# SET UP FOR 3D MICROSCOPIC IMAGING USING MICROLENS ARRAY AND ESTIMATION OF LASER DIODE BEAM PROFILE

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(MS14047)

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**Certificate of Examination**

This is to certify that the dissertation titled "SET UP FOR 3D MICROSCOPIC IMAGING USING MICROLENS ARRAY AND LASER DIODE BEAM PROFILE ESTIMATION." submitted by Mr. Avinash Kumar (Reg. No. MS14047) for the partial ful- filment of BS-MS dual degree programme of IISER MOHAL, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Ananth Venkatesan Dr. Sandeep K. Goyal Dr. Samir K. Biswas

(Supervisor)

Dated: April 22, 2019

## Declaration

The work in this dissertation has been carried out by me under the guidance of Dr.Samir K. Biswas at the Indian Institute of Science Educa- tion and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgment of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Avinash kumar

(Candidate)

Dated: April 22, 2019

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr.Samir K. Biswas

(Supervisor)

**Acknowledgement**

I would like to thank my Project guide Dr. Samir K. Biswas, for his valuable suggestion and sincere involvement in my project. He provided me all the materials required for this project and helped me while handling instruments and also supported me throughout the duration of the project. I would also like to sincerely thank all the members of Bio-Nanophotonics lab for their kind co-ordination. I also Acknowledge IISER Mohali community for their infrastructure and INSPIRE for their fellowship thought the course.

AVINASH KUMAR

# List of Figures

**Notation**

| Symbol | Name |
|---|---|
| ***LFM*** | Light Field Microscopy |
| ***LSM*** | Light Sheet Microscopy |
| ***L*** | Light Field |
| ***MLA*** | Micro-lens Array |
| ***PSF*** | Point Spread Function |
| ***NA*** | Numerical Aperture |
| ***X*** | Objective Magnification |
| ***CCD*** | Charged Coupled Device |
| ***CMOS*** | Complementary Metal-oxide Semiconductor |
| ***ADC*** | Analog To Digital Converter |
| ***GPU*** | Graphics Processing Unit |
| ***FT*** | Fourier Transformation |
| ***IFT*** | Inverse Fourier Transformation |

# Contents

**Abstract**

Most conventional.....provide only 2D images where 3D information are either buried in 2D image or missing.Development of 3D microscopic image is a burning deasure in all branch of research domain so there is a great need for the microscopes that can capture the 3D image for use in biological research like studying neuron activity, brain structure of small biological specimens and cellular morphology. By now the most commonly used 3D imaging microscope is the confocal microscope. But this technique gives rise to bleaching and hard to apply to move and light sensitive samples. Light sheet microscopy was also developed based on the concept of an ultra-thin light sheet to image 3D dynamics. By aligning a microlens array between the object and image plane to a conventional microscope ,we can record a 4D light field and this microscopy technique is known as Light Field Microscopy .In this work, I have implemented a setup for 3D imaging of micrometer size sample(ex.neurons, zebrafish brain, optical fiber)using microlens array. This technique is known as light field microscopy which is different from conventional microscopy. In this setup, both 2D positional and 2D anglular information of the incident light is captured by aligning a microlens array between the camera sensor and image plane. I have captured 100um and 1mm diameter optical fiber and then tried to reconstruct the volume of the fiber. In the other half part, I tried to estimate the beam profile of laser diode using my designed setup.

# Chapter 1

# Introduction

## 1.1 Motivation

It has been around 500 years when the use of microscopes was reference(2).All the developed techniques within this period were not capable of capturing 3D object without scanning it and they were not good enough to obtain a high resolution images . All these techniques require to scan the specimen to obtain a high-resolution image. Obtaining a 3D image of any object in an only capture using the above techniques is quite hard. To overcome this problem single shot 3D light field image capturing using lens array was first developed by Lippmann in early 20th century and in 1996 ,Levoy solved the 5D plenoptic function and simplified it to a 4D light field parameterization L(u,v,s,t) and images can be described as two dimensional cut of the four dimensional light field. In this technique known as light field microscopy, a microlens array is placed between object and image plane to capture 4D light field,using single line series 2D images.This technique is great in use not only for macro size specimens but also micro size specimens.

## 1.2 Light Field Microscopy

Light field microscopy(LFM)(1) is newly emerged scanning free 3D imaging technique.This technique is basically follow the theory of light.LFM can be applied mainly to stable and weakly scattered objects . It allows the user to collect three-dimensional information about a scene in a single shot.

By placing a microlens array between the camera sensor and objective lens or between the object plane and image plane(2),4D light field of 3D object can be obtained in an only shot. Compare to conventional image , light fields allow manipulation and refocus of any viewpoint after the shot has been captured.

4D light field(3): In optics ray is the basic carrier of light. The radiant flux emitted passing along all rays in a 3D region illuminated by a invariant composition of a light source has been dubbed the plenoptic function(5D function that represents the intensity of light from each position and direction in a 3D space). since ,In 3D spcae light rays can be represented by its three coordinates and two angles so this function is 5D. In a region that is free of occluders (one-dimensional redundant information) give a 4D function that is known as a light field.

