring the displacement.[12]

Once the particle is trapped, bright scattering from a red laser allows the trapped particle to be recognized with the naked eye, as shown in Figure 1 (inset photograph). Also, real-time images of the substrate can determine if the particle has been trapped since it is at a different height (focus) from the other microparticles adhered to the substrate (Figure 3). The microparticles can be trapped in two positions: a trapping position and a levitation position. In the trapping position, optical forces stabilize the particle in all directions. In contrast, in the levitation position the particle is only stabilized transversely by optical forces. In the vertical the upward force from radiation pressure is balanced by gravity. With our loading method, the selected particle is generally delivered to a levitation position. At the levitation position, the vertical location of the suspended particle is much more sensitive to variations in the optical power than at the trapping position near the focus.[18] One can vertically move the particle repeatably between these two stable positions by varying the optical power. The levitation position also has higher sensitivity to external forces than the nominal trapping position because the trap stiffness becomes softer as the light propagates away from the focus. Therefore, the levitation position can also be used for more sensitive measurements when displacement noise is not dominated by brownian motion. When the position noise is thermally limited as it is here, decreasing the stiffness increases both sensitivity and noise so there is no gain for precision measurement.

The motion of the trapped particle is monitored by a QPD and recorded by a DAQ board. The QPD signal is recorded in the time domain (Figure 4 (c)) and Fourier transformed (Figures 4 (a) and (b)). The overall alignment can be conveniently checked by comparing the power spectra of two radial channels (X and Y). If they are not superimposed (Figure 4 (a)), the optical alignment has to be corrected until superposition occurs (as shown in Figure 4(b)).

The particle trajectory shows both Brownian and ballistic motion as shown in Figure 4. Time and frequency domain analyses can be used to interpret these measurements. We have introduced two approaches to force measurement which allow more complete understanding of the optical trap by comparing Brownian motion to the Ballistic motion induced by an electrostatic force. The particle trajectory for Brownian motion under no electrostatic field is converted to the power spectral density which can then be analyzed by a nonlinear least square fit the solution of the full Langevin equation.[19] This analysis of the PSD yields the resonant frequency and damping near the trap center. The resonant frequency is converted to the trap stiffness using the known mass in the formula $\omega_o = \sqrt{k/m}$. The measured displacement then gives the optical force using the formula for a spring $F = -kx$.

The ballistic motion induced by a step change in the electrostatic field can also yield the resonant frequency of the trap and damping of the medium.[12] As we remove the electrostatic field from the trapped particle, the particle will be released to return to the field-free tapping position.as shown in Figure 4 (d) and (e). The displacement as function of time can be fit to the general solution of a damped harmonic oscillator to give the resonant frequency, damping, and steady-state displacement. Both of these approaches assume that the particle in the trap acts as a linear spring. These measurements can be extended to general (non-linear) forces using the parametric force method.[12] The details of the PSD analysis and parametric force analysis are not the focus in this protocol but they can be found from the literature.[12,19]

Figure 1: Schematics of the experimental setup used for selective optical trap loading in air.
A single-beam gradient force optical trap is developed on an inverted optical microscope. Abbreviations used in the schematic are listed below: EOM, electro-optic modulator; HAL, halogen illuminator; MFS, motorized focusing stage; NIR-LWD objective, infrared corrected long working distance objective lens; TS, translation stage (x−y); PZT, piezoelectric transducer; ESM, electrostatic field modulator; ND, neutral density filter; QPD, quadrant-cell photodetector; DM, dielectric mirror; ITO, indium tin oxide coated coverslips; CCD, charge coupled device camera; HeNe, helium neon laser (633 nm); Nd:YVO_4, 1064 nm laser for trapping.[12]

Figure 2: Fabrication of the piezoelectric launcher assembly.
(a) Rendered images of a PZT holder using CAD software package in a "-.SLDPRT" format and (b) "-.STL" format for 3D printing. (c) A rendered image of the final assembly of the piezoelectric launcher: sample enclosure (with ITO coated coverslips), PZT holder, ring spacer, ring-type PZT, aluminum plate, coverslips. (d) Picture of the final assembly.

Figure 3: Step by step demonstration of selective optical trap loading of a 20 µm PS particle.
(a) locating the focus of the trapping beam, (b) levitating the particle above focus (The particle image is a dim blur because the levitation position is well above the nominal microscope focus) , (c) transitioning into the trapping position (nominally in focus), and then (d) moving the trapped particle to the central area for data acquisition. The particle is trapped at a fixed location of the beam focus whereas the sample stage is moved as indicated with a yellow arrow in Figure 3 (d)

(Scale bar = 100 μm).

Figure 4: QPD captured particle trajectories both in frequency and time domain.
(a) A poorly aligned experimental setup shows low-frequency noise and noise peaks at specific frequencies whereas (b) well-matched PSDs of the x and y-axis indicate correct optical alignment. (c) A QPD records the Brownian motion of the trapped particle in the time domain. (e) A step change in applied electric field across the trapped particle is synchronously recorded with the induced (d) ballistic motion through the data acquisition (DAQ) system.

