Constructing a digital in-line holography (DIH) set-up and analyzing particles with different characteristics

by

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Abstract

Atmospheric aerosols play a significant role in our understanding of climate change, processes taking place in the atmosphere and certain biological transport systems. Imaging these aerosols and determining their distinct characteristics, like size and shape, is a challenge. We need a way to sample and resolve the size and shape of aerosols in real-time. One way this has been done successfully in the past is through Digital In-line Holography (DIH). A simple DIH set-up was constructed with stationary particles as samples. I first learned how to align the laser beam used in the set-up. Following that, I optimized the beam width by selecting appropriate lens and pinhole. I could then use the CCD sensor to get the images of the beam with or without particles present. Without particles present, the image formed was named "reference measurement" and the image formed with particles was named "raw measurement". A contrast hologram is the reference measurement subtracted from the raw measurement. This contrast hologram can then be computationally processed to obtain an image of the particle. This was done using a Mathematica code which transforms the contrast hologram using Fresnel equations, solved using Fourier transforms, into an image of the particle. Different micro-spheres of known diameters (50μm,40 μm,30μm, and 15μm) were imaged to gauge resolution of the set up. To verify the results, these particles were also imaged using an optical microscope. The resolution limit of the set-up is more than 15μm. Following this, I checked the accuracy of the setup by imaging samples containing a mix of various sizes and shapes and comparing them to digitally calibrated images of the same particles using the optical microscope. 10 such samples were analyzed out of which I have discussed Fe_2O_3, NaCl, and Road Dust. Thus, while the resolution of the set-up is less than the theoretical limit, accurate results can be obtained in certain ranges.

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Introduction

Atmospheric aerosols play a significant role in our understanding of climate systems [1,2]. However, we are not able to use our current climatic models effectively because we have limited knowledge of the characteristics of aerosols. This is primarily due to the simplified shapes (mainly spheres or ellipsoids) of aerosols that form the inputs of these models [3,4]. To obtain accurate information about aerosols we first need to obtain *in situ* samples because if samples are collected on slides or substrates their characteristics may change. If we target the coarse mode aerosols ranging from about 1um to 100um, we could analyze most atmospheric aerosols, including volcanic ash, mineral dust, pollen, etc. [5-7,9]. For these aerosols, the basic defining characteristics would be to know their shape and size. One of the easiest, effective, and non-invasive methods is to use optical light scattering for flowing aerosols [8,10]. While such a technique is useful, we run into the inverse problem. The inverse problem states that using only light intensity measurement we cannot uniquely determine the size and shape [11]. There are other methods to resolve this problem but there are always constraints due to the large range of sizes and the complexity in shape of our samples [12, 13,14]. To avoid the inverse problem entirely, we can explore the method of digital in-line holography or DIH [15,16]. This method uses a coherent and collimated beam of light as the optical light source which is then incident on free-flowing aerosols, the incident light and the scattered light from the aerosol gives rise to an interference pattern. This interference pattern can then be used to obtain the image of the aerosol. The rendering of image takes place on a digital sensor and the processing of the obtained pattern is done computationally; hence it is digital holography [17,18]. The advantage of this method is that we resolve images at different focal lengths so one image file can have multiple aerosols at a given time instance and we would be able to resolve all of them without loss of information [18, 19]. So, the work discussed here is about my understanding of the working of a DIH set-up and constructing such a set-up followed by the imaging of various particles using a Mathematica code.

Holographic Measurement

Hologram measurements follow a two-step process like scattering measurements. When the expanded and collimated beam is incident on the sensor in absence of a particle, the sensor receives what is termed as the reference beam. Whereas, when there is a particle present in the beam, there is some part of the incident beam that is scattered (called object wave) which then interacts with the incident beam and forms an interference pattern on the sensor.

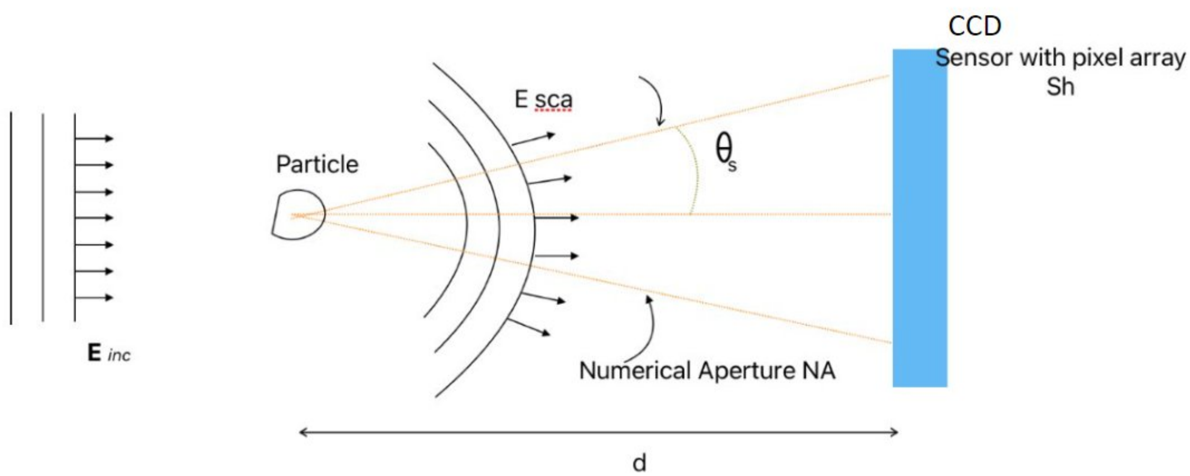

Figure 1 The distance between the particle and the sensor plane (S_h) is denoted by d. The numerical aperture NA is the measure of how much area of the sensor is receiving the interference pattern due to a particular particle.

