MiniMeta-Miniature Imaging System with Meta-optics for Biomedical Applications

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Abstract

MiniMeta:Miniature Imaging System with Meta-optics for Biomedical Applications

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This thesis delves into the world of meta-optics for biomedical applications imaging. Mission to improve the diagnosis, prevention and treatment of diseases by developing novel imaging technology is booming in the research globe. Medical imaging in hospitals and laboratories has shown growing benefits around the globe. In recent years plenty of research articles have been published highlighting the need of medical imaging in solving problems of healthcare institutions. Size of the optics limits the imaging functionalities. For example neurons are inherently distributed in 3D, which leads to a need of probing upto an extended depth of focus. This is the first motivation we are moving towards meta-optics in biomedical. The work begins with the foundation in metasurfaces, examining issues such as narrow depth of field in metalenses and the development of EDoF called extended depth of focus in metalenses with larger working distances. Alternative solution for miniature imaging system called Miniscope developed by UCLA researchers. It further delves into the integration of meta-optics with different versions of Miniscopes. Subsequent chapters explore the extension of different metalens capabilities with miniscope, so called MiniMeta. It further delves into the more

imaging features required for microscopic images and how the size of the optics limits the imaging features like, resolution, wide field of view and simultaneous multiple imaging functionalities with minimal distortion of metalenses. The thesis concludes with multiple resulting solutions in microscopic images for biomedical applications. Overall this thesis looks at the future opportunities to share discoveries and advanced imaging research with metalenses for multiple medical applications.

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Dedication

to my parents
and my brother

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Chapter1: Introduction to Meta-optics

1.1 Metasurface overview

Light is the basis of human exploration and understanding of the world, because of carrying an enormous amount of information that can be perceived by people or machines. Flexibly and effectively controlling light beams has always been a significant goal that people aspire to achieve.

We are familiar with the tools that we use to control light waves. A great example of one is a magnifying glass - a lens. We also see those lenses in eyeglasses. How these lenses work - we know from high school physics. When we think in terms of ray paths the direction of light travels, and it's bent by the shape of the glass so that on the other side, the light converges and creates a focus as shown in the Fig:1.1.

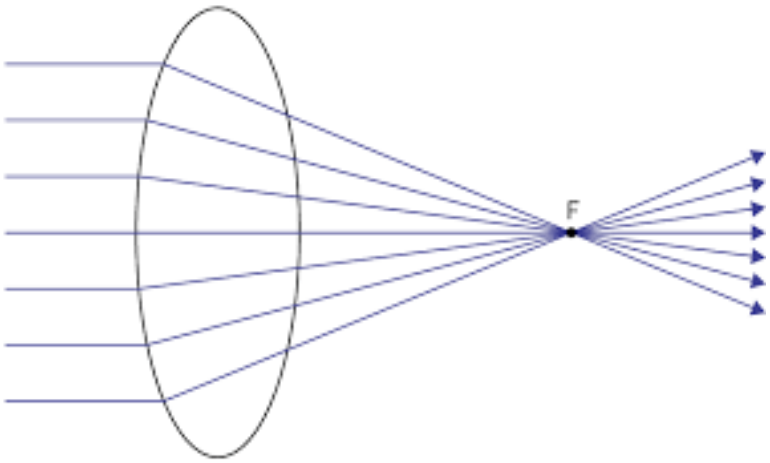


Fig:1.1 Ray of light

From the perspective of how waves travel, there's an alternate view of how lenses work. The wave front of the light waves that are traveling and hitting the lens, and what happens is: in the middle of the lens the light is slowed down, while on the edges of the lens where the lens is thinner, the light is slowed down comparatively less. So the shape of the wavefront as it emerges from the lens is curved - slowed down or delayed, and it creates a converging wavefront that also creates a focus at spot. It is kind of the opposite of what happens when you throw a rock in a pond, creating an expanding wave. This is a converging wave to image. Fig:1.2 can be analyze in the similar manner:

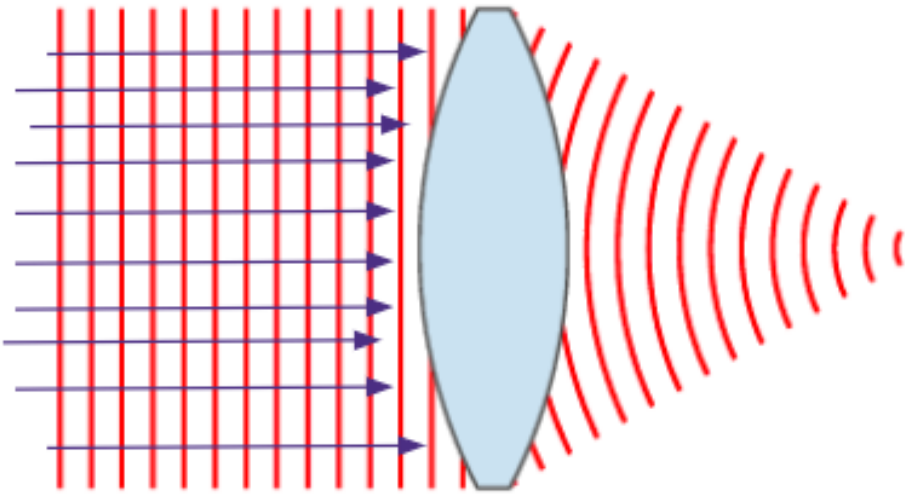


Fig:1.2 Wave nature of light

Conventional lenses work because of their specific shape. They are curved in order that the incident light waves are slowed down in the middle where the lens is thickest and slowed down the least at the edges where the lens is thinnest. That is what creates the converging wavefront and an image. The reason lenses are expensive and complicated is because we are stuck with the material properties of glass, and the only way we can create this spatially varying light propagation is with the shape of the lens. Lens shape has to be controlled very precisely in optical devices. We could ask ourselves, what if we were no longer stuck with material properties like glass that are fixed? If we had more flexibility in the material properties, then we could imagine creating a lens that has a much simpler shape, and we simply need different material properties in the middle of the lens in order to slow down the light waves there and allow the light waves to travel faster at the edges. If we have the ability to do that, we could create a lens that does exactly the same thing - creates a converging wavefront and an image over a point. It has a much simpler shape and actually could be much easier to manufacture. The basic idea of how can we control those light wave controlling material properties, that is the basic idea of metamaterials. Now, what are the essential features of a wave? How do we describe it? Well there are a couple of fundamental properties of waves. One is the amplitude of the wave - how high it is from peak to valley, and that's linked to how bright light is in a light wave. The more important feature for metamaterials is the wavelength, which is the distance between one peak

and the next peak. That is tied to the color of the light. So how do we design metamaterials? We start by looking at the wavelength we hope to control, and if the wavelength is stretched then we need a metamaterial structure that is significantly smaller. So we then need to create an array or grid of metamaterial structures whose size is smaller. Now if we are creating something with conventional materials like glass, we would be stuck with the same material properties in each one of these squares (referring Fig: 1.3).

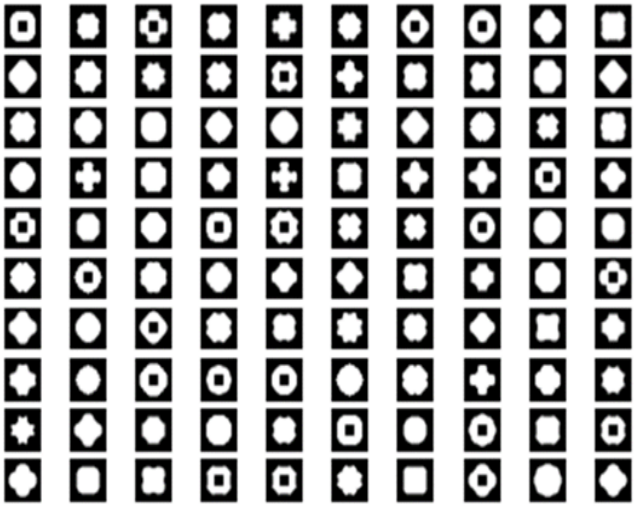


Fig: 1.3 Front View of the metamaterial with different scatterer

With metamaterials, we can put different material properties in each one of those squares as shown in the Fig:1.3. That enables us to do things - creating a flat lens where the wave travels slowly through the middle of the structure and more quickly through the edges. But what is really interesting with metamaterials is we can put anything at all arbitrarily inside each one of these material building blocks. And then we do something like that, we then have the ability to create wave control that can take an input wave and not just create a converging wave on the other side, but a completely arbitrarily complex wavefront. And that enables us to do much more powerful wave processing with metamaterials. Fig:1.4 shows wave propagation in meta-optics.

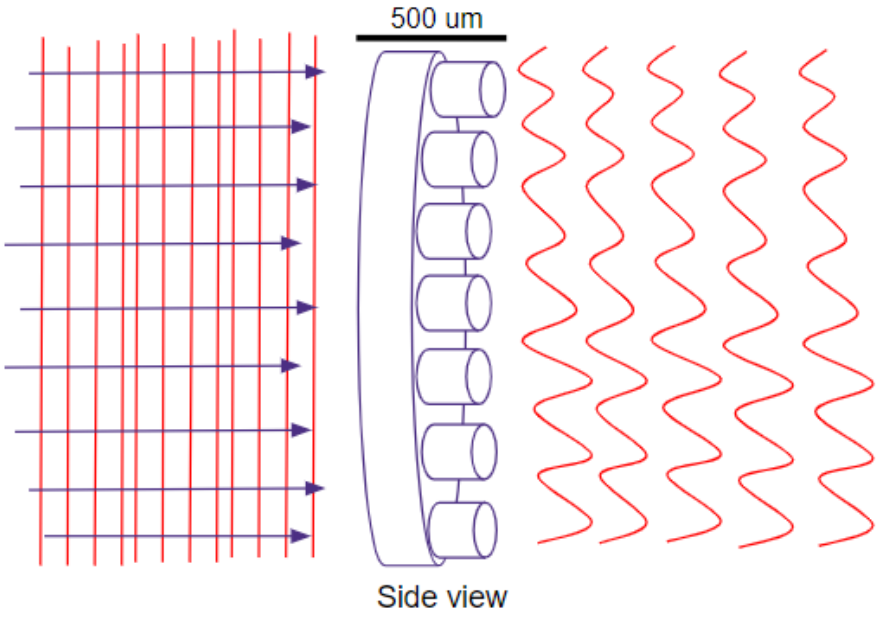


Fig: 1.4 Wave propagation in metamaterial

1.2 Metasurface design process

The main steps for designing the meta-lenses are:

A. Design the scatter which is the main building block of a metasurface.
B. After designing the scatter we need to design the phase mask.

A. The main building block of a metasurface is a grating composed of scatterers arranged in a subwavelength periodic lattice. To design the scatterer based on the wavelength of the lens, first we need to choose the materials and match the refractive index, then shape of the scatters (we usually use pillar, circle, cross as scatterers.) and the dimension of the scatterer including height (t1), width (W1) and periodicity (P) as shown in the Fig:1.5.

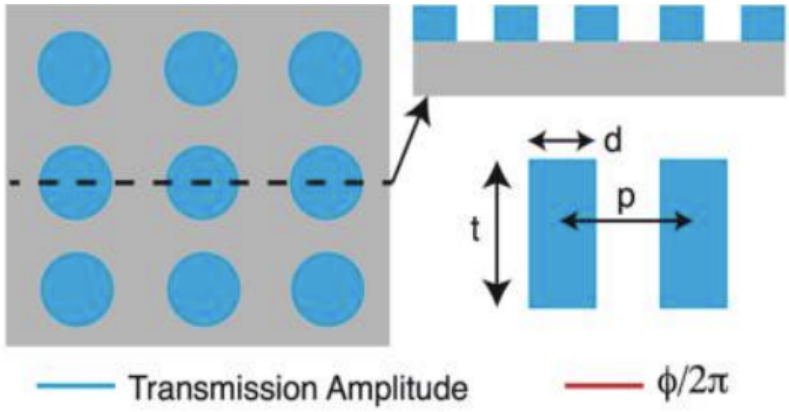


Fig: 1.5 Scatterer Overview [1]

After setting the parameters of the scatterer or the nanopillars, sweep the width of the pillar called halfwidth to get the phase response as a function of pillar width.