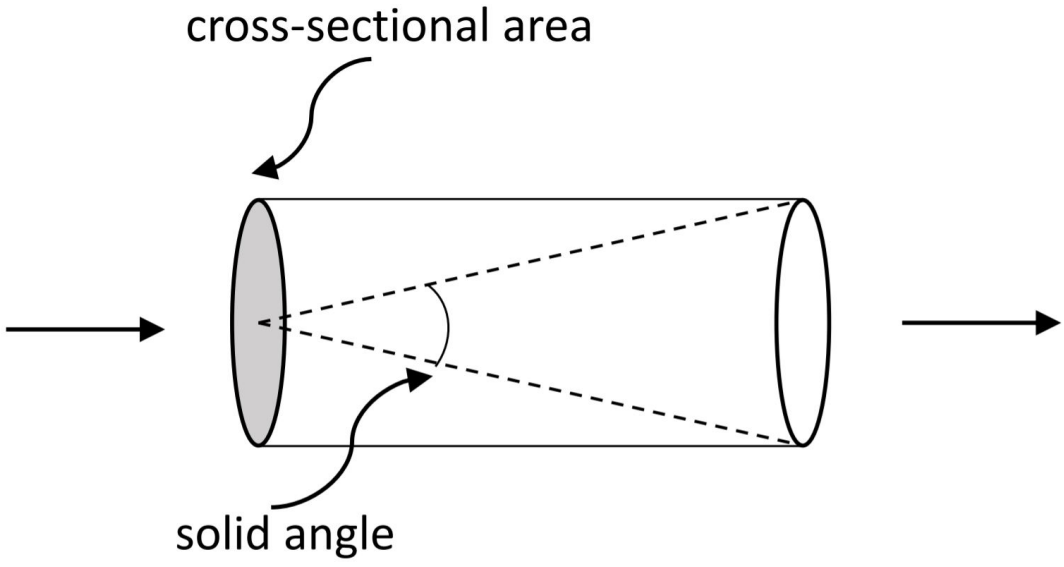
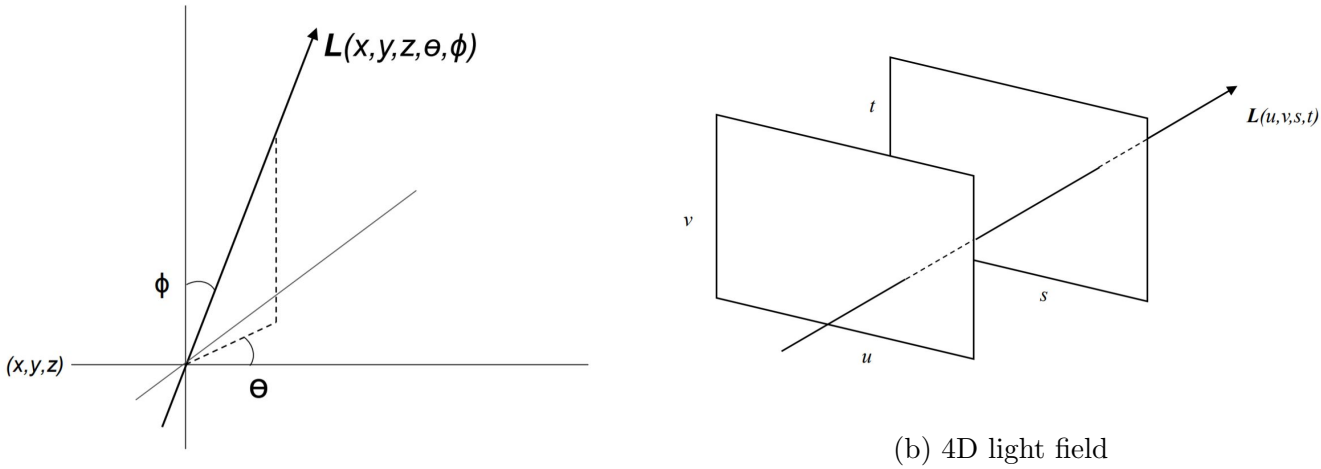



Figure (1.1) Radiant flux along a ray(2)

The radiant flux is equal to the amount of light passing along all rays through a tube for a solid angle and area (a)Plenoptic 5D function in 3d space.Rays can be




(a) 5D plenoptic function

(b) 4D light field

Figure (1.2) 5D Plenoptic function and 4D light field(2)

represented by three coordinates(x,y,z) and two angles (b)D function is simplified to a 4D light field in the regions that are free of occluders.(u,v) represents ray direction and (s,t) represents position on some imaging surface(2)

**4D light field in LFM:** A light field microscope can be designed by aligning a microlens array between object plane and image plane of a conventional microscope.In LFM design plane (u,v) corresponds to plane of objective lens and plane (s,t) corresponds to plane of microlens array.That image is captured by camera sensor in the sub sampled (s,t) plane that is a st array of the uv images of the light field.

