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***published in***
Review of Scientific Instruments
2008

***DOI (link to publisher)***
10.1063/1.2898407

***document version***
Publisher's PDF, also known as Version of record

# Twin optical traps for two-particle cross-correlation measurements: Eliminating cross-talk

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(Received 17 February 2008; accepted 25 February 2008; published online 18 April 2008)

The correlated motions of two micron-sized particles reflect the (micro-) rheological properties of a fluid and can be conveniently detected using two optical traps in combination with interferometric displacement detection. When the correlations become small, cross-talk between the two beams becomes important. We have used dual optical traps created by either two orthogonally polarized laser beams derived from one laser source, or by two independent lasers of different wavelengths for microrheology experiments. High numerical aperture lenses (objective and condenser) in the optical path can introduce depolarization, and polarizing beam splitters are not perfect, both of which can lead to optical cross-talk. We have characterized the cross-talk in our setup and demonstrate that the use of two independent laser eliminates cross-talk entirely. © *2008 American Institute of Physics.*
[DOI: 10.1063/1.2898407]

## I. INTRODUCTION

A tightly focused laser beam can trap a dielectric object in a three-dimensional "potential well" if the size of the object is on the order of the laser wavelength. Laser-based optical trapping was first introduced by Ashkin and coworkers[1] and is now used in a large range of applications from atomic physics to medicine. Optical traps (or optical tweezers) are usually built into a light microscope with a high numerical aperture (NA) objective. Possible trapping forces on micron-sized objects range from fractions of piconewtons to several hundred piconewtons, and the displacement of the trapped particle can be measured with subnanometer precisions by laser interferometry.[2–4]

High-precision position detection by lasers with and without optical trapping is, among many other applications, used to study the mechanical properties of motor proteins,[5–7] of single biopolymers[8,9] or to measure the viscoelastic properties of complex fluids in microliter volumes and with high bandwidth in so-called microrheology techniques.[10,11] Passive microrheology is a commonly used variant of microrheology based on fast and accurate tracking of Brownian particles. The motions are tracked by laser interferometry, and auto- or cross-correlations of position fluctuations of one (one-particle microrheology) or two colloidal particle(s) (two-particle microrheology) are calculated. Viscoelastic properties, e.g., shear elastic moduli can then be derived from the single-particle or interparticle response functions which are related to the position fluctuations via the fluctuation-dissipation theorem.[12–16]

A variety of optical trapping experiments are performed using not just one, but a pair of traps, which hold a pair of colloidal particles at a defined distance. These include so-called three-bead assays in molecular motor experiments,[5–7] two-particle microrheology experiments,[17–21] or experiments measuring hydrodynamic correlations in fluids or gels.[15,17,22] The position fluctuations of both particles are in that case typically simultaneously detected using quadrant photodiodes (QPDs).[4,23,24] Two optical traps are commonly created using one linearly polarized laser beam, which is split into two orthogonally polarized and independently steered beams using a polarizing beam splitter.[13,23,25,26] Typically, the laser creating the traps is also used for position detection with QPDs.

Using polarization to split a single beam into two, creates a potential cross-talk problem, which can become relevant when very weak cross-correlations need to be detected (as typically in two-particle microrheology). Polarized light is partially depolarized when passing as a collimated beam through a highly curved surface or as a divergent or convergent beam through a flat surface.[27,28] When optical traps are generated by high-NA lenses, a portion of the light will thus be depolarized both at the internal lens surfaces and at the coverslip surfaces. The measured depolarization of the electric field in a high aperture objective lens (NA=1.4, oil immersion) can be substantial, up to 10% of the integrated focal intensity.[29,30] Likewise, polarizing beam splitters are never perfect in separating the two orthogonal polarizations. Beam-splitting cubes which are frequently used can be particularly bad, with specified polarization ratios of only $\sim 10^{-2}$ in the deflected beam and $10^{-3}$ in the beam passing through. Glan-Laser and Glan–Thompson polarizers are better ($10^{-4}$–$10^{-5}$ polarization ratio in both beams), but have to be well aligned and cannot cope with noncollimated beams. The depolarization introduces cross-talk in the position (force) detection when the same laser light is used for trapping and detection. In the applications mentioned, one typically is interested in real correlations between the position fluctuations

 