DISCUSSION:
The piezoelectric launcher is designed to optimize the dynamic performance of a selected PZT. Proper selection of PZT materials and management of ultrasonic vibrations are the key steps to yield a successful experiment. PZTs have different characteristics depending on the type of transducer (bulk or stacked) and component materials (hard or soft). A bulk type PZT made of a hard piezoelectric material is chosen for the following reasons. First, hard piezoelectric materials have lower dielectric losses and higher mechanical quality factor than soft materials. Second, the bulk type PZT represents a lower electrical load and is easier to drive at high frequencies than a stacked type transducer. Under dynamic operation, high amplitude oscillation can cause tensile forces on an unloaded PZT ceramic that result in mechanical failure. A mechanical preloading structure is used to provide a constant load to reduce backlash and enhance dynamic performance of the PZT. A metallic ring spacer is inserted between the PZT holder and the ring-type PZT. This metallic ring spacer concentrates the ultrasonic power and distributes it evenly around the ring (Any local (uneven) stress can easily break the coverslip.). With a well-designed PZT launcher, proper alignment of the particle to the trapping beam in both axial and radial directions determines the efficiency of trap loading. If the particle is not successfully levitated after pulsing, repeat the substrate alignment and move the focus a little below the particle to find the optical loading position. For the near-infrared corrected objective lens, the focus of the trapping beam is set to be a few micrometers below the sample plane that is focused onto the CCD. The optimal trapping power required to trap microparticles varies as the size of the target microparticle changes.[13] The optimal trapping power can be found empirically through trial and error. The power required here (140 mW) is relatively high due to the low NA and long working distance used.

Here we demonstrated reversible trap loading of a 20 μm PS particle. However, our approach can be extended to smaller particles. For smaller microparticles, our current PZT launcher may not able to provide enough ultrasonic power to detach the particles. Use of a faster PZT driving circuit has been shown to release smaller particles.[20] In addition, a low-adhesion surface can be an alternative approach.[21] Reduction of the adhesion between microparticles and the substrate will mitigate the minimum ultrasonic power required to detach the particle thus our current PZT launcher can also be used to detach smaller particles.

Most conventional loading techniques are random processes in which numerous aerosol droplets with solid inclusions are continuously generated until one of them is trapped by chance near the trap center. Thus this conventional technique may not be appropriate for trapping samples with a limited quantity or maintaining uniform sampling. In the protocol, we demonstrate reversible optical trap loading which includes repeated cycles of trap loading and landing. This enables unique experiments, for example the study of charge accumulation on the particle.[22] The charge on the trapped particle can be measured by fitting the transient response (Figure 4 (d)) to the ideal solution of harmonic oscillator in a nonlinear least square manner. The induced displacement multiplied by trap stiffness gives the electrostatic force which allows calculation of charge from the known electric field strength (given by the applied voltage divided by the distance between the two parallel ITO coated plates).[12] This simple charge measurement can be extended to study particle-surface interaction when combined with the reversible trap loading technique demonstrated here.[22]

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DISCLOSURES:
The authors declare no competing financial interest.