B. After designing the scatterer, the next step is to design a phase mask. The use of metasurfaces to build optical components is primarily motivated by the observation that functionality of many such components, such as lenses and focusing mirrors, is determined by a spatial phase profile imparted on an incident beam. Reproducing these devices using a metasurface involves selecting the correct parameters to achieve the desired spatial phase profile, arranging the scatterer on a subwavelength lattice, and spatially varying their dimensions. To design an arbitrary transmission phase profile, we must be capable of producing phase shifts spanning the whole 0 to 2pi range while maintaining large transmission amplitudes. Such phase variations have been demonstrated with high refractive refractive index scatterers. Using numerical simulation called rigorous coupled-wave analysis (RCWA), it is possible to select parameters to achieve a phase variation with a low contrast grating. In these simulations, we can calculate the achievable phases and transmission amplitudes by varying the diameter d of the posts for a fixed periodicity P.

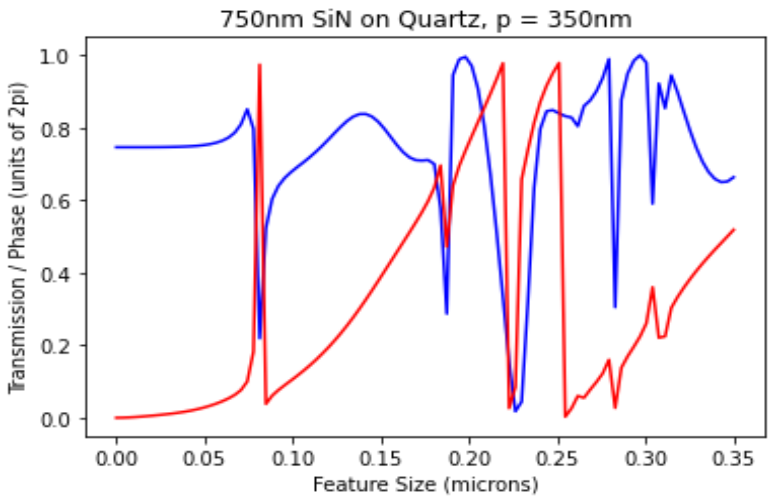


Fig: 1.6 Phase profile of one of the lens designed for Miniscope

1.3 Thesis Outline

The contributions of this thesis can be summarized as follows:

This thesis explores the capabilities of meta-optics, a technology that leverages artificially structured sub-wavelength meta-surfaces, opening up new paradigms in the world of optics and imaging.

The work begins by delving into the motivations behind using meta-optics for various imaging applications, discussing their potential to revolutionize traditional optical systems.

Step1: Assemble and install V4 miniscope on the optical table vertically so that we can change the sample (2um beads and 100um Kidney).

Step2: Focus the objective module of v4 miniscope to replace it with meta-optics. The idea behind this is that the size of the optics limits the imaging parameters.

Step3: Characterization of different meta-optics with miniscope to check the different imaging capabilities.

Step4: Find the methods to analyze the real biological fluorescence samples of 2um beads.

Step5: Check the focal efficiency of the lenses by using the point spread function.

Step6: Imaging with Hyperboloid to check resolution, EDoF called extended depth focus to achieve highest depth of focus ~ 140um, WFoV to cover a wide field of view and MO Array called Meta-optics array to achieve multiple functionalities at the same time including large field of view with less distortion.

Chapter-2 : What has been done with the Meta-optics

This chapter deals with application of meta-optics. You will get familiar with areas where can we see meta-optics. Different capabilities of meta-optics for different imaging applications.

Meta-optics, also known as metasurfaces or metadevices, have opened up new possibilities in optics and photonics research. Here are some notable advancements and applications that have been achieved using meta-optics.

There are few examples of the advancements and applications of meta-optics discussed below. The field continues to evolve rapidly, and researchers are exploring new possibilities in areas such as quantum optics, photovoltaics, and biomedical imaging, among others. Meta-optics holds significant promise for revolutionizing various aspects of optical science and technology.

2.1 Biomedical Imaging with Meta Optics

I can start discussing this concept by describing the first paper of our group members on meta-optics fiber endoscopy for biomedical devices [2].

In this paper we looked into the endoscope project and we showed potential of meta-optics for integration and miniaturization of biomedical devices towards minimally invasive surgery. Furthermore, we aim to extend meta-optics for applications with miniaturized fluorescence microscopes for brain imaging. Here a meta-optic can augment the existing platforms, such as the MiniScope, which is a miniaturized head- mounted microscope used for in-vivo fluorescence imaging.

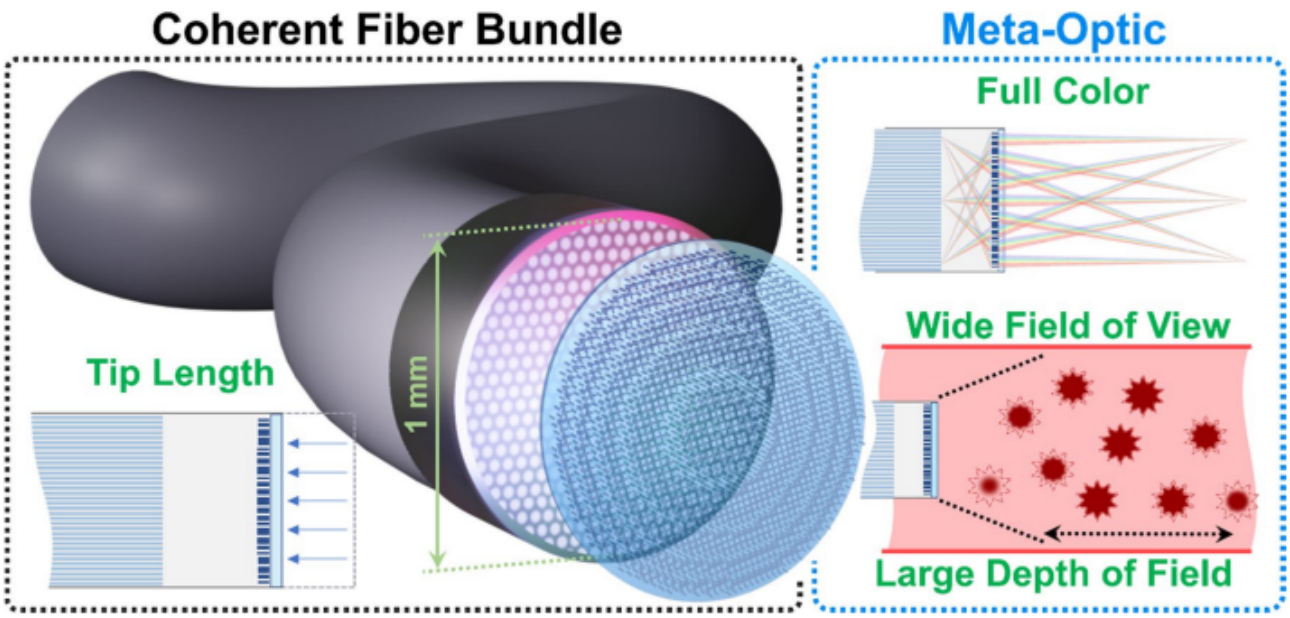


Fig: 2.1 Schematic of the meta-optical fiber endoscope [2]

Biomedical imaging with metaoptics refers to the application of metasurfaces or metastructures in biomedical imaging techniques to enhance imaging capabilities, improve image quality, and enable new imaging modalities for biomedical research and clinical applications. Metaoptics offers unique advantages in terms of miniaturization, flexibility, and the ability to manipulate light at subwavelength scales, making it well-suited for various biomedical imaging modalities.

Here are some examples of how meta optics can be applied in biomedical imaging:

Super-resolution imaging: Metasurfaces can be designed to manipulate the wavefront of light and overcome the diffraction limit, enabling super-resolution imaging in microscopy. By introducing engineered phase profiles using metastructures, it is possible to achieve subwavelength resolution, allowing for the visualization of fine cellular structures and nanoscale details.

Label-free imaging: Metasurfaces can enable label-free imaging techniques by selectively enhancing the contrast of specific biological structures or molecules. For example, metasurfaces can be used to enhance the scattering or fluorescence signals from certain biomarkers, enabling the detection and imaging of specific cellular or molecular targets without the need for exogenous labels.

Polarization imaging: Metasurfaces can control the polarization state of light, which is valuable in biomedical imaging for characterizing tissues and biological samples. By selectively manipulating the polarization components of light, metaoptics can enhance contrast, reveal hidden features, and provide insights into the structural and optical properties of biological samples.

Multispectral imaging: Metasurfaces can be designed to manipulate light across different wavelengths, enabling multispectral imaging in biomedical applications. By selectively manipulating the spectral components of light, metaoptics can enhance the discrimination and visualization of specific biological structures, such as differentiating between healthy and diseased tissues based on their spectral signatures.

Optical coherence tomography (OCT): OCT is a widely used imaging modality for non-invasive imaging of biological tissues. Metaoptics can enhance the resolution and sensitivity of OCT systems by manipulating the coherence properties of light, improving depth resolution and enabling high-speed volumetric imaging. Metasurfaces can also be used to correct aberrations and enhance image quality in OCT systems.

Endoscopy and minimally invasive imaging: Metaoptics can enable the development of compact and miniaturized imaging systems for endoscopy and minimally invasive procedures. By integrating metasurfaces into the optical components of endoscopes or catheters, it is possible to manipulate light rays, enhance imaging capabilities, and enable real-time visualization of tissues or organs inside the body.

These are just a few examples of how metaoptics can contribute to biomedical imaging. The precise design and engineering of metastructures allow for tailored optical functionalities, enabling new imaging techniques, enhancing image quality, and expanding the capabilities of existing imaging modalities. Ongoing research in this field is expected to lead to further advancements and practical implementations in biomedical imaging with metaoptics.

2.2 Polarization Imaging with Meta Optics

Polarization imaging with meta optics refers to the use of metasurfaces or metastructures to manipulate and control the polarization properties of light for imaging applications. Metasurfaces are artificially engineered surfaces composed of subwavelength structures that can manipulate the properties of light, such as its polarization state, phase, and amplitude, with high efficiency and flexibility.

Polarization imaging plays a crucial role in various fields, including remote sensing, biomedical imaging, materials characterization, and optical communications. Traditionally, polarization imaging has been achieved using bulk optical components, such as polarizers and wave plates. However, metasurfaces offer several advantages over conventional methods, including compactness, lightweight, and the ability to manipulate polarization properties at subwavelength scales. [3]

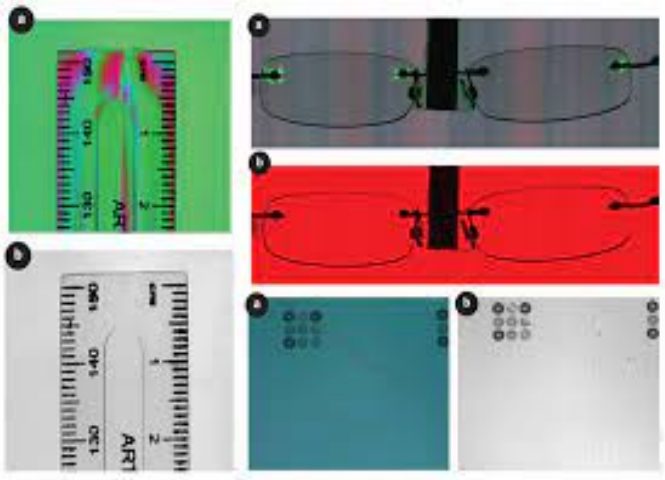


Fig: 2.2 Polarization Imaging showing surface roughness & stress detection respectively [4]

By designing the geometry, arrangement, and orientation of subwavelength metastructures, metasurfaces can control the polarization state of light with high precision and achieve functionalities not possible with conventional optics. For polarization imaging, meta optics can be used to enhance the contrast and sensitivity of polarization signals, enable polarization selective detection, and even create novel imaging modalities.