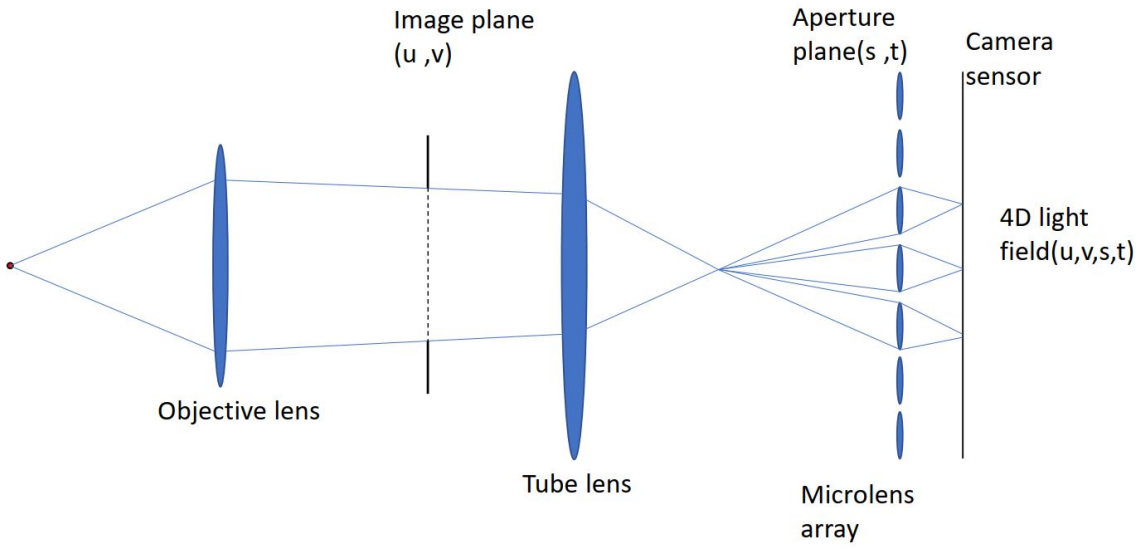



Figure (1.3) 4D light field in LFM

**Advantages and Limitations of LFM:**

(i)Advantages:

(a)LFM can obtain 3D structure in a single shot without scanning the object.Most of the other imaging techniques (confocal microscopy,light sheet microscopy) need to scan the specimen to obtain a 3D image.

(b)LFM allows us to adjust the view point and refocus of a point after shot has been captured.

(c)LFM provides a superb temporal resolution

(ii) Limitations:

(a)Although LFM can obtain a 3D structure but we have to compromise with image size and the resolution.If microlens array subimages have N*N pixels then the reconstructed image will have N*N pixels less than without microlens array captured image.

(b)This technique is not that effective for imaging moving object because there is no such arrangement to refocus the object if it moves

# Chapter 2

# Optical Components And Designed Setup

## 2.1 Optical Components

(a)Collimated light source: I have used a laser diode as a light source.As we know beam of a laser diode is very divergent so a collimating lens is mandatory thing to make it parallel beam . Since the aspheric lens does not have any spherical aberration, they are mostly used. By placing a laser diode at the focus point of the lens, I collimated the beam.

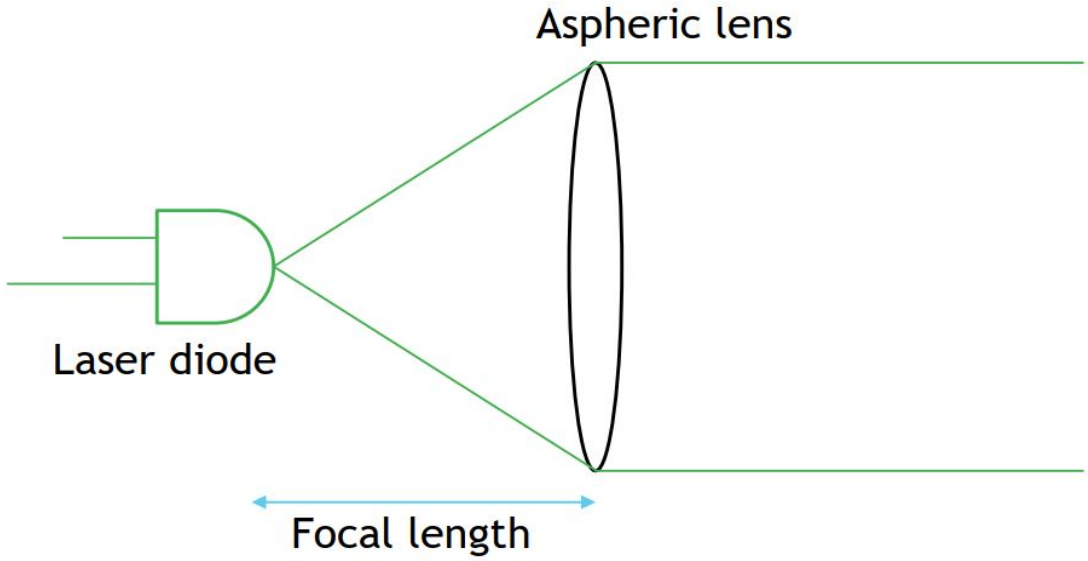



Figure (2.1) Collimated light source

For the experiment a red laser diode(wavelength=650nm ) and aspheric lens with focal length=11mm was used

(b)Infinity corrected objective lens and tube lens: To form an image with an infinity-corrected objective lens a focusing lens have to be used to focus the image ,in my setup that lens is tube lens. Objective lens forms an image at infinity and tube lens focus that image on microlens array.If we use tube lens with a infinity corrected objective in our setup then there is one benefit One advantage that there can be a gap between the objective and tube lens so that other optical components can be used into the setup, such as optical fibers ,diffusers or beam splitters.

