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A comprehensive software suite for optical trapping and manipulation

Daryl Preece, Richard Bowman, Graham Gibson and Miles Padgett

University of Glasgow, Department of Physics & Astronomy, University of Glasgow, Glasgow
G12 8QQ, United Kingdom

ABSTRACT

We present a comprehensive laser tweezers software package, comprising the software components for any laser tweezers system. This includes fast hologram generation software, implemented on a graphics card, thereby allowing 100 hundred independently moving traps at video frame rate. The software also includes comprehensive camera suite and image recognition software for multi-particle tracking and analysis. The software is freely available from the authors and online(http://www.physics.gla.ac.uk/Optics/).

Keywords: Optical trapping, Spatial light modulator, Software, OpenGL

1. INTRODUCTION

Optical tweezers are a way by which small micron sized dielectric particles may be trapped and manipulated.[1] A focused laser beam is used to create an optical gradient force which pulls dielectric particles in towards the focus of the beam. Most often this focus is created by the use of a microscope objective which may also be used for imaging the particle. Holographic optical tweezers extend this idea by the use of a spatial light modulator (SLM) which allows the user to impart arbitrary phase characteristics to a beam, dynamically with a computer.[2] Thus the SLM is used as a diffractive optical element capable of creating multiple spatially controllable diffracted beams serving as optical traps. However more often than not, optical tweezers systems have been confined to an optics laboratory although there are wide spread applications,[3] rather than being used in other disciplines where they be useful such as cell biology or rheology.[4]

In this paper we seek to present easy to use software for both hologram generation and particle tracking which will allow the non-specialist user to effectively use a holographic optical tweezers system without the complex and time consuming task of designing software.

The system was initially developed for use in a holographic optical tweezers setup with a fast camera (Prosilica GC640) and spatial light modulator(Boulder non linear systems), however the goal is to create a usable software package suitable for a variety of tweezers architectures which may be employed as a first step in creating a tweezers system. To this end the software has been developed in a modular fashion. Each module may be used separately or in combination with the others. Hence the user may pick and choose as to what elements of the system may be appropriate to the experiment they wish to carry out. We have divided the system into three parts. Camera software for use with Ethernet and IEEE1394/firewire cameras, software for use with DVI/SVGA based SLMs hologram design and trap control, Analysis software to analyse particle trajectories on the fly . All the software is written within the LabVIEW programming environment. A further goal was to develop a system which performed all processing completely online and did not rely on any post processing. The combination of fast camera and analysis techniques with the ability to generate multiple optical traps at very high speed provides the opportunities for many interesting experiments. The software was applied in a position clamping experiment. Many applications of optical tweezers require a force or position stabilised bead[5]this is usually done with an active feedback often from the combination of a photo-diode and AOM or fast mirror device[6].[7] Many such devices work with a bandwidth well into the 10's of kHz range (currently far higher than that of an SLM). However these devices lack the ability to work with many optical traps and may only be used in 2 dimensions.[8]

Further author information: (Send correspondence to: Department of Physics & Astronomy Kelvin Building University of Glasgow Glasgow G12 8QQ Scotland United Kingdom)
E-mail: d.preece@physics.gla.ac.uk, Telephone: +44 (0)141 334 6432, Web:http://www.physics.gla.ac.uk/Optics/

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2. CAMERA SOFTWARE

At the most basic level the job of the camera software is to take in an image from the camera, analyse that image and export a set of co-ordinates which corresponds to the centres of the particles within that image for further processing.[9] Firstly, data must be grabbed from the camera. Completing this operation successfully requires a number of practical considerations be taken into account. Since in this context of this paper we are dealing with a fast Ethernet or Firewire camera, we must fully utilise the camera's high speed capabilities (in the case of a prosilica GC640 Gigabit Ethernet camera the frame-rate is up to 2000 FPS). Therefore, careful design of all software associated with the camera is required such that the camera may run uninterrupted as much as much as possible and at a constant frame rate.[10]

Once extracted, images must be analysed as quickly as possible. Since all camera software was mandated to run in real time the camera must output co-ordinates in less than a millisecond. thus,the image processing proves both a technical and a mathematical challenge. Many algorithms are available for object recognition. However due to the time taken for them to process only a small subset of these are useful for real time processing. Though it is possible to save image directly to disk within the software for later processing this operation require large amounts of disk space and may also slow the speed of acquisition.

