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Andrew Resnick

Cleveland State University, [a.resnick@csuohio.edu](mailto:a.resnick@csuohio.edu)Follow this and additional works at: [https://engagedscholarship.csuohio.edu/sciphysics\\_facpub](https://engagedscholarship.csuohio.edu/sciphysics_facpub) Part of the [Physics Commons](#)**How does access to this work benefit you? Let us know!**

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## Introduction

An optical tweezer apparatus has been developed as part of a microscope that will fly on the International Space Station beginning in mid 2005 [1]. The development of optical tweezers as a tool to manipulate microscopic particles began in the early 1970s with the use of a focused laser beam to levitate transparent glass microspheres [2]. Later developments have included the use of laser tweezers to manipulate microspheres for use in colloid studies [3–5]. The laser tweezers discussed here will be used for materials science studies in microgravity, specifically to probe the physics of colloidal crystals [6]. The tweezers will be capable not only of measuring crystal properties such as yield strength [7], but also of manipulating crystals by creating seeds or defects through the creation of multiple traps [1,8,9].

This paper presents results from a series of measurements designed to accurately determine the force applied by the tweezer beam onto a colloidal microsphere. The majority of previous work with tweezers used polystyrene in water as a calibration sample (see for example [10–13]). Furthermore, quoted refractive index values were generally at the sodium D-line. Our apparatus uses oil-immersion lenses and solvent-based systems, and so aqueous-based calibration samples are inappropriate. Given that the index mismatch between polystyrene and water is large, differences

between the refractive index mismatch at the sodium D-line and the tweezer wavelength are expected to be a small perturbation to the calculated trap strength. However, our systems use highly index-matched colloidal suspensions, and as will be shown, small changes in the relative index mismatch can cause large changes in the applied force. Finally, there has been a persistent and significant difference between measured trap forces and theoretical calculations (see, for example [14–16]), precluding the use of a useful model for the apparatus used here.

A detailed description of the tweezer apparatus has been discussed elsewhere [1]. Briefly, the laser tweezer unit is part of a microscope that will be installed on the International Space Station (ISS) in mid 2005 and will be in operation for approximately 2 years. The microscope (designated the Light Microscopy Module, or LMM) is housed within the Fluids and Combustion Facility, which consists of three adjoining equipment racks within the U.S. Lab portion of the ISS.

The manifested experiments that make use of laser tweezers will be using the device to perform various functional tasks. The experiments concern themselves with the statics and dynamics of (hard sphere) colloidal crystal growth. Typically, the colloidal system consists of nearly index-matched polymer microspheres and a solvent solution. Different samples will lie on different parts of the hard sphere phase diagram, from liquid through coexistence to crystalline to glassy. However, some samples will contain exotic constituent materials to be used to grow materials with use-

ful (and novel, e.g., photonic bandgap) photonic properties. Other samples will contain novel states of colloids (e.g., gels, binary alloys) whose physical properties are not well understood at this time. The optical tweezer apparatus will primarily be used to probe the viscoelastic properties of the various flight samples. In addition, the tweezers could be used to create crystalline defects or seed crystals to perform “colloidal engineering.”

Because of the unique opportunities afforded by spaceflight experiments, it is desirable to perform science on as many different types of samples as possible. For example, previous protein crystallization experiments [17] have flown hundreds of different proteins at once in order to maximize the science return. In terms of tweezer operations on various types of colloids, it is necessary to know the force applied to a colloidal microsphere of a general composition and size within a generic fluid matrix. To this end, trap force measurements have been performed on a suite of dilute suspensions to determine the trapping force as a function of particle radius and index mismatch. Unless otherwise noted in this paper, the refractive index is only of quantitative interest at the tweezer wavelength (1064 nm).

