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.

Your revision is due by **Jul 05, 2016.** Please note that due to the high volume of JoVE submissions, failure to meet this deadline will result in publication delays. To submit a revision, go to the [JoVE Submission Site](http://www.editorialmanager.com/jove" \t "_blank) and log in as an author. You will find your submission under the heading 'Submission Needing Revision'.

Sincerely,

Nam Nguyen, Ph.D.  
Science Editor

[JoVE](http://www.jove.com/)

1 Alewife Center, Suite 200, Cambridge, MA 02140

tel: 617-674-1888

[https://www.jove.com/files/img/signature/jove_signature_twitter.png](https://twitter.com/JoVEJournal) [https://www.jove.com/files/img/signature/jove_signature_facebook.png](https://www.facebook.com/JOVEjournal) [https://www.jove.com/files/img/signature/jove_signature_linkedin.png](http://www.linkedin.com/company/jove)

[https://www.jove.com/files/img/signature/jove_signature_jove.png](http://www.jove.com/)

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**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 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 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.16 One can vertically move the particle repeatably 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