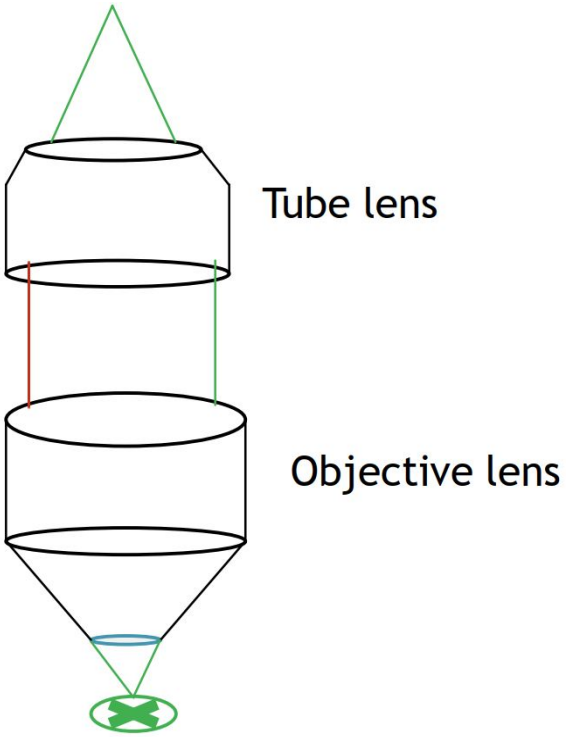
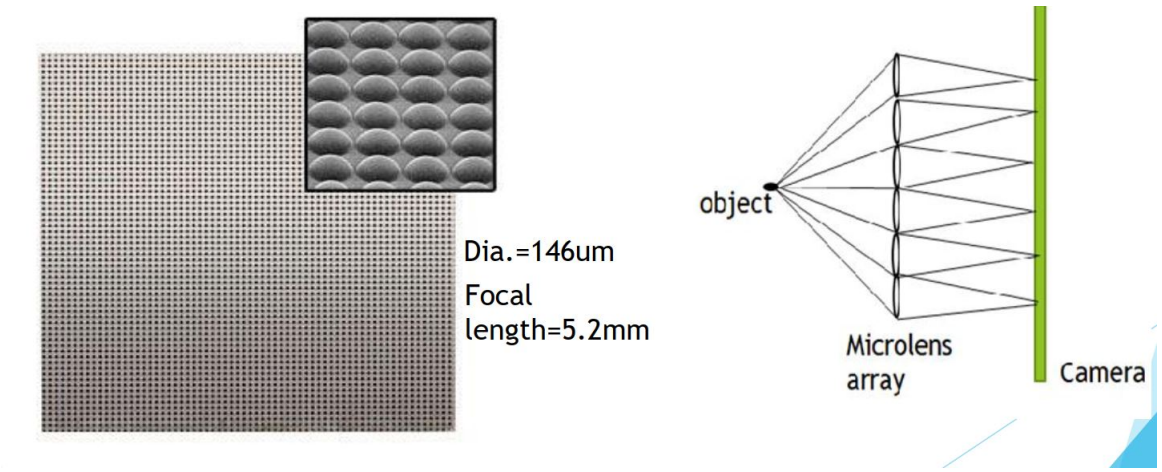



Figure (2.2) Objective lens with tube lens

For the experiment an infinity corrected objective lens(NA=0.65, magnification=40x) and a tube lens(focal length=200mm) was used

(c)Microlens array: The single 2D image captured by the camera consists of n*n sub-images, one per microlens, each representing the different part of the sample.Pixels of each sub-image representing a different point on the objective lens and hence a unique direction of view of the sample.




*https* : *//www.thorlabs.com/Images/GuideImages/*2861*MicrolensArray*3*.jpg*

Figure (2.3) Microlens array

For the experiment a microlens array (plano-convex lens,focal length=5.2mm,dia=146um,pitch=150u lens was used

(d)Digital camera: A digital camera is used to capture all the sub-images into a single 2D image.Nikon D3000(10 megapixel,DX-format CCD image sensor) is used in the setup.CCD sensor transfer the charge through the chip and reads it at the corner of array.By measuring the total amount of charge and using the analog to digital converter(ADC) each pixel value is converted in to digital form.

(e)Post-processing software: To reconstruct the 3D volume of the sample by rendering all the sub-images present in the single 2D image a software LFDisplay, which code was written in MATLAB is used. LFDisplay(5) is an open source,cross platform,GPU accelrated software which is used for viewing light fields in real time.Using with light field microscope it allows to specimen under microscope through the use of tilting and refocusing.It uses GPU to finish light field rendering and refoucsing.

## 2.2 Schematic Diagram

After placing and aligning all the above component according to their role in setup, I designed the following setup for capturing the single 2D image.