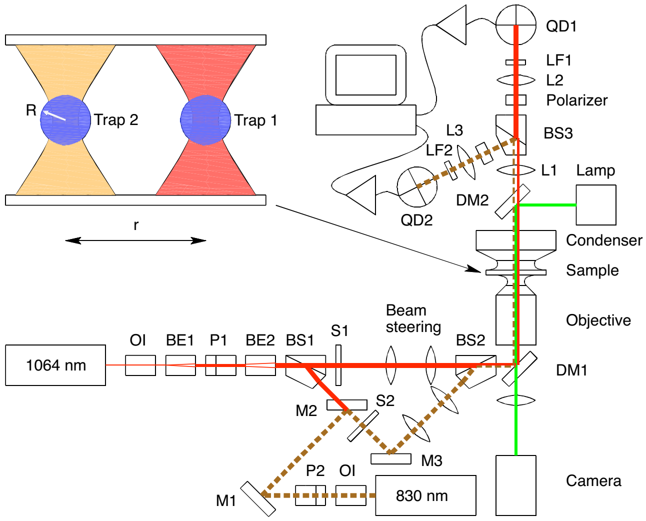


FIG. 1. (Color online) Sketch of the experimental setup, in the two alternative configurations, as described in the text. (i) One laser (1064 nm) is used and split into two beams with orthogonal polarizations: beam 1 (solid lines, trap 1) and beam 2 (dotted lines, trap 2). (ii) Two independent lasers are used by flipping up mirror M2: 1064 nm (solid lines, trap 1) and 830 nm (broken lines, trap 2).

of the two beads and the cross-talk thus introduces an error that becomes the more important, the weaker the real correlations become.

We have used here a dual optical trap setup made with a 1064 nm wavelength laser which we mainly use to perform one- and two-particle microrheologies with high bandwidth (up to 195 kHz sampling frequency).[16,17,21] Cross-talk in this setup initially became a problem since hydrodynamic correlations between two particles decay rapidly with particle distance, and cross-talk even on the scale of a few% is a hindrance.

We explain here the technical construction of the setup used for one- and two-particle microrheology measurements. We quantify the cross-talk and present a method to reduce it. Finally, we describe an alternative method eliminating cross-talk altogether, using a second laser.

## II. EXPERIMENTAL SETUP

### A. Twin optical traps with one laser

Figure 1 shows a sketch of our custom-built inverted microscope[13,31] which is equipped with two optical traps. We use a linearly polarized near infrared laser (ND:$YV0_4$ cw, $\lambda=1064$ nm, maximum power=4 W, Compass, Coherent Inc., Santa Clara, CA), protected against back reflections to enhance stability with an optical isolator (OI) (37 dB isolation, Optics for Research, Caldwell, NJ). A 3× beam expander (BE1) (CVI Laser Corp., Albuquerque, NM) is used to increase the beam diameter to ≈3.9 mm and to extend the Rayleigh range. The laser is usually operated at a constant power of about 2 W for maximum stability. A combination of $\lambda/2$ plate and polarizer (P1 in Fig. 1) is used to adjust the power. The laser beam is then split into two orthogonally polarized beams by a Glan–Thompson polarizing beam splitter (BS1) (all beam splitters from Zeta International Corp., Mount Prospect, IL). The ratio of powers in the two beams depends on the orientation of the polarizer in P1 relative to the beam splitter and can thus be altered when necessary. In the experiments described here, the polarizer in P1 was mostly set at an angle of 45° with respect to the vertical and therefore both traps had close to equal strength. Two shutters S1 and S2 are placed in the laser paths on the optical table, which allows us to switch on and off each of the traps independently. In this one-laser configuration, mirror M2 is flipped down, so that the deflected laser beam reaches mirror M3 and is directed toward the second Glan–Thompson beam splitter BS2 that recombines the two beams. The lateral and axial positions of both laser foci in the specimen plane are independently adjustable using two pairs of telescope lenses ($f=80$ mm). The second telescope lens images the point about which the beam is bent by moving the first lens into the back-focal plane of the objective. This guarantees that the intensity distribution in the back focal plane of the objective and all conjugated planes including the detector plane remains unchanged when the traps are moved in the specimen plane.

