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Design and construction of a space-borne optical tweezer apparatus

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A compact optical tweezer package has been developed for use on a microscope to be flown on the International Space Station as part of a series of experiments in colloid crystallization. A brief introduction to the principles of single-beam optical tweezer operation will be presented, after which a detailed system layout will be shown. Special design requirements due to the spaceflight nature of the hardware will also be discussed. The tweezer apparatus is capable of trapping many particles through use of a two-axis acousto-optical deflector. The trap strength is sufficient to perform the required science (50 pN at $\Delta n = 0.2$). The trap beam behaves approximately as a diffraction limited single mode Gaussian beam of numerical aperture, $NA = 1.4$, as shown through spot size measurements and confocal-type images of the focal region. This is the first time optical tweezers will be deployed in a microgravity environment. © 2001 American Institute of Physics.
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I. MOTIVATION

The development of optical tweezers as a tool to manipulate microscope particles began in the early 1970s with the use of a focused laser beam to levitate transparent glass spheres.¹ Although early studies used two counterpropagating beams, it was realized that a single beam could be used to form a stable trap.² Optical tweezers found their first application within the field of biology to manipulate single cells as well as subcellular structures.^{3,4} Later developments have included laser scissors, which operate on the same principle as tweezers but use a short-wavelength source to sever molecular bonds,⁵ and the use of laser tweezers to manipulate microspheres for use in colloid studies.^{6,7} The laser tweezers discussed here will be used for materials science studies in microgravity, specifically to probe the physics of colloidal crystals.⁸ The tweezers will be capable of not only measuring crystal properties such as yield strength,⁹ but also be capable of manipulating crystals by creating seeds or defects through the creation of multiple traps.^{10,11}

The laser tweezer unit is part of a microscope that will be installed on the International Space Station (ISS) in early 2005, and will be in operation for approximately 2 yr. It is anticipated that future flight experiments will desire to take advantage of the unique capabilities of the microscope in general, and the laser tweezers in particular. The microscope [designated the light microscopy module (LMM)] is housed within the Fluids and Combustion Facility (FCF), which consist of three adjoining equipment racks within the U.S. Lab portion of the ISS. Each rack is approximately 6 ft high, 3 ft wide, and 3 ft deep. The LMM resides within the fluids integrated rack, one of the three FCF racks. Although some aspects of flight-qualifying hardware pose special challenges, hardening the laser tweezer package is not anticipated to be especially difficult. For example, all polyvinylchloride coated wires must be replaced with Teflon® coated wires, all

connectors must be push-on, pull-off quarter-turn connectors, and the hardware in general must conform to a MIL-SPEC type specification. Because (passive) convective cooling is absent, heat sources must be carefully placed away from critical components, and the heat must be carried away either by forced air or by use of a cold plate. A safety constraint is that the microscope may not be powered when a crew member is, for example, changing sample trays or replacing hardware. Thus, crew members cannot be used to align critical optical components should they become misaligned during launch. The current plan is to motorize all components that may need to be aligned, and to verify alignment remotely via down linked data and images.

The manifested experiments that make use of laser tweezers will be using the tweezers to perform various functional tasks. The experiments concern themselves with the statics and dynamics of (hard sphere) colloidal crystal growth. Different samples will lie on different parts of the hard sphere phase diagram, from liquid through coexistence to crystalline to glassy. Primarily, the tweezers will be used to simply trap a particle and move it. The amount of applied force required to displace a particle provides information about the yield stress or viscosity of the material. In addition, the tweezers will be used to manipulate the growth environment. For example, by creating an array of traps, the tweezers can create a seed crystal that the colloid will then grow around. The seed crystal can be matched or mismatched to the natural habit of the hard sphere packing pattern. In the event of a mismatch, the strain field as the crystal grows around the seed can be directly visualized. Also, given a crystallite, defects can be created by removing a particle or dislocating a row of particles, and the effects on crystal properties can be evaluated. Finally, because the tweezer beam can be a source of thermal energy, a projected use for the tweezers is to perform local melting of a crystal by heating a thin gold layer deposited on the coverslip. It should be emphasized that although the trapping functions of the tweezer have been evaluated, this application is still in early development.