Although determining the trap force as a function of particle radius is straightforward, the dependence of trap force on index mismatch is complicated by several factors. Primarily, the refractive index of the microspheres is unknown at the tweezer wavelength (1064 nm). Although refractive index measurements of some bulk polymers can be found in the literature [18], it is not clear that the refractive index of polymer microspheres should be the same. In addition, solvent can (and does) leach into the microspheres over time. Furthermore, the colloidal microspheres will have a steric coating to prevent flocculation, and this could affect the (apparent) refractive index [19,20]. Also, many of the flight sample particles will be impregnated with a fluorophore for use with confocal imaging to obtain the full three-dimensional structure. In addition, novel materials will be flown such as encapsulated liquid crystals whose optical properties at 1064 nm have not yet been measured. This paper presents a method of accurately obtaining the refractive index of colloidal microspheres when immersed in a fluid of known refractive index.

Because it is not possible to directly measure the trapping force, but rather it is calculated assuming that Stokes flow conditions exist, it is desirable to extend these results into dense colloidal suspensions where sphere–sphere hydrodynamic interactions are non-negligible. It has been shown in our lab that the trap strength is high enough to drag a single sphere through a hard sphere colloid of volume fractions slightly below the fluid–crystal coexistence region ( $\phi < 0.494$ ), and in this case it is not clear what value should be used for the “viscosity” of the fluid. The work presented here can be used as a baseline against which future work on these dense suspensions can be compared.

## Materials and methods

### *Colloidal suspensions*

As indicated above, the key to performing these measurements is the creation of a “standard” sample. Ideally, this sample will be well characterized and stable. Four model systems were used, and each presents its own difficulties. For all measurements, the ambient temperature was controlled to  $23 \pm 0.5$  °C. In addition, care was taken not to heat the samples during the measurements by using the minimum amount of illumination necessary to view the samples.

The first sample series tried consisted of borosilicate glass microspheres (Duke Scientific 9000 series, diameters of  $2.5 \pm 0.5$   $\mu\text{m}$  and  $5.0 \pm 0.7$   $\mu\text{m}$ ) in a series of Cargille immersion oils. This system was chosen partly due to convenience (various immersion oils were present in the lab as part of the LMM development cycle) and partly due to the chemical compatibility between the immersion oils and glass. However, it was found that the glass microspheres were extremely inhomogeneous, both in composition and shape, and furthermore it was postulated that residual stresses in the microspheres greatly affect the refractive index in a nonpredictable manner [21]. All of these issues were reflected in the unacceptable scatter present in these trap strength measurements [1].

The next system chosen was polystyrene (PS) microspheres (Bangs Laboratories Estapor and Dynospheres) in Cargille (Cargille Laboratories, Inc., New Jersey) refractive index fluids. The specific composition of the microspheres was 45% PS and 55% divinylbenzene, as stated by the manufacturer. The index fluids were of a proprietary composition and varied with refractive index but were composed of aliphatic/alicyclic hydrocarbons and hydrogenated terphenyls. Four different diameter microspheres were used ( $5.10 \pm 0.04$   $\mu\text{m}$ ,  $1.80 \pm 0.02$   $\mu\text{m}$ ,  $0.93 \pm 0.01$   $\mu\text{m}$ , and  $0.49$   $\mu\text{m}$ ) to measure the trap strength as a function of particle size. A series of refractive index liquids was obtained to provide well-controlled index mismatches between the fluid and microspheres. The PS spheres had the advantages of homogeneity in size and composition but were chemically incompatible with the refractive index fluids. However, fresh solutions could be used, and this system was used the most. Trap force data taken with this system will be presented here.

A third system consisted of 0.92- $\mu\text{m}$ -diameter polyhydroxystearic acid (PHSA) coated polymethylmethacrylate (PMMA) microspheres in *cis*-decahydronaphthalene (*cis*-decalin) supplied by the PHaSE-II team. This system is close to the “typical” colloidal system that will be flown in space, and it has been shown that long-term storage of PMMA in *cis*-decalin does not alter the physical size of the PMMA microspheres. However, although the refractive index of *cis*-decalin was known, the refractive index of PHSA-coated PMMA was not. Thus, a fourth system was used, namely PHSA-coated PMMA in Cargille index fluids. Again, PMMA is incompatible with the index fluids, so fresh

Table 1  
Selected physical properties of Cargille index fluids used in this study