One example of polarization imaging with meta optics is the development of polarimetric cameras. These cameras utilize metasurfaces to selectively detect and analyze different polarization components of light, providing additional information about the objects or scenes being imaged. By capturing and analyzing the polarization state of light reflected or transmitted by the subject, polarimetric cameras can reveal hidden features, enhance image contrast, and improve object detection in challenging environments.

Metasurfaces can also be designed to act as polarization filters or beam splitters, selectively transmitting or reflecting specific polarization states. This property can be exploited in polarization imaging systems to separate and analyze polarized light from different sources or to selectively image different polarization components of a scene.

Moreover, the combination of metasurfaces with other imaging technologies, such as computational imaging and machine learning algorithms, can further enhance the capabilities of polarization imaging systems. By leveraging the unique polarization manipulation capabilities of meta optics and advanced data processing techniques, it is possible to extract valuable information from complex polarimetric data and achieve high-resolution, real-time polarization imaging.

Overall, polarization imaging with meta optics holds great promise for a wide range of applications, enabling the development of compact, lightweight, and high-performance imaging systems with enhanced capabilities for analyzing the polarization properties of light. Ongoing research in this field is expected to lead to further advancements and practical implementations in the near future.

2.3 Phase Imaging with Meta Optics

Phase imaging can be summarized by the below paper which focuses on the phase control by second harmonic light for nonlinear wavefront shaping and showing various imaging applications. [5]

Phase imaging is an amazing source of visualization of transparent objects in real-time. This imaging can be visualized by a metasurface that converts variations in a wavefield of intensity to create a pseudo-3D image of a phase object.

Phase imaging with meta optics refers to the utilization of metasurfaces or metastructures to control and manipulate the phase of light for imaging applications. Phase is a fundamental property of light that describes the relative position or timing of the peaks and troughs of a light wave. Manipulating the phase of light enables various imaging techniques, such as phase contrast imaging, holography, and wavefront sensing.

Below figure shows the phase imaging by contrast detection in the specimens. This can be achieved by phase contrast yields image intensity values as a function of specimen optical path length magnitude.

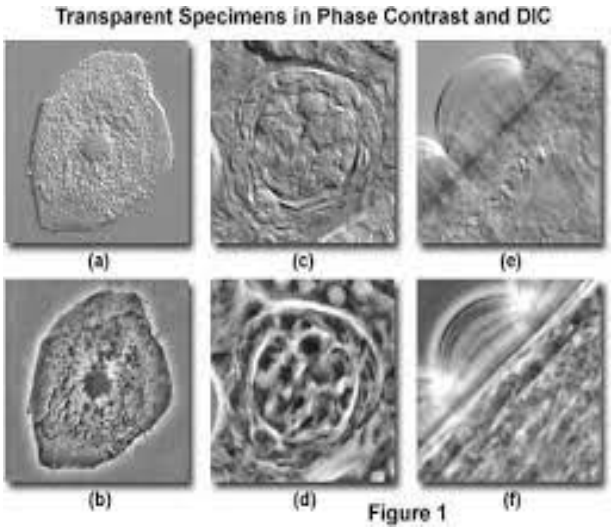


Fig: 2.3 Phase Imaging showing by contrast detection of specimens [6]

Metasurfaces are engineered surfaces composed of subwavelength structures that can control the phase of light with high precision and efficiency. By carefully designing the geometry, arrangement, and properties of the metastructures, metasurfaces can introduce local phase shifts to incident light, shaping the wavefront and enabling advanced imaging capabilities.

Phase imaging with meta optics offers several advantages over traditional methods based on bulk optical components. Metasurfaces provide compact and lightweight solutions, allowing for integration into miniaturized imaging systems. They also offer the flexibility to manipulate phase

across a wide range of wavelengths, making them suitable for multi-spectral or broadband imaging.

One application of phase imaging with meta optics is phase contrast imaging. In conventional microscopy, transparent samples with minimal inherent contrast can be challenging to visualize. Phase contrast imaging techniques, such as Zernike phase contrast or differential interference contrast (DIC), enhance the visibility of transparent structures by detecting variations in the phase of light passing through the sample.

Metasurfaces can be used to create phase plates or optical elements that introduce controlled phase shifts to the incident light, enabling phase contrast imaging. By integrating metasurfaces into the microscope objective or other imaging components, it becomes possible to enhance the contrast and visualize transparent samples with high resolution.

Another application is digital holography, which captures and reconstructs the entire complex wavefront of light using interference patterns. Metasurfaces can be designed to manipulate the phase and amplitude of the incident light, enabling the precise control of the wavefront in holographic imaging systems. By introducing specific phase patterns through the metasurface, it becomes possible to shape the wavefront and generate holographic images with high resolution and accuracy.

Wavefront sensing is another area where phase imaging with meta optics finds applications. Wavefront sensing techniques are used to measure and analyze the distortions or aberrations present in optical systems. Metasurfaces can be employed as adaptive optics elements to correct these aberrations by manipulating the phase of incident light and compensating for wavefront distortions. This leads to improved imaging quality, especially in applications such as astronomy or high-resolution microscopy.

In summary, phase imaging with meta optics harnesses the unique capabilities of metasurfaces to control and manipulate the phase of light for advanced imaging applications. By introducing precise phase shifts using metastructures, it is possible to enhance contrast, enable phase contrast

imaging, perform digital holography, and correct aberrations through wavefront sensing. Continued research and development in this field are expected to yield further advancements and practical implementations in phase imaging.

2.4 Light-field Imaging with Meta Optics

Light field imaging in meta-optics can be summarized by the beautiful article written by stanford on “Light-field imaging using a gradient metasurface optical element” with various applications of imaging like; microscopy, digital imaging, wearable devices, displays, etc. [7]

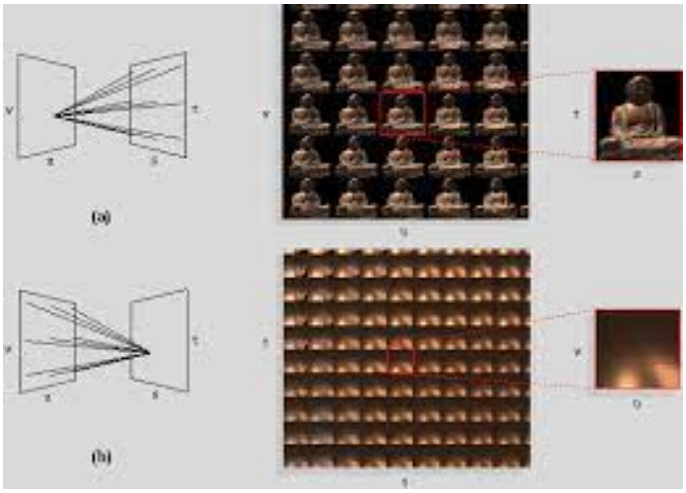


Fig: 2.4 light-field imaging with spatial frequency domain analysis [8]

Light-field imaging is also a developed method for 3D imaging. This method can be used simultaneously for 3D with super high resolution by multifunctional metalens. It can replace the conventionally used microlens array, used in displays, digital cameras. This invention changed the way of light field microscopy imaging. Using metalens sacrifices of spatial resolution with 3D imaging can be overcome for microscopy imaging.
Some of the applications can be: light-field imaging, light-field display, light-field digital camera, light-field microscopy and wearable devices.

The advantages of using a multiplexed metalens array over a conventional microlens array can be seen here: [7]

(i) The metalenses are easily fabricated using conventional thin film deposition and patterning techniques.
(ii) The metalenses are flat, ultrathin, and features are small footprint, affording easy incorporation in microscope systems.
(iii) Metalens can be created on any substrate, e.g. a glass slider or a semiconductor wafer.
(iv) Metalens can achieve simultaneous 3D imaging with super high spatial resolution.
(v) Random spatial multiplexing of phase profiles of different microlenses can suppress the higher diffracted order between focal spots, which can be a problem in microlens arrays.
(vi) Metalens can feature a large numerical aperture.
(vii) A phase plate function can be easily integrated into the metalens design.

Light-field imaging with meta optics refers to the use of metasurfaces or metastructures to manipulate the properties of light in order to capture and reconstruct the light field information of a scene. The light field represents the intensity and direction of light rays in a given scene, capturing both spatial and angular information.

Traditional light-field imaging systems often involve complex setups with multiple lenses and sensors to capture the required angular information. However, metasurfaces offer a promising approach to simplify and miniaturize light-field imaging systems by enabling the manipulation of light rays at subwavelength scales.

Metasurfaces can be designed to control the phase, polarization, and direction of light rays, which are key parameters for light-field imaging. By carefully engineering the metastructures, it is possible to control the propagation and distribution of light rays, enabling the capture of the complete light field information with a single optical element or sensor.

One application of light-field imaging with meta optics is the development of single-shot light-field cameras. These cameras utilize metasurfaces to selectively direct and focus light rays onto a single image sensor, capturing both spatial and angular information simultaneously. The metasurface acts as a micro-optical element that redirects the light rays, allowing the reconstruction of a 3D scene or the refocusing of the image at different depths.

Metasurfaces can also be designed to enable the capture of polarized light fields. By incorporating polarization-sensitive metastructures, it is possible to selectively capture the polarization state of light rays in different directions. This capability can provide additional information about the scene and enable polarization-resolved light-field imaging, which finds applications in material characterization, object detection, and remote sensing.

Furthermore, meta optics can enhance the resolution and quality of light-field imaging systems. Metasurfaces can be used to manipulate the wavefront of light rays, enabling super-resolution imaging and aberration correction in light-field setups. By introducing phase shifts and controlled optical path differences, metasurfaces can compensate for aberrations and improve the overall image quality in light-field reconstruction.

The combination of meta optics with computational imaging techniques, such as image reconstruction algorithms and machine learning, further enhances the capabilities of light-field imaging systems. By leveraging the precise control of light rays provided by metasurfaces and advanced data processing techniques, it is possible to extract depth information, refocus images, and generate immersive 3D representations from captured light-field data.

In summary, light-field imaging with meta optics leverages the unique capabilities of metasurfaces to manipulate light rays and capture the complete light field information of a scene. This approach offers the potential for compact, single-shot light-field cameras, polarization-resolved light-field imaging, and enhanced resolution through aberration correction. Continued research and development in this field are expected to lead to further advancements and practical implementations in light-field imaging systems.

Chapter-3 : Concept of MiniScope and Integration of Meta-optics

This chapter deals with the history and evaluation of Miniscope. Followed by the integration idea of MiniScope with meta-optics. The MiniScope concept is given by the UCLA researcher: [9]

The Miniscope project was first introduced by the UCLA researcher in mid January, 2016. The idea behind this project was to understand how the brain links memories across time in nature and later neuroscience.

The very stage of Miniscope was V3. The Miniscope system was designed to be easy to build and use.