A dichroic mirror (DM1) (590DCLPxt, Chroma Corp., Rockingham, VT) is placed below an oil-immersion infinity-corrected objective (100×, 1.3NA; Neofluar, Carl Zeiss, AG, Jena, Germany) and couples the beams into the microscope imaging beam path which is perpendicular to the optical table. After passing through the sample, the laser light is collected by an oil-immersion condenser (1.4NA, Zeiss) and passes through a second dichroic mirror (DM2) (740DCSPxr, Chroma Corp.) toward the detectors. The light from the two perpendicularly polarized traps is separated by BS3, providing independent and simultaneous position detection for both traps. A further lens $L1$ ($f=50$ mm) is placed below the polarizing beam splitter BS3 to collimate the divergent beams and to, in combination with lenses L2 and L3, image the condenser back focal plane onto the quadrant photodiodes (QD1 and QD2). QD1 is a specialized silicon *p-i-n* quadrant photodiode operated under a reverse bias voltage of 100 V (10 mm diameter, YAGG444-4A, Perkin–Elmer, Vaudreuil, Canada) to guarantee fast detection at 1064 nm (Ref. 32) and QD2 is standard silicon *p-i-n* photodiode, operated under a reverse bias voltage of 15 V (10 mm diameter, Spot9-DMI, UDT, Hawthorne, CA). Laser line filters LF1 and LF2 (D1064/10, Chroma Corp.) are placed in front of both detectors to block room light. Lenses L2 and L3 are also used to center the beams on the detectors.

For imaging the samples, we use the microscope in differential interference contrast mode, with Köhler illumination[33] using a fiber coupled 100 W mercury arc lamp (546 nm line). The illumination light is coupled into the condenser via DM2 (740DCSPxr, Chroma Corp.), which transmits the laser light, but reflects the illumination light. Images are recorded by an Ultricon tube camera (model VT1000, Dage-MTI, Michigan City, IN). Focusing in the sample is controlled by a dc motor which moves both objective and condenser with respect to the fixed sample. The sample is mounted on a three-axis piezostage (Nano-LP-100, MAD CITY LABS Inc., Madison, WI), with 0.7 nm precision.

The signals from the quadrant diodes are converted to voltages and amplified by low-noise preamplifiers[13,31] and combined by analog electronics to obtain signals proportional to the $x$ and $y$ positions of the trapped particles with respect to the centers of the traps in the plane normal to the optical axes. The four displacement signals are finally sampled via an A/D converter (200 kHz, ChicoPlus board with AD16 A/D module, Innovative Integration, Simi Valley, CA). The digitized data are processed by a custom-written LABVIEW (National Instruments, Austin, TX) data acquisition program. Every set of data in the experiments reported here was recorded at the chosen sampling frequency to a length of about $8.5 \times 10^6$ data points ($>2^{23}$), and the recorded position fluctuation data were processed off-line. Both traps were calibrated using the power spectral method,[34] using bead diameter and solvent viscosity as input parameters to calculate a conversion factor to actual displacement from the high-frequency Brownian bead fluctuations in water.

### B. Twin optical traps with two independent lasers

In this alternative configuration, two different laser wavelengths are used to create the two optical traps. The first laser is the near-infrared 1064 nm laser described above. The second laser (broken line in Fig. 1) has a wavelength $\lambda = 830$ nm (diode laser, cw, maximum power 140 mW, IQ1C140, Laser 2000, BeNeLux C. V., Vinkeveen, Netherlands). It is coupled into the path of the deflected 1064 nm beam by mirror M2 (dotted line in Fig. 1).

The choice of wavelength ($\lambda = 830$ nm) avoids the need for a specialized infrared detector. The rest of both optical paths is identical to those in the single-laser configuration. The 830 nm laser is polarized vertically to the table, as was the deflected 1064 nm beam in the other configuration. As for the 1064 nm laser, an optical isolator (IO-3-820-LP narrow adjustable Isolator, 760–860 nm, Optics for Research, Caldwell, NJ) is used in front of the 830 nm laser. Power is adjusted with a combination of $\lambda/2$ plate and polarizer (P2). LF2 is in this configuration replaced by a 830 nm laser line filter (D830/10, Chroma Corp.).

In this two-laser configuration, the laser intensities and the trap strengths of the two optical traps are entirely independent. QD1 measures through a narrow-band laser line filter the 1064 nm signal from trap 1, and QD2 measures through a narrow-band laser line filter the 830 nm signal from trap 2. Signals are processed and data are recorded to hard disk, as described above. Both traps are calibrated separately for each of the wavelengths as described above.