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Figure

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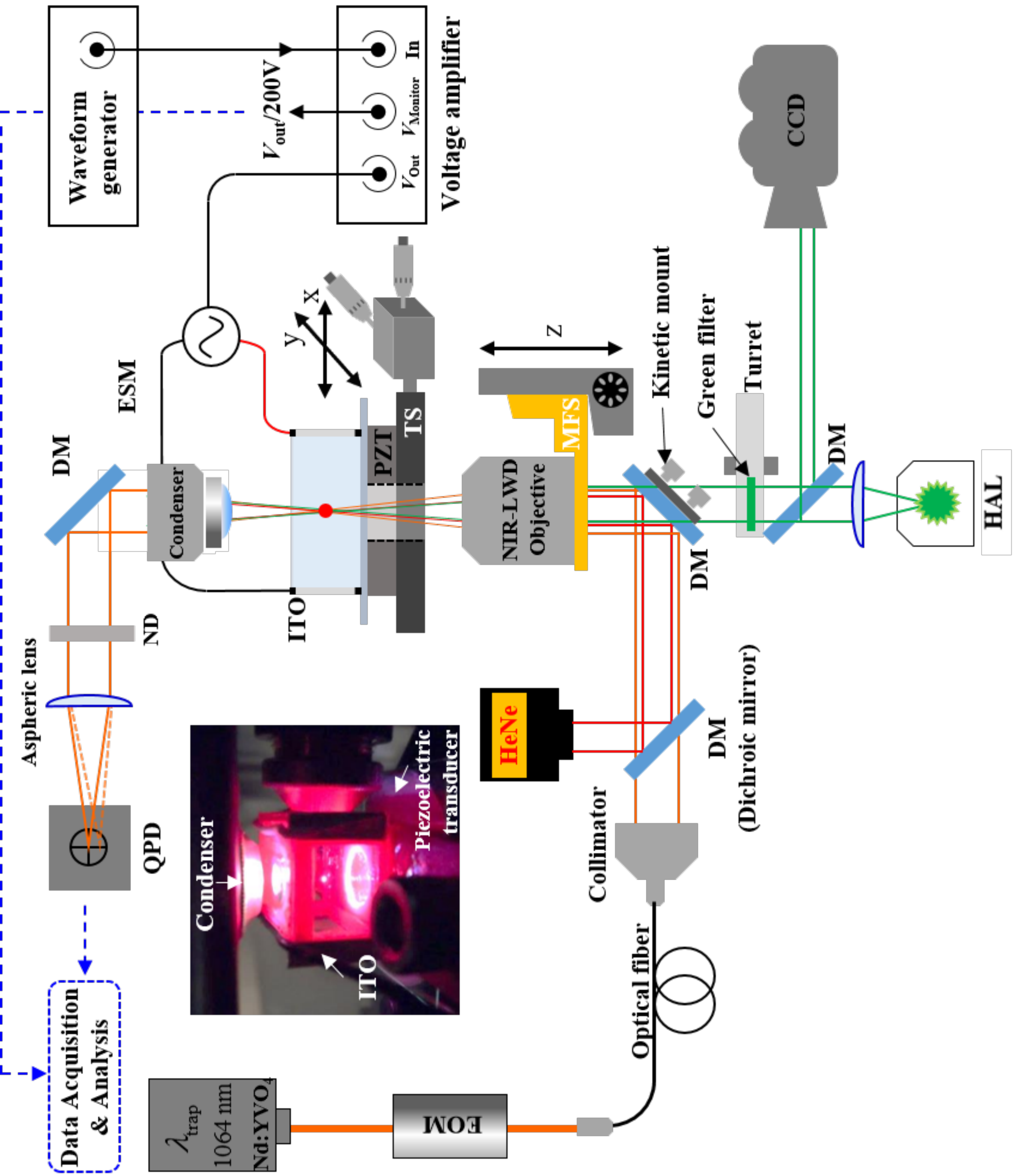
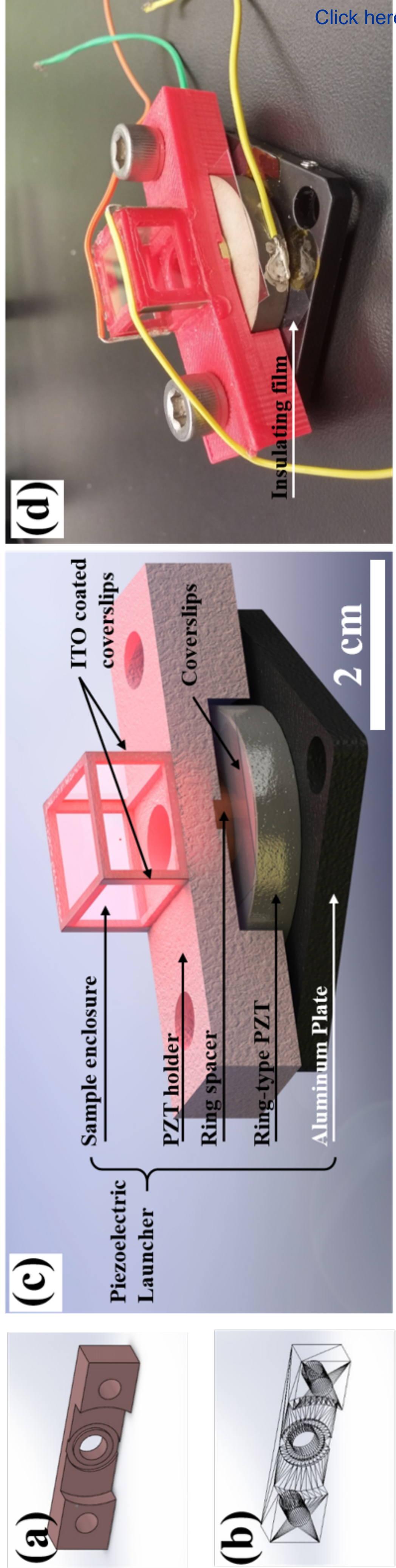
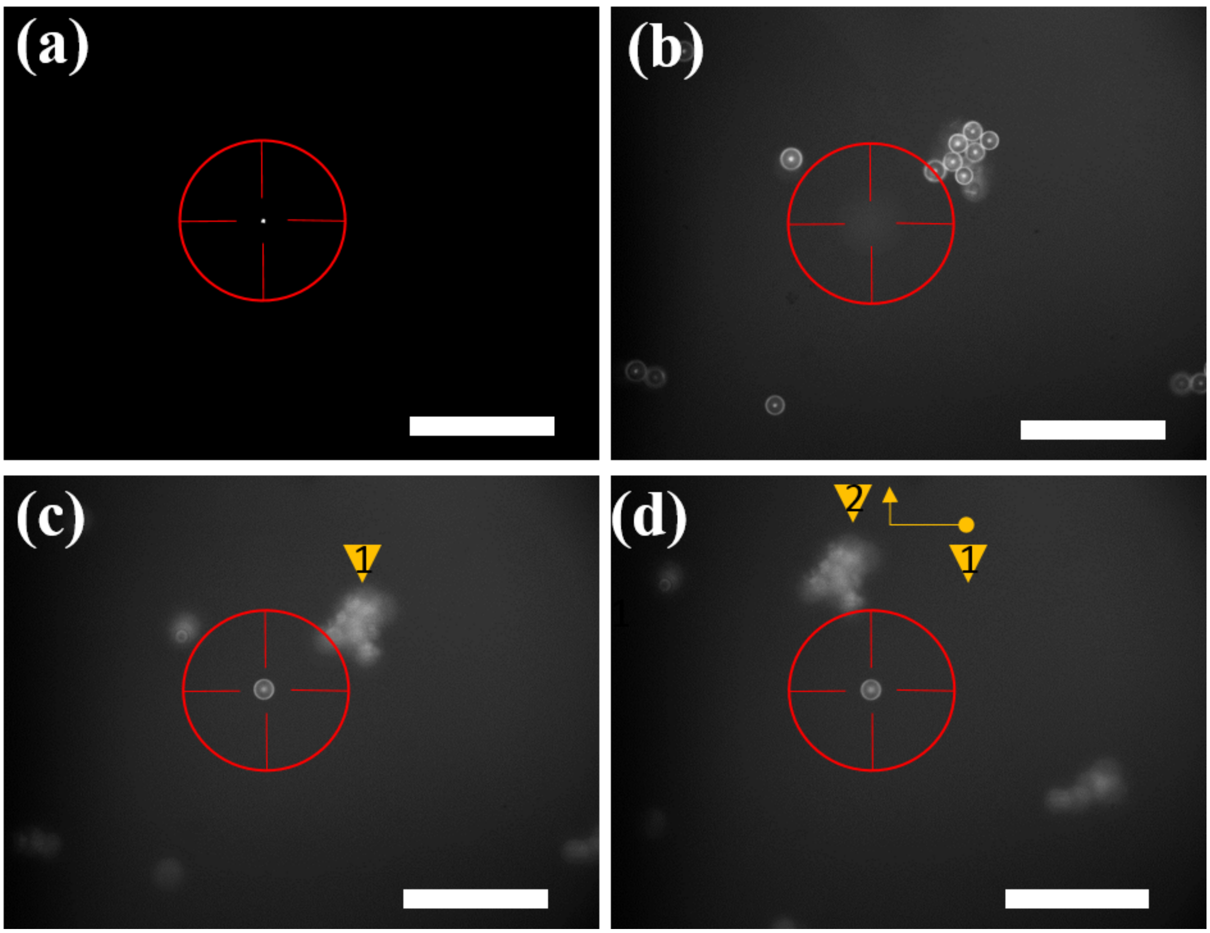