Most fast particle tracking techniques rely on some type of invariance in the characteristics of the image to be processed in order to reduce the search space. This may be spatial symmetry, constant morphology, rotational invariance, spatial invariance, size invariance or colour invariance. Polystyrene or silica beads in this context are therefore easily identifiable being both rotationally invariant and having constant shape and size. Furthermore they often have easily definable intensity characteristics. Tracking cells is far more difficult due to the variable morphology both between species and among cells of the same type.

Several different imaging algorithms were implemented within the software and are as follows:

Centre of mass(COM)[11]first thresholds and then centroids each particle. Using the formula

$$xcentroid = \sum(xV)/\sum(V); ycentroid = \sum(yV)/\sum(V) \qquad (1)$$

Where V is the pixel value and is regarded as a 8bit number (0-255). x and y are the distances in pixels from the top left hand corner of the image. This algorithm is assumes the image of the particle is slightly defocused such that a the intensity at the centre of the particle is higher than the surrounding media. It has been found to work extremely well for silica beads or polystyrene as long as the illumination is correct.

Dark COM uses the COM algorithm as above however with inverted intensity values. This is particularly useful when the illumination is such that it is easier to see the edges of particles than the bright spot in the centre or when the particle is defocused so as to give a dark central spot.

Fourier truncated COM is used as, often due to aberration or poor alignment, camera images may have intensity gradients across the image. Further more in images with low light levels pixel noise may become a problem when gauging intensity characteristics. By truncating the Fourier transform (FT) of the image and then performing an inverse Fourier transform both of the aforementioned problems may be reduced. The truncated image then undergoes the same COM operation as before. However since the FT is dependent on the number of pixels in the image, the result is dependent on the size of the image being processed meaning that it is predominantly useful for large regions of interest.

Cross correlation relies on an image template being taken of an ideal particle. The algorithm then compares the template with the acquired image and performs a 2D cross correlation. This technique is quite effective at giving a good estimate of the position of more complex particles such as cells however it is quite computationally intensive particularly when a large search image is used. This makes it suitable for a real time environment when a low frame rate <500 Hz or a small region of interest is used.

Circle Recognition uses a native LabVIEW Sub VI (Imaq detect circles) to search for the presence of circular shapes within the image. This technique gives a good estimation of the position of beads particularly if the image contains concentric rings.

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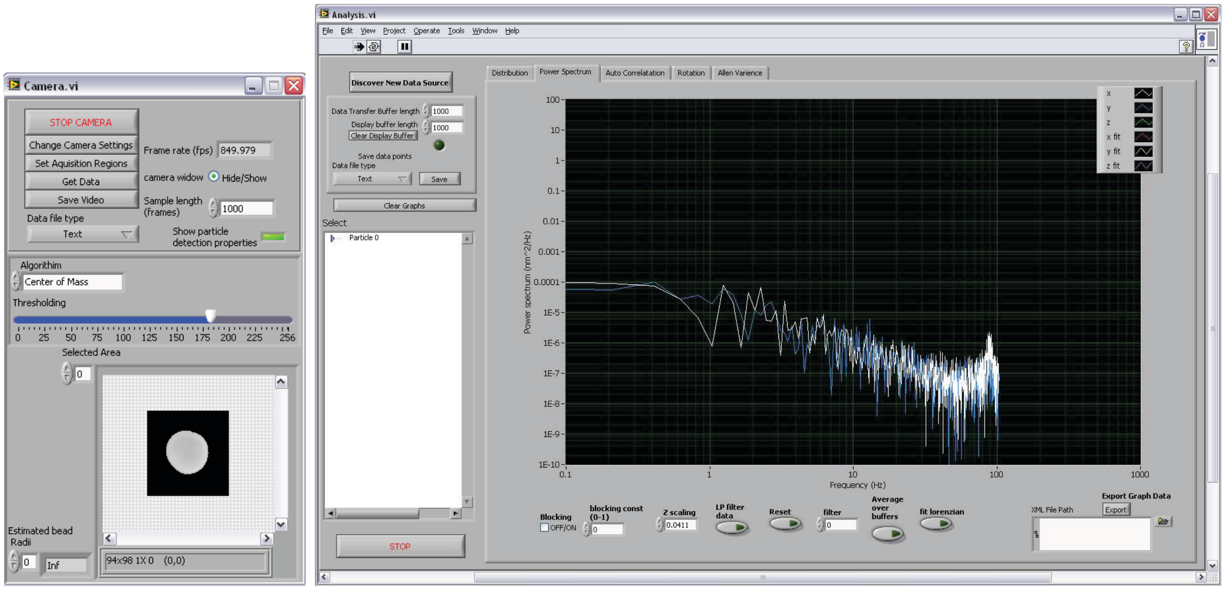
Frame rate (fps) 849.979

camera widow ⊙ Hide/Show

Sample length (frames) 1000

Data file type Text

Algorithim Center of Mass

Selected Area 0

Estimated bead Radii 0 Inf

94x98 1X 0 (0,0)