When I was taking measurements in my set up, I had the particles mounted on an anti-reflection coated transparent glass (called window) which was at a set distance from the CCD. I wanted as much uniformity as possible in the conditions of measurement of the reference file and the raw file (containing the interference pattern). The easiest way to do this was to take the image formed with particles present on the window and then take another image after removing the particles from the window using an electronics duster. This way I did not have to change any part of the optical set up and hence the alignment stayed intact.

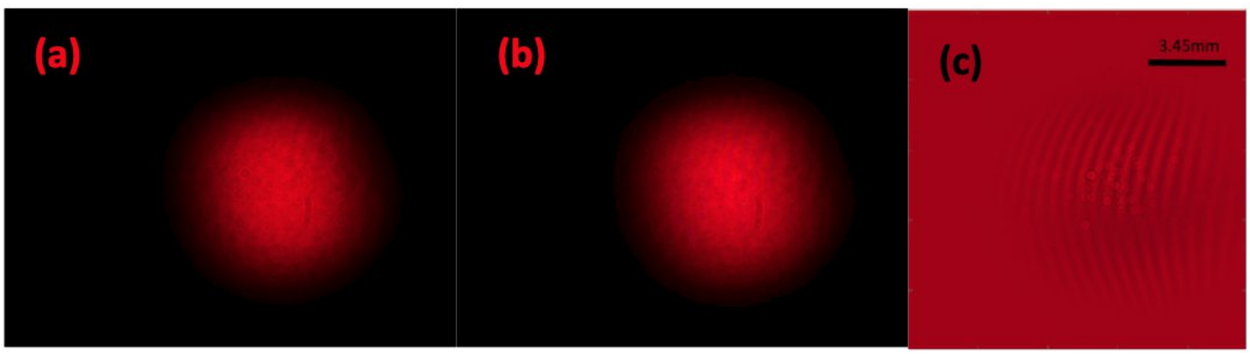

Figure 2 In the figure above, 30μm sized micro-spheres were mounted on the window. The sensor used is of pixel size p= 3.45μm. Image (a) is the raw image which contains an interference pattern formed due to the interaction of the reference wave and the object wave. Image (b) is the reference image which shows the incident light when no particle is present in its path. Image (c) is of the contrast hologram, obtained computationally by subtracting the reference image from the raw image.

Experimental Set-Up

I decided to make a Digital In-Line Holography set up based on [19] to image aerosols placed on a window

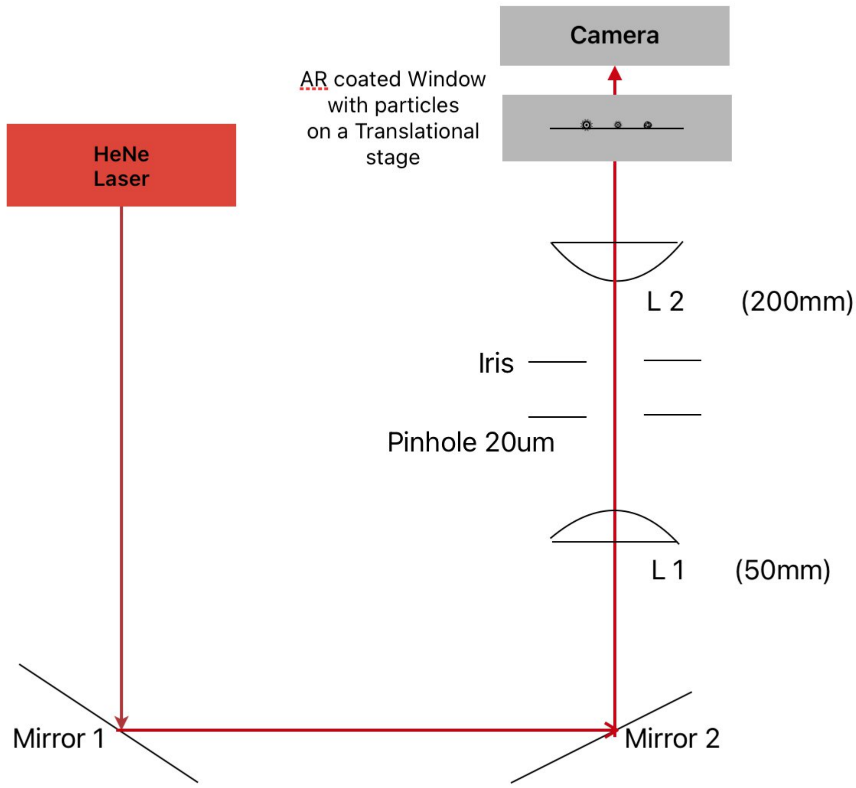

Figure 3 A schematic diagram of the set up. The red rays show the beam path, but they don't show the changing beam width. Lens 1 and lens 2 are depicted as L1 and L2.