(a)
(b)
(c)
Piezoelectric Launcher
Sample enclosure
PZT holder
Ring spacer
Ring-type PZT
Aluminum Plate
ITO coated coverslips
Coverslips
2 cm
(d)
Insulating film


(a)
(b)
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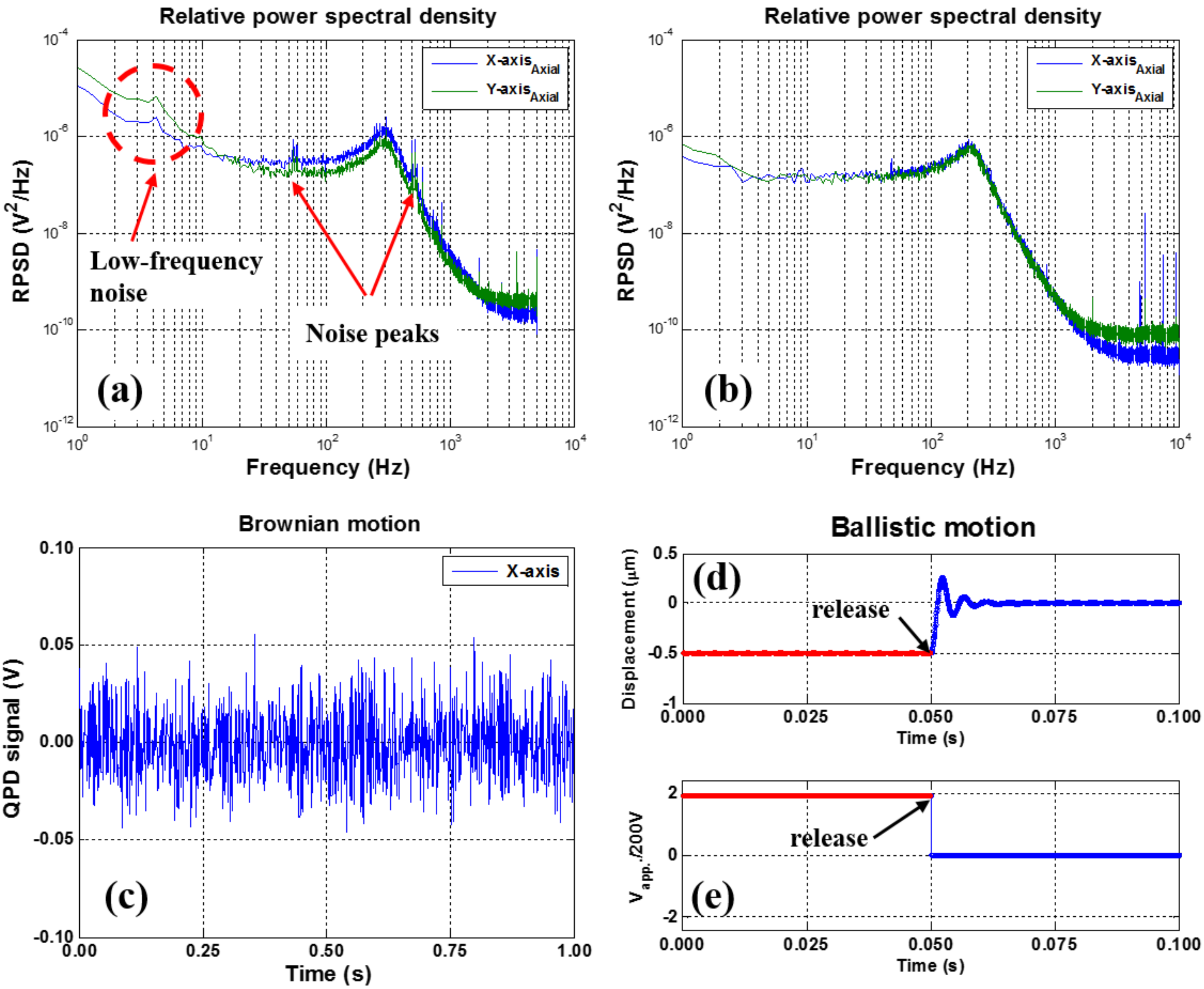
1
(d)
2
1