Data Transfer Buffer length 1000

Display buffer length 1000

Data file type Text

Particle 0

Blocking ☐ OFF/ON

blocking const (0-1) 0

Z scaling 0.0411

filter 0

Figure 1. Figure showing screen shots of the camera and analysis software. Camera software is shown using the centre of mass to tracka a bead while While analysis software displays the power spectra of a stuck bead.

Radius estimation uses the Dark COM to invert the image and apply a threshold. It then extends lines from the centre of the image and via edge detection, estimates the radius of the circle and the position of the centre of the particle.

The above algorithms provide x -y plane positional data. However z data may also be achieved by looking at the defocus of the particle in the microscope objective. Currently this is done by calculating the ratio between pixels above and below threshold value of the algorithm in question. However this method only gives an estimate of the exact displacement.

Once the particle's position has been ascertained the data must be fed out to other applications. A number of options exist within the software for this operation. In an experimental setting it is often very useful to write the data directly to disk. In a multi-core machine this can be done in parallel with other processes and hence there is little disruption to the rest of the camera software. Furthermore, the data is saved so that the often more computationally expensive analysis of the data may take place at another time. Another way to output the data is via a queueing operation. This is native to LabVIEW and easily integrable into other VIs it also solves the problem of the disparity between the rate at which the data is read out from the camera VI and read into another VI (which may operate with different timing). UDP data transfer provides a way in which to transfer data across a network. This means that data may be transferred between machines freeing up processing power on both machines and making it easy to monitor data from a remote location.

3. POSITION ANALYSIS

There are a great number of useful mathematical techniques for the evaluation of data in optical tweezing experiments. In the analysis module some of the most common have been implemented to try and speed up the processing of data and allow the user to get some real time feedback on the progress of an experiment. The analysis module also incorporates some of the data transfer methods outlined above which allows real time use with the camera as well as off-line use with saved data. Several common metrics have been implemented such as position histograms to give information about the position of the particle, power spectra and autocorrelation for the frequency characteristics of the particle[12]and its mean squared displacement. Cross-Correlation between X and Y data reveals rotational movement and Allan variance gives information about the time evolution of the data. Clearly it is not possible to anticipate the all the mathematical operations that each user will wish to

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perform however the analysis module is intended only to provide a basic tool box for quick online analysis. The queueing operations mentioned above allows users to develop their own data analysis programs if necessary.

4. HOLOGRAM CONTROL SOFTWARE

Holographic optical tweezers use dynamic holograms to generate optical traps. Previously, a number of algorithms have been used to try and create holograms each with its own advantages and disadvantages. Iterative algorithms[13]for instance have been able maintain good homogeneity between traps however they tend to be computationally slower than other algorithms. In designing the hologram engine we focused on designing software which can be used in real time and which the user can operate with little technical knowledge.

We generate optical traps by applying an algorithm used by liesner[14].[15] The algorithm uses the complex sum of wedge and lens shaped phase holograms to create optical traps in the far field of the SLM. These traps are controlled positionally in three dimensions by refreshing the phase pattern displayed on the SLM. For one trap the phase on the SLM is :

$$\phi_i = k_x x + k_y y + k_z(x^2 + y^2) \tag{2}$$

Where ϕ_i is the phase value on the SLM between 0 and 2π for a single spot. We then add the complex holograms for each spot, and the final phase-only hologram is given by:

$$\phi_T = \mathrm{Arg}\left[\sum_i \exp\left(\mathrm{i}\phi_i\right)\right] = \mathrm{Arg}\left[\sum_i \exp\big(\mathrm{i}(k_x x + k_y y + k_z(x^2 + y^2))\big)\right] \tag{3}$$