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II. PRINCIPLES OF LASER TWEEZER OPERATION:

Optical tweezers work by creating a potential well due to the spatial gradient of the electromagnetic field.^{12–18} Other developments include the effects of trapping a nonspherical object.¹⁹ The specific forces involved are well understood, but their complete mathematical expression remains problematic. The so-called scattering force is proportional to the intensity and is given by

$$\mathbf{F}_{\text{scat}}(r) = \frac{n_2}{c} C_{\text{pr}} I(r) \hat{\mathbf{z}}, \quad (1)$$

where n_2 is the index of refraction of the medium within which the particle resides, C_{pr} the scattering cross section of the particle, and I is the intensity of the incident beam. In this formulation, the beam is assumed to propagate in the z direction. This force can naively be thought of as momentum imparted to the particle by the impact of photons. The second force acting on the particle can be derived from the Lorentz force, which is simply the dipole interaction with an electric field. The interaction energy is

$$U = -\mathbf{p} \cdot \mathbf{E} = -\alpha(\mathbf{E} \cdot \mathbf{E}), \quad (2)$$

where p is the dipole moment and α is a constant of proportionality. The gradient force is simply the gradient of this interaction energy:

$$\begin{aligned} \mathbf{F}_{\text{grad}} &= -\nabla U \\ &= \alpha \nabla (E^2) \\ &= \alpha \nabla I. \end{aligned} \quad (3)$$

Reference 2 has an explicit expression for α . Note that the gradient force derived in this way holds only for linear dielectric nonmagnetic materials ($\mathbf{p} \sim \mathbf{E}$) and also for the case where the particle can be treated as a dipole, e.g., the focused spot of the tweezers is much larger than that of the particle, and the index mismatch between the particle and solvent is small. Although this formulation would not hold for the overwhelming majority of implementations of optical tweezers using a microscope objective to focus the beam, it should be emphasized that the error introduced by the dipole approximations is at most only several percent.¹² Even so, improved approximations, such as the generalized Lorentz–Mie theory¹⁷ have been developed.

III. SYSTEM LAYOUT

The basic laser tweezer design closely follows the layout in Refs. 10, 20, and 21 and is shown in Figs. 1 and 2. Figure 1 shows a photo of the laboratory breadboard, while Fig. 2 is a solid model of the flight configuration. The system consists of a laser, a two-axis acousto-optic deflector (AOD) for beam steering, two lenses $L1$ and $L2$ for beam expansion and imaging, and a few mirrors to shrink the overall package size. Note that Fig. 2 contains two polarizers ($P1$ and $P2$) that will be used to control the beam intensity and thus the trap strength. This is important for yield strength measurements. The two lenses merely expand the beam to overfill the entrance pupil of the objective lens, while at the same time

FIG. 1. Breadboard configuration of laser tweezer module.

mapping a plane at the AOD to the entrance pupil plane. This is done following the same guidelines in Ref. 20.

The laser chosen was a Crystalaser IRCL-1W-1064, a diode-pumped Nd:yttrium–aluminum–garnet continuous-wave single mode laser providing 1 W optical power from a 10 W electrical power supply. This laser was chosen primarily because of the small size of both the laser head and the efficiency of the power supply. The beam steering is provided by a two axis AOD produced by IntraAction Corporation. Plano-convex singlets (Newport Corp.) were used for the lens elements. The first lens has a 38 mm focal length, while the second has a 250 mm focal length. Both lenses were antireflection coated for 1064 nm. The focal lengths were chosen for convenience: the distance between the entrance port of the microscope and the objective lens is 140 mm, and the laser beam needed to be expanded a factor of about $6\times$ to fill the aperture. The objective lens used was a Leica 100 $\times$ oil immersion numerical aperture (NA)=1.4 planapochromat with a 90 μm working distance.

A Leica DM RXA upright microscope was chosen for use aboard the ISS. A solid model rendering of the entire LMM is shown in Fig. 3. The tweezer couples into the microscope through an existing lateral port. A side-looking dichroic mirror (Chroma) mounted within the fluorescence turret provides the ability to perform normal microscope viewing or confocal microscopy while the tweezers are operating. The small amount of leakage from the backscattered tweezer light through the dichroic allows observation of the

FIG. 2. Solid model of LMM optical tweezer module.

FIG. 3. LMM in operational configuration.

tweezer spot by a camera during alignment and operation. It should be noted that the confocal unit will not pass the tweezer beam to the confocal camera due to the presence of numerous edge filters within the confocal apparatus, in addition to the fact that the tweezer source is not confocal to the pinhole array of the confocal unit. In addition to the tweezer package, the solid model also shows the confocal microscope unit (Yokogawa) at the top of the microscope and a fluid containment box with gloveports located on the front.