Relative power spectral density
RPSD (V²/Hz)
Frequency (Hz)
X-axis Axial
Y-axis Axial
Low-frequency noise
Noise peaks
(a)
(b)
Brownian motion
QPD signal (V)
Time (s)
X-axis
(c)
Ballistic motion
Displacement (μm)
release
(d)
V app./200V
(e)

Name of Material/ Equipment	Company	Catalog Number
ScotchBlue Painter's Tape Original	3M	3M2090
Scotch 810 Magic Tape	3M	3M810
Function/Arbitrary Waveform generator	Agilent	HP33250A
Power supply/Digital voltage supplier	Agilent	E3634A
Ring-type piezoelectric transducer	American Piezo Company	item91
Electro-optic modulator	Con-Optics	350–80-LA
Amplifier for Electro-optic modulator	Con-Optics	302RM
Mitutoyo NIR infinity Corrected Objective	Edmund optics	46-404
LOCTITE SUPER GLUE LONGNECK BOTTLE	Loctite	230992
3D printer	MakerBot	Replicator 2
Polylactic acid (PLA) filament	MakerBot	True Red PLA Small Spool
Data Acquisition system	National Instruments	780114-01
Quadrant-cell photodetector	Newport	2031
Translational stage	Newport	562-XYZ
Inverted optical microscope	Nikon Instruments	EclipsTE2000
Fluorescence filter (green)	Nikon Instruments	G-2B
Flea3/CCD camera	Point Grey	FL3-U3-13S2M-CS
Diode pumped neodymium yttrium vanadate($Nd:YVO_4$)	Spectra Physics	J20I-8S-12K/ BL-106C
Indium tin oxide (ITO) Coated coverslips	SPI supplies	06463B-AB
Fast Drying Silver Paint	Tedpella	16040-30
Dri-Cal size standards	Thermo Scientific	DC-20
Optical Fiber	Thorlabs	P1–1064PM-FC-5
Aluminium plate	Thorlabs	CP4S
High voltage power amplifier	TREK	PZD700A M/S

Comments/Description

Manufactured by Mitutoyo and Distributed by Edmund optics

Trapping laser

Polystyrene microparticles

bottom plate

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CORRESPONDING AUTHOR:

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Institution: National Institute of Standards and Thechnology

Article Title: Optical trapping of a selected dielectric microparticle in air

Signature: tl

Date: 04/15/2016

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Dear Dr. LeBrun,

Your manuscript JoVE54862R1 "Optical trap loading of dielectric microparticles in air" has been peer-reviewed and the following comments need to be addressed. Please keep JoVE's formatting requirements and the editorial comments from previous revisions in mind as you revise the manuscript to address peer review comments. Please maintain these overall manuscript changes, e.g., if formatting or other changes were made, commercial language was removed, etc.

Please track the changes in your word processor (e.g., Microsoft Word) or change the text color to identify all of the manuscript edits. When you have revised your submission, please also upload a separate document listing all of changes that address each of the editorial and peer review comments individually with the revised manuscript. Please provide either (1) a description of how the comment was addressed within the manuscript or (2) a rebuttal describing why the comment was not addressed if you feel it was incorrect or out of the scope of this work for publication in JoVE.

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Editorial comments:

The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (54862_R1_051616.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

1. Formatting: All figure legends should have a title and a brief description.

→ We have revised the title and brief description for each of figures as shown below and in the manuscript.

Figure 1: Schematics of the experimental setup used for selective optical trap loading in air. A single-beam gradient force optical trap is developed on an inverted optical microscope. Abbreviations used in the schematic are listed below: EOM, electro-optic modulator; HAL100, halogen illuminator; MFS, motorized focusing stage; NIR-LWD objective, infrared corrected long working distance objective lens; TS, translation stage (x−y); PZT, piezoelectric transducer; ESM, electrostatic field modulator; ND, neutral density filter; QPD, quadrant-cell photodetector; DM, dielectric mirror; ITO, indium tin oxide coated coverslips; CCD, charge coupled device camera; HeNe, helium neon laser (633 nm); Nd:YVO4, 1064 nm laser for trapping.[12]

Figure 2: Fabrication of the piezoelectric launcher assembly. (a) Rendered images of a PZT holder using CAD software package in a "-.SLDPRT" format and (b) "-.STL" format for 3D printing. (c) A rendered image of the final assembly of the piezoelectric launcher: sample enclosure (with ITO coated coverslips), PZT holder, ring spacer, ring-type PZT, aluminum plate, coverslips. (d) Picture of the final assembly.