Previously this technology has been implemented with a refresh rate of <60 Hz however with the increase in graphics card technology thanks mainly to a demand from computer games we have been able to develop software which can generate multiple optical traps in under 1 ms. This is possible thanks to the parallel processing abilities of the graphics card.(see Fig:3) In order take advantage of this we use OpenGL Shader Language to calculate the hologram and then output it from the graphics card directly onto the SLM. Recently SLM technology has improved to the point that has been able to refresh faster than video frame rates (60 Hz). This means that holograms may be generated on the graphics card at a speed a of >200 Hz and then output directly to the SLM.[16]

This fast update rate provides a number of benefits to the tweezers user. Such as completely fluid operation of the tweezers system. and faster trap movement minimising the probability of losing control of the trapped object. This also means that it is possible to use SLMs for applications that require faster frame rates such as feedback systems.

The LabVIEW software communicates via UDP with the OpenGL engine by sending packets of trap co-ordinates. Thus the LabVIEW only generates co-ordinate information and is therefore free to work solely as a user interface. The interface provides the capability to create and move spots in real time in 3D. It also allows the user to move and rotate groups of spots as well as overlay spots on a video image produced by the camera software. This can all be done directly with the mouse allowing a very intuitive feel to the software.

5. EXPERIMENTAL APPLICATION

In order to suppress Brownian motion any deviation from the centre of the trap must be corrected for as soon as possible. To do this, the time between sensing the displacement and acting to correct it must be as small as possible. This is done firstly by updating the SLM at the maximum possible speed, but also by calculating the hologram on the graphics card as quickly as possible. A nVidia Quadro 5600 graphics card was used to calculate the holograms. The custom shader program used during rendering allows the entire hologram algorithm to be executed in parallel. As can be seen from Fig:2, the sum in Eqn:3 was executed for each pixel, eliminating the need to calculate and sum ϕ_i as large arrays. Furthermore the resultant hologram is then directly rendered to the frame buffer. This means holograms may be calculated and output in a single pass, in under a millisecond. The SLM used was a Boulder Nonlinear Systems (XY Series)[17]which operates via a Dual-link DVI socket allowing frame rates up to 203Hz. setup shows the experimental apparatus used in the experiment.

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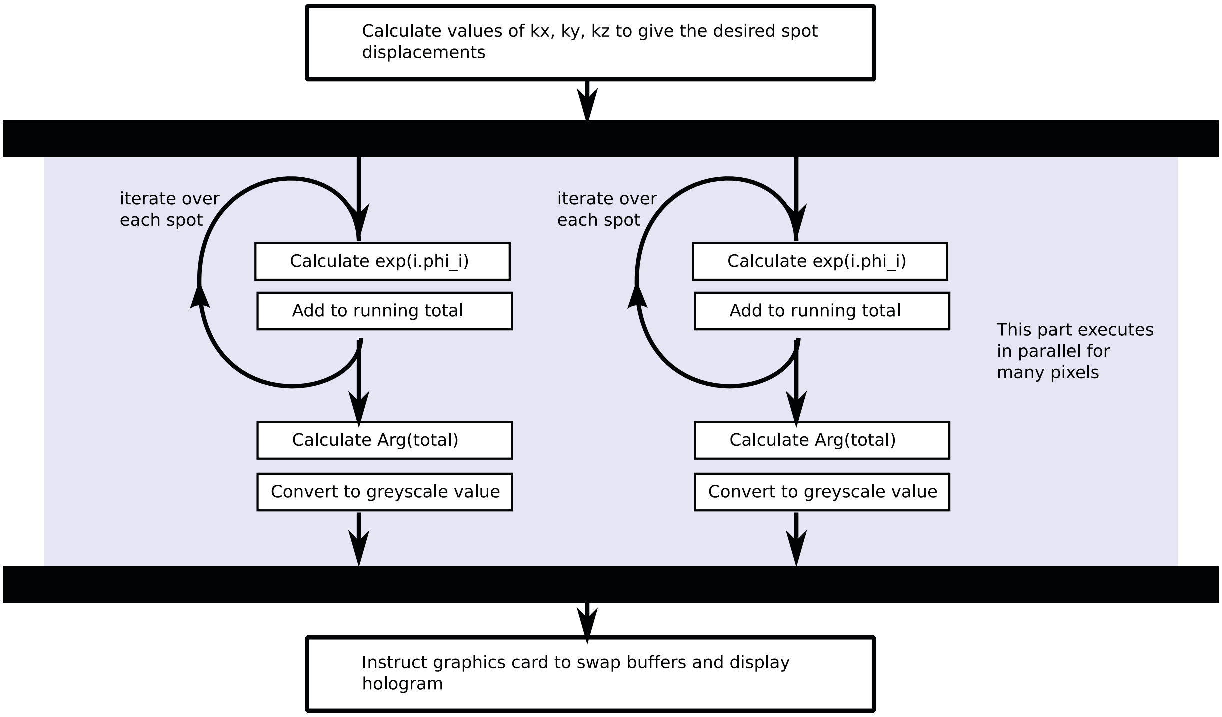