As shown in the image, I used a He-Ne Laser with λ =637nm as incident light. I then aligned the beam coming out of the laser using two mirrors. Both mirrors are mounted on mounts which allow the mirror to be adjusted in all three directions to make the alignment more precise. Once I had a linear and collimated beam, I could then introduce the first lens into the set up. This lens is a plano convex lens which will collimate the beam at its focal point. The focal length of L1 used here is 50mm. Next, I placed the pinhole at the focal point of L1. The purpose of the pinhole is to diffract the beam and produce a pinhole diffraction pattern. The first maxima of this diffraction pattern is centered around the middle of the beam. This increases the beam diameter and makes it more coherent. I now only need the first maxima of the diffraction pattern to be incident on the second lens L2 as the first maxima is the widened light beam. So, I used an iris to block the rest of the diffraction pattern out and only allow the first maxima beam to continue. The L2 is placed in such a way that the pinhole is at its focal point. Here the focal length of L2 is 200mm. (based on the pinhole diffraction formula to obtain a beam diameter of 1cm.) This serves to further collimate the beam and increase the beam width to 1cm. Finally, an anti-reflection (AR) coated window is placed in line with the set up. I will be using this window to mount aerosol particles. Close to this window I placed our sensor which is a camera with pixel size p=3.45μm and a data array of N=1544.

Image Reconstruction

In analog holography, the diffraction pattern is obtained on a film which can then be used as a grating to obtain an image. In digital holography, the diffraction pattern is incident on the sensor and hence one obtains a file with pixels which have certain values attached to them. This file can then be treated as an input to evaluate diffraction processes and obtain an image. This computational process is called image reconstruction.

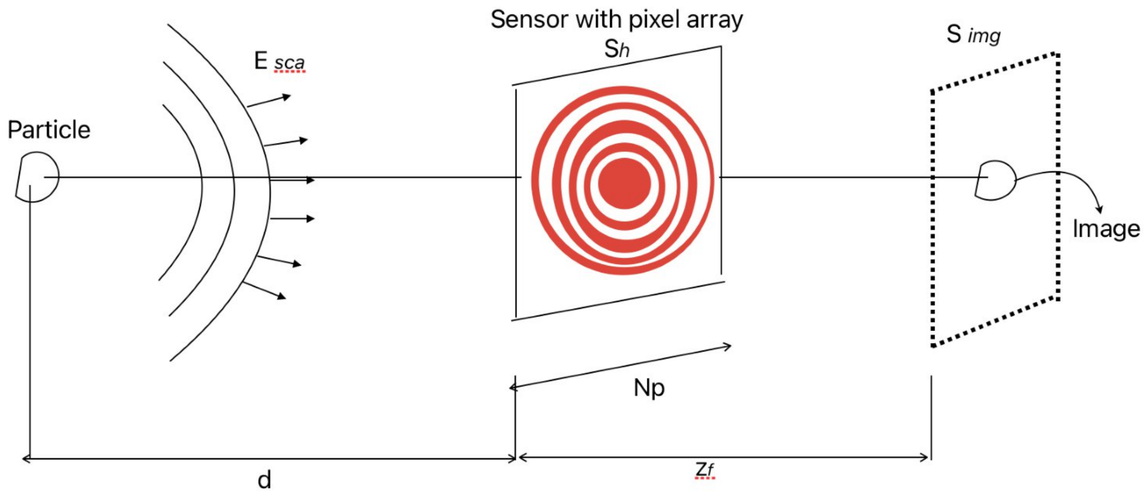

Figure 4 At distance d from the particle the scattered and incident waves interfere on the sensor S_h. Computationally, we define a plane where the image from the diffraction pattern on S_h is formed at distance z_f called S_{img}. S_{img} has the same pixel array as S_h. All the components are centered around the axis connecting them which is in the z-direction.

I could now use a diffraction formula that can explain the image reconstruction as shown in Fig. 4. As we can see, the light is incident on the square plane of the sensor which is labeled Sh. The image is formed on the plane S_{img} which is parallel to S_h. The distance between the particle and the sensor plane is d and the distance between the sensor plane and image plane is z_f . After some calculations [19], the Fresnel transform is the most essential factor in obtaining an image. The evaluation of Fresnel transform by the Fourier transform is the fastest way to render an image.

For a spherical wave emanating from a point (ξ ,η) which is any point that is not the lying on the center of the plane S_h, the Fresnel diffraction integral will satisfy the paraxial wave approximation [21, 19]:

$$U(x,y,z)= \frac{e^{ikz}}{(i\lambda z)}\left(\iint Uo(\xi,\eta)\times \exp\left\{ \frac{ik}{2z}\left[(x-\xi)^2+(y-\eta)^2\right]\right\} d\xi d\eta\right) \tag{1}$$

U(x,y,z) is the diffracted-field amplitude while $U_0(\xi, \eta)$ is the transformation of the function on image plane.

We know the forward and inverse Fourier transform as the following where v_x and v_y are wavenumbers in the x and y direction and *A* is the defined variable for *a* after transformation in one of its dimensions to the wave number domain [22].

$$A(v_x,v_y)= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} a(x,y)\exp[-i2\pi(v_x x+v_y y)]dxdy \tag{2}$$

$$a(x,y)= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} A(v_x,v_y)\exp[i2\pi(v_x x+v_y y)]dv_x dv_y \tag{3}$$

Convolution theorem states that the convolution of two functions is equal to the inverse Fourier transform of the product of two functions which means that our Fresnel equation can be calculated with two Fourier transforms and one inverse transform.

Hence, the only unknown is the distance in z-direction between the sensor plane and image plane which will be the focal point for image formation.