The microscope is mounted to an optics bench within the rack. The Leica microscope uses so-called infinity-corrected objectives, that is, the objective lenses form their intermediate image at infinity. This greatly simplifies introduction of auxiliary optics into the light path of the microscope, because light coupled to the objective need merely be collimated to produce proper behavior. Thus, by mapping a tilted plane wave to the back pupil plane of the objective lens, the focused spot is translated along the front focal plane where the sample resides.

Although several schemes exist for steering an optical trap,^{10,11,20} beam steering in this system is accomplished through use of a dual axis (AOD), produced by IntraAction Corp. The AOD consists of two orthogonal TeO₂ crystals, each excited by an ultrasonic pressure field, inducing a spatial periodicity in the density, and through the photoelastic effect of the index of refraction. Thus, the AOD essentially serves as a programmable diffraction grating with a period that can be changed on the millisecond timescale. By suitable manipulation of the AOD, several different configurations of the trap can be created: a single movable trap, a line or bar trap, and an array of discrete traps, where the laser scans an array of discrete locations with very fast motion between the individual locations. Examples of various trap geometries are shown in Figs. 4 and 5. The overall beam steering must occur very fast because the motion of particles due to Brownian motion must not allow the particles to wander out of the trap location during times the laser is not

FIG. 4. A circle of trapped PMMA fluorescently dyed particles, as viewed through a confocal microscope.

incident on them. Although use of a dual-axis AOD potentially causes problems due to the fact that beam steering does not occur in a single plane, the benefit gained is tighter control over the beam steering as well as a faster response. This design was chosen over gimbaled mirrors due to speed considerations, and over fixed diffractive optical elements due to the need to dynamically control the trap location. However, the main drawback of the AOD is a lack of a well-defined steering plane. This is partially mitigated due to the high f number of the beam expansion/mapping system.

The typical sample used for this study to simulate flight samples was a mixture of fluorescently dyed polymethylmethacrylate (PMMA) particles, 2.3 μm in diameter, in a nearly index-matching bath of decalin and tetralin. The samples were supplied by the PCS-II team. The total sample thickness was over 150 μm , and thus the total thickness was not accessible to the microscope. An image of a trapped array of particles is shown in Fig. 4. Samples used for trap strength measurements consisted of borosilicate glass spheres in various index fluids supplied by Cargille. These samples were different because it was desired to have control over both the index mismatch and the volume fraction of the particles. In trap strength measurements, low volume frac-

FIG. 5. Example of tweezer capabilities: 1 μm diam PS particles in water.

tions were required while for performing PCS-II science evaluations, much higher volume fractions were needed.

Use of oil as an immersion medium potentially precludes use of the tweezers with aqueous samples. This is due to the increasing amount of spherical aberration present as the focal plane is moved further and further into the sample. This is not expected to pose problems for very thin samples of the type typically encountered in biology, but does represent a fundamental constraint when thick (greater than several tens of microns) colloidal samples are used.

An additional design constraint is due to the AOD mechanism. Rapid scanning results in a nonuniform grating pitch within the crystal, causing astigmatism in the focused spot. As a result, there are situations where the trap is non-symmetric and is unable to trap a particle in three dimensions, although the particles will still be confined in the two lateral dimensions. The constraint places an upper limit on the number of discrete points that can be scanned by the AOD.

The optical tweezer breadboard layout was constructed using Linos Microbench® optomechanical mounts. This was chosen based on the small size, tight tolerancing, and rigidity provided by the lens mounts. The system will be vibration tested to determine the degree to which it will stay in alignment during launch aboard the space shuttle. It may be possible to replace the mirrors with prisms, thus providing a more rugged package.

Alignment of the tweezer then occurs in two stages. First, the optical elements of the tweezer are aligned relative to the source. Then, the optical axis of the tweezer package must be made coincident with the optical axis of the microscope. The optic axis of the laser is used to create a well-defined optical axis through the entire system, with the AOD turned off. This process centers all lenses and properly locates and orients all mirrors. When the AOD is turned on, the diffracted beam of interest is now tilted about the defined optic axis. The mirrors are then repositioned to vignette the central (undiffracted) beam while recentering the deflected beam. Then, the entire tweezer apparatus is repositioned to send the beam into the microscope. In the laboratory, for the breadboard stage of development, a 5 degree of freedom mount holds the optical platform on which the tweezer is mounted. This platform provides independent control of 3 orthogonal axis of translation and 2 orthogonal axis of rotation (pitch and yaw). The third axis of rotation (roll) is not required due to symmetry considerations of the optical elements. The 5 axis positioner allows us to determine the total alignment budget for the tweezer package relative to the microscope optics. The flight hardware will be mounted to the microscope through use of a dovetail slide with a locator pin to delineate the final location of the package.