## III. SAMPLE PREPARATION

We used pure water as a simple fluid (viscosity $\eta = 0.969$ mPa at 21.4 °C). Spherical silica particles (radius $R = 0.58$ $\mu$m, Van't Hoff Laboratory, Utrecht University, Utrecht, Netherlands) were added to 20 $\mu$l of water at a final dilution of $10^{-5}$ weight/volume. Sample chambers were made of a coverslip attached to a microscope slide with two narrow strips of double-stick tape, giving an inner chamber height of $\sim 70$ $\mu$m. In order to avoid an increase of effective friction due to the vicinity of the glass surfaces, particles were trapped near the surface and then moved to a distance of $\sim 20$–$40$ $\mu$m above the bottom surface (coverslip). The laboratory temperature was stabilized to $21.4 \pm 1$ °C.

## IV. RESULTS

Cross-talk measurements were first performed in the one-laser setup, using orthogonally polarized beams from the 1064 nm laser. For detection, the two beams were separated by the polarizing beam splitter BS3. Leakage through the beam splitters and depolarization in the microscope path as well as misalignment of the polarizers can, in principle, generate cross-talk, i.e., can lead to light from one trap ending up on the detector belonging to the other trap. Depolarization of light by the trapped particle itself can cause further cross-talk. We label the leakage of light from trap 1 into detector 2 as the cross-talk from 1 to 2 and vise versa. Cross-talk can be characterized in different ways. We first measured the relative integrated leakage power in the respective "wrong" channels without any trapped particles in either trap and with the main beam of the measured channel blocked before the microscope. This can be done with a calibrated power meter in the place of the detectors or from the average current measured by the QDs themselves, which is linearly proportional to the laser intensity. Here, we have used the latter method. We used two different types of QDs, as mentioned before, and we controlled that the sensitivity of both detectors were the same.

Cross-talk from 2 to 1 is measured when light with polarization vertical to the optical table (trap 2) is on (shutter S2 open) and the other laser beam with polarization horizontal to the optical table (trap 1) is off (shutter S1 closed). The average voltage read by QD2 is proportional to the trapping light intensity, and the voltage read by QD1 is proportional to the leakage due to incomplete polarization or due to depolarization of beam 2. The light thus measured on QD1 can come both from trap 1 and from trap 2. Only the part passing through trap 2 will be contributing to actual cross-talk between displacement signals of trapped particles. Similarly, the cross-talk from 1 to 2 is measured when S1 is open and S2 is closed. The measured amounts of cross-talk are listed in Table I as ratios of the averaged voltage signals read in the respective detectors. Changing the incoming laser intensity did not affect the relative amount of measured cross-talk. An average result for seven different laser intensities is presented in column 2 of Table I. Cross-talk from 1 to 2 was 1.9% and from 2 to 1 was 7.6%. The asymmetry of the measured cross-talk points to leakage in BS3, in the sense that light with the polarization that should be totally internally reflected off the internal interface of the splitter is not entirely prevented from passing straight through the splitter. This is likely due to the fact that the laser light can, in practice, not be perfectly collimated going through BS3. A similar problem does not exist for the deflected beam, i.e., there is no reason for the wrong polarization to be deflected out sideways, hence the asymmetry. In these measurements the laser beam diameter, quantified as full width at half maximum intensity, was measured with a beam profiler (WinCam-PCII, Melles Griot, Carlsbad, CA) to be 3.9 mm right before the objective. This is smaller than the back aperture of the objective of 4.2 mm. Therefore, the back aperture was not overfilled. When the back aperture was overfilled by using an additional 3× beam expander BE2, placed

TABLE I. Comparison of measured cross-talk from trap 1 to 2 and from trap 2 to 1 in the different configurations used. For the one-laser setup, cross-talk is expressed as the ratio of total light intensity measured by the detector corresponding to the dark trap, for which the laser beam is blocked before entering the microscope, and the total light intensity measured by the illuminated detector (no bead trapped). Twin traps were either made with one laser (1064 nm) and orthogonal polarizations, with a laser beam underfilling the objective back focal plane or with an expanded beam overfilling the objective, using additional polarizers after BS3, or twin traps are generated with two separate lasers of wavelengths of 1064 and 830 nm.

| Cross-talk | One laser underfilled objective | One laser overfilled objective | One laser with additional polarizers | Two lasers (V) | Detector dark noise (V) |
|---|---|---|---|---|---|
| 1 to 2 | 1.9% ±0.2% | 1.6% | 1.5% | 0.009 | 0.009 |
| 2 to 1 | 7.6% ±0.2% | 10.6% | 1.1% | 0.017 | 0.017 |

after the $\lambda/2$ plate and polarizer in the 1064 nm laser path (in Fig. 1), the cross-talk from 2 to 1 increased as it is shown in column 3 of Table I.