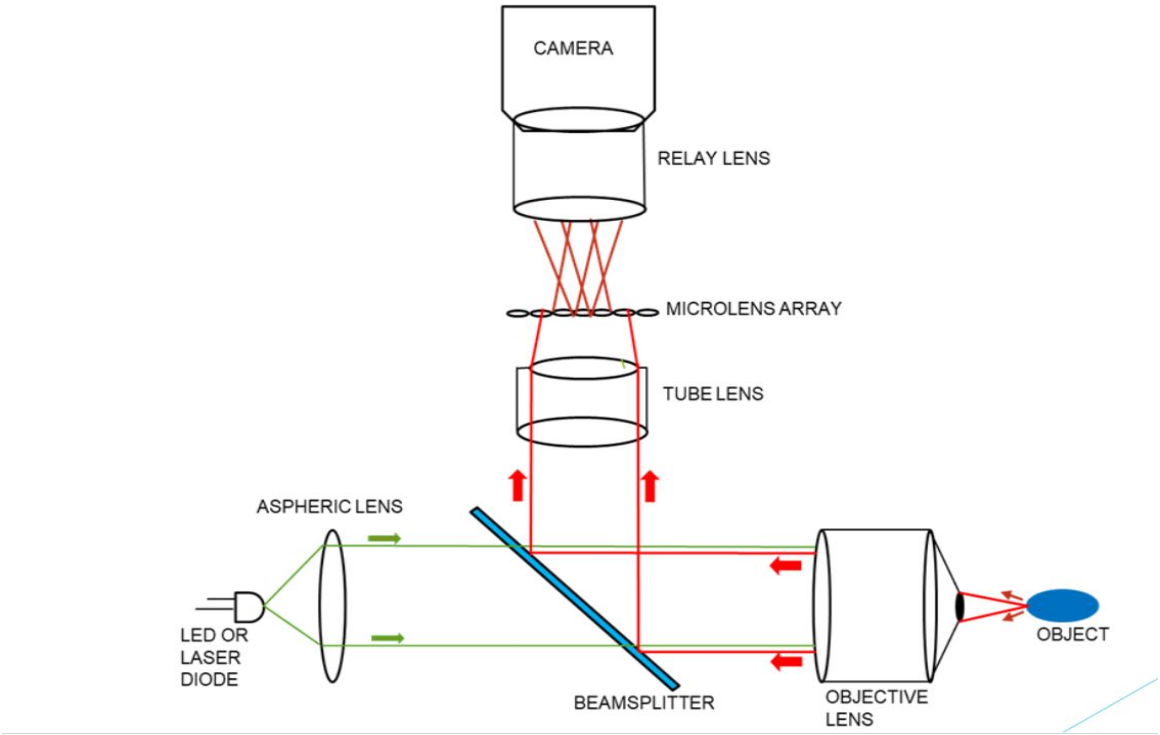



Figure (2.4) Schematic Diagram

## 2.3 Final Setup And Results

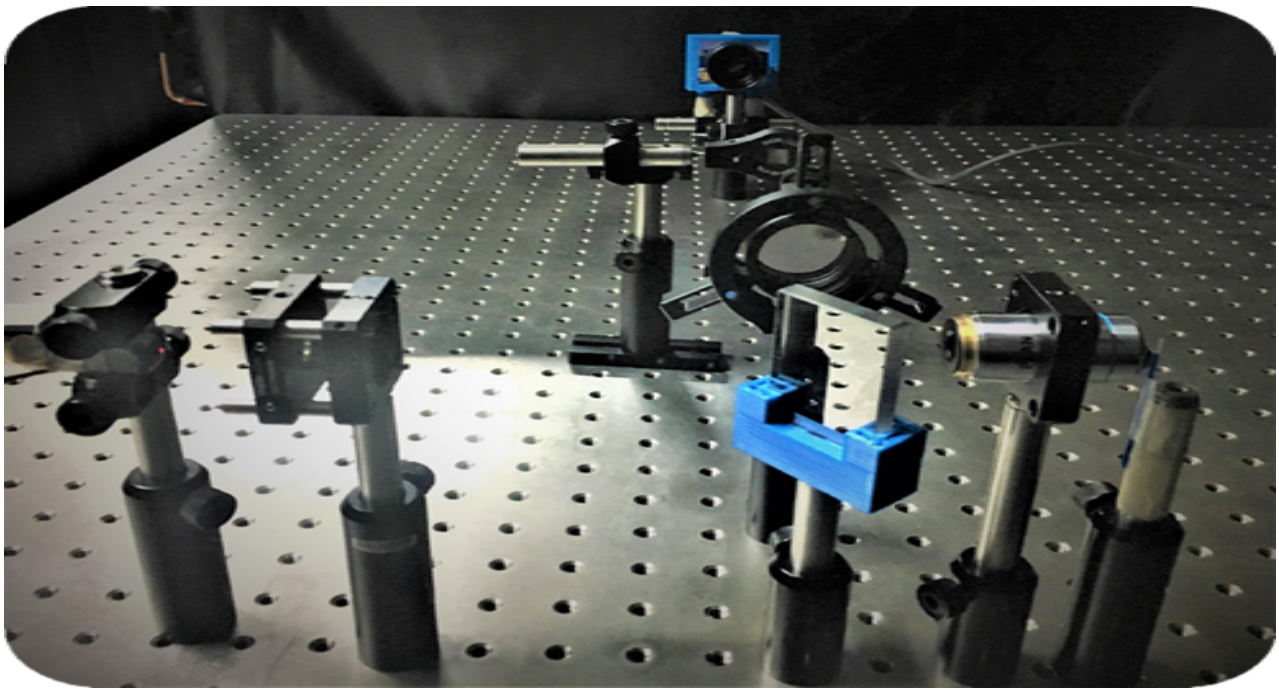
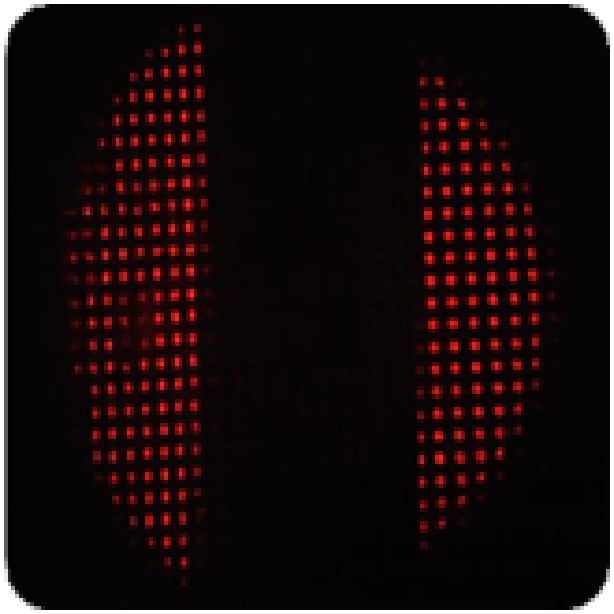