IV. SOFTWARE INTERFACE

The software interface as currently written simply moves the trap by changing the drive frequency of the AOD. Currently, the program is capable of producing a single steerable trap, an ellipse of varying radii and angle, a line of varying length and angle, and an array of traps of varying spacing

FIG. 6. Oscilloscope trace of focused tweezer beam spot. Time scale has been converted to travel distance.

and angle (i.e., rectangular or hexapolar). The program is also capable of reading a list of trap locations from a file. The program computes a list of points from the shape (or reads a file into an array), and moves from point to point at a user-defined speed. For example, the user could either have the trap beam trace out a complete circle or produce a discrete number of traps about the circumference. The resolution of the AOD frequency driver allows the trap position to be set to within $2 \times 10^{-4} \mu\text{m}$. It should be noted that this level of positional accuracy cannot be verified optically. The overall plan in software development for the tweezers, as well as for the LMM, is the use of “scripts” which will predefine specific functions for the tweezers to perform without assistance from a human operator. Examples of scripts are, e.g., trap a particle and oscillate it back and forth at a known frequency and known displacement.

V. PERFORMANCE

Two measurements of the optical performance of the tweezer system were performed: a measurement of the beam profile through focus, and trap strength as a function of sphere size.

A. Spot size measurement

The tweezer beam was measured using a Photon SpotScan system. This consists of an array of apertures which oscillate back and forth at 10 Hz. Essentially, the system performs as a scanning slit apparatus capable of measuring beam widths as small as $0.3 \mu\text{m}$. The apparatus was calibrated at the factory prior to use. In order to provide an accurate measurement, a piece of No. 1 1/2 thickness coverglass (nominal thickness 0.17 mm) was placed over the aperture array and held in place with a thin film of immersion oil. This allowed the $100\times$ microscope objective to work with the proper coverslip correction during the course of the measurement. The data taken are shown below, in Figs. 6 and 7. Data were taken at two trap locations: the “home” location, and at a location near the edge of the field of view of the camera, corresponding to a trap displacement of approximately $15 \mu\text{m}$. The SpotScan provided $1/e^2$ beam waist diameters in two orthogonal directions, averaged over three passes of the mask array. Figure 6 presents a sample output

FIG. 7. Measured tweezer beam spot sizes. (a): On-axis, (b) displaced in y 14 μm , and (c) displaced in x 14 μm .

scan from the instrument, when the aperture is located at a focus. This scan shows a near-Gaussian profile, with minimal sidelobes.

The data shown in Fig. 7 indicate that the tweezer beam behaves approximately like a diffraction-limited NA 1.4 Gaussian beam, except in the immediate neighborhood of focus. For this system, the calculated diameter of the Airy disk is 0.97 μm , while the Gaussian beam waist is calculated to be 0.48 μm . It is thought that spherical aberration is present, accounting for the larger than predicted beam waist. This is a plausible explanation given that the wavelength of the tweezer beam (1064 nm) is well outside the waveband for which the objective lens is corrected (400–700 nm). No significant astigmatism was detected. A consequence of the beam profile is that the trap strength is expected to be different in the z axis as opposed to the in-plane axis. The behavior of the beam, when displaced from the home position, shows little evidence of distortion, coma, or astigmatism.

In addition to the SpotScan instrument, a series of images was taken of the tweezer beam incident on a flat mirror (with a coverglass) at different beam positions within the field of view. The resulting set of images is similar to an “image cube” of the type obtained through confocal imaging. A slice in z taken through the stack is shown in Fig. 8. The contrast has been adjusted for this article, but it clearly shows the intensity gradient of the beam near focus. Note the difference in scale in x and z . Also seen in Fig. 8 is the increasing asymmetry of the trap as the position is moved away from the center of the field of view. Figure 8(a) is an

FIG. 8. Intensity profile of tweezer beam in vicinity of focal spot (x - z slice): (a) on-axis, (b) displaced in x 7 μm , and (c) displaced in x 14 μm .

x - z slice of the beam in the neutral position, while Fig. 8(b) is a slice taken of the beam when displaced in the x direction 7 μm and Fig. 8(c) when displaced in the x direction 14 μm . The spot position in Fig. 8(c) is near the edge of the usable range of motion for the tweezers, as observed during testing. The tweezers can cover approximately the field of view of a 1/3 in. format charge coupled device array using a 1.6 $\times$ tube lens.

