

Integrated 3D imaging, trapping, and dynamic particle tracking

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Introduction

This paper describes a three-dimensional tracking system setup that has been integrated with a three-dimensional holographic optical trapping setup. The paper also will provide a basic guide to calibrating the system. The three-dimensional dynamic tracking system described in this report determines the position of a trapped particle and tracks its movement at video rates. The setup utilizes a spatial light modulator, a point spread function imposed on a phase grating developed at the University of Colorado at Boulder, lenses, mirrors, a high speed CCD camera, and an Olympus Inverted Fluorescence Confocal Microscope. The setup is used in conjunction with the three-dimensional holographic optical trapping setup previously designed by REU student Gabriel L. Stockdale from the University of California Santa Cruz. The tracking system images particles in three dimensions while simultaneously the trapping setup can manipulate these particles. These functions are achieved by using separate ports in the Olympus microscope. This report concentrates on the tracking function.

Background

Three-dimensional (3D) dynamic tracking of micrometer sized particles is made possible by using the double-helix point spread imaging system. Prior to this advance, particle imaging used slow scanning mirrors that did not allow real time 3D particle tracking. In the holographic optical trap setup, there is 3D trapping capability, but it is unable to track the particle position without using the slow method of fluorescent confocal polarization microscopy (FCPM). The scanning mirrors used in FCPM can not track movement necessary for the study of dynamic processes, such as for particles under the influence of Brownian motion, because of the time required to sweep through the sample. This short

coming was overcome by implementing a phase only spatial light modulator (SLM) in conjunction with a double-helix point spread function phase mask (DH-PSF).

When a particle is imaged using a standard point spread function applied to the SLM the resulting image appears as a sphere that goes in and out of focus as the focal plane is swept across the position of the particle. Applying the DH-PSF phase mask to the SLM causes the particle to appear as two lobes that rotate around each other as the focal plane is swept across the position of the particle. The lobes are horizontal when the particle is directly in the focal plane. The angle between the lobes is directly proportional to the position of the particle relative to the focal plane. We can analyze the angle between the lobes using a Matlab program that picks the center of each lobe by finding the brightest pixels and then calculates the angle of the line between the bright centers of the lobes.

Requirements

Illumination Laser

Enough light must illuminate the sample so that in the integration time of the CCD camera there is enough back scattered light to resolve the location of the particle

SLM

The angle at which the laser is reflected off the SLM needs to be minimized

DH-PSF

There must be two distinct and bright lobes that have sufficient contrast from the background

Restrictions

There is limited working space around the laser port and amongst the laser trapping components. M1 and M2 are used to fold the beam and reduce the setup length to fit on

the optical bench.

Limited lens focal lengths in lab to work with and only a slight magnification desired (1x to 3x).

Illumination laser output power is limited

Speed of the SLM is limited

Experimental Setup

Given the restrictions and requirements, the following setup was devised and constructed.