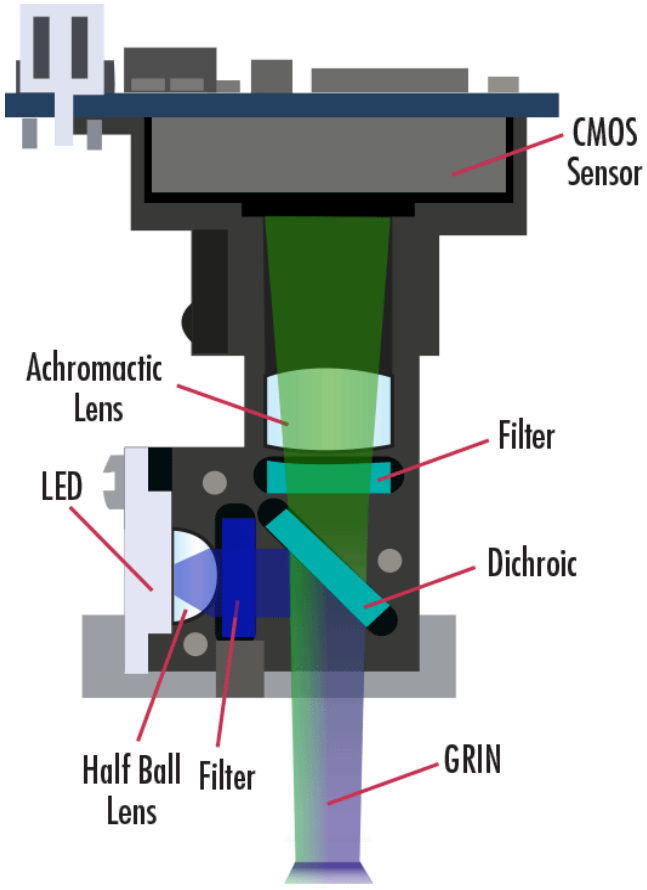


Fig: 3.1 Schematic shows the V3 Miniscope with different working module [9]

The above pictures show the schematic of the v3 miniscope. This miniscope is divided into three modules: i) Objective ii) Emission & iii) Sensor module.

The Objective module was designed to be equipped with lenses required for microscopic imaging, called GRIN lenses, was specially designed for v3 miniscope.

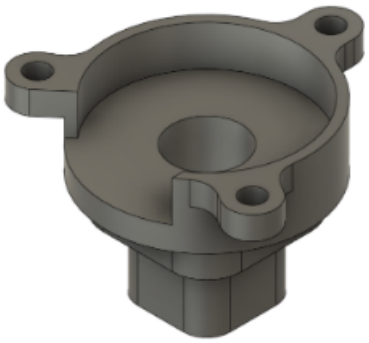


Fig: 3.2 Objective Module

Second is the emission module with dichroic mirror and emission filter. The main function is to convert high energy of light with low wavelength into low energy of light with higher wavelength.

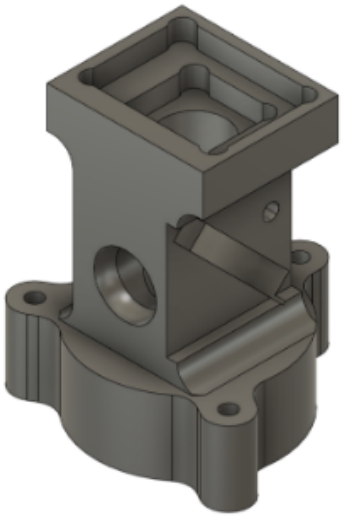


Fig: 3.3 Emission Module

Sensor module is designed to create an interface between the objective and emission module.

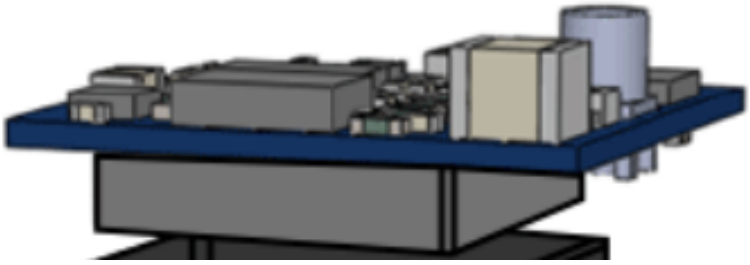


Fig: 3.4 Sensor Module

To read the v3 Miniscope, first we need the coax cable to connect the miniscope with DAQ hardware. Next is to interface the image sensor with the PC, for the interface we need to install CYPRESS Control Center from Infineon technology. Once you install the CYPRESS Control Center, you will be able to see the control center software. To run the control center you need to program it.

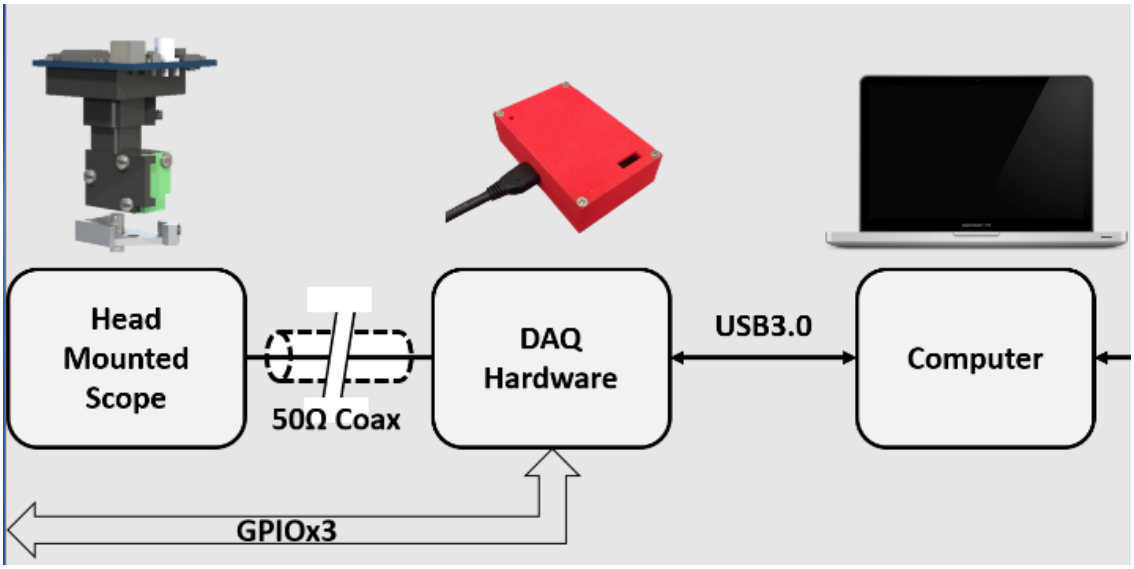


Fig:3.5 Complete System to read v3 MiniScope [9]

Engineers and researchers are meant to improve and innovate new technology. Head-mounted miniscope or miniaturized fluorescence microscopes have become an invaluable tool for interrogating neural activity in freely behaving animals by employing 3D printing and shelf miniature optics to enable low-cost and light weight one-photon neural imaging across various research. So the idea comes to the next generation of miniscope or so called new

versions of the Miniscope series available from V4.0 to V4.4 to fulfill high resolution imaging with fast live neural tracking activity.

My V3 miniscope experimental setup with the three axis controller.

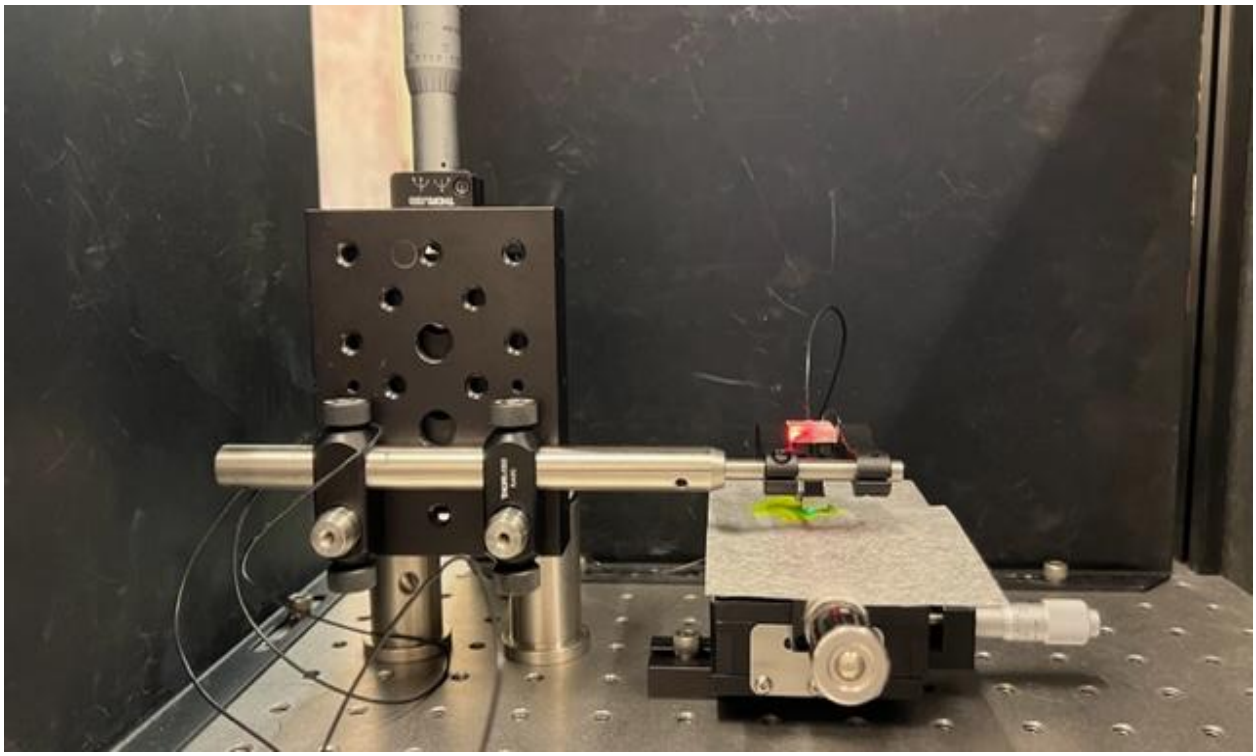


Fig: 3.6 In-lab V3

My next chapter is an in-depth full description of V4 Miniscope. (Chapter 4)

3.2 Concept for integration with Meta-optics

The Miniscope is a compact and lightweight imaging device that combines a microscope and a miniature camera system. It is designed for portable and in vivo imaging applications, particularly in the field of neuroscience research. The Miniscope allows for the imaging of neural activity in live animals, providing valuable insights into brain function and dynamics.

The Miniscope typically consists of a small lens, a gradient refractive index (GRIN) lens, and a camera sensor, all integrated into a single unit. The GRIN lens is a key component that enables the miniaturization of the system while maintaining high-quality imaging capabilities. It provides a compact solution for capturing high-resolution images from deep within biological tissues, such as the brain.

Integration of Meta-optics:
The integration of meta-optics into the Miniscope further enhances its imaging capabilities. Meta-optics, also known as metasurfaces or metadevices, involves the use of nanostructured materials to manipulate light at the subwavelength scale, enabling precise control over its properties.

By incorporating meta-optics into the Miniscope, several benefits can be realized. Here are some potential advantages:

Improved Imaging Resolution: Meta-optics can be used to design metasurfaces that manipulate the phase and amplitude of light, enabling improved imaging resolution. This can be especially beneficial for capturing fine details in neuroimaging, enhancing the clarity and quality of the acquired images.

Compact and Lightweight Design: Meta-optics allows for the creation of ultrathin and lightweight optical components. By integrating meta-optical elements into the Miniscope, the overall size and weight of the device can be reduced, making it more portable and easier to handle during experiments.