Figure 2. Flowchart showing the operation of the OpenGL algorithm.

6. EXPERIMENTAL RESULTS

For a single 5 μm trapped bead moving orthogonally to the propagation direction of the beam, a Lorentzian distribution can be fitted to the power spectrum. From this we can calculate a trap stiffness $k = 2.1 \times 10^{-6} N m^{-1}$. When feedback was applied to this system the mean squared displacement was decreased by 44%. By using Maxwell Boltzmann statistics we see this corresponds to an effective increase in trap strength of 77%, effective trap strength $k' = k_B T / \langle x^2 \rangle$. In the axial direction a decrease was also observed. Good suppression of low frequency motion was observed as well as similarly shaped power spectra however decrease in mean squared displacement was only 8 percent. This may be due in part to lower trap strength in the axial direction. It is anticipated that with more sophisticated image analysis this result could be improved. For three beads trapped using multiple optical traps,three decrease of MSD was calculated to be 44%.

7. CONCLUSIONS

We have created a comprehensive software package for holographic optical tweezers comprising of fast camera software, analysis software and hologram generation software capable of generating holograms at KHz speeds and displaying them on an SLM at hundreds of Hz. Video images may be acquired at 1kHz and the data analysed in real time. Experimentally the system was able to position clamp multiple 5μm silica beads reducing the mean squared displacement of a single bead by 44% and increasing the trap stiffness by 77%. Though the system outlined above currently lacks the bandwidth of other approaches based on photodiodes and acousto-optic modulators. Technology which is driven by consumer demand such as cameras and graphics cards can be expected to increase in both ability and complexity. We then may expect higher bandwidth SLM based systems to be future. The software will be freely available from the authors and online at: http://www.physics.gla.ac.uk/Optics/

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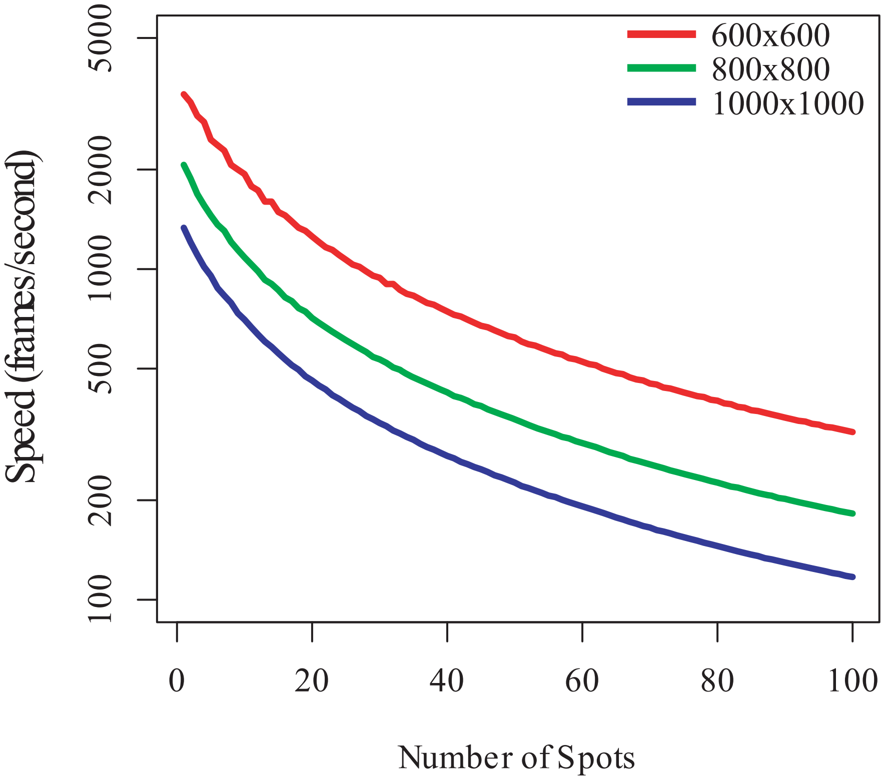

Figure 3. The graph shows the speed response of the gratings and lenses algorithm calculated via openGL to the number of traps calculated. Three different sized holograms are used 1000x1000, 800x800 and 600x600 pixels.