A simple way to clean up leakage in BS3 is to use two additional prism polarizers right after BS3, before the lenses L2 and L3, which focus the beam again. This modification reduced the cross-talk and also made it symmetric. The results of integrated intensity measurements as described above are listed in column 4 of Table I. The remaining ~1% cross-talk can still introduce artifacts in cross-correlation measurements, in particular, when weak correlations are studied between relatively far separated particles.

To also eliminate this remaining cross-talk in the instrument, we used two independent lasers of different colors as explained above to construct the twin traps. The control measurements of cross-talk signals, again measured as integrated intensities, in this configuration are presented in column 5 of Table I. In this case, the absolute voltage reading is given measured with maximal laser powers in the respective other beams (10 V for highest power) and compared to the dark noise signal from the detector and amplifier produced without any light on the detector. As can be seen, there was no detectable cross-talk. The dark noise level (column 6) was subtracted from all voltages before calculating ratios in columns 2–4.

Depolarization of the polarized laser beam is expected either when a collimated beam hits a strongly curved interface or equivalently when a strongly convergent or divergent beam hits a flat interface between media with different indices of refraction. To examine what portion of the cross-talk is due to the depolarization from the glass-water-glass interfaces in the sample chamber, we replaced the water in the sample chamber by index-matching oil. The amount of cross-talk measured with this sample chamber was slightly lower than with a water-filled chamber: cross-talk from 1 to 2 was 1.5% ±0.1% and cross talk from 2 to 1 was 6% ±0.2%. This was not a major contribution, suggesting that the main effect was not due to the interfaces.

Furthermore, we tested for additional depolarization by the trapped particles with laser powers of ~20 mW (measured after shutters S1 and S2 in the laser path) with both laser beams on. We did not observe any additional contribution from trapping particles to the cross-talk.

Cross-talk between the two traps shows up in the data as a dynamic signal from the particle trapped in one beam in the other detection channel. To demonstrate this effect, we calculated power spectral densities (PSDs) from the displacement signals recorded from both detectors while only one trap was occupied by a bead, i.e., one particle is trapped in one of the laser foci (e.g., trap 1), while the other trap (e.g., trap 2) is on and empty, and vice versa. The cross-talk signal adds to the noise spectrum that one obtains in the respective detector channel with the laser of this trap switched on, but without a trapped particle in it, and with the other laser beam blocked. The PSDs were calculated as the Fourier transforms of the autocorrelations of the measured voltage fluctuations after converting these to (apparent) displacements $u(t)$ by multiplying with the appropriate calibration factors: $S(\omega) = \int\langle u(t)u(0)\rangle e^{i\omega t}dt$, where $\omega = 2\pi f$ is the radial frequency.

The results are plotted in Fig. 2. No additional polarizers were used after BS3. The PSDs of a trapped particle in water are, apart from noise contributions, expected to be Lorentzians.[34] Noise that adds to the Lorentzians is predominantly $1/f$ noise from laser beam pointing fluctuations at low frequencies and (white) shot noise at high frequencies. Figure 2 shows that cross-talk from the loaded trap adds an extra component to the PSD of the empty trap consisting of an attenuated version of the Lorentzian characterizing the fluctuations of the bead in the occupied trap. The use of additional polarizers after BS3 reduced the cross-talk in this case so that there was no more Lorentzian discernible in the

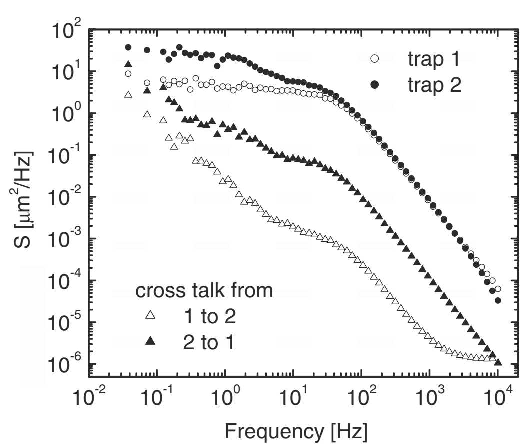


FIG. 2. Power spectral densities of displacement fluctuations of particles (0.58 $\mu$m radius silica beads) held in trap 1 or trap 2, respectively, (circles) when twin optical traps were made from orthogonally polarized light derived from one laser. Apparent displacement power spectral densities from the respective empty trap reflecting noise and cross-talk from the filled trap are plotted as triangles. Laser powers (~20 mW) corresponding to trap stiffnesses of ~0.9 $\mu$N/m were equal in traps 1 and 2. The sampling frequency was 20 kHz. Without additional polarizers after BS3, cross-talk was not symmetric as also observed in the integrated intensities (Table I).