Material	Refractive index at 1064 nm and 25 °C	$dn/dT$ ( $10^{-4}$ )	Viscosity at 25 °C (cS)	$\rho$ (g/cc)
Cargille index fluid 1.460	1.452	-3.89	18	0.832
Cargille index fluid 1.462	1.453	-3.89	18	0.835
Cargille index fluid 1.464	1.455	-3.90	19	0.838
Cargille index fluid 1.466	1.457	-3.91	19	0.842
Cargille index fluid 1.470	1.461	-3.92	20	0.848
Cargille index fluid 1.480	1.470	-3.95	23	0.863
Cargille index fluid 1.482	1.472	-3.95	24	0.866
Cargille index fluid 1.486	1.475	-3.96	25	0.873
Cargille index fluid 1.488	1.477	-3.97	26	0.876
Cargille index fluid 1.490	1.479	-3.98	27	0.879
Cargille index fluid 1.492	1.481	-3.98	28	0.882
Cargille index fluid 1.494	1.483	-3.99	28	0.885
Cargille index fluid 1.510	1.497	-4.04	35	0.910
Cargille index fluid 1.550	1.534	-4.15	62	0.972
Cargille index fluid 1.554	1.538	-4.17	66	0.978
Cargille index fluid 1.558	1.541	-4.18	69	0.984
Cargille index fluid 1.570	1.552	-4.21	82	1.003
Cargille index fluid 1.578	1.559	-4.27	63	1.053
Cargille index fluid 1.580	1.561	-4.28	59	1.063
Cargille index fluid 1.584	1.564	-4.30	51	1.085
Cargille index fluid 1.590	1.570	-4.33	42	1.117
Cargille immersion oil FF	1.470 <sup>a</sup>	-3.70	170 <sup>a</sup>	0.877

<sup>a</sup> Measured at 23 °C, from manufacturer.

samples were constantly generated during the measurement process. These data will be presented here.

It should be noted that the fluids used, in particular the index fluids, can cause contact dermatitis and should be handled with care. In addition, exposure of the index fluids to ultraviolet light causes degradation of the fluid and alters the physical properties of the fluids. For convenience, physical properties of interest of the index fluids used are presented in Table 1. Note that the values presented are from the manufacturer and were not measured in house (although selected refractive index values were verified)

The refractive indices of various index fluids at 1064 nm were provided by Cargille but was also verified by use of an Abbe refractometer (Model WY1A, Xin Tian Optical Instrument Corporation, China), with a Nd:YAG laser as the illumination source. Within the accuracy and precision of the instrument, the provided values were correct. The refractive index of *cis*-decalin was not found in the literature and was measured to be  $n_{\text{decalin}} = 1.4769 \pm 0.0005$  at 1064 nm and 23 °C. Tetralin, another fluid commonly used in the light samples, had a measured refractive index of  $1.5295 \pm 0.0003$ , also at 1064 nm and 23 °C. However, due to the effects of tetralin on the polymer microspheres (primarily absorption and swelling) [22], tweezer measurements of colloidal solutions containing tetralin were not performed at this time, but will be performed as this work progresses.

Control of the temperature of the sample should be mentioned. It is known that residual infrared light from normal halogen bulbs can affect the temperature of the sample even if filters are used due to the extremely high levels of flux present in the sample plane. Thus, the lowest possible level of light was used to visualize the sample, and a hot mirror

(Edmund Industrial Optics) was added to the optical train of the microscope to supplement the IR filters already present. Even so, when the light level was increased near its maximum value, flow was induced in the colloid, indicating temperature gradients. It must be emphasized that this did not occur when data were acquired.

#### *Trap strength measurements*

Because the trapping measurements were performed on dilute suspensions, the method used to calculate the trap strength followed that commonly found in the literature [14]. The objective lens used was a 100X Plan Apo NA = 1.4 Leica objective (Part No. 11506038) that was epi-illuminated by the tweezer beam. A particle was trapped in a static trap and the microscope stage (Märzhäuser) was translated at a known speed. If Stokes flow conditions are obeyed (spherical particle in an infinite viscous fluid) then the trap strength is proportional to the speed at which the particle “falls” out of the trap due to the drag force of the viscous fluid. Care must be taken to ensure that the trapped particle is far enough from the sample cell walls so that Stokes flow conditions are obtained and far enough from other particles so that any hydrodynamic sphere–sphere interactions do not affect the measurements. In practice, the trapped particles were at least 20 diameters from either wall and 10 diameters from any other particle. From [14], any error introduced by the walls or other spheres is then less than 1%. It should be noted that as the refractive indices of the microspheres and fluids approached each other, it became impossible to visualize the microspheres through the microscope eyepiece, and images

from a camera (QImaging Retiga 1300) were used to allow visualization of a trapped particle.