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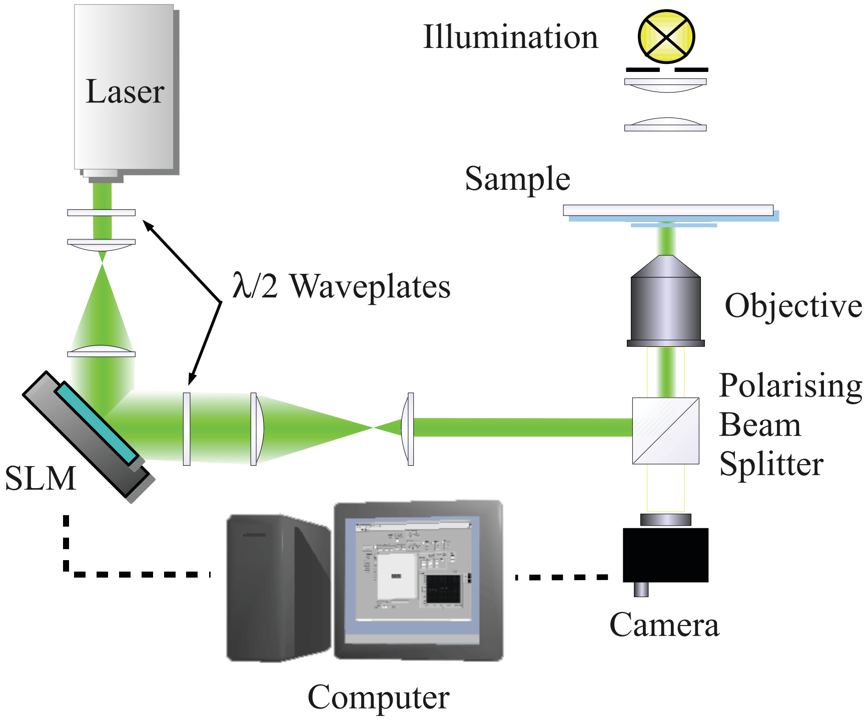

Figure 4. The diagram shows the experimental setup used for the position clamping experiment.

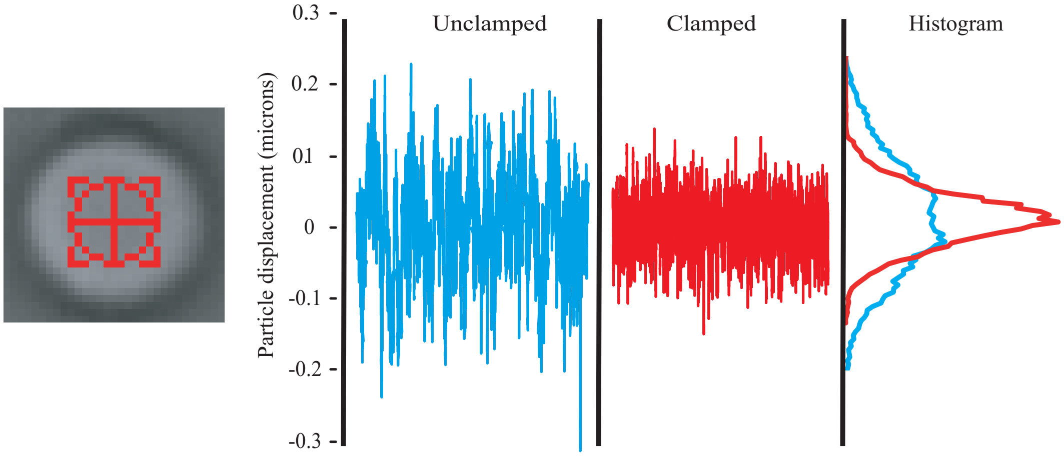

Figure 5. Figure showing the displacement in microns of a trapped bead In first the unclamped and then the clamped states.

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