So, I need to know the exact z_f in order to obtain a focused image of the particle. In my experiments I have always used a window with particles on it which was then mounted on a translational stage (For eg: 40μm particles on the window which is 15cm away from the CCD). So, the d, is known. I needed the focus z_f to be as precise as possible because even a minor change of 1mm could result in a blurry image. To do this, I set the program in a loop. So, I specify the range of z_f which can be estimated to be the same as d (in this case, I can set the estimated z_f to be between 15cm-18cm) and then specify in what increments of z_f the image is to be displayed (I chose to see the image formed at every 5mm starting from 15cm and ending at 18cm). The Mathematica code can then give a series of images at different focal lengths. The images can be zoomed in on the particles that we wanted to see and adjust the focal length further (I then edited the plot range to see the particles that I was interested in. This might show that the focal length is still off by a few millimeters. So, I can fine tune the estimated z_f further and get my z_f value to be 17.2cm where the image is focused best). Finally, I obtained the correct value of z_f and the focused image of the particle.

I then used an optical microscope (Dino-lite hand-held microscope) to confirm if the particles I was seeing were present. I could also calibrate the microscope so it could tell me the size of the particles. This can further verify my results.

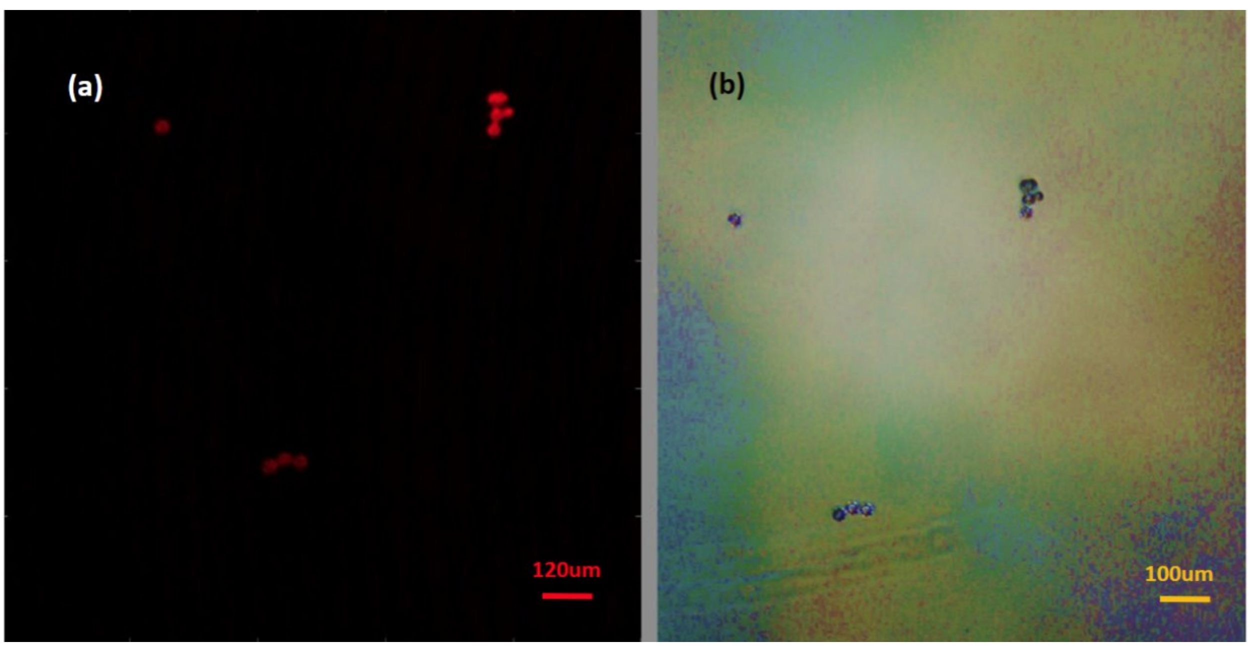

Figure 5 In the figure above, picture (a) is the focused image of the 40μm glass spheres rendered using the DIH set up and the Mathematica code. The picture (b) is from Dino-lite handheld microscope.

Optimizing *d*

For each setup, there is a range of d that gives a focused image at z_f. If the particles are too far away the interference pattern will not converge into a clear image, however if the particles are too close to the sensor, we get an aliasing effect in the image obtained. To know this working range for my setup, I used a delay stage and mounted the particle window on that stage. Till now I was using a translational stage which can move in small increments and is highly accurate, but it is operated manually. The delay stage spans a longer distance (25cm) and it is operated via a computer. Placing the sensor at the 0cm mark of the delay stage and mounting the window onto the stage, I could specify the distance between the two through the computer. I could also move the window at fixed increments similarly and obtain data for the same sample of particles at different d values. A reference image was taken once the raw images had been obtained for 50μm sized micro-spheres from 20cm to 1cm with increments of 1cm.