Figure (2.5) Final Setup

(a) Micro lens array with 10x scan lens

(b) Optical fiber

(a) Optical fiber(dia=100um



(b) Optical fiber(dia=1mm)

In all optical fiber images,Black portion is the clading part and bright part is the core part of fiber.These images are captured with 2MP CCD sensor camera.

# Chapter 3

# Beam Profile Estimation and 3D Image Reconstruction

## 3.1 Beam Profile Estimation

After designing the above microscopy setup, using that I have tried to estimate the beam profile of a laser diode. In this experiment, the light beam is passed through the optical fiber, and then the intensity profile is captured by the camera into a single 2D image.

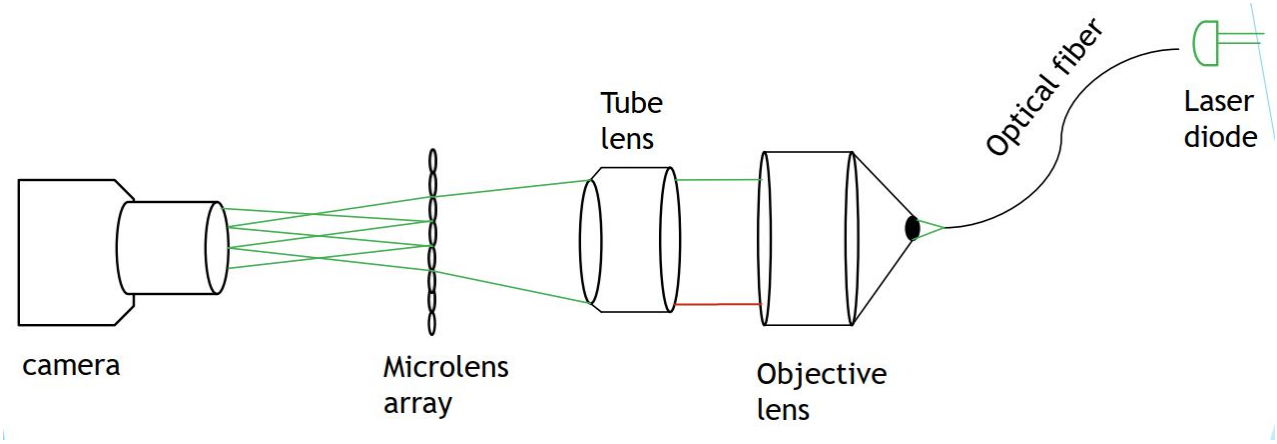
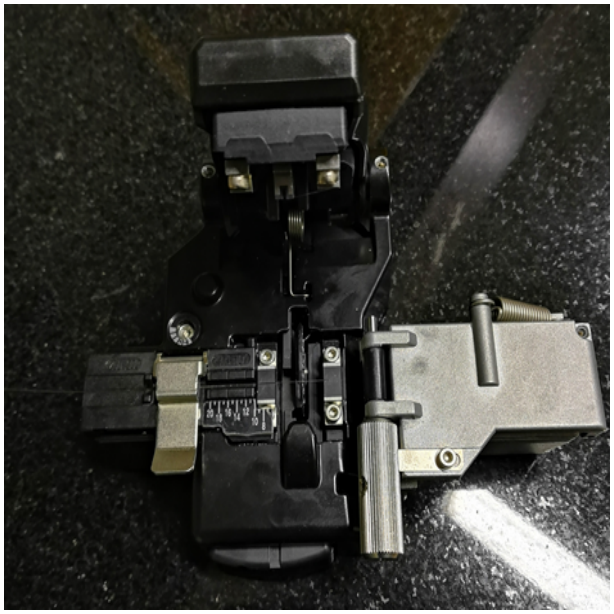



Figure (3.1) Setup for estimating beam profile

**Perpendicular cut:** To avoid the scattering at the end of optical fiber because of its irregular surface,fiber was cut perpendicularly using fiber cleaver.