Enhanced Light Collection Efficiency: Meta-optics can optimize light collection and transmission, maximizing the amount of light captured by the Miniscope. This results in improved sensitivity and signal-to-noise ratio, enabling more accurate and precise measurements of neural activity.

Multifunctional Capabilities: Meta-optics offers versatility in functionality. By integrating different metasurfaces or metadevices into the Miniscope, it can be tailored for specific imaging modes or applications. For example, metasurfaces can be designed to control polarization, enabling polarization-sensitive imaging or analysis.

Customizable Imaging Properties: Meta-optical components can be designed with specific optical properties to meet the requirements of different imaging modalities. This customization allows for flexibility in adapting the Miniscope to various experimental needs, expanding its potential applications.

The integration of meta-optics into the Miniscope enhances its imaging capabilities, enabling high-resolution imaging, compact design, improved light collection, and customizable functionality. This integration holds promise for advancing neuroscience research by providing researchers with a powerful and versatile tool for in vivo imaging of neural activity.

Furthermore, if we focus on the existing objective module of each miniscope then we can conclude that both the generation of the miniscope needs at least 5 mm of space for lenses to interact with light. Size of the optics limits the imaging functionalities. For example neurons are inherently distributed in 3D, which leads to a need of probing an extended depth of focus. That's the first reason we move to meta-optics. Below picture compares the dimensions of the optics and working distance.

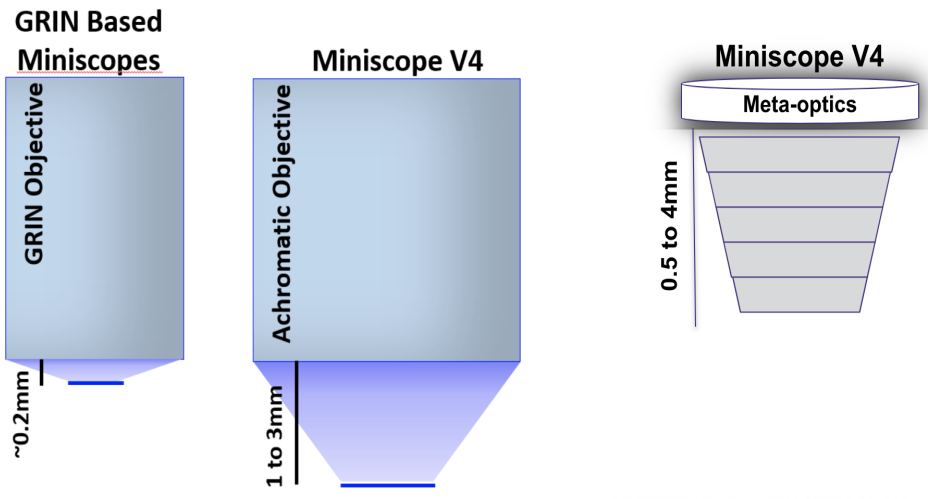


Fig: 3.7 Size & the working distance comparison for different optics used for

3.3 Biological Imaging concept with flat optics

Biological imaging with meta-optics combines the principles of optics and the unique properties of metasurfaces to enable advanced imaging techniques in the field of biology and life sciences. Meta-optics, also known as metasurfaces or metadevices, involves the use of nanostructured materials to manipulate light at the subwavelength scale, offering precise control over its properties.

Overall, the integration of meta-optics in biological imaging opens up new possibilities for high-resolution, multispectral, polarization-sensitive, label-free, and functional imaging. These advancements provide researchers with powerful tools to explore and understand biological systems at various length scales, from subcellular structures to tissue-level interactions, enhancing our knowledge of biology and enabling advancements in medical diagnostics, drug discovery, and fundamental biological research.

As of now we are all familiar with the size of the optics used for Miniscope, it's always been true that the size of the optics limits the imaging features due to small working distance.
Below schematic shows the microscopic imaging features through metamaterial. Metamaterials have the capability for multiple functionalities of imaging at a time, i.e, Super high resolution, wide field of view and extended depth of field with minimal distortion.

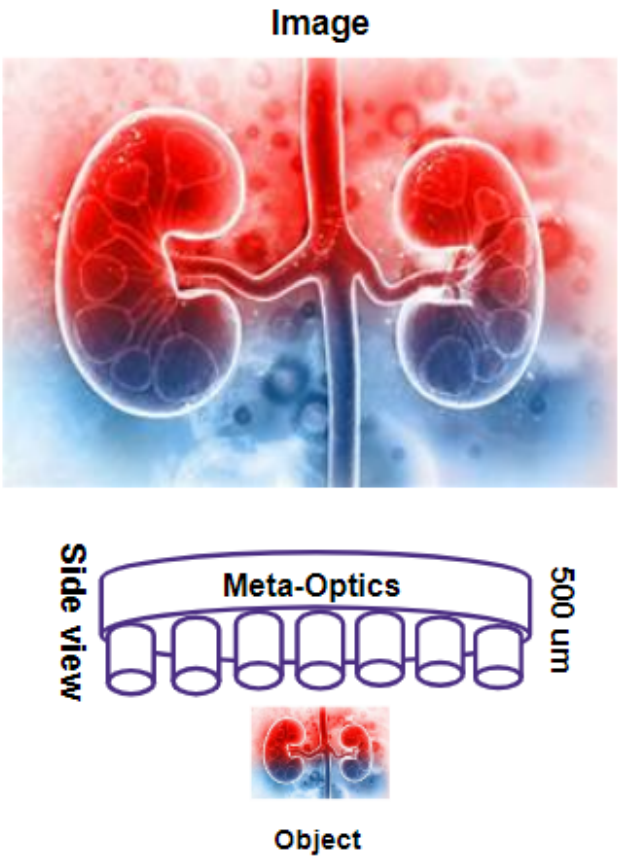


Fig: 3.8 Microscopic Imaging by Metamaterial

Chapter-4 : How V4 Miniscope works

This chapter mostly focused on the updated version of Miniscope i.e, 4th generation.

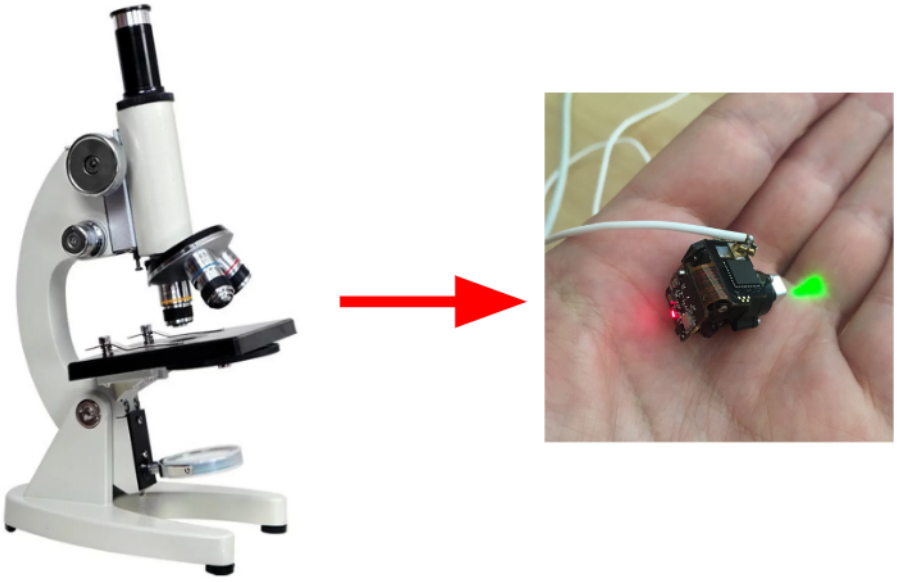


Fig: 4.0 Overview of Microscope to Miniscope

4.1 Microscope to miniscope overview

In this chapter you will get familiar with the working principle and construction of microscope/miniscope. Miniscope is the miniaturized version of microscope. Unlike the fluorescence microscope working principle - It absorbs high energy of light with low wavelength and emits low energy of light with higher wavelength, miniscope works on the principle of absorbing blue light and emitting green light.

Fluorescence microscopy uses a high-intensity light source that excites a fluorescent molecule called a fluorophore in the sample observed. The samples are labeled with fluorophore where they absorb the high-intensity light from the source and emit a lower energy light of longer wavelength.

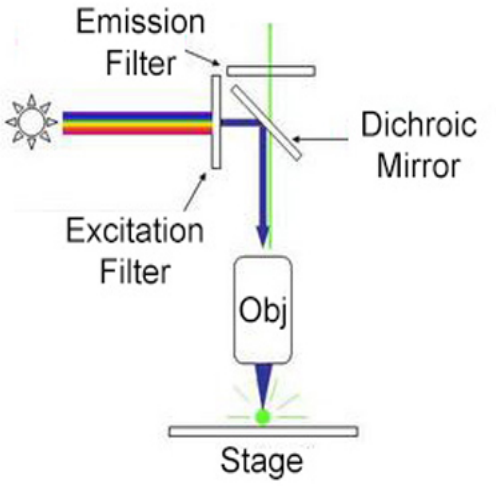


Fig: 4.1 Internal parts of MiniScope [10]

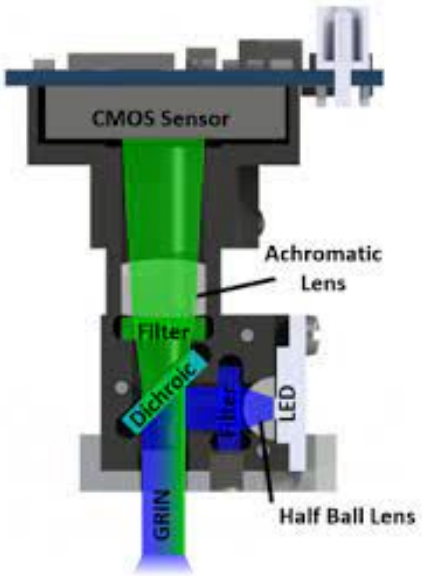


Fig: 4.2 Miniscope [9]

4.2 Construction of V4 Miniscope: [11]

Below fig shows the closer look of the V4 Miniscope, which describes each module entirely of the latest version of miniscope called V4 series.

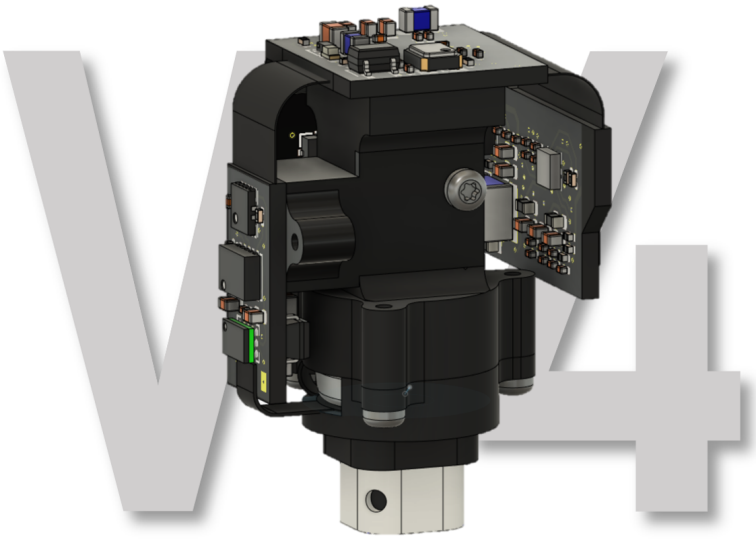


Fig: 4.3 Closer look of V4 Miniscope [9]

The V4 Miniscope system typically consists of several key components that work together to enable in vivo imaging of neuronal activity in small animals. Here are the main parts of a typical V4 Miniscope setup:

Construction of Miniscope:

Miniscope Body: The miniscope body is the main housing unit that contains the essential components of the system. It typically includes the imaging sensor, miniaturized objective lens, and various electronics required for data acquisition and control.