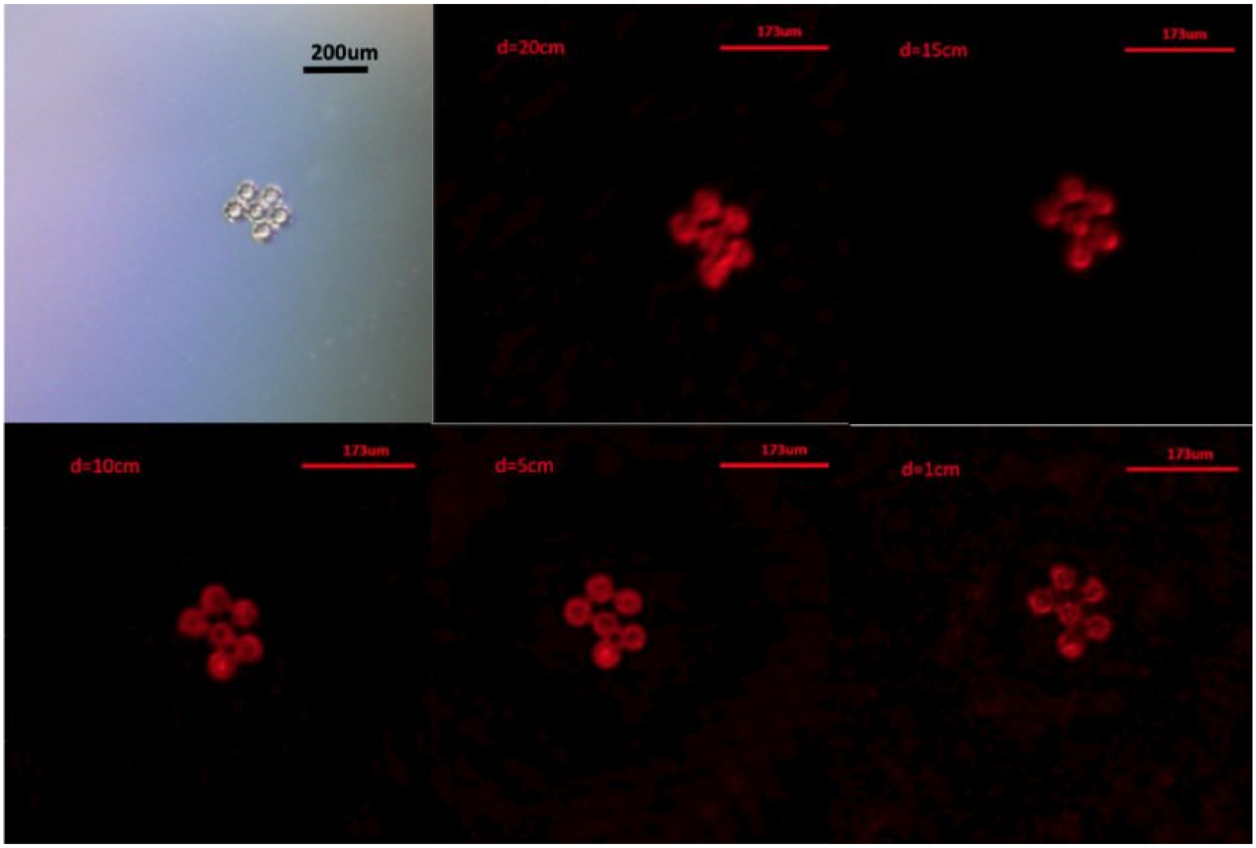

Figure 6 The image on upper far left is taken through a microscope. The same cluster of particles were then imaged using the DIH set up. At 20cm, the image formed is a little fuzzy. We can see the different spheres but not separately. At 15cm the image grows sharper, and we can also see the changing surface of the sphere. From 10cm to 5cm the particles are well focused, and we can also see the distance between two spheres for some of them. But if we image at 1cm the image becomes hazy again and the edges are not sharp.

Different Sized Particles

To better understand the resolution and resolution limits of the set-up, different sized particles were imaged and then focused computationally. This would also test the resolution of the hand-held optical microscope.

Rayleigh criterion was used to find the theoretical resolution limit of the set up. Rayleigh's criterion says that to obtain a good image, point sources must be sufficiently far apart that their diffraction patterns do not overlap. To achieve this, the minimum distance between images must be such that the central maximum of the first image lies on the first minimum of the second and vice-verse. Such an image is said to be just resolved.

Using Rayleigh's criterion, we can define the limit of resolution as

$$(\Delta l)_{min} = \frac{1.22 f\lambda}{D} \tag{4}$$

Where, $(\Delta l)_{min}$ is the distance between the centers of the two spheres, f is the focal length of L2, λ is the wavelength of the incident light and D is the beam width which is 1.75cm for this setup [20].

Hence, the theoretical resolution limit obtained for my set up is 8.8μm. Particles having diameter less than of equal to 8.8μm cannot be completely resolved.

To verify the resolution limit, I imaged particles of different but known sizes and obtained the corresponding image of each in the optical microscope. Particles used were 50μm, 40μm, 30 μm and 15μm diameter micro-spheres. The images are shown in **Fig 5**, **Fig 7**, **Fig 8**, and **Fig 9**.

