Investigations on Imaging of Biological samples through Random Media

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I declare that this written submission represents my ideas in my own words, and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources that have thus not been properly cited, or from whom proper permission has not been taken when needed.

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Approval Sheet

This thesis entitled "Investigations on Imaging of Biological samples through Random Media" by Suheel Mohd Zargar is approved for the degree of Master of Technology from IIT Hyderabad.

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Finally I want to thank and acknowledge my parents for their constant love and motivation throughout my course of study at IIT Hyderabad.

Dedicated to

My Grandparents

Abstract

Imaging through a highly random media e.g. biological tissue has been one of the prime challenges in the field of optical imaging. Developing optical techniques for imaging through highly turbid media has potential applications mostly in the areas of astronomy and biomedical research and medical care. Over the years numerous efforts have been laid down in the direction of developing optical techniques which can detect the area of interest hidden in the scattering layers. One of the solutions for imaging under turbid media can be derived from the statistical properties of the laser speckle. Shape and Size of the laser speckle carries significant information of scatters and thus can provide a solution to the challenges of optical imaging in highly random scattering media. From Statistical approach, incoherent source is related with far field speckle distribution by Van-Cittert Zernike theorem. A method for imaging through the scattering medium is suggested and demonstrated using principle of speckle holography and intensity correlation technique. Here, spatially averaged far field provides only amplitude of complex coherence function where phase is also important. Lost phase is recovered by averaging speckle hologram with frequency domain spatial filtering i.e. Fourier fringe analysis. The complex coherence function is further explored for the reconstruction of object in the scattering medium in a single shot. In the present research work, we have concentrated on theoretical understanding of practical problem to achieve object information through multiple scattering by simulation study. An experimental study based on intensity correlation is practically demonstrated and object is being recovered with theoretical approach which finds numerous applications in optical imaging science mainly in biological imaging.

Nomenclature

λ	Wavelength
I	Intensity
$t(x, y)$	Transmission function
$\Delta I_0(r)$	Object intensity
$E_0(r)$	Electric field distribution
$K_0(\Delta r)$	Covariance function
$C(\Delta r)$	Complex coherence function
k	Wave number
μ_A	Complex coherence factor
$\mathcal{G}_1$	Power spectral density
c	Speed of light
T_c	Temporal correlation function
β	Bandwidth
Δt	Time delay
GG	Ground Glass
CCD	Charge-coupled device
BS	Beam splitter
MO	Microscope objective
SLM	Spatial light modulator

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Chapter 1

Introduction

1.1 Motivation

Optical Imaging methods form the backbone of various fields of research and engineering. Due to its non-invasive nature and use of non-ionizing light, the applications of optical imaging has grown in the fields of metrology, life sciences, biomedical studies etc. The main goal of these modalities has been to replace the conventional imaging techniques that are costly and which use ionizing radiations for investigation. The optical imaging removes the ionizing effects of conventional imaging techniques by making use of infrared and invisible regions of the electromagnetic spectrum. Even after several advances and development of various types of techniques, imaging through a highly scattering media e.g. biological tissue has been one of the prime challenges for optical imaging.

Scattering media is a media that scatters the incident light randomly due to the random nature of medium. Tissues or biological samples are made up of organic fibrous materials from which incident light waves get scattered thereby the presence of any malignant tumor, cancer or object information cannot be detected easily. The study of wave propagation through a random or scattering media has profound applications in biomedical optics and imaging through random media is basically aimed to obtain the relevant information regarding the shape, depth and size of objects hidden in a tissue but being able to image through the scattering media is problematic as the disordered turbid media distorts the incoming light wavefront thus degrading the image formation of the optical system. Due to the extensive research efforts to study the phenomenon of light propagation through the random media several breakthroughs in the field have been established. Over the years many techniques have been investigated to tackle the fundamental problem of imaging through turbid media but only recently methods have been investigated which make use of the coherent propagation of laser light for focusing and imaging through a scattering medium and the subsequent progresses made in the development of advanced digital numerical reconstruction methods has shown their significant progress and applications in biomedical research.

The coherent light scattering resulting from the inherent inhomogenity of complex media results in the formation of complex speckle pattern. Speckle phenomenon is fundamentally a statistical process and can be completely understood from the perspective of statistical optics. The phenomena of laser speckle can be utilized for extracting the information about the illuminated surface that generates it. From optical imaging perspective Van Cittert-Zernike theorem in coherence theory of light plays a significant role and defines a relation connecting incoherent source structure with the far field speckle properties through a Fourier transform. The two point correlation between the two points at far field is related to the spatial fluctuating random object field. When the medium is highly scattering, the phase and amplitude of the field coming from it becomes fluctuating thus hiding any determinable information.

The invention of lasers also marked the arrival of a revolution in the holographic imaging technique. Holography is a novel imaging method by which with suitable coherent scattered and un-scattered light from an object source, it is possible to record and reconstruct the complete information of the object in the recording media which responds only to the intensity of light. Holographic imaging with the coherent light has opened doors of possibilities for imaging through free space and scattering layers. Two main steps are mainly involved in the holography; recording and reconstruction of the complex waves that emerge from the object. Gabor originally developed the earlier form of holography technique known as in-line holography but it had a main drawback. Holographic imaging causes the object under investigation to produce two images; one real and one virtual on the camera plane. The real image in in-line holography gets superimposed by un-diffracted part of the reconstruction wave resulting in 'twin image' problem. In order to remove this drawback, Leith and Upatnieks introduced an off-axis holography technique in 1962.

By combining the principles of holographic imaging with the statistical nature of laser speckle and by making use of their second order properties mainly autocorrelation of the speckle intensity, a new method can be developed to image through the scattering media. One of the main advantages of such a technique would be its single shot operation which is of utmost importance in bioimaging applications. Since the human tissue is highly turbid and scatters the light, the role of complex correlation function in optical imaging and the subsequent recovery of the object in the scattering media in a single shot are highly advantageous with its applicability in various research fields such as microscopy, biomedical science, astronomy, and so on.

1.2 Scope of the thesis

The primary objective of the thesis is to introduce the speckle statistics along with the concept of two point intensity correlation and its application in random inhomogeneous media. The development of optical imaging technique which is capable of imaging through scattering layers is carried out by measuring the second order correlation i.e. complex correlation function of a spatially fluctuating speckle at far field using the principle of speckle holography. An experimental geometry is discussed and further demonstrated from application point of view of complex correlation function. An object placed in a scattering regime would be recovered and reconstructed. Initially I will discuss a method that retrieves the complex coherence function of the object in the single scattering regime. Later on, I will focus and develop the experimental basis of recovering the complex coherence function and its subsequent use in recovering the object hidden behind the multiple scattering layers (white paint in our case).

1.3 Organization of the thesis

Chapter 2 of this thesis mainly deals with the introductory work. In this chapter the challenges and basic theory of imaging through random media will be presented. Next, we will look into few techniques which also attempt to solve the fundamental problem of optical imaging through random medium along with their limitations and drawbacks. Also the method of holographic imaging and its applications in optical imaging will be explained and in the concluding part of the chapter an insight into the basic understanding of digital holography imaging technique is discussed.

Chapter 3 of the thesis will be devoted to the coherence theory of light and the effect of laser light when passing through a scattering medium. First and second order speckle statistical properties will be discussed. Also an experimental method based on speckle holography to retrieve the complex coherence function will be demonstrated through which both phase and amplitude of the randomly varying speckle field can be recovered.

Chapter 4 will mainly deal with the recovery of an object in a multiple scattering medium. The experimental setup for imaging through the scattering layers will be presented and the speckle field complex coherence function of an object in the scattering medium will be recovered and the object information will be reconstructed.

Chapter 5 gives the summary and future work for the given project.

Chapter 2

Random Media and Holography Imaging

2.1 Historical overview of Optical Imaging

Our current knowledge of optics and its related phenomenon has a very interesting background and history. In order to visually perceive things, humans and other animals have been bestowed by nature with the wonderful natural organ called eye. The Eye in collaboration with brain nerves lets us visualize objects around us with the help of light. The Greeks coined the term 'optikos' for such ability which simply means vision [1]. Though Archaeological findings suggests that the knowledge of optics was also known to ancient human civilizations but it was not well developed and fully understood. The works of Leonardo da Vinci (1452-1519) also reflect his knowledge of optics but it was not till 17th century that the development of scientific optics picked pace. The fundamental works in the field of optics was done through the contribution of many. Works done by notable scientists like Snellius, Isaac Newton, Descrates, Galileo, Huygens and others laid the foundation of present day optics [2].

The development of scientific theory and understanding of light was further enhanced throughout 18th and 19th century through the works of many great scientists. In the 19th century, Fraunhofer (1787-1826) known as the father of astrophysics and spectroscopy constructed the first spectroscope which he used for the study of sun's spectrum. Fresnel (1788-1827) during the same time furthered the wave theory of light and explained the dependence of transmission on the incidence angle. The breakthrough works of Albert Einstein (1879-1955), Max Planck (1858-1947) and the formulation of Maxwell’s equations further broadened our understanding of light [3] and finally led to the invention of first laser in 1960 by T. Maiman. Ever since the laser was invented, optical imaging has seen a tremendous growth and many techniques have been researched and developed across various fields especially in the field of bio-imaging.

The optical imaging techniques help in removing the harmful ionizing effects of conventional imaging as they make use of “Near Infrared Region” (650-1350nm γ) of the electromagnetic spectrum, additionally optical techniques are also cost effective and can measure various properties of the tissue or organ. Since the human tissue is highly turbid and scatters light randomly, developing optical imaging techniques for imaging through highly turbid media has potential applications in the field of biomedical imaging. Before I go to the problem of imaging through the turbid media using speckle holography which is our ultimate goal, I would like to cover the introductory theory of the scattering medium and discuss few techniques which have been developed over a period of time to image through the scattering medium along with their limitations. We will also discuss the concept of holographic imaging technique in this chapter which will be utilized for the development of our imaging method.

2.2 Optical Imaging through random media

Random/scattering media is a media through which we cannot see clearly for e.g. clouds, milk and human tissue as light waves which are incident on them get scattered due to the random nature of the inhomogenous medium, therefore the detailed study of light wave propagation through an inhomogenous media has wide range of applications in biomedical research. Imaging through disordered nonhomogenous media is difficult because the inhomogenity of the medium makes changes in wavefront of incoming light and degrades the quality of image formation of an optical system [4]. Also, the phase, polarization and the direction of propagation of the light is altered while travelling through the scattering media [5]. This uncertain and random deformation in the wave that has negative impact on the performance and efficiency of the optical imaging systems has been investigated using different optical methods over the period of time. The underlying idea has been that any medium, even a complex multiple-scattering medium, performs a transformation on the light field that you can undo by wavefront shaping thereby being able to image through a scattering medium. The optical imaging techniques through a random media can be broadly classified as incoherent imaging and coherent imaging depending upon the nature of light they use for investigation. In the coming section we will discuss some of the imaging methods which attempt to deal with the same optical problem of imaging through turbid media along with their limitations to get an idea of the fundamental problem of optical imaging through such a media.

2.2.1 Transmission matrix Method

The behavior of a complex media can usually be described by their average macroscopic properties which can be obtained through intensity measurements. There is an alternative approach for the study of scattering media which lies in the retrieval of transmission matrix (TM). Transmission matrix is the subpart of main scattering matrix as described in [6]. Within this foundation, basically the Green's function is recorded between an array of sensors and an array of sources during transmission. The knowledge of the TM brings more explanatory insight into the scattering medium for example one can extract the multiple and the multiple single-scattering components from the recording of TM. The distribution of the singular values of the TM can also be made to relate with the diffusion properties and may exhibit a coherence effect beyond the scattering transport regime like weak and strong localization effects. Such techniques which are based on the reversibility and the reciprocity of the wave equations have found applications in various areas ranging from telecommunication to acoustic imaging and even seismology. For acoustic waves oscillation frequencies are compatible with electronic detection and transducers (antennas) are natural local receivers hence the TM can be measured in a straight forward way.

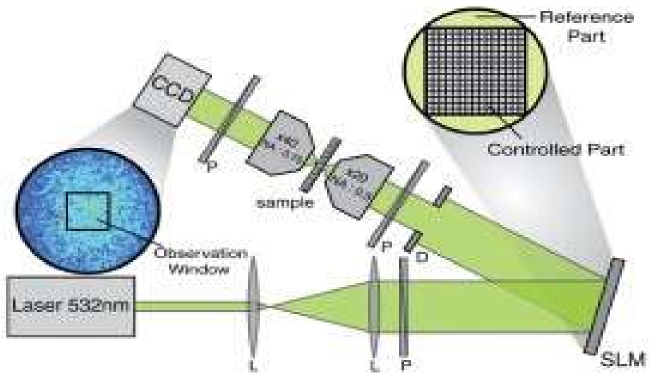


Figure 2.1: Schematic of the apparatus for measurement of transmission matrix [8].