Imaging Sensor: The imaging sensor is a high-sensitivity camera or sensor that captures fluorescence signals emitted by calcium indicators in neurons. It converts the optical signals into digital data for further processing and analysis.

Objective Lens: The miniscope is equipped with a miniaturized objective lens that focuses the excitation light onto the tissue and collects the emitted fluorescence signals. The lens is typically designed for two-photon fluorescence imaging, allowing for high-resolution imaging of neuronal activity.

LED Light Source: The V4 Miniscope uses light-emitting diodes (LEDs) as the light source for fluorescence excitation. LEDs emit light of specific wavelengths to excite the calcium indicators in the neurons, which subsequently emit fluorescence signals that are captured by the imaging sensor.

Optics and Filters: The miniscope may include various optical components, such as filters and beam splitters, to control the excitation and emission light paths. These components help ensure that only the desired wavelengths reach the tissue and are detected by the imaging sensor.

Data Acquisition and Control Unit: The V4 Miniscope requires a data acquisition and control unit to interface with the imaging sensor, control the LED light source, and manage the data acquisition process. This unit typically includes hardware and software components for real-time data acquisition and control.

Battery and Power Management: Since the miniscope is designed for use in freely behaving animals, it is powered by a lightweight battery that provides the necessary power for the entire system. Power management components ensure efficient power usage and allow for long-term imaging experiments.

Head-mount and Connectors: The miniscope is typically attached to the animal's head using a custom head-mount, which secures the system in place while allowing the animal to move freely. Connectors and cables connect the miniscope to the data acquisition and control unit.

Analysis Software: The V4 Miniscope is accompanied by specialized software for data analysis and visualization. This software allows researchers to process and analyze the acquired neuronal activity data, extract signals, and perform various analyses to gain insights into the functioning of neural circuits.

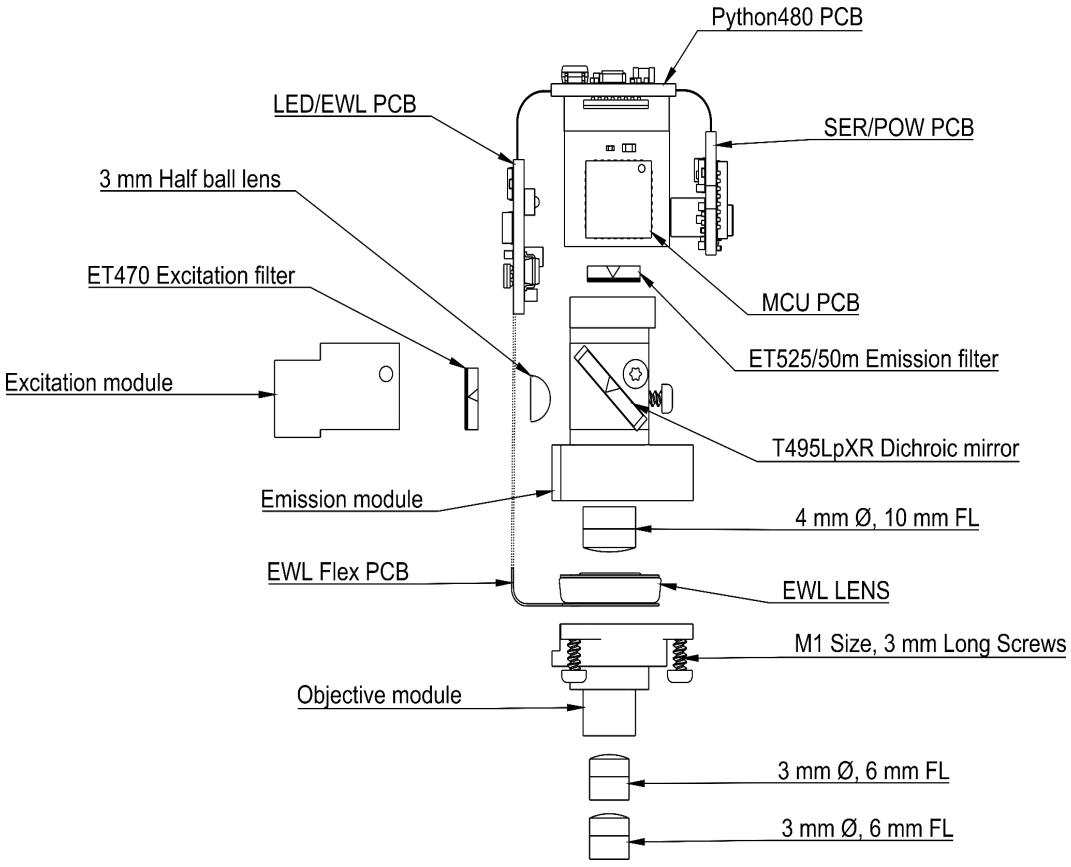


Fig: 4.4 Internal parts of the V4 Miniscope [11]

Recent modifications by Miniscope researchers to improve the diagnosis, prevention and treatment of diseases by developing novel imaging technology can been explored here [12]
[12] gives motivation for integrating miniscope with meta-optics. Amazing imaging features of this paper motivates me to replicate the results with meta-optics.

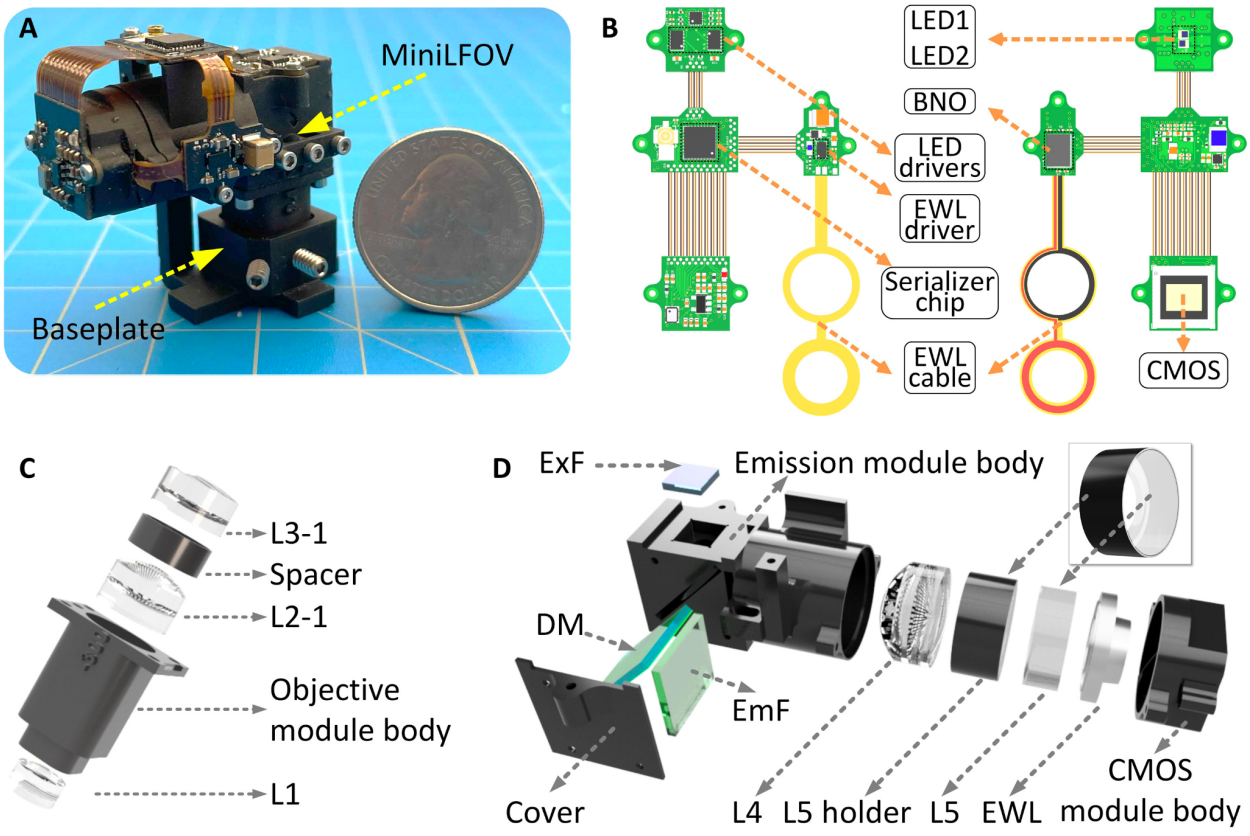


Fig:4.5 Updated Schematic of V4 Miniscope with internal part of each module [12]

V4 miniscope experimental setup with the three axis controller.

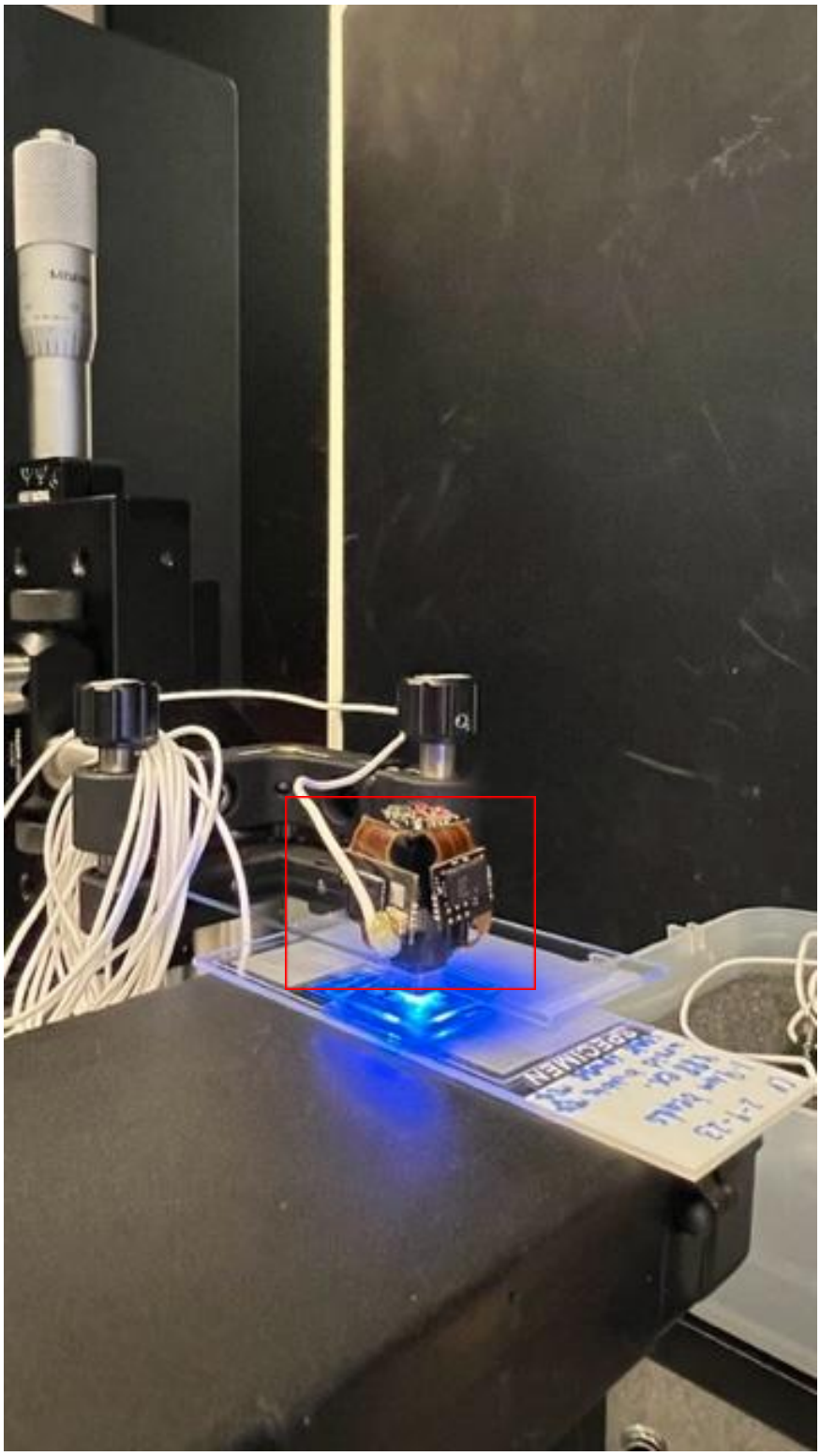


Fig: 4.6 In-lab V4

Chapter-5 : Comparison of GRIN lens, Achromatic lenses and Meta Optics

This chapter is based on the history of lenses used in the miniscope to extract microscopic imaging features. Furthermore, this chapter deals with the lens compatibility for miniscope. Subsequently, it describes different lens configurations with the miniscope which includes recent events. At the very end of this chapter, comparison of Meta-optics with two other lenses called achromatic (normal diffractive lenses) and GRIN lenses are available which are used for MiniScope.