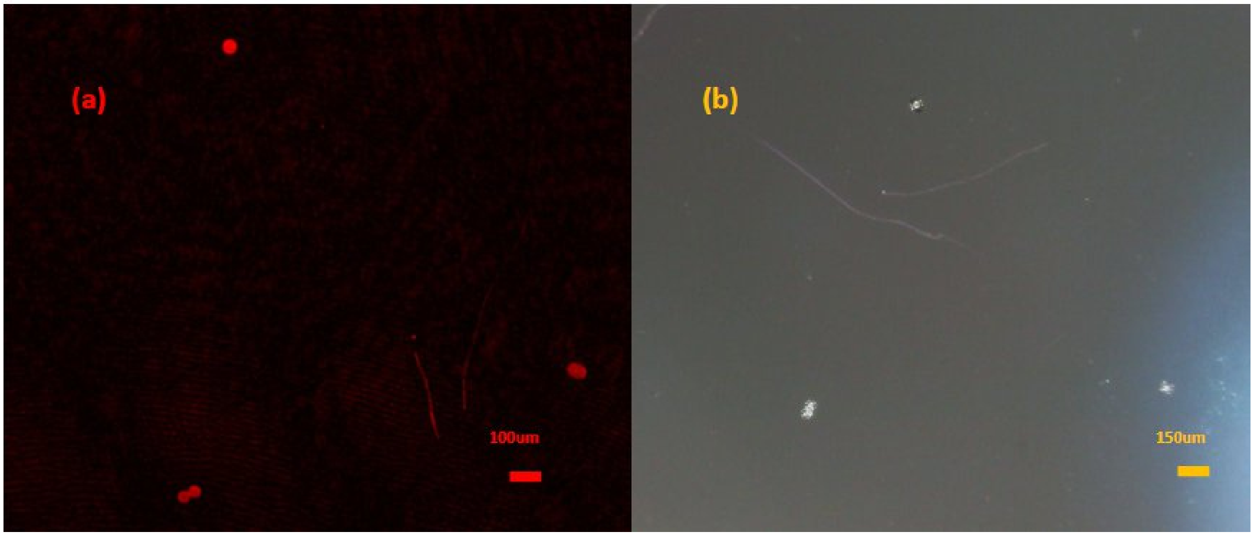

Figure 7 Holograph of 50μm spheres. The bi-sphere is clearly visible and can be resolved. The optical microscope image is also clear and shows the bi-sphere.

As previously shown in Fig 5, the 40μm spheres form a focused image and we can resolve each particle from another. The distance from the sensor was d=15cm and the resulting focal point was 17.2cm.

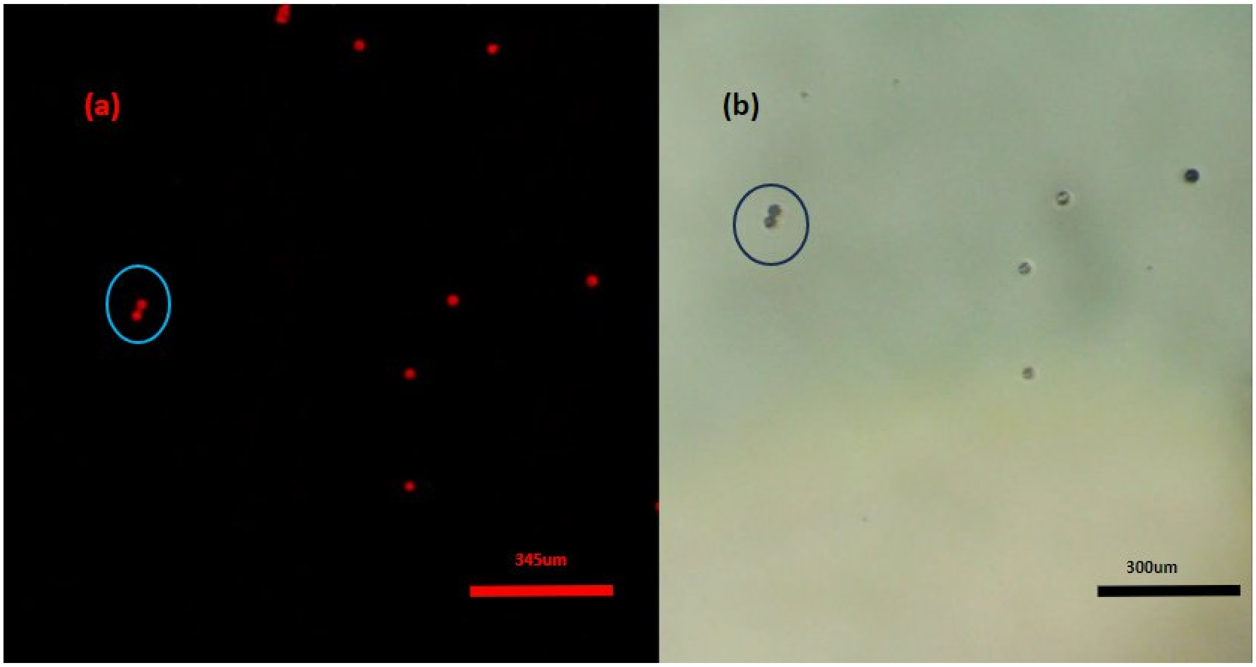

Figure 8 30μm particles imaged with d=8cm and focused at 9.15cm. Circled part shows a bi-sphere which is still clearly resolved.