On the contrary, the transmission matrix reconstruction of a turbid medium is still an elusive problem in the optical imaging domain. Nevertheless few recent experiments have demonstrated that it is possible to manipulate light in a scattering medium in order to focus light through a turbid medium as well as efficiently couple it to the open channels of a complex disordered sample [7]. Figure 2.1 shows the experimental setup of a transmission matrix based imaging technique. These experiments were made possible mainly due to the emergence of spatial light modulators (SLM) but the technique has its own limitations such as less resolution, high processing time and need of extensive sequencing of the smaller matrix.

2.2.2 Time-Gated Imaging Methods

This technique works on the gating mechanism and is utilized for separating the backscattered light from the host medium. In order to directly detect the object hidden in a highly turbid media the light component that diffuses is sorted out from earlier arrived snake and ballistic photons [8]. Figure 2.2 shows the different classes of photons in a random media. The backscattered light imaging techniques have one major difference from the transmission method approaches. In the transmission approach the photons that arrive earlier at the detector array carry the information about the object hidden in the scattering media directly while as in backscattered investigational methods the photons that arrive at the detector first will be the photons that have been backscattered by the host material.

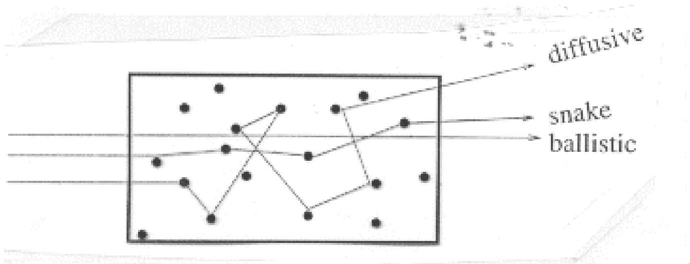


Figure 2.2: The different classes of photons in a turbid medium [10].

The backscattered ballistic photons that arrive later from the host medium in which the object is hidden are mixed with other photons like ballistic, snake and diffusive [9].

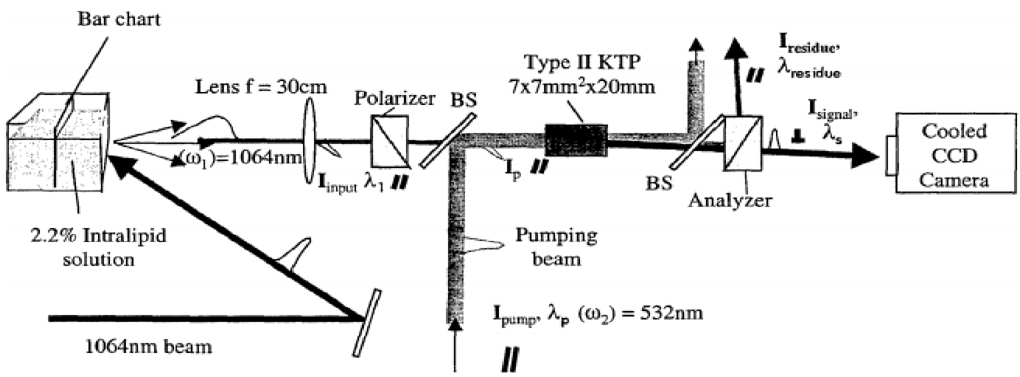


Figure 2.3: Experimental setup of a back-scattered imaging system using NLOG [9]

In time gated imaging technique a nonlinear optical based gated (NLOG) is applied for acquiring the backscattered time resolved images. The main principle that governs the NLOG implementation is based on the non-linear second order optical coefficient of materials like KTiOPO4 (KTP) under the condition of phase matching [9]. The schematic experimental setup of the NLOG imaging system is shown in Figure 2.3. In time gating method a mode-locked laser system is used to emit a laser pulse for illuminating the host medium. The second harmonic of the NLOG system is used as the gating pulse. It also uses a long crystal as a gain medium. Additionally, it makes use of a pair of cross polarizers for adding them into the scattered signals after and before the crystal. In the absence of the gating pulse, the input signal polarization remains the same and light beam is blocked by the analyzer (Second polarizer). The amplified output polarization is perpendicular to the input signal of the system. In this way due to the gradual delaying and synchronizing the gated pulse with respect to the input signal, we can selectively slice the scattered intensity signal profile and can also reject the diffusive portion simultaneously. The output signal of the time sliced NLOG signal can be amplified and then further passed towards the analyzer. After this a narrow band centered filter is used to select the signal [9]. Though backscattered imaging based on NLOG system drastically improves the signal to noise ratio and sensitivity of the image by making use of parametric amplification but time gating methods suffer from various drawbacks like acquisition speed and complexity of hardware requirements.

2.2.3 Continuous wave light source Imaging

This method makes use of low coherence continuous wave sources along with the combination of temporal and spatial Fourier transforms to image through the scattering medium. In this technique, diffusive light is separated from the ballistic light by making use of their propagation and direction and uses a low power continuous source like diode laser or He-Ne laser which forms the input and is polarization modulated in time [10]. The need for a step scan in this method is also not required as partial discrimination formulated on the propagation direction is achieved by incorporating spatial filter instead of the pair of pin holes which allows for the 2-dimensional CCD camera recording. The input polarization modulation is done to record the sequence of images and the fixed analyzer is used for passing the output light. By doing temporal Fourier transform on the sequence of images, the signal is extracted and the signal has the same modulation time as that of the input light. During post processing, polarization discrimination is achieved [10].

The experimental setup based on the continuous wave method is shown in Figure 2.4. In the experimental analysis a linearly polarized light whose polarization plane rotates at an angular frequency ω is generated by passing it through rotating half-wave plate or by passing through the rotating polarizer. The light after this is incident on the object under study and the light emerging out is spatially filtered by using a lens aperture system. This is done to significantly reduce the diffusive light component. Now, at the focal plane of the lens, light waves that are propagating along various directions are brought into focus at several points.

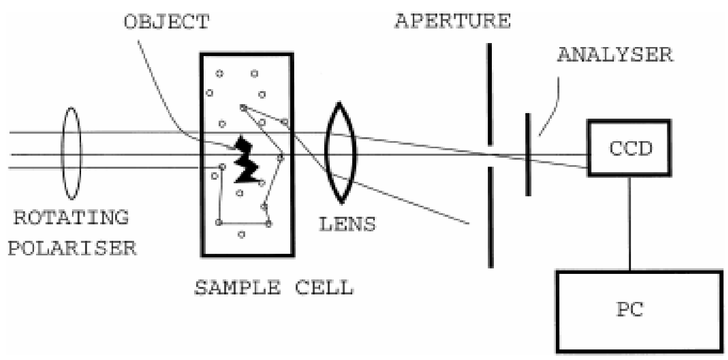


Figure 2.4: Schematic diagram of the experimental setup [10].

The ballistic component of the light is focused at the focal point and is transmitted through the aperture; meanwhile the diffusive components are focused off-axis and thus will be blocked. Following this the signal that has been spatially filtered consists of snake, ballistic components with diffusive component reduced to a large amount. The signal is then made to pass through an analyzer which is fixed and finally recorded on to the CCD camera. Through this a sequence of images are recorded. These images differ as they have different input polarization. The input polarization is rotated in steps and images are captured when the input modulating polarization element is stationary. Since ballistic component undergoes only coherent scattering therefore it maintains its polarization and on passing through the analyzer exhibits a variation in intensity [10].

Similarly, snake component preserves its input polarization to a large extend but the diffuse component has a random variation both in polarization and the direction of propagation. The snake and ballistic photons are discriminated by the formation of time series for each pixel and then time series is Fourier transformed. At last, an image is formed by making a 2-dimensional gray scale plot. The intensity at each pixel is equal to the square of amplitude component at that pixel. The continuous light wave imaging methods as a whole are very sensitive and also fail to differentiate between tissue absorption and tissue scattering.

Above mentioned techniques are based on estimation of the random scattering at different levels and they try to solve the problem of imaging through scattering media by using transmission matrix elements or by taking the ballistic, snake or diffusive light into consideration. Having provided an insight into the problem of imaging through turbid media, now I would like to discuss about the highly useful imaging technique named holography which is of utmost importance in our work.

2.3 Basics of Holography Imaging

Holography is an imaging technique which was invented in 1948 by an English scientist named Dennis Gabor as a method for recording and reconstructing amplitude and phase of a wave. He invented it while working to improve the resolution of an electron microscope [11]. He named the techniques as 'holography' from the Greek words 'halos' which means complete and 'graphein' which means to write. Although Gabor had developed the technique in 1948 but the practical application of holographic principles was not possible due to non-availability of coherent sources but after the invention of laser in 1960 by T. Maiman, the field of holographic imaging got revolutionized. Since laser light releases powerful light bursts therefore it was ideal to develop the holograms. A hologram is the photographically recorded interference pattern between a wave field scattered from the object and a reference wave [12]. Holograms can record entire 3-dimensional wave field on a flat surface. The development of holograms and computationally fast reconstruction methods paved a way for wider application of holographic principles. The invention of electronic capture devices further brought holographic principles to the forefront of optical imaging.

According to the principle of holography, with suitable coherent scattered and un-scattered light from an object source it is possible to record and reconstruct the complete information of the object i.e. amplitude and phase in recording media which responds only to intensity of light [13]. Holographic imaging involves two main steps namely recording and reconstruction of complex waves that emerge from the object. The data is recorded in the form of a "hologram" which contains high spatial frequencies and cannot be seen by human eyes.

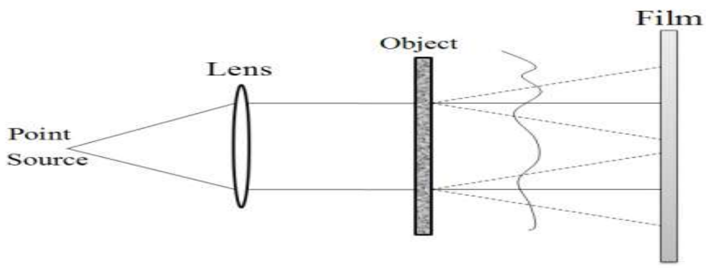


Figure 2.5: Gabor's In-line Holography setup [14].

Reconstruction of the image is carried out by illuminating the hologram by the reference wave again. The reconstructed image can be recognized by effects of perspective and depth of focus. Initially, Gabor had illuminated the hologram by a parallel beam through the object known as in-line holography. Gabor's original in-line setup for recording the hologram is shown in Figure. 2.5.

In in-line holography a monochromatic source after collimation by the lens is used to illuminate the object. The light that passes through the object is made up of two fields, scattered field (U_1) and unscattered field (U_O). Behind the object at some distance "z", the distribution of intensity from the interference of these two fields can be written as [14]:

$$I(x,y) = |U_o|^2 + |U_1(x,y)|^2 U_o{}^* U_1(x,y) + U_o U_1{}^*(x,y) \qquad 2.1$$

Thus the object and reference beam will interfere at the photographic film. At a distance z from the object, a plane un-scattered wave and scattered field interfere and the detector records an intensity distribution generated by the interference of these two fields. The intensity distribution of two complex waves depends on the amplitude and unknown phase of waves.

Now with an assumption of a linear response to the associated photographic plate intensity, we can find the transmission function which takes the form of:

$$t(x,y) = a + bI(x,y) \qquad 2.2$$

Here, a and b are the constants. The hologram gets written and contains all the relevant object information in its transmission function. Reconstruction the image information is done by illuminating the hologram once again as if it's a new object. Now the field that is scattered by the hologram can be represented as the multiplication of transmission function and the illuminating plane wave which can be represented as:

$$U(x,y) = U_o t(x,y)$$

$$= U_O(a + b|U_o|^2) + bU_o(|U_1(x,y)|^2) + b|U_o|^2 U_1{}^*(x,y) \qquad 2.3$$

In above equation 2.3, the first term is spatially constant and the second term is negligible in comparison with the final two terms as for a transparent object the unscattered field is much stronger than the scattered field, therefore the last two terms are of utter importance. Significantly, these two terms contain the complex far field and its conjugate; hence an observer standing behind the hologram at a position z will see the image resembling the object.