(a) Fiber cleaver


(b) Perpendicular Cut at the end of fiber

Figure (3.2) Perpendicular Cut

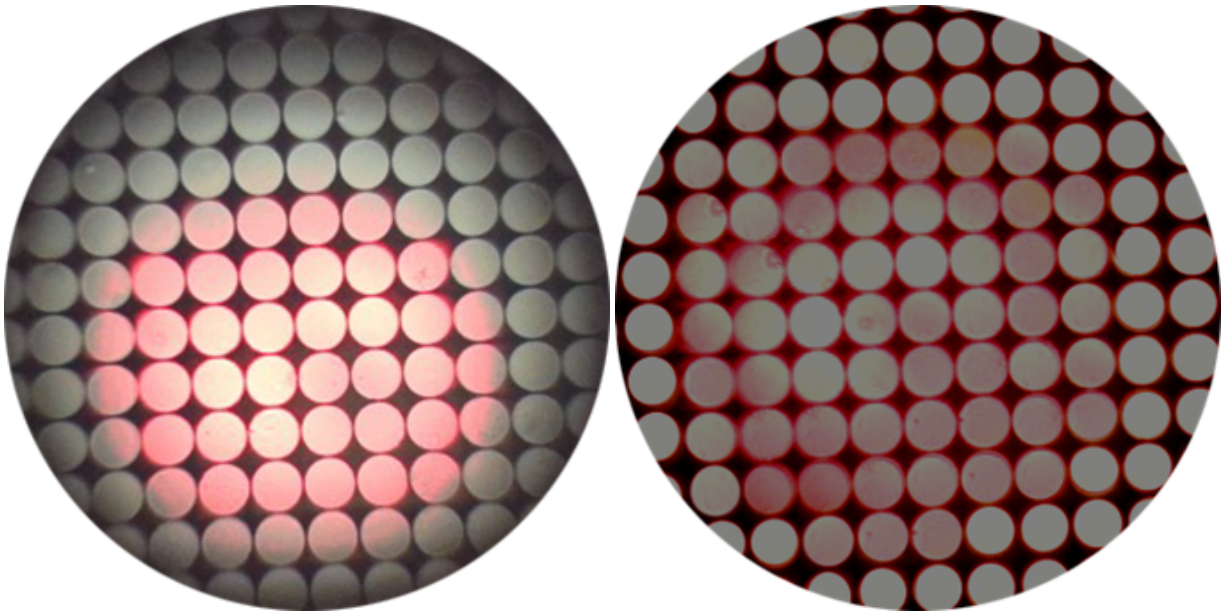
**Microsphere:** Microsphere in an optical fiber works as a converging lens and it focuses light beam to a particular point.Using the splicing machine and by giving electric arc to the perpendicular cut,I created a microsphere.


(a) Splicing machine electric arc


(b) Microsphere in optical fiber

Figure (3.3) Microsphere

## 3.2 Results:



(a) (b)

Figure (3.4) 2D Image of beam intensity distribution

(a)intensity distribution of red laser diode(b)intensity distribution is captured by placing pinhole between beam and objective lens to calculate PSF.These images are captured with 10MP Nikon D3000 digital camera.

## 3.3 3D image reconstruction

**Point Spread Function:** PSF is the response of imaging setup to a point object or point light source. It shows how a point is spreading in our imaging setup. It is calculated by imaging a micro-size bead or pinhole at a different position. I calculated the psf by imaging a light source through a pinhole(figure(3.4)(b))

if we have a fluorescent bead of nanometer size or even a single particle.If we observe bead under the microscope even though it is very small compare to the resolution boundary of the microscope ,still it can be see .Resolution boundaries of a microscope is determines by the diffraction of light.Because of this a point like object blurs out to a certain size and the shape of point is known as PSF. Basically PSF is a 3D diffrac-

tion pattern caused by a very small point source or fluorescent bead and then carried to the image plane through an objective lens with high numerical aperture.When light is emitted by a point source ,objective lens gathered some fraction of light and converged at a particular point.In a PSF diffraction pattern of circular rings(same center but different radius) is formed by convergance and interference of light rays. Resolution of any objective can be found using psf and basic principle behind deconvolution is also a psf convolved with some function.Deconvolution can be seen as a operator or transformation that removes the unwanted light or blurred signal from convolved function using concept of fourier transformation and inverse fourier transformation.In three dimensional microscopic imaging ,lower value of image resolution is mostly caused by obscure light or noise signal or some diffraction pattern forming in signal.To remove these unwanted effects from original image ,deconvolution is mainly used in 3D imaging.

construction of an image begins with assuming this process is linear and independent of shift.Condition of linearity states that addition of two images of two particular objects is similar to the image of joined object. If the process in shift independent then image of a point source will be same in field of view.Shift independency is the ideal condition but in real systems very hard to achieve.and only a fair assumption for developed research equipments by now.

Convolution represents mathematically the link between the object and its optical image.Every point of the object is expressed by a obscured image of the object which is nothing but the psf. By adding all the psf values and multiplied by a intensity function that represents the intensity of light coming out from its corresponding point object,a convolved image is formed.

**Affect of optical components on PSF:** PSF value varies with the wavelength of the light source.Short wavelength light source (blue light,440nm) create a small psf while long wavelegth light source(red light,660nm) create a larger psf.As point spread function increases resolution of the setup decreases.Numerical aperture(NA) also affects the size and shape of psf.An objective lens with high NA result in to a small psf therfore better resolution of images with objective lens which has higher value of NA .Although psf does not depends on the magnification of an objective lens

which is quite surprising.

followings are the mathematical properties of a PSF:

(a)PSF is also known as Fraunhofer diffraction pattern or impulse response ,can be calculated by squaring its fourier transform.

(b)PSF shape varies with the boundary conditions .

(c) maximum value of radiant flux emitted varies with wavelength.

**3D Deconvolution Theorem:** Deconvolution is a mathematical process used in image processing to remove the effects of convolution on captured image with psf. In the field of signal and processing ,devolution principles are mostly used to cover the original volume from some mixed signal.