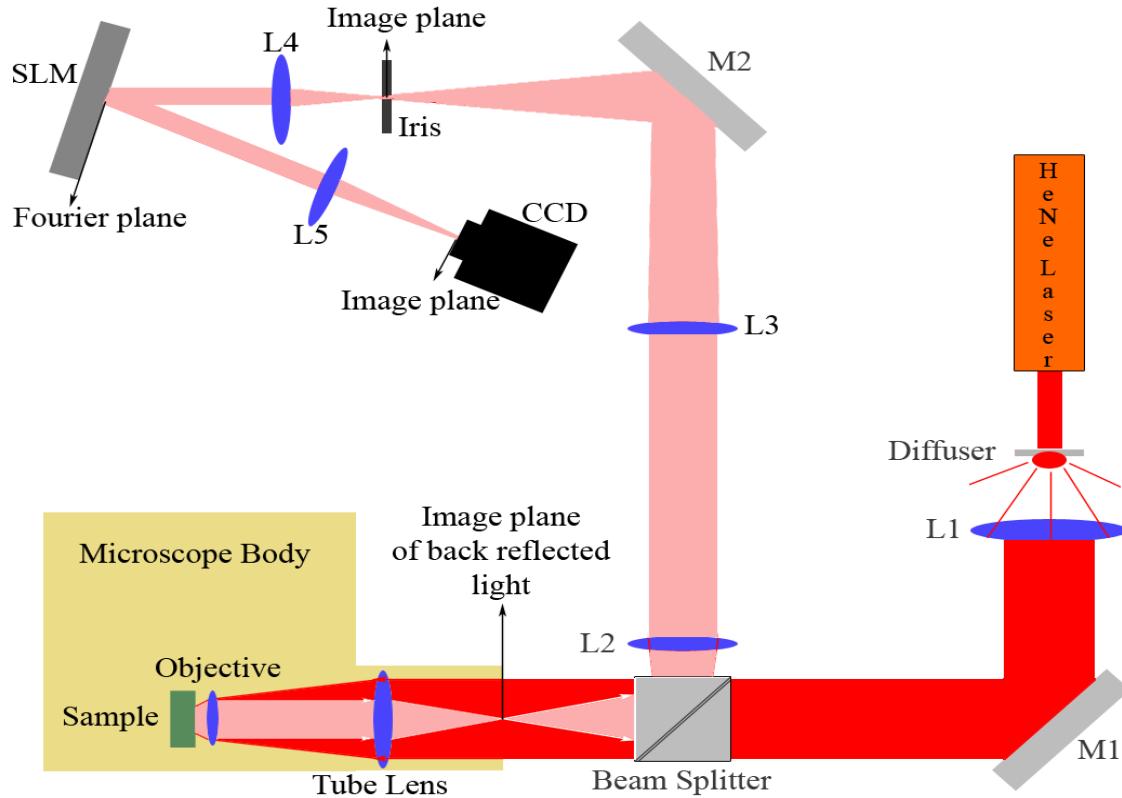


Figure (1) shows the detailed schematic of the setup. Appendix (A) lists more details about the components used in the setup.

The illumination laser is directed into a spinning piece of sandblasted glass used as a diffuser. The light is dispersed and creates a point source at the diffuser. This helps to

make an even illumination that reduces graininess. Speckle patterns cause a grainy texture but the spinning diffuser causes the speckle patterns to change during the integration time of the CCD camera, leading to an averaging out of this unwanted noise. L1 is a lens that is located very close to the diffuser to collect as much of the light as possible. M1 directs the beam to the side port of the microscope body and to the tube lens. The light is focused to the area of the sample that is being investigated. The back reflected light is focused by both the objective and the tube lens to an image plane. The light is not imaged here but is allowed to continue to the beam splitter. The light is redirected to L2 and L3 which collimate the beam and then focus it to a second image plane where there is an adjustable iris. This iris is used to block out the unwanted light and only allow through light from the area of the image that we want to image. L4 recollimates the beam and at one focal distance from L4, at the Fourier plane, is an SLM. This is where the DH-PSF phase mask is applied. The angle of incidence to the SLM should be minimized in order to optimize the quality of the resulting image. L5 is located a second focal distance from the SLM and focuses the beam to an image plane on the CCD in a high-speed camera.

Procedures

Calibrating the tracking system

1. First prepare a slide with 1 μm diameter polystyrene particles.
 - a. To do this mix 30 μm glass beads into some UV cure epoxy glue.
 - b. Place drops of a dilute particle water solution on a cover slip and let dry.

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Appendix A

Hardware

Note: Components refer to the parts labeled in Figure 1

- 1) Laser – Helium neon laser or 100mW Green 532 nm laser for high speed imaging
- 2) L2 and L3 – Telescope 1 Focal lengths 300mm and 500mm respectively
- 3) L4 and L5 - Telescope 2 1:1 ratio, 200mm focal lengths
- 4) Iris - with adjustable aperture
- 5) Collimating Lens* - 250mm focal length
- 6) Mirror - Silver coated with 2 inch diameter
- 7) XY Phase Flat 512 Spatial Light Modulator (SLM) - from Boulder Nonlinear Systems