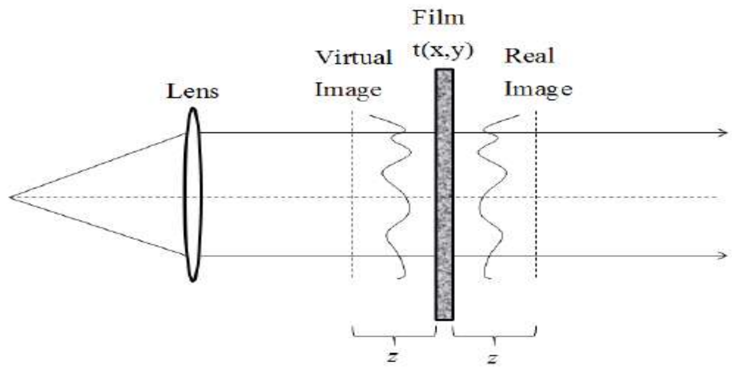


Figure 2.6: Image formation and Reconstruction in In-line Holography [14].

Figure 2.6 shows the formation of real and virtual images from Gabor's in-line method. In-line holography provided a new method of imaging objects but it did suffer from few drawbacks as will be briefly explained.

The main drawback of in-line holography was that the real image got superimposed by un-diffracted part of reconstruction wave, resulting in "twin image" degrading the image quality and thus rendering a significant problem in the Gabor's technique. Due to the formation of twin image the virtual image is out of focus when the real image is brought to the focus thereby marking a physical constraint in holography. Since twin problem posed a significant problem in the practical applications of holography. Over the period of time various attempts and studies were done to remove and eliminate this problem in the in-line holography. A well established method of holography known as off-axis holography is used to remove the effects of twin image in the inline holography [15].

Two scientists namely Juris Upatnieks and Emmett Leith while working at the University of Michigan's Radar and Optics lab invented a new modified technique to remove and solve the problem of twin image of the in-line holography [15]. Most of the limitations of the in-line method were overcome by going to an off-axis geometry that allows various image components to be separated.

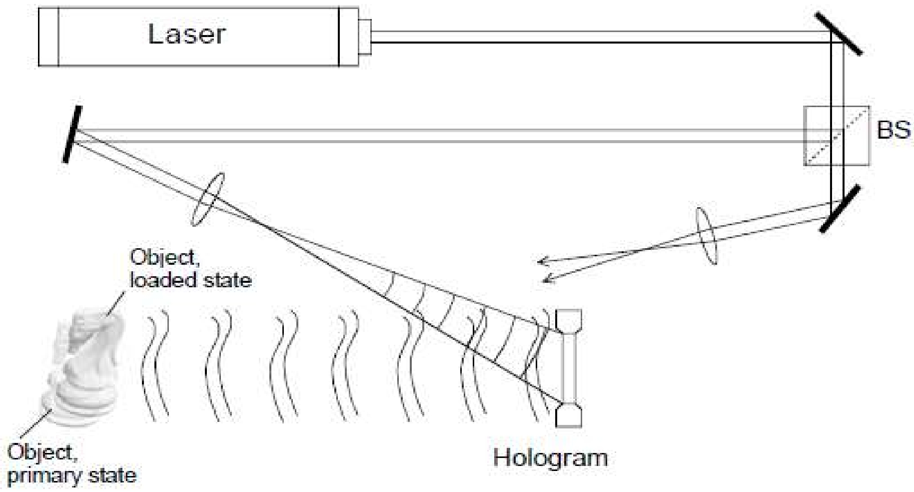


Figure 2.7: Basic setup for recording of Off-axis Hologram[16].

The main difference in off-axis method of holography is that a mutually coherent wave spatially separated from the object wave is used to record the hologram. In the analogy of the radio communication methods, off-axis holography basically adds carrier frequency to the field of interest. Recording of off-axis hologram is similar to the in-line holography.

The geometry used for the recording of hologram is shown in above Figure 2.7. Here, laser is used as a source. The reference laser beam is the unaltered wave and object beam is the wave field that is scattered from the object under investigation, therefore we get the interference pattern in the form of a hologram on the photographic plate. Figure 2.8 shows how the object is reconstructed back. The mathematical basis for the off-axis holography can be found in [17] for more elaboration on the off-axis holography.

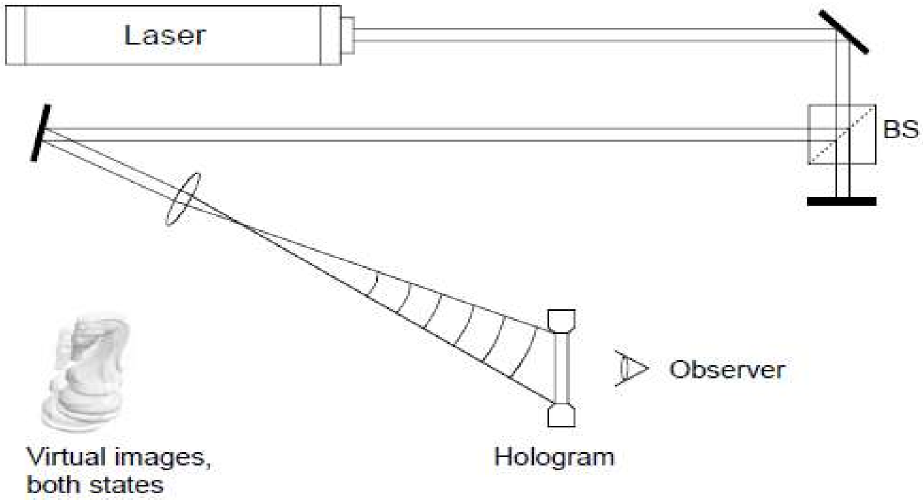


Figure 2.8: Reconstruction of images from a Leith-Upatnieks Hologram[16]

The initial holographic techniques were mainly done optically meaning that both the hologram recording and recovering of the object field is done optically. The main disadvantage of optical holography is that the object that is reconstructed cannot be characterized properly. In order to remove it, digital holography technique is used which is explained in detail in the following section.

2.4 Digital Holography

Over the decades, the wonderful principles of holography had been known to many but the practical application of holography was hindered by the troublesome procedures and stringent reconstruction requirements. Traditionally recording the interference pattern of light in holography was done with the help of photographic plate. Digital holography replaced the conventional photochemical procedures of holography with the computationally efficient and fast electronic imaging. In digital holography the recording of holograms is mainly done with a charge coupled device (CCD) camera or a similar device. In 1994, Schnars and Jueptner became the first scientists to use a CCD camera directly connected to a computer as the input in a holography setup [18]. The reconstruction of the image or rendering of object data is done numerically by digitized interferograms. The optical field propagation is efficiently given by the diffraction theory and Lawrence and Goodman in 1967 explained the numerical reconstruction of the image from its Fourier hologram which is detected by a videcon camera equipping us with the numerical reconstruction of the image as an array of complex numbers which represent the phase and amplitude of a given wave. [18]. Presently available CCD/CMOS cameras and fast processing computers make it easy to reconstruct and numerically focus the object field thereby making the technique highly efficient and has opened the doors for wide range of applications of holographic principles. Apart from it, several recording and processing schemes have been developed to assess the optical wave characteristics such as amplitude, phase, and polarization state, which further makes digital holography a very powerful method for metrology and biomedical applications. Figure 2.9 shows the digital holographic method of imaging.

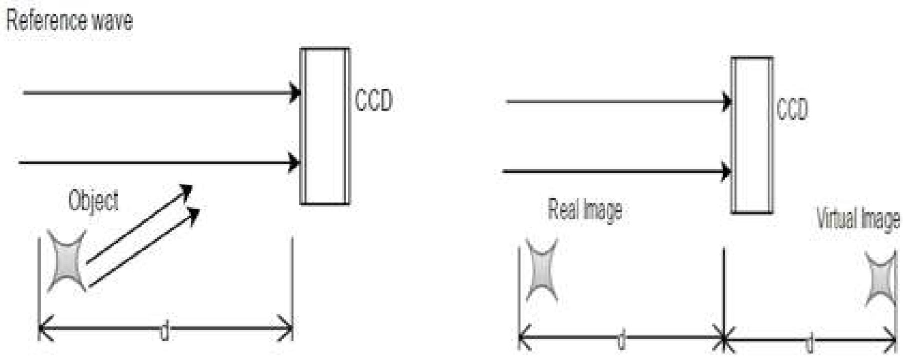


Figure 2.9: Digital holography recording and reconstruction.

For several research areas, the real-time operation is of utmost importance but real time imaging is very difficult with the traditional holography imaging but digital holography makes real time imaging possible by electronically recording the holographic interference and due to the high computational speed that it offers. The detailed study about the holographic techniques and its application in various areas of research can be found in [19-20]. The digital holographic imaging principles are highly useful in imaging through a random media and the method of speckle holography which we will see in the next chapter is utilized in our imaging technique and has potential applications in biomedical research.

Having discussed the holographic imaging techniques, I would now like to focus on developing the theoretical basics of our method for imaging through random media which will utilize the principles of holography imaging. In the next chapter, coherence theory will be discussed along with the speckle statistics which lays the foundational basis of our method.

Chapter 3

Coherence Theory and Speckle Statistics

Statistical knowledge plays a fundamental role in the analysis of physical and engineering problems. Though the statistical methods are somewhat complex but in the long run, they are far more superior and powerful than the deterministic methods. The interaction between the light and matter is also basically a statistical phenomenon which can be very well understood with the statistical analysis thereby statistical fluctuations of the light waves are of high value in optical imaging and plays an important role in determining the nature of the image formed [21]. Statistical optics basically involves the study of random effects of light. For random light, field dependence on the position and time cannot be predicted completely and one has to resort to the statistical methods. The random nature of light arises mainly due to the unpredictable and random nature of the medium in which the light wave is propagating or due to the randomness of the light source. Another factor that can cause randomness in the light waves is the scattering of light from the rough surfaces. While imaging through a vacuum, the statistical aspects of the imaging system do not change but if the medium is highly fluctuating then several other statistical aspects are added to the imaging problem which needs to be taken care of.

In a statistical process we make use of statistical measurements like standard deviation, correlation and averages etc to describe the optical fields. In the first order characterization of optical fields, the field behavior is observed in one point of space or time but statistical optics mainly makes use of second order statistics for the characterization of partially coherent fields [22]. If the coherent light is incident on the scattering layers then laser speckles are formed which will be discussed in detail in this chapter. When the coherent laser light is incident on a biological tissue or in-vitro tissue samples speckles termed as biospeckles are formed on the observation plane however there isn't any noticeable difference between the properties of the biospeckles and the speckles generated from non biological scattering layers and both can be characterized by same optical properties [23]. In second order speckle statistics, the field is characterized by the field correlation at two separate points and complex coherence (Correlation) function can be used to describe the random field fluctuations. As already stated, coherence plays a vital role in the understanding of the field randomness and speckle coherence functions are completely adequate for imaging through the scattering media as the random field coherence function is connected to the source structure by the Van Cittert-Zernike theorem [24]. The theorem and recovering the complex coherence function in the far field can be used to image through scattering layers which is the area of high interest in the biological studies and the upcoming sections will be devoted to the understanding of coherence theory and demonstrating the method to retrieve the speckle complex coherence function by using speckle characteristics in a scattering medium and utilizing the off-axis holography.

3.1 Coherence

Light waves have a property of interference; fundamentally the interference that is associated with the light waves emerges when individual electromagnetic fields of the combining waves interact with each other. When two light waves interfere with each other then depending on their relative phase they can add up in such a way that the amplitude of the resultant wave will be greater than their individual amplitudes and such type of interference is said to be constructive interference. Similarly, two waves can also interact with each other such that the resultant wave possess amplitude lesser than the individual amplitudes of the two waves and this type of interference is known as destructive interference. An ordinary light source e.g. Light bulb is not able to produce the interference effects mainly because the waves from such sources do not maintain zero or constant relative phase and such types of light sources are known as incoherent light sources therefore waves are said to be coherent when they have a constant relative phase relationship, similarly light sources are coherent if the waves emitted by them have same frequency and are phase linked with constant or zero phase difference [25]. It was very difficult to produce the coherent waves earlier but after the invention of lasers, the coherence theory has been developing rapidly as one of the main characteristic properties of laser light is the coherence of its emitted radiations. Laser an acronym for "Light Amplification by Stimulated Emission of Radiation" is a device that emits light through the method of optical amplification based on stimulated emission of photons. As the common stimulus is used to trigger the emission events in the laser that provides the light amplification therefore photons that are emitted have a definite phase relationship with each other. The emitted laser light has got high degree of coherence which is unachievable using other sources. Also, laser beam can be focused on very minute spots thereby attaining very high irradiance and can be used as a beam of low divergence in order to concentrate their power at a large distance.