The MiniScope, a compact imaging device used in neuroscience research, typically incorporates different types of lenses to achieve specific imaging capabilities. Here are some of the possible lenses that can be used in a MiniScope:

5.1 GRIN Lens for Mininiscope

GRIN lens: A GRIN (Gradient Refractive Index) lens is a type of lens that has a varying refractive index across its volume. Unlike conventional lenses that have a uniform refractive index, GRIN lenses are designed to gradually change the refractive index from the center to the periphery. This variation in refractive index allows the lens to bend light rays and focus them, similar to how a traditional lens operates.

The gradient refractive index profile in a GRIN lens is typically achieved by doping the lens material with different concentrations of refractive index modifiers or by using a composite material with varying refractive index properties. The refractive index gradient enables the lens to effectively focus light without the need for complex lens systems or multiple lens elements.

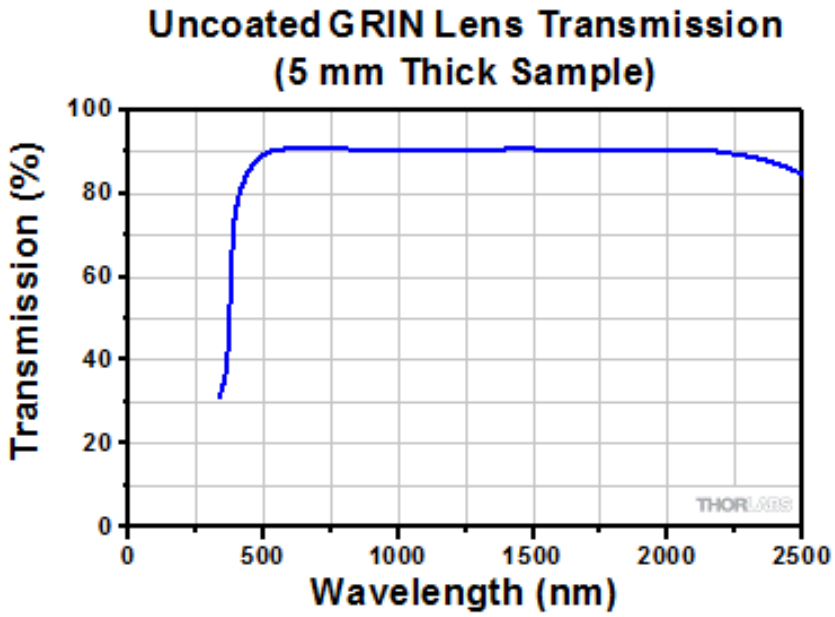


Fig: 5.1 Transmission of one of the uncoated GRIN Lenses as a function of wavelength which can be used for microscopic images [13]

5.2 Achromatic/Diffractive Lens for Mininiscope

Achromatic lenses: In the design of a MiniScope, the combination of achromatic lenses is often employed to minimize chromatic aberration and achieve high-quality imaging. Achromatic lenses are specifically designed to reduce the effects of chromatic aberration, which is the inability of a lens to focus different wavelengths of light to the same point. By combining multiple lens elements with different dispersive properties, achromatic lenses help to bring different wavelengths of light into focus at a common plane, resulting in sharper and more accurate images.

The specific combination of achromatic lenses used in a MiniScope can vary depending on the desired optical performance and imaging requirements. However, a typical combination may include the latest upgrade in the lens configuration. [14]

Three achromatic lenses make up the majority of the optical imaging path. Two of these lenses are 3mm diameter lenses and sit in the Objective Module, the third is a 4mm lens in the Emission Module and acts as a focusing tube lens. By mixing and matching the focal lengths of the two lenses in the Objective Module, a range of working distances and fields-of-view can be achieved.

Fig 5.2 represents the lens configuration of the objective module of v4 miniscope. Lens "1", lens "2", and lens "3". Lenses "1" and "2" comprise the Objective while lens "3" acts as the achromatic tube lens. It achieves single cell resolution, ~1mm diameter FOV, and ~1 mm working distance.

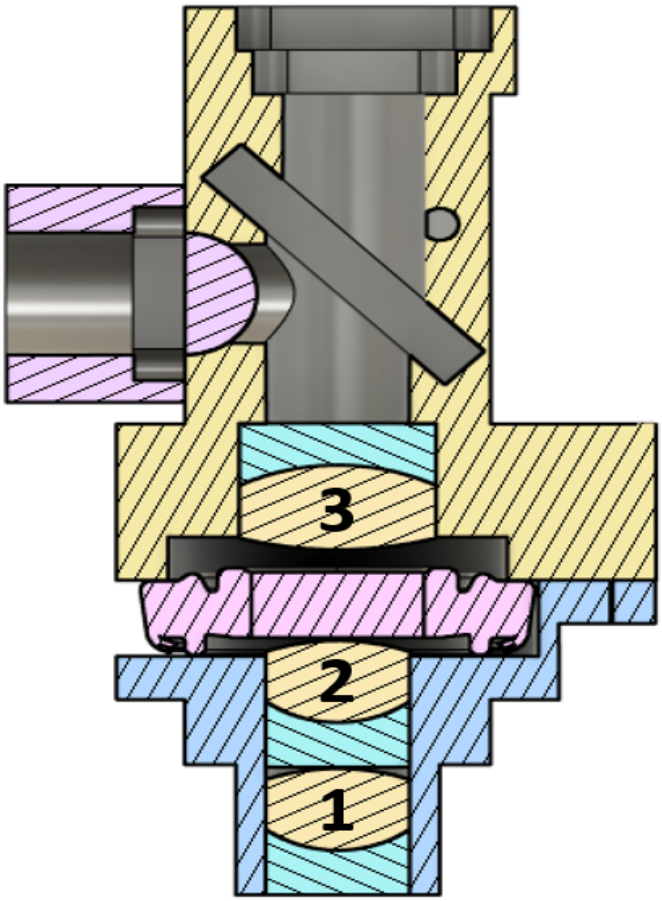


Fig: 5.2 Objective lens configuration of V4 MiniScope, this schematic shows the configuration of the three objective lenses used in V4 MiniScope for Imaging [14]

Lens Number	Vendor	Part Number	Description
1	Edmund Optics	45-089	3mm diameter, 6mm FL achromat used in the objective module

2	Edmund Optics	45-089	3mm diameter, 6mm FL achromat used in the objective module
3	Edmund Optics	63-691	4mm diameter, 10mm FL achromat used in the emission module

5.3 Meta-optics designed for Mininiscope with microscopic capabilities

Meta-optics: For this project all Meta-optics are designed on SiN material with refractive index 2 and multiple focal length 0.5, 1, 2 & 4 mm. Shape of the scatterer used for these lenses is square.
Hyperboloid - This is to achieve single cell high resolution imaging.
EDOF - This lens is designed to achieve a larger depth of focus for multiple layers of biological samples at a time with higher resolution.

5.4 Lens comparison

Comparison Table with different lenses used for Miniscope

	Meta-optics	Grin lens	Achromatic lens
Thickness	500 um	4-5 mm	6-7 mm
Diameter	2 mm	2 mm	3 mm
Working distance	0.5 to 4 mm	0.2 mm	1 to 3 mm

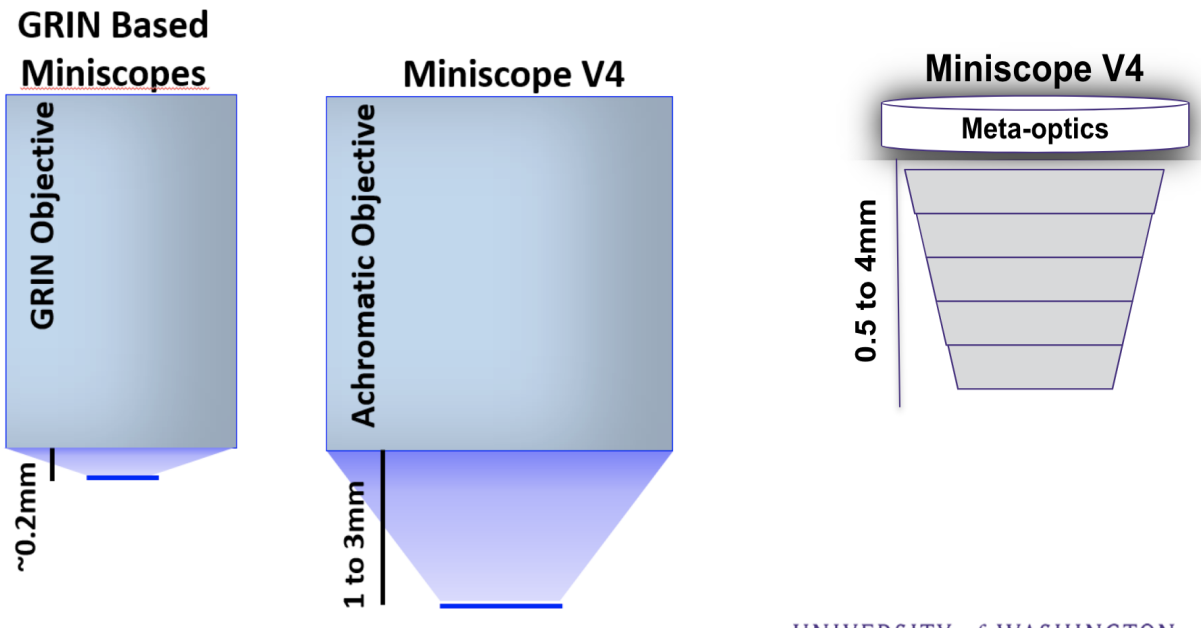


Fig: 5.3 Schematic compares the size of the optics & working distance of different lenses used in the MiniScope

Idea behind the replacement of objective lenses with Meta-optics can be understood by Fig: 5.3. Size of the optics limits the imaging functionalities. For example neurons are inherently distributed in 3D, which leads to a need of probing an extended depth of focus and larger working distance. This is the first motivation we are moving towards meta-optics in biomedical. The work begins with the foundation in metasurfaces, examining issues such as narrow depth of field in metalenses and the development of EDoF called extended depth of focus in metalenses with larger working distances.

It is clear from the fig: 5.3 that the dimensions of the meta-optics is very thin or you can say it's flat as compared to the other two optics (Diffractive & GRIN).

Biggest advantage in terms of dimension is the thickness of the lens and the working distance is quite larger as compared to other lenses.