## Results

Figure 1 presents applied force measurements on polystyrene spheres of various radii in a fixed-index fluid ( $n_{\text{fluid}} = 1.497$ ). The fit curve represents the fraction of the trap beam that is contained within the trapped particle. Assuming a plane wave at the back aperture of the microscope objective lens, the intensity in the focal plane at the sample is given by the Airy function

$$I(w) = \left[ \frac{2J_1(\alpha w)}{\alpha w} \right]^2 I_0, \quad (1)$$

where  $w$  is the radial coordinate,  $\alpha = kd$ , where  $k$  is the wavenumber  $2\pi/\lambda$  and  $d$  the diameter of the aperture. It should be noted that the radial coordinate  $w$  is actually an angle, specifically, the subtended angle as referenced to the exit pupil of the optical system. To convert from  $w$  to a conventional linear distance measured in an image plane requires a simple scale factor. The fraction of the total intensity contained within a cone of angle  $w$  is given by

$$L(w) = 1 - J_0^2(\alpha w) - J_1^2(\alpha w). \quad (2)$$

It is seen that the applied force corresponds well for an Airy disk with the first dark ring at  $1.1 \mu\text{m}$ , in agreement with previous measurements [1]. This result also agrees with the analysis carried out in [23,24]. It should be noted that discrepancies from this result is expected for particles much larger than the beam waist [24].

Figure 2 presents trap force measurements of  $1.8\text{-}\mu\text{m}$ -diameter polystyrene microspheres in a series of Cargille index fluids. As expected from Rayleigh-Gans scattering results [25], the applied force goes as the index mismatch squared. The extrapolated index of refraction of the microspheres, taken at the value of an applied force equal to zero, is  $n = 1.579 \pm 0.005$  at  $1064 \text{ nm}$  and  $23^\circ\text{C}$ , in reasonable agreement with a previously quoted value of  $n = 1.569$  for the bulk material [18]. Given that the microspheres are not pure PS, the discrepancy is not surprising. Note that the data point from Fig. 1 has not been included for clarity.

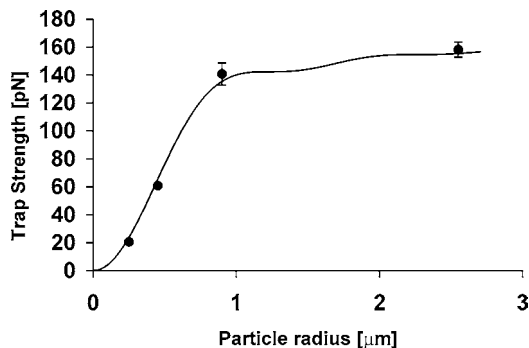


Fig. 1. Variation of applied trapping force with particle radius.

Force measurements on  $0.924\text{-}\mu\text{m}$  diameter PMMA/PHSA particles in a series of Cargille refractive index liquids indicate that the refractive index of these particles is  $n = 1.481 \pm 0.005$  at  $1064 \text{ nm}$  and  $23^\circ\text{C}$ . Again, this corresponds reasonably well with previous measurements [18]. Note that it was substantially more difficult to perform this measurement at nearly index-matched conditions due to the small particle size. Furthermore, the increasing mismatch between the immersion oil and the index fluid as the fluid index decreases causes significant complications in trap geometry (see for example [26]) and the concomitant increase in measurement error. It is possible to determine the role, if any, of the steric coating on the microspheres by performing this measurement for various particle sizes. That is, because the steric coating thickness (approximately  $10\text{--}12 \text{ nm}$ ) is constant, varying the PMMA sphere diameter could measurably change the apparent refractive index. This measurement was not performed at this time.