The light coherence can now be defined in a much precise way as the correlation of the fields either in time or space. Coherence shows how similar (correlative) two time varying electric field distributions are. The coherent light intensity distribution is the absolute square of the field at the observation plane in terms of time and position and can be mathematically represented as:

$$I(x,t) = |E(x,t)|^2 \quad 3.1$$

Also, the Average Intensity for a random light at an observation plane can be represented by:

$$I(x,t) = <|E(x,t)|^2> \quad 3.2$$

Where <> represents ensemble average.

Before 1950's the coherence concept was limited to the electromagnetic field interference however in the mid and latter half of the 1950's Hanbury Brown and Twiss were able to perform and demonstrate the effects of coherence through the correlation of light intensities [26]. The phenomenon developed rapidly after the invention of lasers and led to the development of quantum coherence theory which is now an integral part of modern optics. In our work we will be using the similar concept of two point intensity correlation for imaging through the random media.

The coherence is basically of two types namely spatial coherence and temporal coherence. Our main interest is on the spatial characteristic of the coherent light and its applications therefore we will concentrate more on spatial properties of light rather than the temporal properties but for better understanding of coherence concepts, we will discuss temporal along with spatial coherence.

3.1.1 Temporal Coherence

The temporal coherence is the ability of light wave to interfere with its temporally delayed version and can be described with the help of a Michelson interferometer as shown in Figure 3.1. In a Michelson interferometer light beam from the source is divided into two by the beam splitter. Beams after splitting reach two mirrors individually placed at different positions. One of the mirrors is moveable so that path difference can be introduced [27]. The light beams after getting reflected by the mirrors travel back towards the beam splitter and the unified beam reaches the detector. For a smaller path differences interference fringes will be formed in the observation plane.

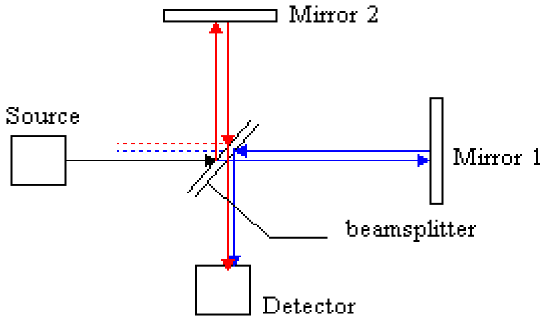


Figure 3.1: Experimental setup of Michelson interferometer [28].

The observed fringes are caused by the temporal coherence that occurs between two interacting light beams. The fringe characteristics depend on the time delay Δt that has been introduced and is known as coherence time which can be given by the following equation:

$$\Delta t = \frac{1}{\Delta \beta} \qquad 3.3$$

Where, β is the bandwidth.

Also, the coherent length can be mathematically represented as:

$$\Delta l = c\Delta t \qquad 3.4$$

Where, c is the velocity of light, Δl is also known as the coherence length.

The coherence time is very important quantity and provides the time interval during which correlations of the light waves at a particular point in the optical field continue to exist and the concept of correlation plays a fundamental role in the understanding of above phenomenon. There exists a strong correlation effect between the interfering waves in succession if the time delay between the light waves is less than the coherence length [21]. On the contrary if the coherence length is greater than the time delay then no correlation exists thereby leading to the absence of interference fringes. In the case of temporal coherence Wiener-Khintchine theorem plays a critical role for measuring the autocorrelation by relating it to the spectral density and the detailed explanation for the same is given in [29].

The temporal autocorrelation function can be represented mathematically by:

$$T_c = [E^*(t)E(t + \Delta t)] \quad 3.5$$

Here, E and E^* represents the field and its conjugate.

The temporal coherence shows how monochromatic a source is. In a polychromatic beam of light, the rate of temporal change is high and higher the change more is the polychromaticity. Thus a temporally coherent monochromatic beam of light has a high coherent length.

3.1.2 Spatial Coherence

Spatial coherence is concerned with the ability of light beam to interfere with its own spatially shifted version or in other words spatial coherence represents the correlation of two spatial points with respect to time thereby giving an insight regarding the uniformity of the phase. The graphical representation of spatial coherent waves is shown in Figure 3.2.

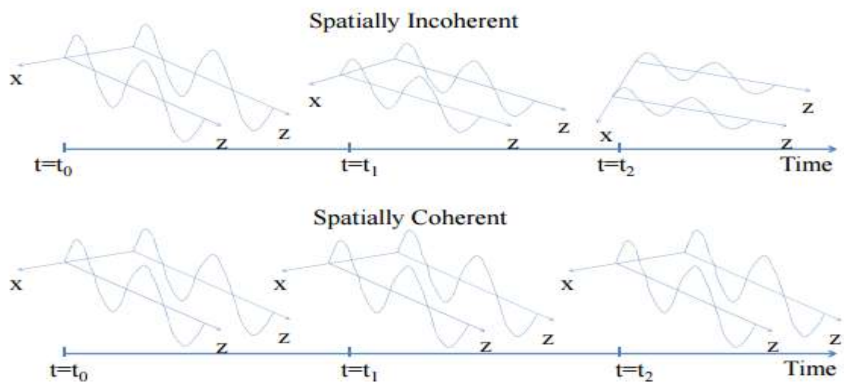


Figure 3.2: Graphical representation of Spatial waves [14].

The phenomenon of spatial coherence can be best explained with the help of Young's experiment as shown in Figure 3.3. Suppose light from the source propagates through the space towards the screen with two slit holes as shown in the

Figure 3.3. When pinholes Q_1 and Q_2 are held close to each other than interference fringes are observed on the screen. The fringe formation is considered as a manifestation of spatial coherence and is the resultant of superposition of two waves that emerge from the slits. Basically two beams form the fringes due to the correlation they share provided the condition of spatial separation is fulfilled [21]. Also, the time delay of the source should be less than its coherence time for observing the fringes. The observed interference fringes are spread throughout the neighborhood and the visibility of these fringes depends on the degree of correlation of the light waves.

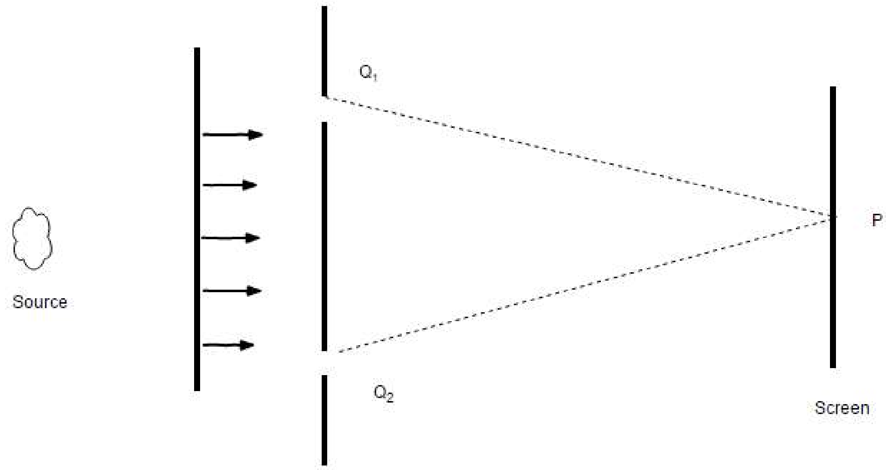


Figure 3.3: Young's double slit experiment

If $E_1(t)$and $E_2(t)$ are the electric fields from the two slits which are superimposed at the detector then spatial coherence or complex degree of coherence γ_{12} can be mathematically represented as suggested in [25]:

$$\frac{\gamma_{12} = \frac{1}{2} < E_1(t)E_2^*(t) + E_1^*(t)E_2(t) >}{2\sqrt{I_1}I_2} \quad 3.6$$

Where, I_1 and I_2 are the intensities of the beams from two slits which are superimposed at the screen or detector.

Further, the intensity distribution at the observation plane can be represented as:

$$I = I_1 + I_2 + 2\sqrt{I_1 I_2}\,\gamma_{12} \cos\theta \qquad 3.7$$

Where, θ is the phase difference in the path at the screen from two slits.

Also, Visibility (V) of interference pattern can be defined mathematically as:

$$V = \frac{I_{max} - I_{min}}{I_{max} + I_{min}} \qquad 3.8$$

For higher values of V fringe contrast will be high whileas when $V = 0$, fringes get washed away. From the above equations we can derive a relationship between the degree of coherence and the Visibility and can be written mathematically as:

$$V = \frac{2\sqrt{I_1 I_2}\ \ |\gamma_{12}|}{I_1 + I_2} \qquad 3.9$$

The values of correlations are of infinite orders and Young's double slit experiment provides the information about the second order spatial coherence of the optical field. Having discussed temporal and spatial coherence in detail, we now focus our attention to the coherent scattering and look in detail the spatial speckle statistics. Though sources like mercury green line or spectral sources do show a certain degree of spatial coherence but their coherence does not match with that of the laser source.

3.2 Laser Speckle - Coherent Scattering

With the arrival of laser in 1960 the world of imaging got revolutionized. Since laser light has a high coherence therefore when an optically rough surface or scattering layer is illuminated with it, the light that is backscattered produces an interference pattern on the recording screen [30]. The interference pattern generated by the laser light on scattering from an optically rough surface or by propagating through the random medium having random fluctuations of refractive index, consists of bright and dark areas which scientists had termed initially as "Granularity" but later it became known as "Speckle" [31]. Figure 3.4 shows the origin of speckle in free space once the incident light on rough surface is scattered.

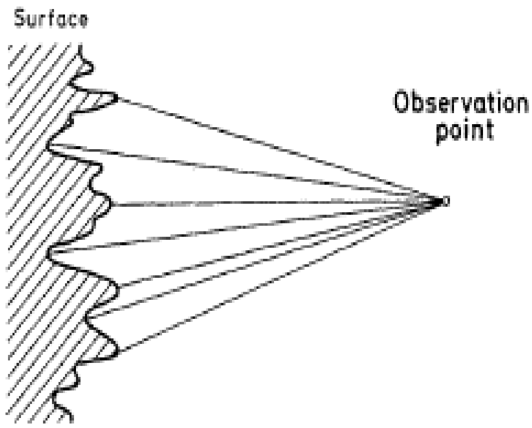


Figure 3.4 Physical origin of speckle pattern in free space [33].

Although the laser speckle phenomenon we are concerned with is the result of coherent light scattering but similar phenomenon can be witnessed due to the scattering of X-rays by fluids and due to the electron scattering from carbon (Amorphous) films [32]. A typical experimentally generated speckle pattern from a coherent laser through diffused media is shown in the Figure 3.5.

Although at first speckle was considered as a noise and nuisance as it used to affect the resolution but as more research efforts were utilized in studying of the laser phenomenon, its utility for imaging especially holographic imaging was found out [33]. The phenomena of laser speckle can be utilized for extracting the information about the illuminated surface that generates it. Even small changes in the conditions of imaging can cause changes in the shape of generated speckle pattern. As already mentioned, the interference intensity resulting at a specific point on the image is due to the superposition of all waves arriving at that point. The maximum amplitude of the recorded intensity at a single point can be observed when all the waves arriving at a point are in same phase and a bright spot can be seen at the point of maximum intensity. Similarly minimum intensity of the recorded intensity at a single point can be observed when all the individual waves interacting at a single point cancel out thereby creating a dark speckle spot.

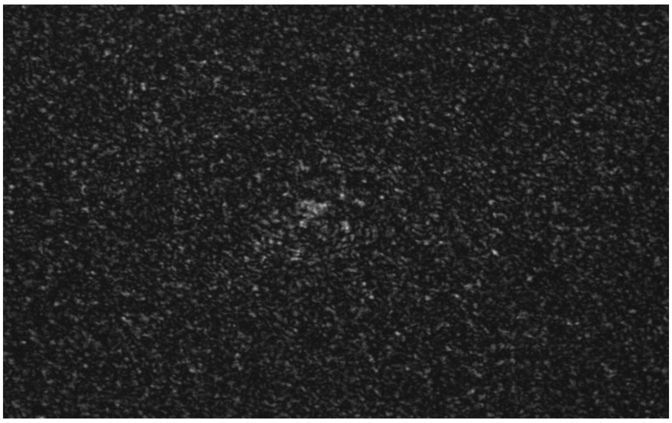


Figure 3.5: Speckle pattern generated from a coherent laser source

It is now established that this fundamental random interference phenomenon of the laser speckle has applications in various branches of engineering and research. Due to the significant applications of laser speckle in other areas of research, the term "speckle" has achieved a far more general meaning than what could have been imagined when laser speckle was first introduced in 1960 [34]. In the next section we will look into the first and second order speckle statistical properties. A brief introduction to first order properties will be presented followed by the second order speckle properties especially intensity correlation function which can be utilized for imaging through the turbid media.