In simpler way, the motive of deconvolution is to extract the original function of a convolution equation of the form:

a*b=c

Usually, c is a recorded signal, and a is captured image through microlens array that we want to reconstruct, but has been convolved with a signal b (in our case b is point spread function ) before we recorded it. If we have b as a function or the form of function b, then we can apply deterministic deconvolution. However, if we do not have b as a function , then we need to do the approximation of function b using statistical approximation.

In physical measured data, the situation is usually similar to (a*b)+e=c

In this case e is noise that has been captured with our captured image . If we approximate the value of function b by assuming that captured image signal is noiseless then this approximation will be wrong. In fact, our approximation of "a" will also be incorrect.Approximation of our deconvolved signal depends on signal to noise ratio.a lower signal to noise ratio results in a bad estimation.Estimation of "a" can be improved by gathering the knowledge of noise type.

Deconvolution is basically performed by measuring the Fourier Transform of the captured signal h and the point spread function g and in the absense of noise in the

fourier domain apply deconvolution.

$A = \frac{C}{B}$

A, B, and C are the Fourier Transforms of a, b, and c . By performing inverse Fourier Transform on A to reconstruct value of the approximated deconvolved signal a.

**Steps of image reconstruction:**

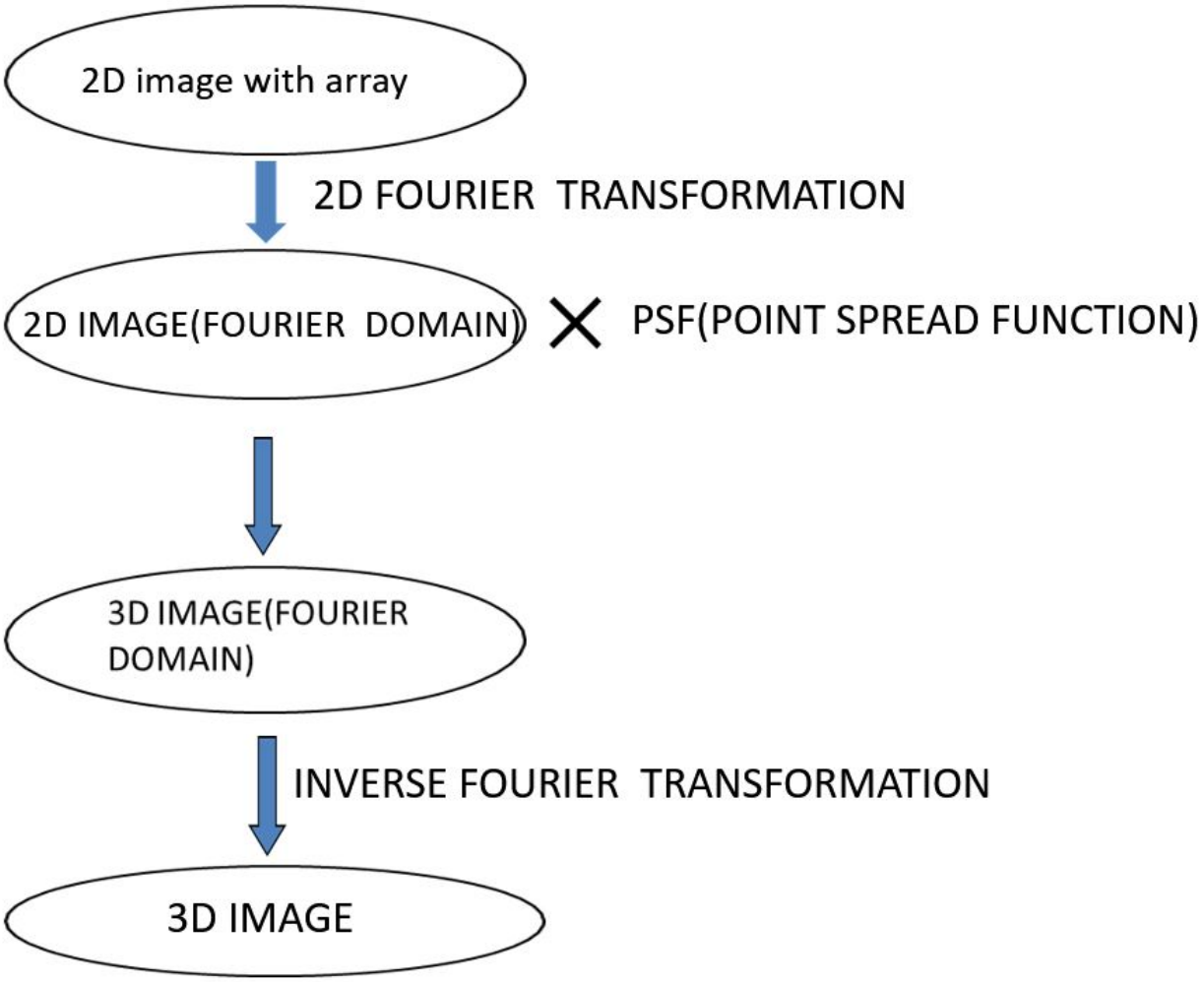



Figure (3.5) Image reconstruction steps flow diagram

(a)Fourier transform of captured single 2D image to be done to transform image in

frequency domain because all the sub-images contains different frequencies so can be rendered only in frequency domain.

(b)A focal stack is formed by convolution of 2D image (contains angular and positional information )with the PSF (depth information)

(c) Using the deconvolution method and inverse fourier transformation, a original 3D image can be reconstructed.

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