Figure 3: Step by step demonstration of selective optical trap loading of a 20 µm PS particle. (a) locating the focus of the trapping beam, (b) levitating the particle above focus (The particle image is a dim blur because the levitation position is well above the nominal microscope focus) , (c) transitioning into the trapping position (nominally in focus), and then (d) moving the trapped particle to the central area for data acquisition. The particle is trapped at a fixed location of the beam focus whereas the sample stage is moved as indicated with a yellow arrow in Figure 3 (d) (Scale bar = 100 µm).

Figure 4: QPD captured particle trajectories both in frequency and time domain. (a) A poorly aligned experimental setup shows low-frequency noise and noise peaks at specific frequencies whereas (b) well-matched PSDs of the x and y-axis indicate correct optical alignment. (c) A QPD records the Brownian motion of the trapped particle in the time domain. (e) A step change in applied electric field across the trapped particle is synchronously recorded with the induced (d) ballistic motion through the data acquisition (DAQ) system.

2. Grammar: 2.4.7 – “above from the substrate”

→ We corrected grammatical error: “above ~~from~~ the substrate”

3. Additional detail is required:
-1.2.8 – How are the coverslips prepared? Are they cut or coated?

→ As explained in 1.2.8, we “use” ITO and glass coverslips, and “cut” them to fit the frame of sample enclosure with diamond cutter. We revised the note associated with step 1.2.8 and 1.2.9 according to reviewer’s comments as following.

“Note: The one pair of ITO coated coverslips are installed on the sample enclosure in parallel (facing each other) to provide uniform electric field and to generate ballistic motion of the naturally charged particle along the electric field. The three conventional coverslip cover the rest of sample enclosure surfaces (top and two other sides) to protect the trapped particle from the external flow of air”

-2.1 – Are the microparticles placed inside of the sample enclosure here? If so, please specify as this is not clear. It sounds like they are just scattered on a coverslip, which is then viewed under a microscope. How do they stay on the coverslip when imaged?

→ In sample preparation step 2.1, the particle is just scattered on a coverslip and imaged with an optical microscope to verify overall arrangement before we insert them between the PZT and PZT holder. Since the van der Waals force is strong enough to hold individual microparticles on the substrate, the adhered particles are secure unless significant external force is intentionally applied.

We included a note to address editor’s concern as below.

“In the sample preparation step, the particle is just scattered on a coverslip and imaged with an optical microscope to verify overall arrangement before we insert them (a coverslip with scattered microparticles) between the PZT and PZT holder. Since the surface adhesion is strong enough to hold individual microparticles on the substrate, the adhered particles are firmly fixed unless significant external force is applied.”

-2.3.5 – How is the frequency scanned?

→ The frequency is manually scanned from zero to 150 kHz by changing the output frequency of a function generator (by turning the front knob of a function generator). We have included additional note as following.
“Note: The modulation frequency is manually changed (scanned) from zero to 150 kHz to find the resonant frequency.”

-2.3.8 – How is verification performed?

→Once the particles are released, it moves to the other spot on the substrate thus one can tell if the target particle is released and moved from the real-time video. We revised the description in step 2.3.8 as following.

2.3.8 Configure the square waveform to generate ~~the amplified~~ voltage signals with an amplitude of 600 V (three times the voltage used for continuous excitation) at the resonant frequency of 64 kHz which has found from the previous step. Verify that the pulsing signal releases the target particle in a repeatable manner by ensuring particles move after each pulse.

-2.4.1 – How is scanning performed? Is this triggered via software?

→We revised the description in step 2.4.1 to make it more clear as following. "~~Scan~~*Move* the motorized focusing block back and forth vertically around the…"

-2.4.3 – How is the sample moved?

→ We included additional note in the step 2.4 as following, "Note: The PZT launcher assembly is installed on the manual linear translation *xy* stage. The particles can be translated relative to the fixed beam focus by moving the translational stage."

-2.4.4 – How is the power required determined?

→We included additional description in the step 2.4.4 as following, "The optimal power depends on particles size and material. The optical power was found through repeated trials to determine the power sufficient to levitate the particle without ejecting it from the beam."

-2.4.5 – How is this triggered? Via software?

→The excitation pulse is manually triggered by pressing the output button on the function generator which is connected to the high-voltage amplifier.

-2.4.6 – How much is the power reduced?

→We include a note for additional information for step 2.4.6. as following.
"Note: The optical power of trapping laser can be modulated by electro-optic modulator (EOM). The EOM regulates the output power with bias voltage supplied through a digital power supply. One can observe the transition from the levitation to trapping position through the CCD while slowly reduces the optical power"

-3.1.3 – How is adjustment/alignment performed? Is it manual? Does one watch the output of something to know that alignment is correct?

→The adjustment and alignment of optical elements (condenser and focusing lens) are manually performed by adjusting the knob of kinetic mount of these elements. The output voltage of QPD signals are Fourier transformed to determine if the alignment is well balanced.

-3.1.5 – How is the wave triggered? How is the "step response" measured? Is this automatically recorded by the software?

→ From the previous step, the QPD signal has been continuously monitored (recorded) through Labview software thus the particle trajectory will show a step response as we supply electric field in square wave. The details of the analysis can be found in our recent literature listed in the reference 12, *ACS Photonics* **2** (10), 1451–1459 (2015).