3.2.1 First-Order Speckle Statistics

As speckle pattern is caused by the superimposition of statistically independent waves due to the scattering media therefore it can be characterized by intensity, amplitude and phase. The values of these quantities in the scattered field vary randomly from one point of detection to another [23]. In first order speckle statistics speckle properties are calculated at one point. In order to calculate these values at a detection point, scattered field probability distribution functions play a vital role as by using them one can calculate the first order speckle statistics of the speckle pattern mainly mean values and variances [22].

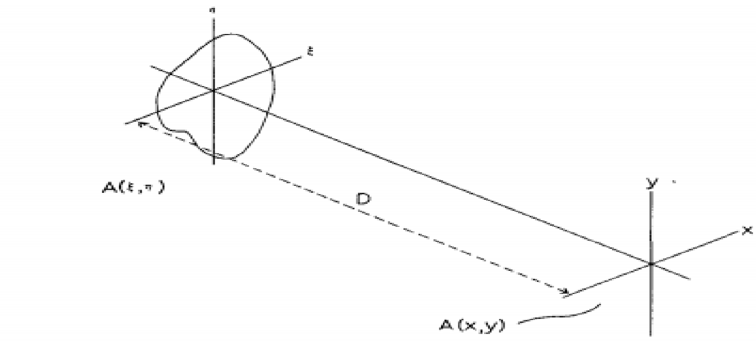


Figure 3.6: Formation of speckle pattern in a fraunhofer plane [35].

Let a speckle pattern be formed from the coherent light in an observation plane as shown in the Figure 3.6.

We can write the complex amplitude of the scattered wave as suggested in [35] as:

$$A(\xi,\eta) = \sum_{j=1}^{N} a_j e^{(ib_j)\delta(\xi-\xi_j)\delta(\eta-\eta_j)} \qquad 3.10$$

Here N is the number of scatterers,

a_j is the modulus of the scattered wave due to scatterer,

$\delta(\xi)\delta(\eta)$is the two dimensional Dirac delta function,

b_j is the phase of the wave.

Taking the Gaussian far field assumptions, the complex amplitude $A(x,y)$ can be written as:

$$A(x,y) = \int_{-\infty}^{\infty}\int A(\xi,\eta)\, e^{[\frac{-2\pi i}{\lambda D}(x\xi+y\eta)]d\xi d\eta} \qquad 3.11$$

From equation 3.10, 3.11 and by making use of Fourier transform we get:

$$A(x,y) = \sum_{j=1}^{N} a_j e^{(ib_j)}\, e^{[\frac{-2\pi i}{\lambda D}(x\xi_j+y\eta_j)} \qquad 3.12$$

Also, the probability density distributions of the scattered complex field amplitude have a Gaussian form which can be derived from central limit theorem [22]. The probability density of the field phase can be characterized by the uniform distribution and accordingly the probability density for developed speckles has a negative exponential form as shown below:

$$\rho(I) = \frac{1}{< I >} e^{-(\frac{I}{<I>})} \qquad 3.13$$

The above property can be manifested as the unit value of the speckle contrast which can be written as follows:

$$C = \frac{\sigma_I}{< I >} = 1 \qquad 3.14$$

The contrast is the ratio between the standard deviation and mean value of the speckle intensity fluctuations. Also, the relation between the developed speckle patterns for first and higher order statistical moments can be written as:

$$\frac{< I^n >}{< I >^n} = n! \qquad 3.15$$

And can be easily calculated from the statistical moments as shown in equation 3.16:

$$< I^n >= \int_0^\infty I^n \rho(I) di \qquad 3.16$$

A scattering surface consisting of many equal sized facets produces non-Gaussian speckle distribution characterized by the dependency of second order normalized moment of intensity fluctuations upon the system parameters as shown in equation:

$$\frac{< I^2 >}{< I >^2} = 2(1 - N^{-1}) + [\frac{k^2\psi^2}{4\pi P(\theta)}]N^{-1} \qquad 3.17$$

In the above equation k is the wave number of the light.

N is the number of facets within the area of illumination.

ψ is the radius.

$P(\theta)$is the probability of finding the facet.

If the speckle is fully developed, the first order statistics of the speckle fluctuations are completely non-dependent on the scattering object structural properties therefore they cannot be utilized for characterization of the scattering but they are enough to explain the brightness fluctuations. The speckle intensity statistics for a known illumination are the basis for several speckle technologies. The detailed study of first order speckle statistics can be found in [36] for better understanding. We now look into our main topic of interest i.e. the second order speckle statistics.

3.2.2 Second-Order Speckle Statistics

The statistical properties of a scattered field can also be characterized by simultaneous analysis of the correlation of the complex amplitude values for two spatially separated observation points and for different moments of time. Van Cittert-Zernike theorem is one of the central theorems in the coherence theory and according to it, in the far field; two-point spatial coherence function shares a definite relationship with an incoherent source structure [37]. Let us consider a monochromatic light that is incident on a rough surface. The scattered field can be observed in the observation plane as shown in Figure 3.7.

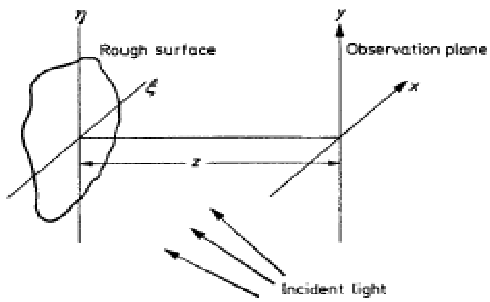


Figure 3.7: Geometry for speckle formation [34].

The scattered fields from the rough surface can be described in the adjacent plane to that surface by a complex valued function (ξ, η). The speckle field of interest is represented by the complex field $A(x, y)$ which is observed across the plane parallel to the (ξ, η) plane. Intensity distribution in the (x, y) can be represented from equation 3.1 and can be reproduced as:

$$I(x, y) = |A(x, y)|^2 \qquad 3.18$$

Our main goal is to calculate the autocorrelation function of the intensity distribution in (x, y) plane. The autocorrelation function in terms of its intensity can be represented by the below equation as in [34]:

$$R_f(x_1, y_1; x_2, y_2) =< I(x_1, y_1)I(x_2, y_2) > \qquad 3.19$$

Here average is taken over ensemble of rough surfaces.

The autocorrelation function in this form provides a considerable measure of speckle width. In order to calculate the above autocorrelation function, we take into consideration the fact that if a surface is rough as compared with the wavelength then the field $A(x, y)$ behaves as a complex random Gaussian variable at each point on (x, y). In case of such fields; the intensity autocorrelation function can be represented by:

$$C_A(x_1, y_1; x_2, y_2) =< A(x_1, y_1)A^*(x_2, y_2) > \qquad 3.20$$

The above equation represents the mutual intensity of the field.

For complex circular Gaussian fields, the relationship between the R_f and C_A after taking into consideration the fact that $C_A(x, y; x, y) =< I(x, y) >$ can be written as:

$$R_f(x_1, y_1; x_2, y_2) =< I(x_1, y_1)I(x_2, y_2) > +|C_A(x_1, y_1; x_2, y_2)|^2 \qquad 3.21$$

From above equation the calculation of R_f is reduced to the measurement of C_A.

Now, from the Fresnel approximation of the Huygens-Fresnel principle as suggested in [38], we can write:

$$A(x, y) = \frac{1}{\gamma z} e^{[\frac{-i\pi}{\gamma z}(x^2+y^2)]} \iint_{-\infty}^{\infty} \alpha(\xi, \eta)\, e^{[\frac{-i\pi}{\gamma z}(\xi^2+\eta^2)]} e^{\frac{i2\pi}{\gamma z}(x\xi+y\eta)]d\xi d\eta} \qquad 3.22$$

Here $\alpha(\xi, \eta)$ is the complex valued function which represents the linear polarization component of the field. Now, after interchanging the orders of integration and averaging we get a relationship between C_A at the observation plane and $C_\propto$ at the scattering plane as follows:

$$C_A(x_1, y_1; x_2, y_2) = \frac{1}{\gamma^2 z^2} e^{\left[\frac{i\pi}{\gamma z}(x_1^2 - x_2^2 + y_1^2 - y_2^2)\right]} \times \iint \iint C_\propto(\xi_1, \eta_1, \xi_2, \eta_2)\, e^{\left[\frac{i\pi}{\gamma z}(x_1^2 - x_2^2 + y_1^2 - y_2^2)\right]} \times e^{\left[\frac{i2\pi}{\gamma z}(x_1\xi_1 + y_1\eta_1 - x_2\xi_2 - y_2\eta_2)\right]} d\xi_1 d\eta_1 d\xi_2 d\eta_2 \qquad 3.23$$

Here, we area mainly concerned with the modulus of C_A thereby we ignore the initial exponential factors in (x_1, y_1) and(x_2, y_2).

Also we have:

$$C_\propto(\xi_1, \eta_1, \xi_2, \eta_1) \cong kP(\xi_1, \eta_1 P^*(\xi_2, \eta_2)\delta(\xi_1 - \xi_2, \eta_1 - \eta_2) \qquad 3.24$$

k here is a constant of proportionality and $P(\xi, \eta)$ is the amplitude of incident field. $\delta(\xi, \eta)$ represents the two dimensional delta function. Now, due to the simplifications we can write:

$$C_A(x_1, y_1; x_2, y_2) \cong \frac{k}{\gamma^2 z^2} \iint_{-\infty}^{\infty} |P(\xi_1, \eta_1)|^2\, e^{\{\frac{i2\pi}{\gamma z}[\xi_1(x_1 - x_2) + \eta_1(y_1 - y_2)]\}} d\xi_1 d\xi_2 \qquad 3.25$$

The above equation clearly depicts that the observed field at the plane is dependent only on the coordinate difference at the (x, y) plane and can be calculated by Fourier transforming the intensity distribution$|P(\xi, \eta)|^2$. The relation is analogous with the Van Cittert-Zernike theorem of the coherence theory. Now, we can define the more convenient normalized version of the mutual intensity which is known as the complex coherence factor as follows:

$$\mu_A(x_1, y_1; x_2, y_2) = \frac{C_A(x_1, y_1; x_2, y_2)}{[C_A(x_1, y_1; x_1, y_1) C_A(x_2, y_2; x_2, y_2)]^{1/2}} \qquad 3.26$$

Comparing it with equation 3.25, the complex coherence factor can be written as:

$$\mu_A(\Delta x, \Delta y) = \frac{\iint_{-\infty}^{\infty} |P(\xi,\eta)|^2 \, e^{[\frac{i2\pi}{\gamma z}[\xi\Delta x + \eta\Delta y] d\xi d\eta}}{\iint_{-\infty}^{\infty} |P(\xi,\eta)|^2 d\xi d\eta} \quad 3.27$$

Finally, we can write the speckle intensity autocorrelation factor as:

$$R_f(\Delta x, \Delta y) =< I^2 > [1 + |\mu_A(\Delta x, \Delta y)|^2]$$

$$=< I >^2 1 + \left| \frac{\iint_{-\infty}^{\infty} |P(\xi,\eta)|^2 \, e^{[\frac{i2\pi}{\gamma z}[\xi\Delta x + \eta\Delta y] d\xi d\eta}}{\iint_{-\infty}^{\infty} |P(\xi,\eta)|^2 d\xi d\eta} \right|^2 \quad 3.28$$

From the perspective of second order speckle statistics, Power spectral density of the intensity distribution $I(x, y)$ is an important quantity. The power spectral density $g_1(v_X, v_Y)$ is given by the Fourier transform of correlation function $R_f(\Delta x, \Delta y)$ which is written mathematically as:

$$g_1(v_X, v_Y) =< I >^2 \{\delta(v_X + v_Y) + \left| \frac{\iint_{-\infty}^{\infty} |P(\xi,\eta)|^2 \, e^{[\frac{i2\pi}{\gamma z}[\xi\Delta x + \eta\Delta y] d\xi d\eta}}{\iint_{-\infty}^{\infty} |P(\xi,\eta)|^2 d\xi d\eta} \right|^2 \quad 3.29$$

In case of a uniform and square scattering spot, power spectral density can be written in the following form:

$$g_1(v_X, v_Y) =< I >^2 \{\delta(v_X + v_Y) + \left(\frac{\gamma z}{L}\right)^2 \exists(\frac{\gamma z}{L} \mathrm{v_x}) \exists(\frac{\gamma z}{L} \mathrm{v_y}) \quad 3.30$$

Here $\exists(\mathrm{x}) = 1 - |x|$ for $|x| \leq 1$ and zero for other values.