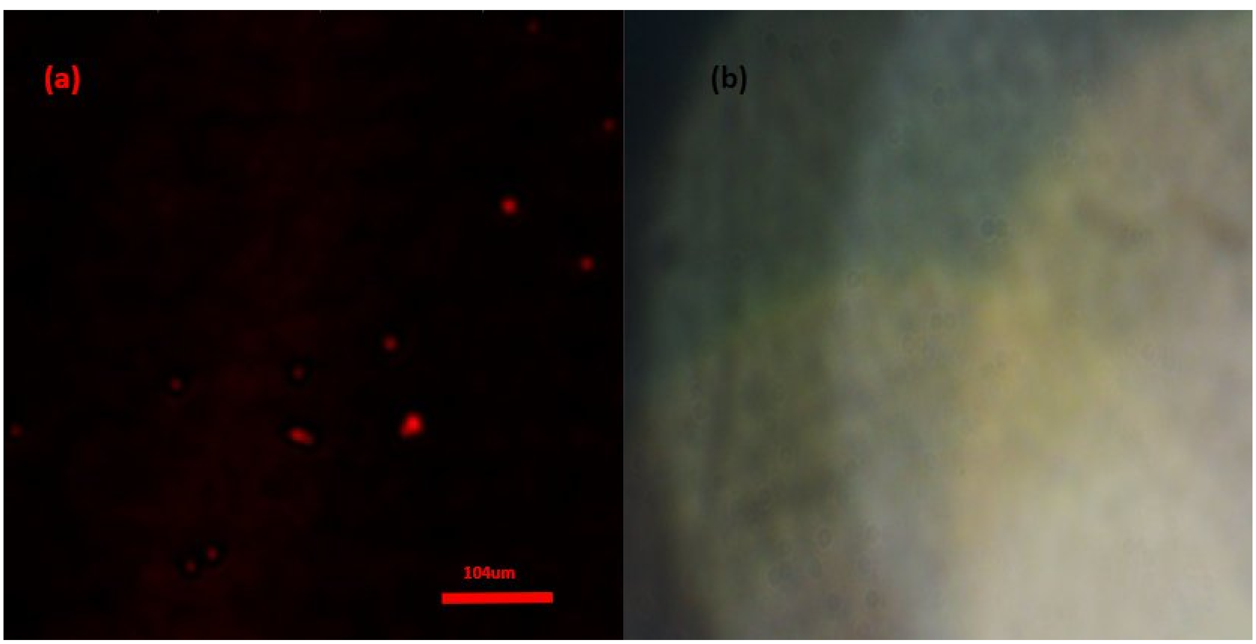

Figure 9 The hologram of 15μm spheres could not be focused beyond this point, d=15cm while z_f=17.23. Even in this image, we cannot differentiate a bi-sphere. The optical microscope has even lower resolution. There are very faint spheres in the image and no particle can be seen**.**

So, the resolution in practice is a little less than 15μm. There could be several reasons for this, the principle one being the numerical aperture value (which in the above calculations is taken as the sensor's surface area) would be less. I was unable to change the value of the numerical aperture because of time constraints.

Different Shaped Particles

The particles used so far have been homogenous and uniform sized but the set up can also be tested using particles of irregular shape and a large distribution of sizes. Some examples are shown below.

Fe_2O_3

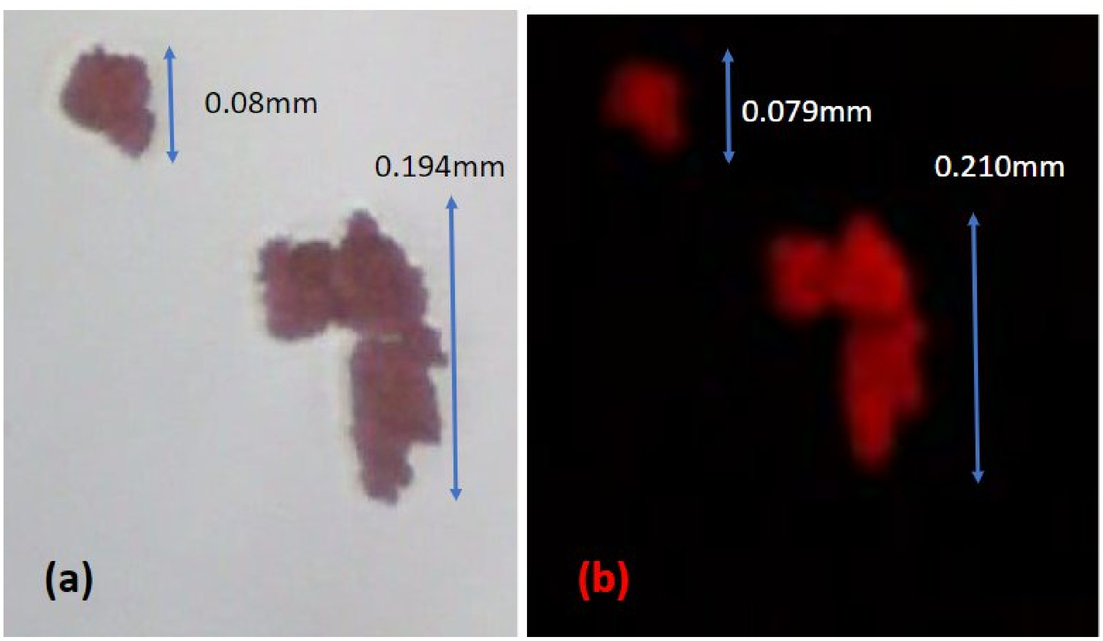

Figure 10 A sample of Fe_2O_3 was imaged and the sizes in (a) have been given by the software of the microscope and the hologram size was resolved by eye with coordinates from the Mathematica code.