Figure 3 presents optical trap strength for both the PS particles and PHSA/PMMA particles collapsed onto one curve. The trap strength has been corrected for differences in particle size as per Fig. 1, and it is seen that the applied

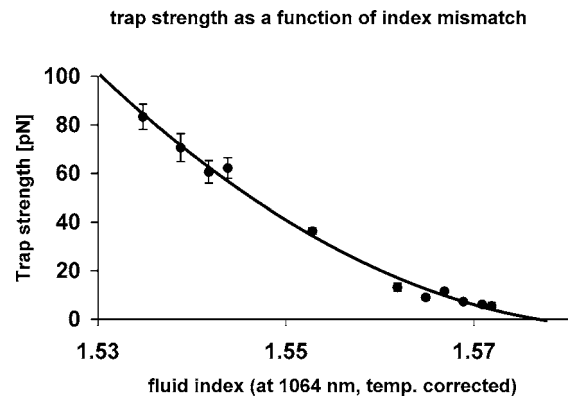


Fig. 2. Variation of applied trapping force with index mismatch ( $1.8\text{-}\mu\text{m}$  PS in index fluids).

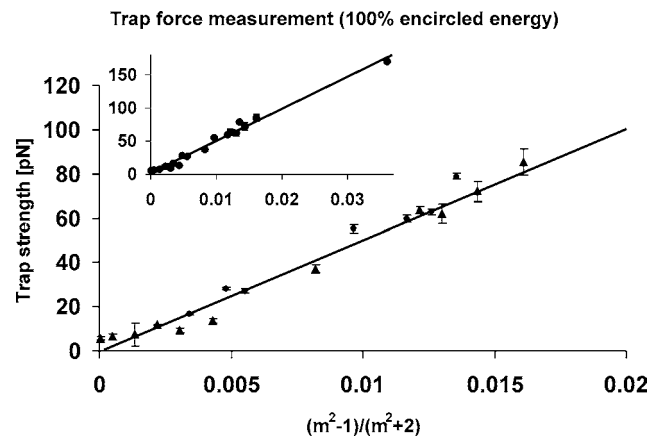


Fig. 3. Variation of applied trapping force with index mismatch (PS and PHSA/PMMA microspheres in index fluids). Inset: same, with extended range of mismatch. Circles are PHSA/PMMA and triangles are PS.

force scales linearly with  $(m^2 - 1)/(m^2 + 2)$ , where  $m = n_{\text{particle}}/n_{\text{fluid}}$ . This result agrees with [23]. The inset graph includes the data point from Fig. 1 ( $(m^2 - 1)/(m^2 + 2) = 0.036$ ) for clarity.

As a check on the above method, using the above measured value of the refractive index of the PHSA/PMMA microspheres, a trap strength was calculated for PHSA/PMMA microspheres in *cis*-decalin and the viscosity calculated by  $\eta = F/6\pi a v$ , where the trap force  $F$  is calculated from the particle size and index mismatch, while  $v$  is measured directly. We obtain a viscosity  $\eta = 2.80 \pm 0.38$  cP at 23 °C, which is in excellent agreement with published data [27] stating  $\eta = 3.04$  cP at 25 °C.

Finally, it should be noted that none of the sample systems strictly met the requirements for a “standard” as set forth in Section 2. Potential drift in the sample properties over time was mitigated by performing measurements on freshly made samples only. For the flight experiment, it is assumed that the samples will have reached equilibrium and that some of the (equilibrated) sample material will be retained for use on the ground.

## Summary

A comprehensive characterization of an optical tweezer apparatus for use in space-borne colloidal investigations has been performed. It has been shown that the refractive indices of materials used for hard-sphere colloidal suspensions at the tweezer wavelength are sufficiently different from previously quoted values to warrant attention when working with suspensions that are highly index matched. In addition, the refractive indices of polymer microspheres are substantially different from the bulk values, a fact that has significant implications when interpreting trap force data. It has also been shown that the applied trapping force for this apparatus can be accurately calculated knowing only the refractive index mismatch and the colloidal particle size, which greatly simplifies the use of the instrument. In addition, it is hoped these results allow proper interpretation of measurements when dense or complex colloidal suspensions are probed.

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