3.3 Measurement of Complex Intensity Correlation Function Using Speckle Holography

Over the period of time various methods have been developed to measure the complex correlation function (Amplitude and phase) so that it can be utilized for various research needs but most of these techniques are inefficient, also phase of complex function is lost in many existing techniques and they require multiple shots for the retrieval of complex coherence (correlation) function but recently an alternate single shot imaging method has been put forward which measures the complex coherence function in a single scattering medium with the help of holographic principles. It works on the principle of two-point intensity correlation and is able to retrieve the phase of the complex function efficiently by utilizing the fact that two point intensity correlation is related with the source structure through a Fourier transform. The imaging methodology has its foundations in the Michelson-Stellar interferometry imaging wherein the image of a distant star is retrieved from the propagated mutual coherence function modulus [22].

In two point intensity correlation the coherence function of the randomly time varying field distribution originating from laser speckle having Gaussian statistics is measured by replacing ensemble averaging with the spatial averaging provided the conditions of spatial ergodicity and stationarity are fulfilled [39]. Here, a method to measure complex correlation function using the two point intensity correlation (fourth order field correlation) with the help off axis holography in a single shot as suggested in [39] is discussed. The major objective of it is to measure the complex coherence function of the laser speckle in a scattering media. This method is highly efficient and recovers both the phase and amplitude of the coherence function by using Intensity interferometer and CCD/CMOS camera which otherwise is very difficult to achieve.

If a quasi-monochromatic coherent source of laser light is incident on a scattering layer, a random spatial distribution of intensity called speckle pattern in the observation plane is generated.

This random distribution of electric field $E_0(r)$ as shown in Figure 3.8 at observation plane can be represented as [39]:

$$E_0(r) = \int E_0(\hat{r})\, e^{[i\emptyset(\hat{r})]} e^{(\frac{-2\pi}{\gamma f} r.\hat{r})d\hat{r}} \qquad 3.31$$

Where r and $\hat{r}$ are the position vectors in observation/Fourier transform plane and object plane/scattering plane.

$E_0(\hat{r})$is the random field distribution at scattering plane $\emptyset(\hat{r})$.

γ is the wavelength,

f being the focal length of Fourier transforming lens,

suffice 'o' for object speckle distribution.

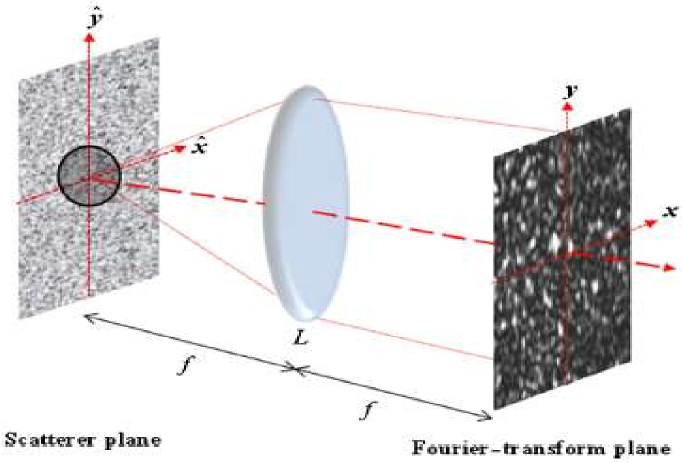


Figure 3.8: Conceptual diagram for generation of speckle [39].

Now we can express the second order correlation $C_0(r)$ after spatial averaging at the observation plane as:

$$C_0(r) =< E_O^*(r_1)E_0(r_2) >_s$$

$$= \int E_O^*(r_1)E_O(r_1 + \Delta r)dr_1 \quad 3.32$$

Where, $r_1 + r_1$; r_1 and r_2 are position vectors in observation plane, and $<>_s$ is spatial average.

In case of a random field obeying Gaussian statistics, there exists a relationship between second order and fourth order correlations which can be expressed as:

$$K_0(\Delta r) =< \Delta I_0(r)\Delta I_0(r + \Delta r) >_s \quad 3.33$$

And spatial fluctuation of intensity can be written as;

$$\Delta I_0(r) = \Delta I_0(r) - < \Delta I_0(r) > \qquad 3.34$$

Where, $K_0(\Delta r)$ is covariance function, $\Delta I_0(r)$ is object intensity at Fourier transforming plane, ΔI is spatial fluctuation with respect to mean value of intensity. By using speckle statistics phenomena, the cross covariance is proportional to the modulus square of second order correlation at the same plane which can be expressed as:

$$K_0(\Delta r) \propto |C_O(\Delta r)|^2 \qquad 3.35$$

In the above equation only the amplitude of the complex coherence function can be calculated while as phase part is lost. The lost phase can be recovered by employing speckle holographic principle suggested in [39], [40] and [41].

By using the off-axis holographic technique, the resultant coherence function at Fourier transforming plane can be expressed as:

$$\begin{aligned} C(\Delta r) &= < E^*(r_1)E(r_1 + \Delta r) >_s; \\ &= < \{E_0(r_1) + E_r(r_1)\}^* \{\{E_0(r_1 + \Delta r) + E_r(r_1 + \Delta r)\} >_s \end{aligned} \qquad 3.36$$

Also, from [39] $< E_o{}^*(r_1)E(r_1 + \Delta r) >= 0$. Therefore, the resultant coherence function can take the form:

$$C(\Delta r) = C_0(r) + C_R(r) \qquad 3.37$$

Hence we get,

$$\begin{aligned} C(\Delta r)|^2 &= |C_0(r) + C_R(r)|^2 \\ &= |C_O(\Delta r)|^2 + |C_R(\Delta r)|^2 + C_0(\Delta r)C_R^*(\Delta r) + C_O^*(\Delta r)C_R(\Delta r) \end{aligned} \qquad 3.38$$

C_O^* and C_R^* are the complex conjugates of the respective coherence functions.

From Equation 3.38, it can be seen that the complex part of the coherence function is encoded in an interferometric equation and with the use of a proper techniques one can recover complex part of correlation function. The optical setup for the retrieval of speckle field coherence function is discussed in the following section and results are shared.

3.3.1 Optical Setup

In the setup a basic model of Mach-Zehnder interferometer is used which is a major tool for an experimental setup working on the principle of off-axis holography as shown in Figure 3.9. Using the holography technique it is demonstrated here how to measure the complex correlation function of the speckle field in the scattering media. A spatially filtered light is collimated by a lens and then the collimated light is divided by a beam splitter $BS1$ into two beams, the transmitted beam emerging from $BS1$ acts as an object arm of the interferometer while as the other beam acts as the reference arm of the interferometer. The transmitted beam from $BS1$ is reflected by mirror $M2$ and illuminates the ground glass GG1 and generates the object speckle pattern. The beam in reference arm is reflected by mirror $M1$ and passes through a microscopic objective and illuminates the ground glass GG2 which generates an independent reference speckle pattern. The speckle pattern from the object and reference ground glasses are coherently combined using beam splitter BS2 and the coherent superposition is Fourier transformed using a common lens. The resultant speckle field is captured by a camera in the form of speckle hologram.

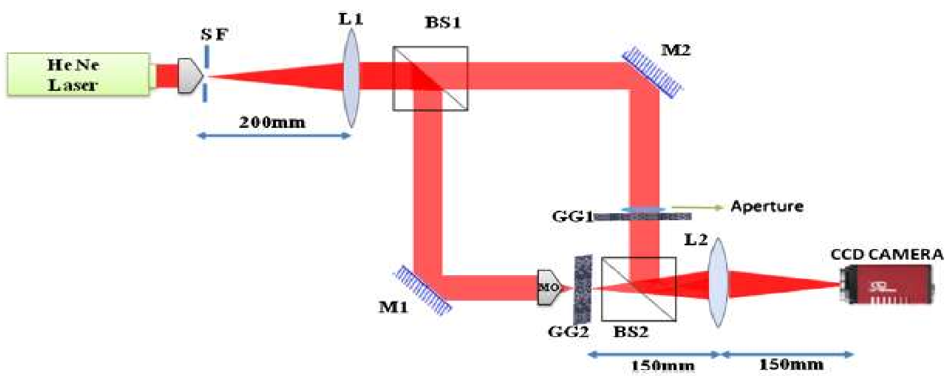


Figure 3.9: Experimental setup for measurement of complex correlation function [39].

By providing a spatial angular shift between the object and reference arm which is adjustable through the microscopic objective, we can separate the object from the direct current (DC) term or central background.

3.3.2 Recovering Speckle Complex Coherence Function

The resultant speckle field after the coherent super position of object and reference speckles is captured in the form of a hologram. The speckle hologram captured by CCD is shown in figure 3.10 for 1.5mm size of aperture introduced on ground glass which generated the spatially fluctuating intensity distribution that is imaged onto the CCD by a Fourier transforming lens. The obtained speckle hologram is spatially averaged for finding out covariance function which is further utilized for the purpose of measuring complex correlation function [39].

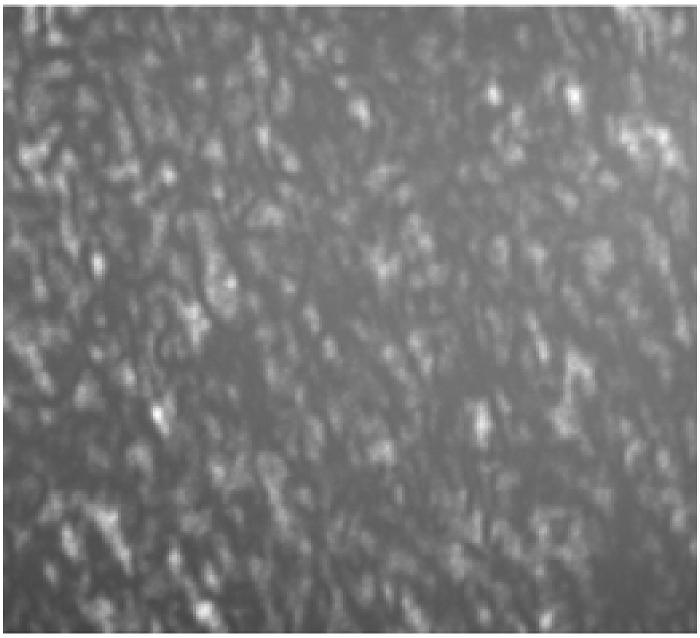


Figure 3.10: Resultant speckle pattern [39].

Once the hologram is averaged out, fringes will appear. The phase of correlation function is encoded in this fringe pattern. By employing a Fourier fringe analysis technique on the obtained fringe pattern we can recover the complex correlation function from two point intensity correlation. A detailed analysis of Fourier fringe technique is given by Takeda in [42].