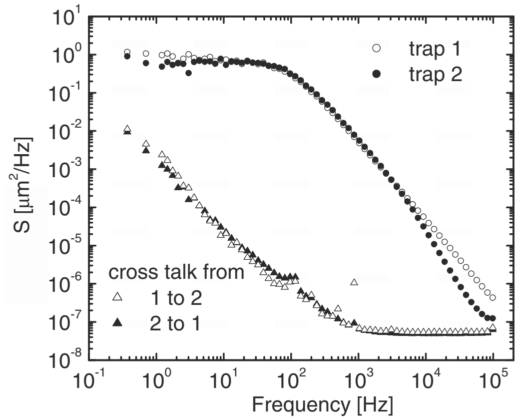


FIG. 3. Power spectral densities of displacement fluctuations of particles (0.58 $\mu$m radius silica beads) trapped in trap 1 or trap 2 (circles) when twin optical traps were made from orthogonally polarized light derived from one laser, but with additional polarizers after BS3. Apparent displacement power spectral densities from the respective empty trap reflecting noise and cross-talk from the filled trap are plotted as triangles. Laser powers were (~26 mW) corresponding to trap stiffnesses of 17$\mu$ N/m in traps 1 and trap 2. The sampling frequency was 195 kHz. The high-frequency additional decay in the apparent displacement power spectral density in trap 2 is due to the slow response of the standard QPD used for that trap at 1064 nm (Ref. 32).

spectra of the empty, trap as shown in Fig. 3. Using the two independent lasers of different colors, likewise eliminated the cross-talk as shown in Fig. 4.

In summary, we have shown that cross-talk on the order of a few percent can occur in twin optical trap/twin detector experiments. If this level of cross-talk is detrimental, for example, for the measurement of weak hydrodynamic or elastic interactions between trapped particles, one needs to be extremely careful in aligning the optics and eliminating the cross-talk by using additional polarizers. Alternatively, and much more simply, one can use two independent lasers with different wavelengths to build the twin optical trap. This approach entirely eliminates cross-talk when the appropriate laser line filters are used.

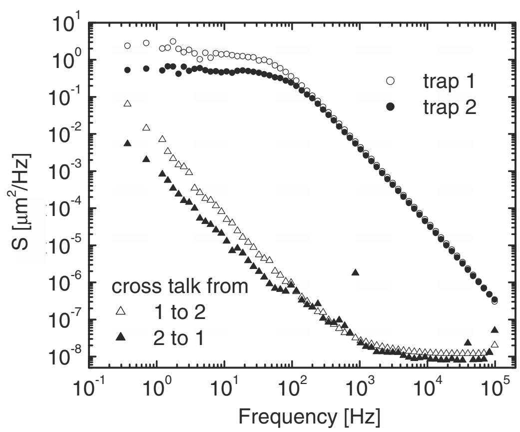


FIG. 4. Power spectral densities of displacement fluctuations of particles (0.58 $\mu$m radius silica beads) held in trap 1 or trap 2, respectively, (circles) when twin optical traps were made from two independent lasers (830 and 1064 nm). Apparent displacement power spectral densities from the respective empty trap reflecting noise and cross-talk from the filled trap are plotted as triangles. Laser powers were (~18 mW) corresponding to a trap stiffness of 1.1 $\mu$N/m in trap 1 and (~30 mW) corresponding to a trap stiffness of 1.8 $\mu$N/m, in trap 2. The sampling frequency was 195 kHz.

## ACKNOWLEDGMENTS

We thank Jens-Christian Meiners, Daisuke Mizuno, and Erwin Peterman for helpful discussions, Joost van Mameren, Frederick Gittes, and Mark Buchanan for help with data-evaluation software. This work was supported by the Foundation for Fundamental Research on Matter (FOM).

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