B. Trap strength measurement

This measurement required the creation of standard samples to tweeze. The trap strength is a function of particle size, index mismatch between the particle and fluid, trap location due to nonuniform AOD response, axial versus transverse movement, and tweezer misalignment to the microscope. Six slides were created: three containing borosilicate glass spheres of average diameter 2.5 μm (Duke Scientific 9000 series size standards), while three others contained glass spheres of diameter 5 μm , also from Duke Scientific. The spheres were immersed in calibrated index fluids supplied by Cargille Industries corresponding to index mismatches at the Sodium D line of $\Delta n = 0.01$, 0.05, and 0.1. The refractive index of the spheres was measured by Duke Scientific to be 1.56, as determined by use of index matching fluids. Because the tweezer operates at 1064 nm rather than the 589 nm D line, the actual index mismatches as seen by the tweezer beam were slightly different, but the difference is expected to be small. Upon filling and sealing, the beads would often aggregate due to van der Waals interactions, and so the slides were immersed into an ultrasonic cleaner bath

for a few seconds to break up the aggregates. Some beads clung to the coverslip or glass slide, presumably also by electrostatic interactions, and served as a marker to indicate the surfaces of the cell. For all measurements, an image of the trapped particle was taken for accurate determinations of radius.

It was found during the course of testing that the sample slides with an index mismatch of 0.05 were ideal for use. The higher index mismatched samples were unsuitable to the lensing effect of the microspheres: the intense field at focus would actually catastrophically burn either the spheres or the fluid. The damage does not appear to be caused by the fluid absorbing the light. The process occurs very quickly and is hard to analyze. It is thought that lensing by the spheres causes an extremely intense focal spot to be generated, perhaps within the spheres. Small imperfections within the glass will absorb this light and heat up, causing the fluid to boil and the glass to blacken, which in turn absorbs more of the laser light, which causes further heating. This will not pose a problem for the flight experiments as the index mismatch for flight samples is several orders of magnitude smaller, and the PMMA spheres much more uniform in composition. The samples at an index mismatch of 0.01 still exhibited trapping, but the mismatch was so small that viewing the particles normally presented difficulties. It is not known at this time if the tweezer beam would damage the optical elements used for the differential interference contrast method of viewing nearly index-matched materials.

Measurements were performed in the following way. The glass beads, being denser than the index fluids, would sink to the bottom of the microscope slide. The tweezer beam would grab a particle, and then the sample stage was lowered to bring the trapped particle halfway between the bottom surface and the coverslip. The approximate distance between the cell walls and the particle was 20 μm above and 20 μm below, which is far enough to neglect wall effects for most of the particles. The microscope slide was then laterally translated at a known speed on a motorized slide stage (Marzhauser) to within 1 $\mu\text{m/s}$ accuracy, for a distance far enough to develop steady state conditions of the flowfield around the particle. The stage control program allowed the identification of two setpoints, and the particle was dragged from one point to the other and back and increasing speed until the viscous drag force of the index fluid overcame the trap strength. The viscosities of the fluids at 25 $^{\circ}\text{C}$, as given by Cargille, were 62, 35, and 18 cS for indices of 1.55, 1.51, and 1.46, respectively. The room temperature was kept constant to within 5 $^{\circ}$ to minimize changes in viscosity of the fluid. It was found that the maximum stage speed before a particular particle was lost was repeatable to within 1 $\mu\text{m/s}$. The measured data are shown in Fig. 9. This measurement was performed with the trap located at the neutral point. It is thought that the large amount of scatter in the measured data is due to structural inhomogeneities from sphere to sphere (for example, inclusions, bubbles, and nonsphericity). The next set of samples will use polystyrene spheres rather than borosilicate glass specifically to address variations in particle properties. It has been seen with other samples that polystyrene spheres are significantly more uniform in both

FIG. 9. Measured tweezer trap strength as a function of particle size. Glass spheres in Cargille index fluid, $\Delta n = 0.05$. Line is a least squares fit to the data.

shape and composition than the glass microspheres. In any case, the data show that the trap strength exceeds the required specification (50 pN) at any particle size of interest.

VI. FUTURE DIRECTIONS

The major thrust of future work will be to fully test the current layout. The existing breadboard meets requirements for small size, low power consumption, and ruggedization. Because the trap strength varies over the field of view, the trap strength measurement will be performed for several different trap positions. Operation of the tweezer in a mechanically noisy environment potentially introduces trap strength asymmetry due to preferred bending modes of the dichroic mirror mount within the microscope housing, leading to high-frequency, small amplitude motion of the tweezer spot. Finally, the tolerance budget for the module alignment will be determined to provide direction for the construction of the module housing.

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