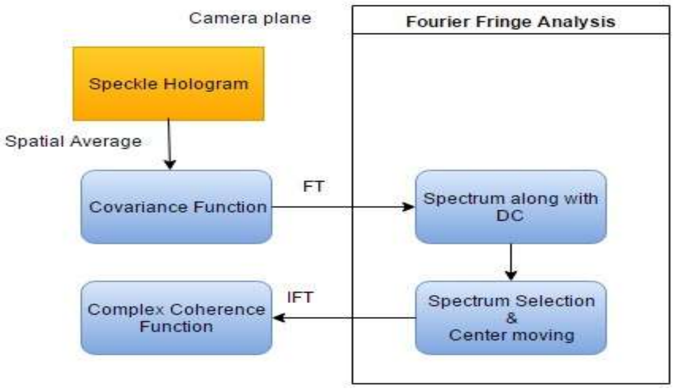
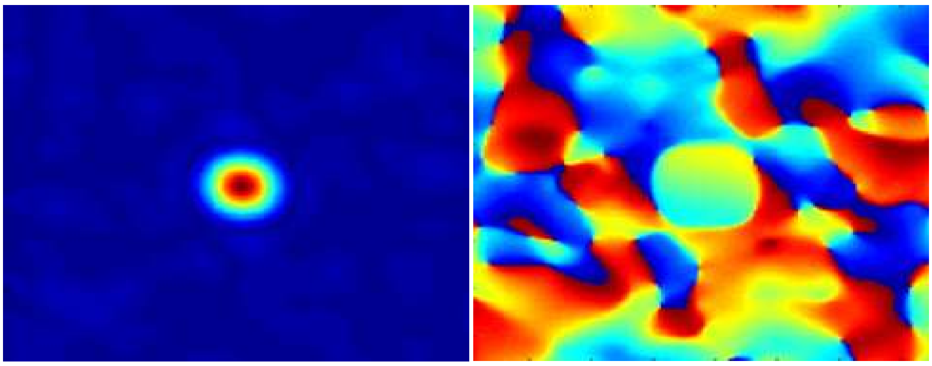


Figure 3.11: Steps involves in retrieving complex correlation function

Figure 3.11 shows the steps for retrieving the complex correlation function of the speckle field. By following these steps one can retrieve the complex correlation function. The retrieved complex coherence function for the aperture diameter of 1.5 is shown in Figure 3.12. Along with the experimental calculation, analytical evaluation of the retrieved complex coherence function by this method is also performed and is shown in Figure 3.13.



(a) (b)

Fig. 3.12 Retrieved complex correlation function (a) amplitude, (b) phase [39].

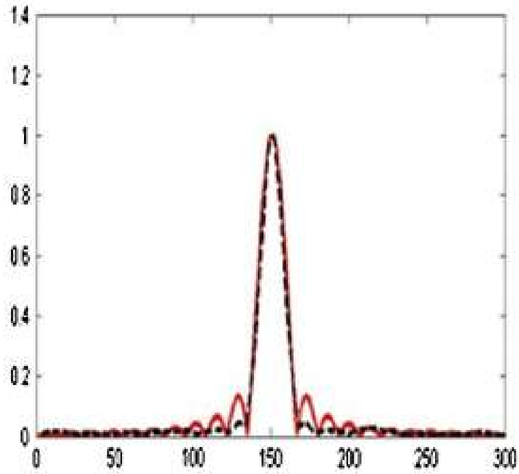


Fig. 3.13 Amplitude profile obtained by analytical and experimental results [39].

In the next chapter we will mainly target the retrieval of correlation function for a multiple scattering regime which will be further utilized for the recovery of an object hidden behind multiple scattering layer. The experimental setup for the reconstruction of object hidden behind multiple scattering layer will be demonstrated which will have applications in various areas of research especially biomedical imaging.

Chapter 4

Experiments and Results

When an object is placed in a random medium, a speckle pattern is generated as already discussed in chapter 3. We will develop a method that will be able to reconstruct the object information in the multiple scattering medium. The recovery of the object information from the randomly scattered field is realized by the Van Cittert-Zernike theorem which connects the statistical light properties in the far field with the source structure.

4.1 Simulations

The computationally generated letter "L" here will act as an object for simulation study. The interferometric technique is utilized by generating the reference as well as the object field computationally. The two point intensity correlation is performed on the obtained speckle hologram. The speckle hologram as shown in figure 4.2 is spatially averaged for measuring the covariance function which is further utilized for the purpose of retrieving complex correlation function along with its amplitude and phase. Computationally generated object "L" is shown in figure 4.1. The covariance function of the hologram is shown in Figure 4.3. Once the spatial averaging has been done, Fourier fringe analysis is performed and spectrum is moved towards the center. Figure 4.4 shows the spectrum along with DC. After spectrum selecting and moving it to center, the complex coherence function can be retrieved. Figure 4.5 shows the reconstructed amplitude and phase of the object.

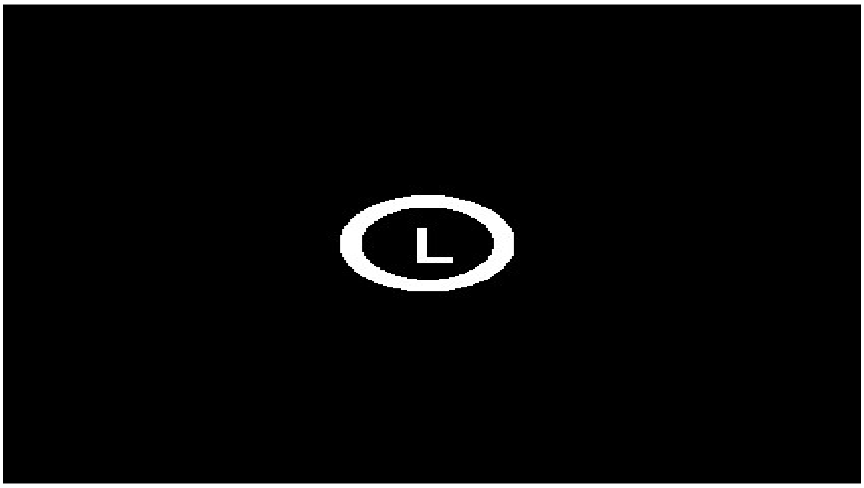


Figure 4.1: “L” Object

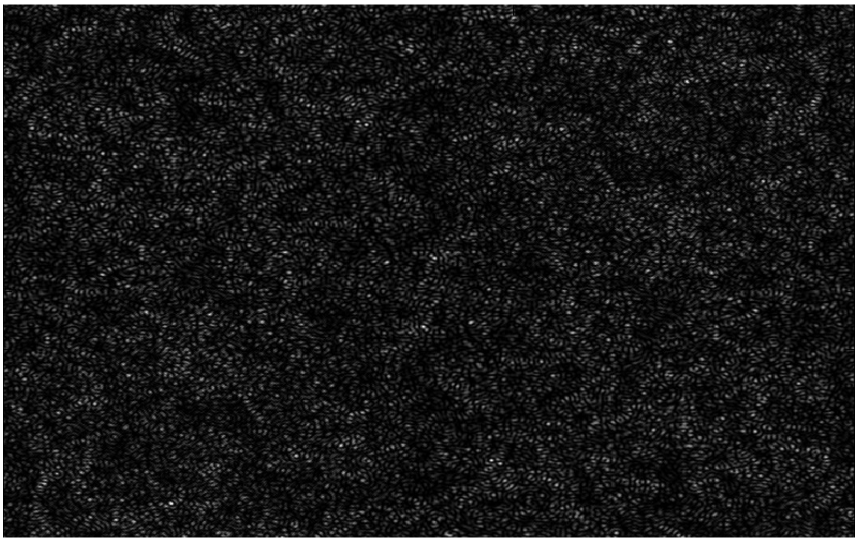


Figure 4.2: Speckle pattern

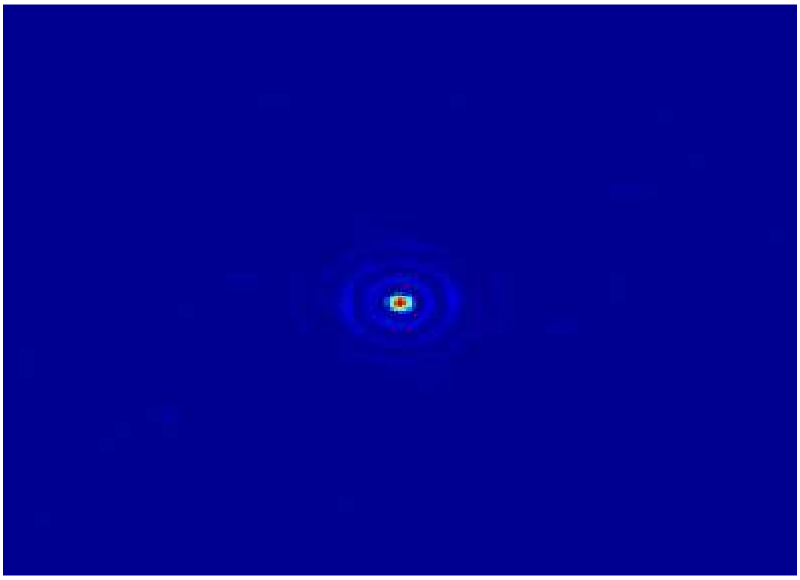


Figure.4.3: Covariance function

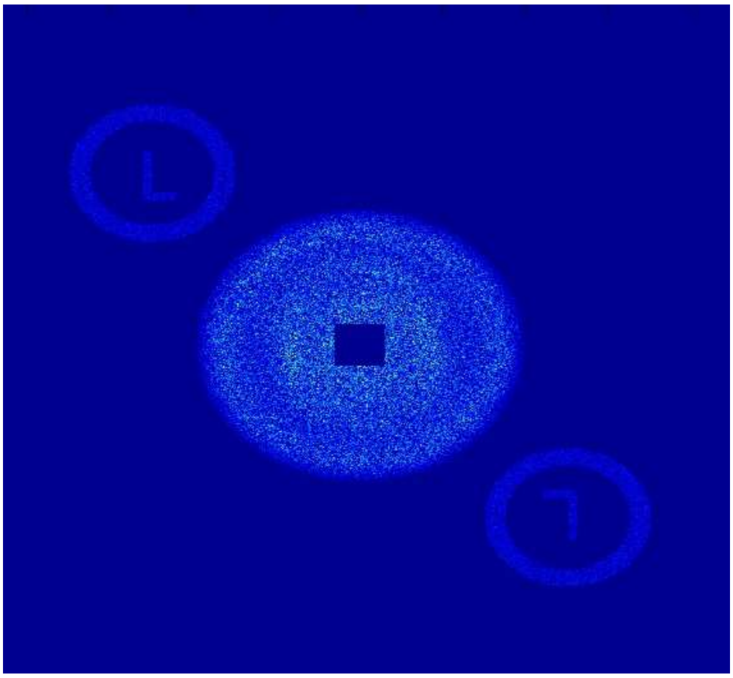


Figure 4.4: Spectrum along with DC

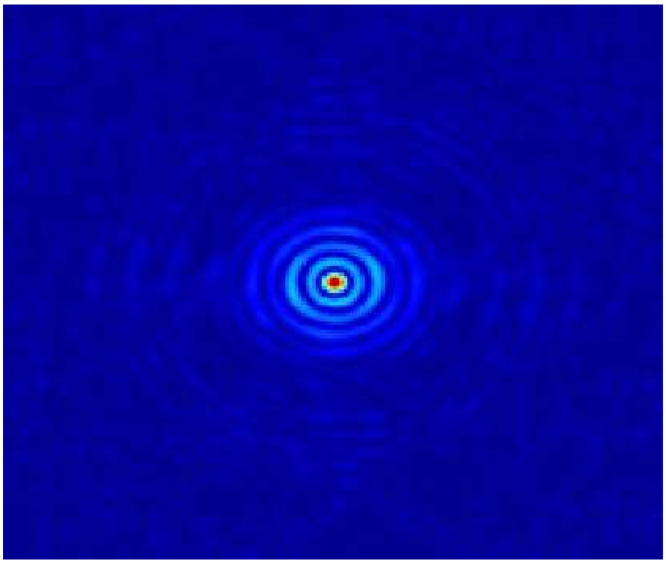
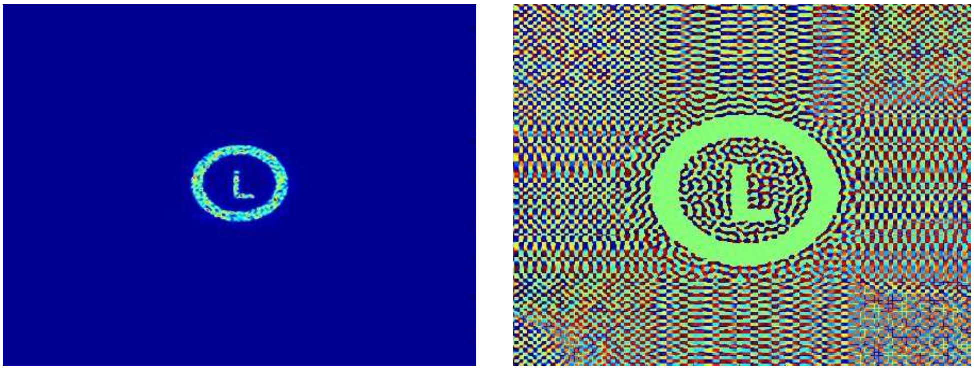


Figure 4.5: Correlation function



(a) (b)

Figure 4.6: (a) Reconstructed object (b) Phase

4.2 Experimental Setup

We devise an experimental strategy based on an interferometric technique. The setup here serves as an off-axis holography method to retrieve the complex coherence function of the speckle field and recover the object placed in a multiple scattering medium. We make use of the basic Mach-Zehnder interferometer that works on the principle of off-axis holography. The white painted glass serves as a multiple scattering layer here. The schematic diagram for the setup is given in the figure 4.7. The laser light of wavelength 780nm has been used in the experimental setup.