4. Branding should be removed from Figure 1, Figure 1 legend - Hal100

→ We removed the brand (HAL100) from the figure 1 and used HAL as an abbreviation of "halogen illumination".

5. Results: Please describe the "release" in the Figure 4 legend for panels c and d. In the results section, please describe what is shown in these panels (i.e. how the data is interpreted).

→We have used "release" to describe the change in the particle motion (figure4(d)) and the applied E-field (figure4(d)) since the particle is being pushed under non-zero electric field and released from the electrostatic force as the E-field is removed.

This protocol is intended to provide information on the selective optical trap loading technique. The interpretation of acquired particle trajectories has been reported in the literatures including our recent publication listed in the reference 12, *ACS Photonics* **2** (10), 1451–1459 (2015). For the sake of reader's interest, we have addressed the usage of particle trajectories for the alignment purpose (for example in Data Acquisition step 3) since it is relevant the scope of this protocol.

We insert additional description in the result section as following. "As we remove the electrostatic field from the trapped particle, the particle will be released to return to the field-free tapping position.as shown in figure 4 (d) and (e)."

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
In this paper, the authors provided a detailed description about an experimental technique for loading an optical trap with dielectric microparticles. This paper is useful for readers who are

new in the field of optical trapping in air. I would like to recommend its publication in the Journal of Visualized Experiments after the authors addressing the following issues:
1. The resolution (and thus the quality) of figures in this paper is too low for publication. For example, I cannot read the words in figure 1 and 4. The authors must increase the resolution and quality of all figures.

→ We have uploaded individual figure files with the best resolution available. However, it seems that the uploading system has some issue during the internal conversion process.

2. It will be useful if the authors can add a scale bar (or provide the size information) in Figure 2.

→We have included scale bar in figure 2.

3. Since this is a detailed technical paper, the authors should provide more details about the experiment to make it useful for readers. For example, the authors should provide information about the model, NA, and focal length of the objective lens, and etc.

→We have included information on the equipment used in this experiment on the separate list of equipment and materials. According to reviewer's suggestion, we have included NA and working distance of our objective lens in the manuscript as following, " … a trapping laser *(wavelength, 1064 nm)* focused by an objective lens (near-infrared corrected long-working distance objective*: NA 0.4, Magnification 20X, Working distance 20 mm*)"

Major Concerns:
N/A

Minor Concerns:
N/A

Additional Comments to Authors:
N/A

Reviewer #2:
Manuscript Summary:
In this paper the Authors describe protocols for the loading of an optical trap in air.
This is based on detachment of particles by ultrasonic vibrations generated by a PZT.
The experimental procedure leading to a selective optical trapping of particles in air is described.

Major Concerns:
None

Minor Concerns:
The methodology and protocols look rigorous and accurate. Additional description and figures in

the paper are clear and text is well written. Thus, my suggestion is to accept the paper to be published Journal of Visualized Experiments.
I only have a minor issue, the Authors should consider the following papers that are also discussions of protocols and methodology for construction of optical tweezers on a fluorescence microscope and advanced optical tweezers and optical manipulation:
-Lee, W. M., et al. "Construction and calibration of an optical trap on a fluorescence optical microscope." Nature protocols 2.12 (2007): 3226-3238.
-Pesce, G., et al. "Step-by-step guide to the realization of advanced optical tweezers." JOSA B 32.5 (2015): B84-B98.

→We have included additional note for providing information on the literature discussing about the protocols and methodology for optical manipulation techniques in the introduction as following.

"Protocols for trapping in liquid media have also been published.[15,16]"

15. Lee, W. M., Reece, P. J., Marchington, R. F., Metzger, N. K., Dholakia, K. Construction and calibration of an optical trap on a fluorescence optical microscope. *Nat. Protoc.* **2** (12), 3226–3238, doi:10.1038/nprot.2007.446 (2007).
16. Pesce, G. *et al.* Step-by-step guide to the realization of advanced optical tweezers. *J. Opt. Soc. Am. B* **32** (5), B84, doi:10.1364/JOSAB.32.000B84 (2015).

Additional Comments to Authors:
N/A

Reviewer #3:
Manuscript Summary:
The manuscript describes method and protocol for launching dielectric microspheres and trapping them in air using optical tweezers. The launching and trapping procedure can be repeated with the same microsphere over a period of time for multiple measurements. Detailed step-by-step instructions were provided to ensure reproducibility for any other interested groups. Overall it is a good protocol paper. There are a few sections can be further improved.

1. Not sure if due to file conversion problem, the words and numbers in the Figures are mostly not illegible.

→ We have uploaded individual figure files with the best resolution available. However, it seems that the uploading system has some issue during the internal conversion process.

2. In 1.2.8, the authors specified using two ITO coated coverslips and three conventional ones, without explicitly describing the configuration and usage. 1.2.9 and the Note kind of imply that afterwards, but it can be stated clearer.

→We revised the note associated with step 1.2.8 and 1.2.9 according to reviewer's comments.

"Note: The one pair of ITO coated coverslips are installed on the sample enclosure in parallel (facing each other) to provide uniform electric field and to generate ballistic motion of the naturally charged particle along the electric field. The three conventional coverslip cover the rest of sample enclosure surfaces (top and two other sides) to protect the trapped particle from the external flow of air"

3. In 2.4, parameters such as the wavelength of the trapping laser (two lasers in use, the He-Ne maybe only for imaging?), and the NA and working distance of objective lens are missing.