NaCl

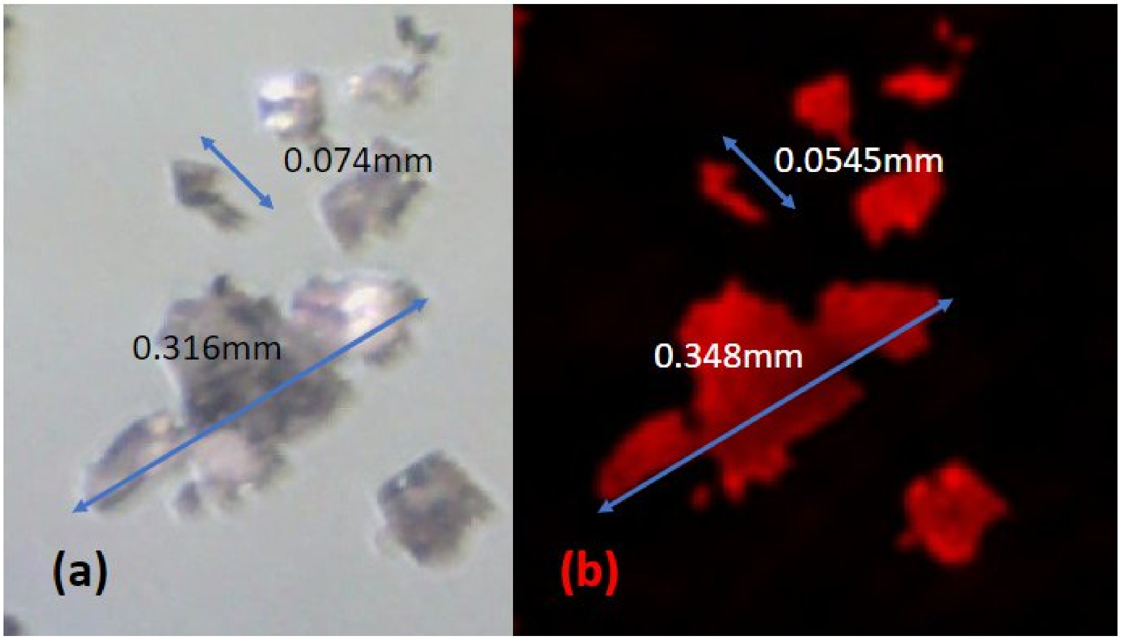

Figure 11 An NaCl sample was similarly analyzed. While Fe_2O_3 was powdery which makes the edges a little hazy, NaCl crystals gave sharper edges which makes the size calculation in holograms easier.

Road Dust 1

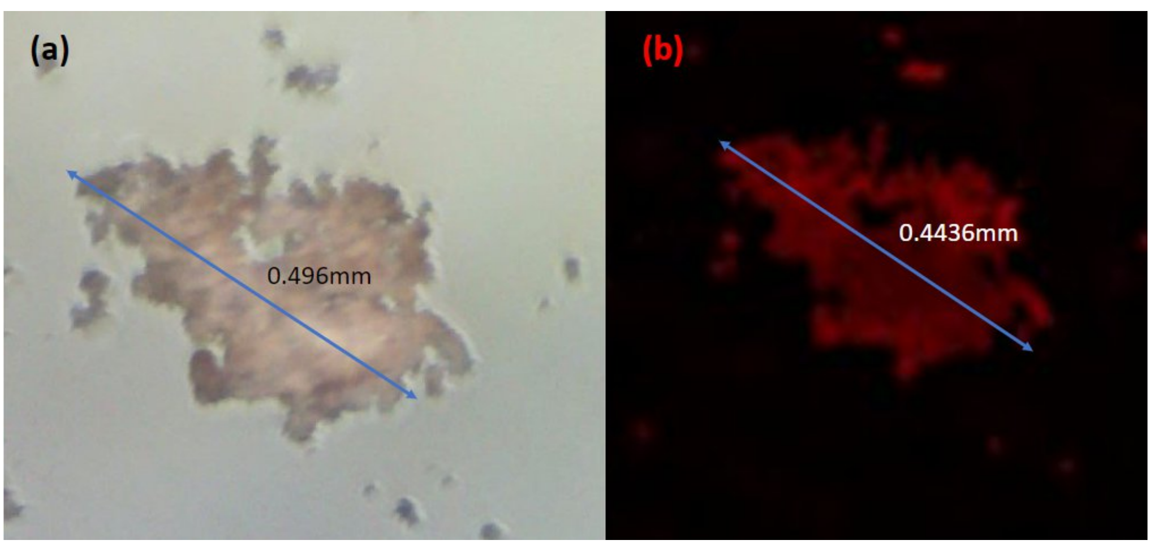

Figure 12 Road Dust samples were similarly analyzed. Road dusts (we analyzed multiple types of road dusts) generally have a very wide range in sizes. I have included the better resolved result here because my set up is more accurate in this range, but we did try to resolve smaller dust particles with varying results.

The microscope length measurements (on the left of each figure) are made by the in-built function in the software for Dino-lite microscope. It was first calibrated using particles of known size and then used to measure samples of unknown shapes. The holography images were measured by counting the pixels by-eye and then multiplying that by the width of the pixels.

Conclusion

Digital in-line holography is an important tool to obtain information about atmospheric aerosols. The setup constructed here is functional and is able to image stationary particles. The working range for the setup is where the distance between the particles and the CCD is 7cm-10cm. The setup can resolve particles with diameter more than 15μm. The setup can also measure the sizes of non-spherical samples in the coarse mode aerosol range. This also includes a wide range of sizes from 500μm to 50μm. So, while the set up might lack resolution, it is accurate in the range that it is operating in. The setup can measure a wide range of different sized aerosols and in such a way that if there are multiple aerosols present, I would be able to resolve them simultaneously by obtaining the focal length for each. This technique can also resolve image shapes with high accuracy while obtaining information about the size of the particle. In future, the resolution of the setup can be improved in order to image finer aerosols.

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