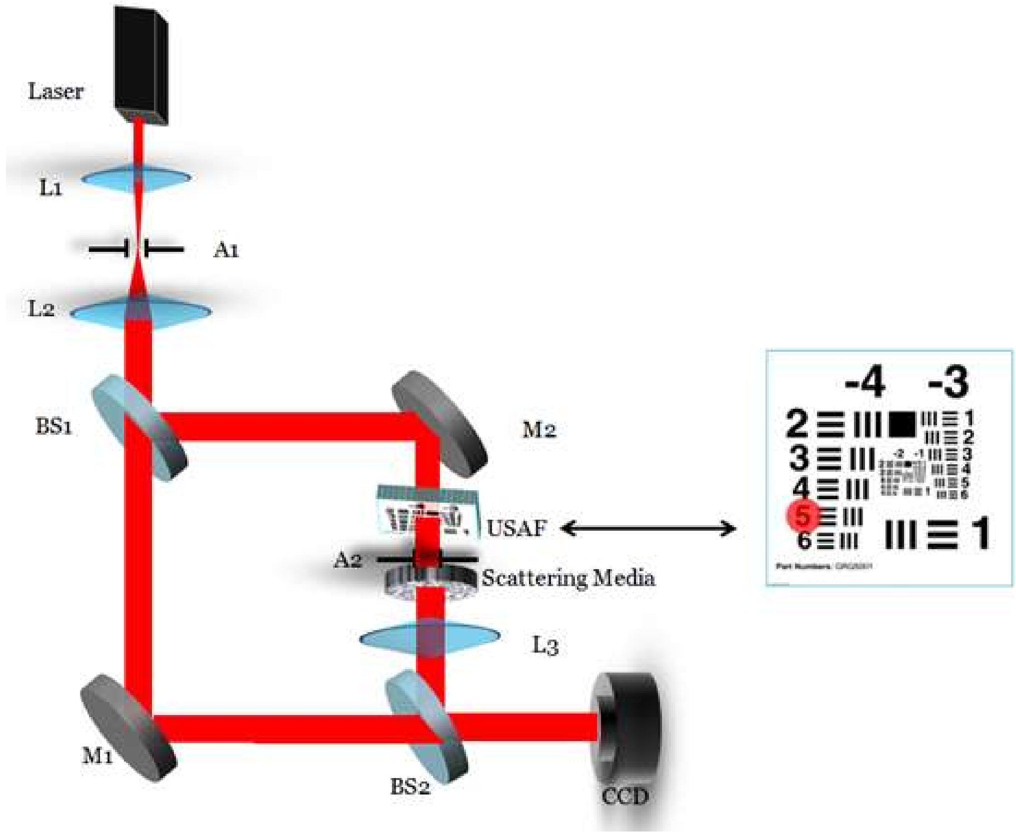


Figure 4.7: Experimental setup

The collimated light from laser source is spatially filtered by the combination of lenses L_1(Microscopic objective) and pinhole of size 15 micron. The spatially filtered light is then collimated with lens L_2 of focal length $150mm$. The collimated beam of light then reaches the beam splitter (BS_1) and beam is divided into two. The laser beam transmitted through the BS_1 acts as the reference arm of the interferometer and the reflected beam from mirror (M_2) acts as the object arm of the interferometer. The reflected beam from M_2 passes through the "5" object and scattering media. On the USAF test chart number "5" serves as the object whose information is to be retrieved. Mirror (M_1) is used in the reference arm to direct the beam towards beam splitter BS_2. The laser beam in the object arm reaches the white painted scattering layer and the object. Beam splitter (BS_2) is used to combine the beams emerging from reference arm and object arm of the interferometer. Here, Fourier transforming lenses are used to make the spatial stationarity over the CCD plane. The coherent superposition is captured by the CCD camera which has the pixel width of 8 micron in the form of a speckle hologram. The obtained speckle hologram is spatially averaged for finding out covariance function which is further utilized for the reconstruction of object.

4.3 Results

The speckle hologram generated by the object in the scattering medium is given in figure 4.8. Here, we make use of spatial averaging to calculate the covariance function of the object which is given in figure 4.9. Fourier fringe analysis is performed then to carry the spectrum towards the center. Figure 4.10 shows the field spectrum along with DC. Further, we then calculate the correlation function of the object hidden behind the scattering layer.

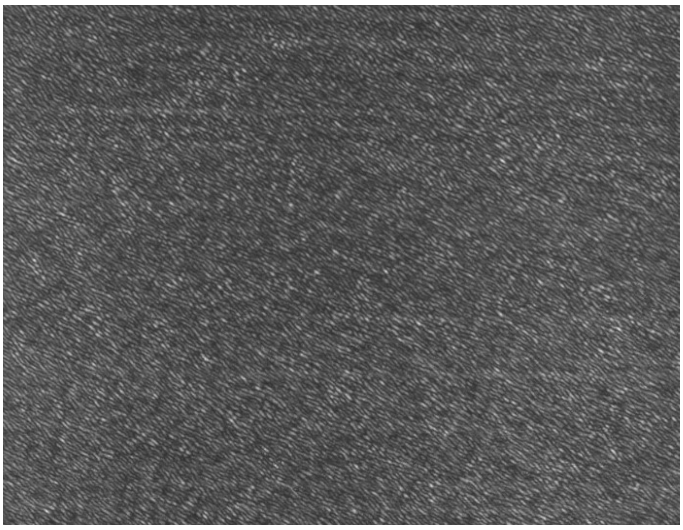


Figure 4.8: Speckle pattern generated by object hidden behind white paint layer

The complex correlation function is shown in figure 4.11. Finally by making use of the coherence function, the object information is reconstructed as shown in figure 4. 12. One of the main advantages of this method is that we can reconstruct the object information in a single shot.

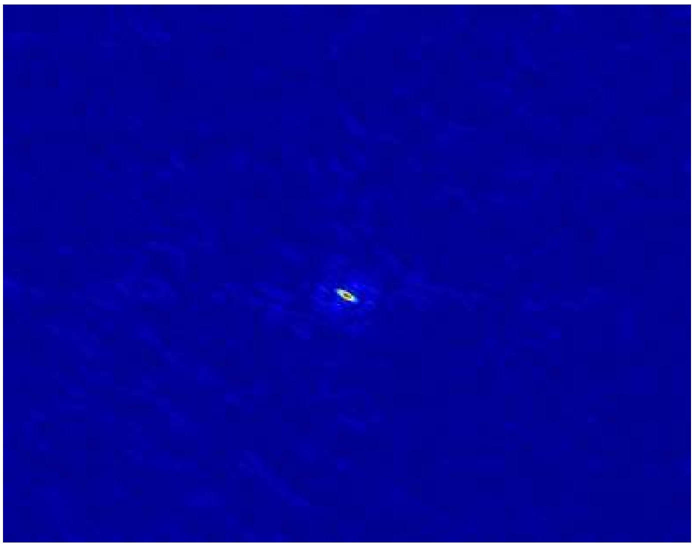


Figure 4.9: Covariance function of recorded speckle

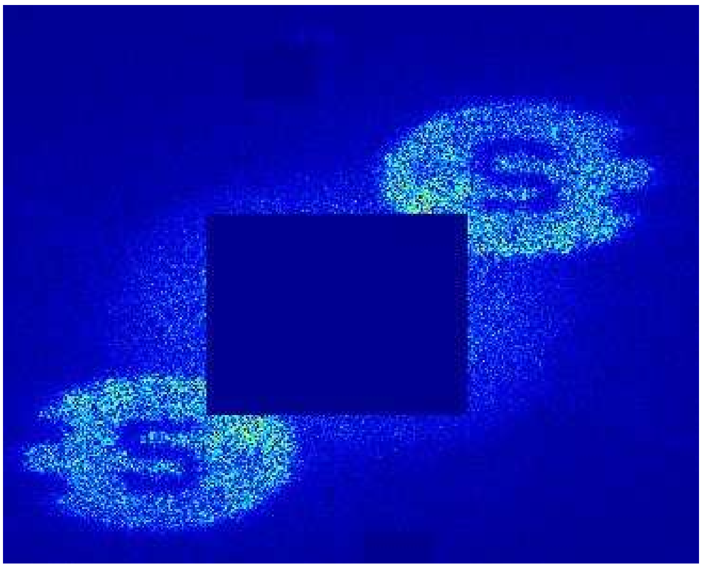


Figure 4.10: Spectrum along with DC

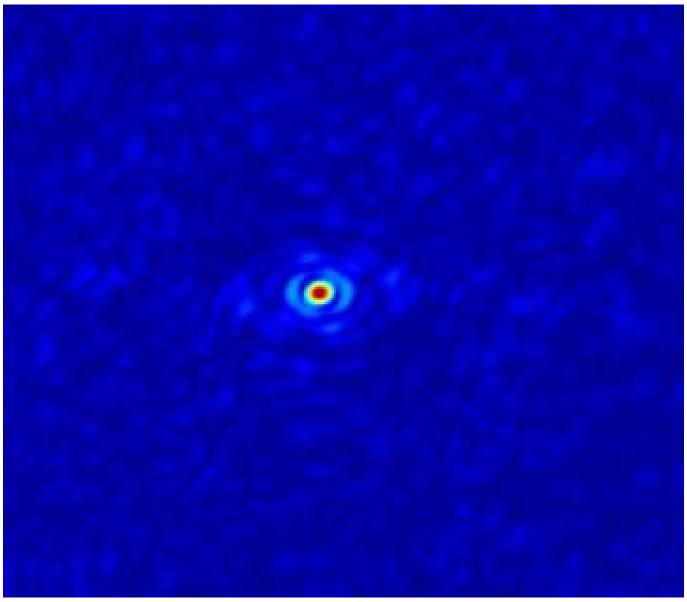


Figure 4.11: Retrieved complex coherence function

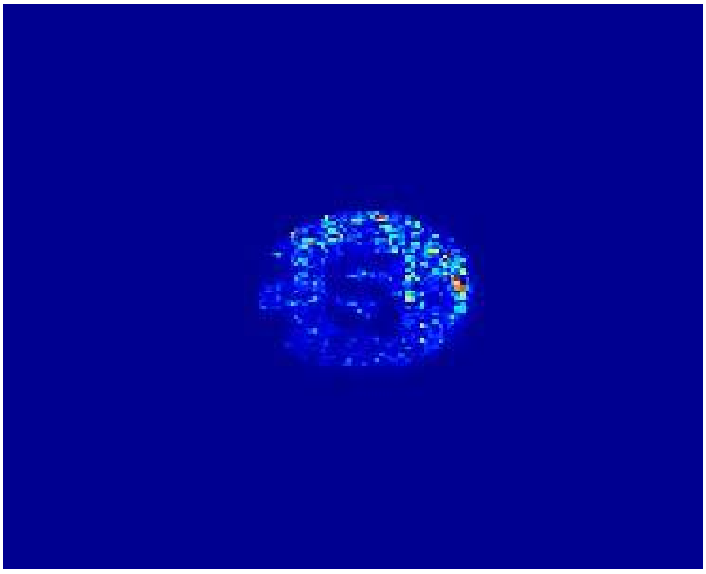


Figure 4.12: Recovered object information of an object hidden behind multiple scattering layer.

Chapter 5

Summary and future work

This thesis presents the problem of imaging through scattering medium. Here we have developed and demonstrated the experimental setup for imaging based on the principle of speckle holography and two point intensity correlation. The detailed theory of complex correlation function is presented and its recovery is shown.

The complex coherence function is further explored for the reconstruction of object in the scattering medium in a single shot. The experimental setup for object hidden behind the multiple scattering medium is also developed and the object is reconstructed. The reconstruction of the object in the scattering medium is achieved by the application of the Fourier fringe analysis technique to the spatially averaged speckle hologram.

Future work can be aimed to develop the technique with appropriate changes in the optical setup for imaging through more turbid media and also for monitoring of the Liquid/fluid flow by taking into consideration the dynamic nature of speckle interference thereby developing the presented technique as a full field flow visualization method.

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