→ According to reviewer's comment, we included additional information including wavelength of trapping laser, NA, working distance of objective lens in the manuscript as following, " … a trapping laser *(wavelength, 1064 nm)* focused by an objective lens (near-infrared corrected long-working distance objective*: NA 0.4, Magnification 20X, Working distance 20 mm*)". The He-Ne laser is only for the visualization of trapped particle with bare eye.

4. In 3. Data acquisition, the steps, especially for data processing and results analysis corresponding to Fig. 4, are not sufficient in details.

→ This protocol is intended to provide information on the selective optical trap loading technique. The interpretation of acquired particle trajectories has been reported in the literature including our recent publication listed in the reference 12, *ACS Photonics* **2** (10), 1451–1459 (2015). Details of data analysis and their interpretation are beyond the scope of this protocol.

5. From Line 335, "The microparticles can be trapped in two positions: a nominal trapping position and a levitation position." The authors can elaborate on this specification. Normally the axial trapping position can be adjusted around the focal point by changing the power. The authors differentiate these two positions by the role of gravity and radiation pressure that provide the restoring force seem not very understandable.

→Ashkin has been classified optical trapping into two category depending on relative strength of gradient force to scattering force: optical tweezer trap and optical levitation trap.[1] The gradient force is larger than scattering force only for optical tweezer trap not for the levitation trap. Thus tweezer trap (or simply optical tweezer) can localize the particle motion in 3D only near the trap center whereas levitation trap cannot localize the particle motion in the direction of light propagation (z-direction, vertical) due to the scattering force.[2] Therefore, we have distinguished these two stable positions as nominal trapping position and levitation position.

[1] A. Ashkin, "History of optical trapping and manipulation of small-neutral particle, atoms, and molecules", IEEE J. Sel. Top. Quantum Elec. **6** (6), 841−856 (2000).
[2] A. Ashkin et al., "Stability of optical levitation by radiation pressure", APL **24** (12), 586−588 (1974)

We revised our manuscript (representative results section) in response to reviewer's comment as following.

"The microparticles can be trapped in two positions: a ~~nominal~~ trapping position and a levitation position. In the trapping position, optical forces stabilize the particle in all directions. In contrast, in the levitation position the particle is only stabilized transversely by optical forces. In the vertical the upward force from radiation pressure is balanced by gravity. With our loading method, the selected particle is generally delivered to a levitation position ~~where gravity provides the vertical restoring force to stabilize the particle whereas in the nominal trapping position the particle is stabilized by radiation pressure alone~~. At the levitation position, the vertical location of the suspended particle ~~varies as~~ is much more sensitive to variations in the optical power ~~increases~~ ~~whereas the nominal~~than at the trapping position ~~is relatively fixed at~~ near the focus.[16] One can vertically move the particle repeatab~~ed~~ly between these two stable positions by varying the optical power. The levitation position also has higher sensitivity…"

6. The 20 μm PS spheres used in the experiments are sufficiently large to support WGM resonance as microresonators, especially in the air. Did authors notice resonant effects of optical forces in experiments with different spheres, as discussed in DOI: 10.1002/lpor.201400237 and 10.1063/1.4895631?

→ As the reviewer points out, Mie/Whispering Gallery Mode resonances are generally present in these particles and Mie scattering calculations show they are likely active here. Measurements of these modes go back some time, including for optical trapping forces [1] and are not our focus here. Because we don't take measurements while varying wavelength or particle diameter, excitation of Mie resonances won't change our results, it would just set the overall force observed. The changes would probably be modest – about 30% in trapping power because the calculated resonances are low Q. None of this changes the procedure, which remains valid as written and the results repeatable.

We see this as an interesting point scientifically, but a distraction for a procedural paper and so have chosen not to address it in the paper. There remains the question of whether the excitation of Mie resonances influences the dynamics of stably loading the trap, but this represents an open research question rather than one that can be addressed here.

[1] Chylek, P.; Ramaswamy, V.; Ashkin, A.; Dziedzic, J. M. Simultaneous Determination of Refractive Index and Size of Spherical Dielectric Particles from Light Scattering Data. Appl. Opt. 1983, 22, 2302.

the 20 µm polystyrene (PS) sphere supports WGM resonance and it can be used to measure the diameter of trapped particle precisely. [1] However, in order to match the resonant condition, either particle diameter or wavelength should be varied (or scanned). Even though the particle has some variation in size (about 3 % standard deviation according to manufacturer's value), a fixed wavelength trapping laser (1064 nm) is not suitable to find the WGM resonances of solid PS microparticles. Moreover, our loading scheme is developed to load a target particle in a repeated manner thus, we have focused on loading of the same particle rather than loading various size of particles. Particularly, we have been using 20 µm diameter PS particle which doesn't allow us to compare the difference in optical forces arising from the variation of particle size.

[1] Chylek, P.; Ramaswamy, V.; Ashkin, A.; Dziedzic, J. M. Simultaneous Determination of Refractive Index and Size of Spherical Dielectric Particles from Light Scattering Data. Appl. Opt. 1983, 22, 2302.

Major Concerns:
N/A

Minor Concerns:
N/A

Additional Comments to Authors:
N/A