Chapter-6 : Meta Optics capability for Miniscope integration

This chapter deals with the different design of lenses for MiniScope compatibility to show the potential of meta-optics in terms of miniaturization and functionalities for biomedical devices.

Optical lenses play a key role in modern optoelectronic applications. Among them, lenses with large numerical aperture (NA), wide field of view, and high focusing efficiency determine the performance of optical systems in some important applications, such as lidar, microscope objectives, and photography cameras. In the design of conventional refractive or diffractive optics, the configurations cascading multiple lenses are commonly used to correct the chromatic aberration of the system. Therefore, the conventional optical systems are too bulky and difficult to adapt to the miniaturization of optical systems and also require high manufacturing costs.

This section describes the meta-optics designed for Miniscope.
Hyperboloid - Resolution test.
EDOF - Extended depth of focus test.

6.1 Hyperboloid - Resolution test [15]

The hyperboloid design is supposed to have better resolution than all other metalens profiles. Hyperboloid metalens will give the best result for resolution tests in terms of single wavelength at a particular focal point. However, slight deviation in the wavelength or focal plane greatly reduces the lens's performance and results in aberration, to overcome that issue we designed EDoF.

There are several improvements in the metalens design to achieve highest resolution imaging. However, this hyperboloid metalens requires complex design techniques and rules, it will provide the phase of transmitted light from 0 to 2pi, which is necessary for a complete control of the optical wavefront. The concept of phase break/cut off which has been used in the demonstration of metasurfaces capable of beaming light in the direction characterized by generalized laws of reflection and refraction, provides a multiple path for designing metalens.

Using this approach, the control of the wavefront no longer depends on the phase accumulated by the propagation of light but it can be controlled by the phase shifts experienced by scatterers, which are an optically thin array of subwavelength-spaced resonators comprising the metasurface.

This design mainly focuses on the optimization for 500 wavelengths ranging from 450 to 600 nm, for set focal lengths of 500um, 1mm, 2mm and 4mm. The design of miniscope metalens is obtained by imposing a hyperboloidal phase profile on the metasurface. Here, the waves emerging from the constructively interfere at the focal plane similar to the waves that emerge from conventional lenses. For a given focal length f, the phase shift φL imposed in every point PL(x, y) on the metalens must satisfy the below equation

$$\varphi_L(x, y) = \frac{2\pi}{\lambda}\overline{P_L S_L} = \frac{2\pi}{\lambda}\left(\sqrt{(x^2 + y^2) + f^2} - f\right)$$ [15]

where λ is the wavelength in free space.

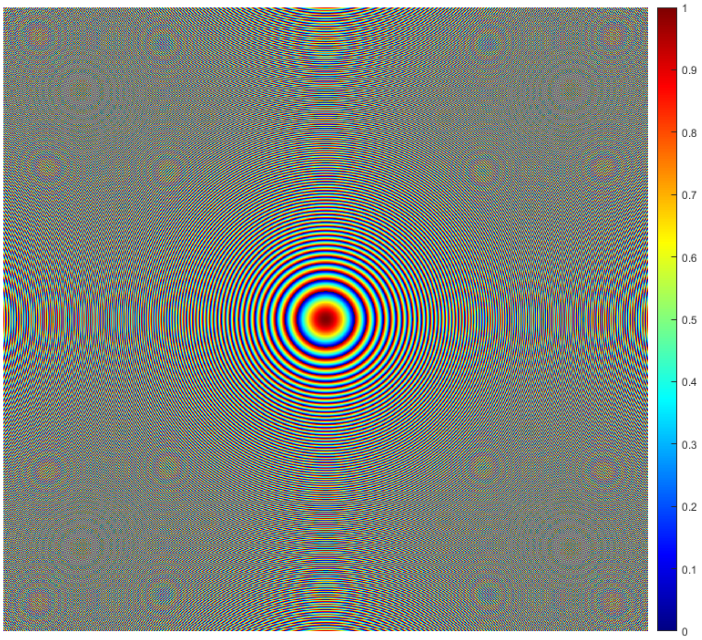
This hyperboloid phase profile is theoretically the best performance you can achieve for normally incident light at a single wavelength. So it works very well over a very narrow field of view and only one wavelength, but breaks down quickly for off-axis light that is not a single wavelength.

6.1.1 Design Parameters

Specifications:

- Nominal wavelength: 525 nm
- Wavelength range: 450-600 nm
- Lens diameter: “2mm”
- Periodicity: 350nm
- N = 1 mm / 0.000350mm ~ 2857.14
- To ensure correct periodicity, use N = 2857 and p = 0.000350mm exactly and adjust radius:
- R = N*p = 0.99995 mm
- Focal lengths: 500um, 1mm, 2mm, 4mm

6.1.2 Final phase profile and MTF plot for different focal lengths lenses



6.2 EDoF - Extended depth of focus

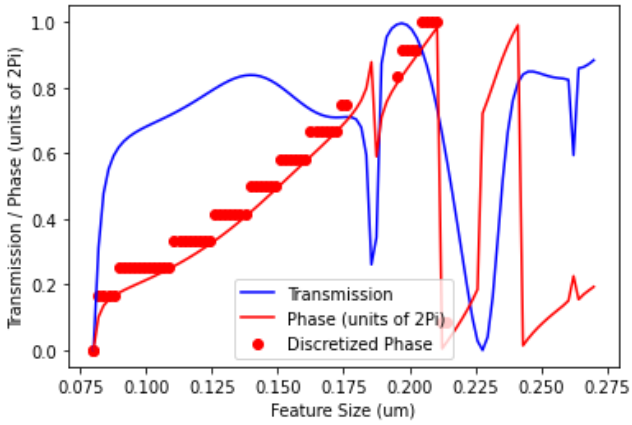
The EDoF design is supposed to have better broadband performance than a standard hyperboloid metalens profile. A standard hyperboloid metalens will give theoretically the best result (diffraction-limited performance) for a single wavelength at a particular focal plane. However, slight deviation in the wavelength or focal plane greatly reduces the lens's performance. The EDoF lens is designed to extend the focal spot into a line so that the lens is less sensitive to changes in wavelength; however, the tradeoff is that the performance is no longer diffraction-limited.

The previous members of our research group looked into analytic forward-designed EDoF lenses in this paper: [16]

However, we later found that inverse-designed EDoF lenses have better performance, and this is the kind of lens we designed for the miniscope: [17]

To design our EDoF miniscope lenses using the inverse-design approach, we assumed normally incident illumination and that the lens is radially symmetric. This allows us to reduce the 2D phase mask to a radial profile, reducing memory requirements and increasing the optimization speed. We define an initial phase mask guess and propagate light to the focal plane using Hankel transform and Rayleigh-Sommerfeld propagation. We iteratively update the phase mask and propagate to the focal plane. We define our merit function to maximize the intensity of the light at the focal plane for a range of wavelengths, resulting in a lens with EDoF properties.

For the miniscope lenses, we optimized for 500 wavelengths ranging from 450 to 600 nm, for set focal lengths of 500um, 1mm, 2mm and 4mm. The subwavelength scatterer has a periodicity of 350 nm, so to achieve a lens diameter of approximately 2 mm, we need to optimize N = 2857 scatterer radially (2857 * 350 nm = 0.99995 mm radius).
Below picture shows the phase versus scatterer plot for EDoF lens and transmission efficiency of the lens.



Phase v/s scatterer plot

6.2.1 Design Parameters

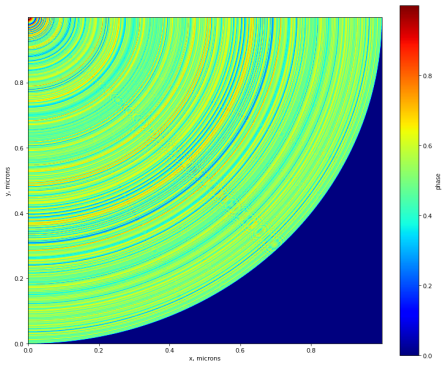
Specifications:

- Nominal wavelength: 525 nm
- Wavelength range: 450-600 nm
- Lens diameter: “2mm”
- Periodicity: 350nm

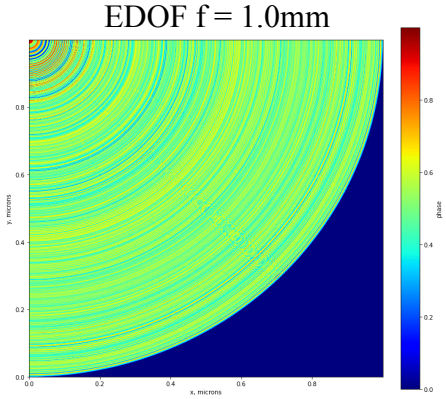
- N = 1 mm / 0.000350mm ~ 2857.14
- To ensure correct periodicity, use N = 2857 and p = 0.000350mm exactly and adjust radius:
- R = N*p = 0.99995 mm
- Focal lengths: 500um, 1mm, 2mm, 4mm

6.2.2 Final phase profile and MTF plot for different focal lengths lenses

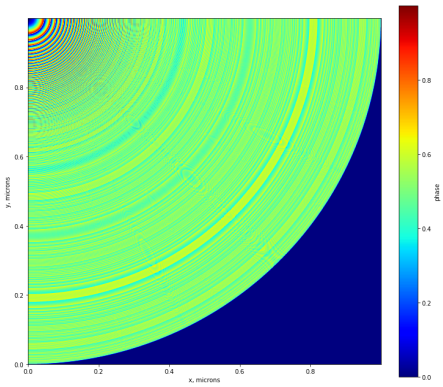
EDOF f = 0.5mm



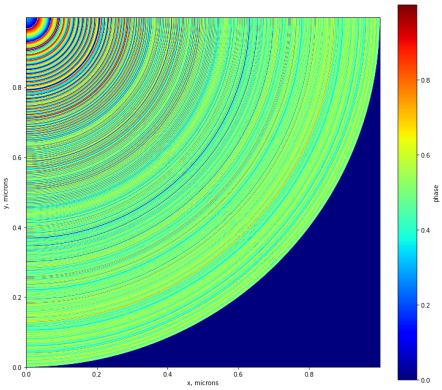
EDOF f = 1.0mm



EDOF f = 2.0mm



EDOF f = 4.0mm



Chapter-7 : Imaging and characterization of meta-optics with MiniScope

This chapter deals with the imaging and characterization of different lenses designed for MiniScope capabilities with biological samples of 2um beads. To demonstrate the potential of metalens for biomedical devices, there are a couple of imaging studies performed. In our preliminary tests, we attached the meta-optics to the Miniscope, which directly allows us to measure test samples of 2um fluorescence beads.

Further we will compare imaging features with different lenses to show potential of Meta-optics in terms of miniaturization and functionalities.

Experimental Demonstration:

I designed the imaging system with high precision control in all the three axes. First I fixed the object plane with the sample of 2um fluorescence beads on three axis controllers with precision of 5um. To define the image plane I fixed the MiniScope just above the object plane as shown in the Fig:7.0. The distance between the lens and the image sensor is 22mm which is fixed. While assembling the MiniScope I have taken consideration of only objective lenses, all other lenses in the MiniScope have been removed. This experimental system allows me to do the direct imaging with objective (Meta-optics) and Image Sensor of the MiniScope which is ~ 22mm away from the lens.

For a particular lens, object distance is equal to its focal length. Image distance is significantly larger than the object distance.

The equation that relates the object distance (do), image distance (di), and focal length (f) in optics is known as the lens formula or thin lens equation:

$$\mathbf{1/f = 1/do + 1/di}$$ [18]

In this equation:

f represents the focal length of the lens,

do represents the object distance (distance of the object from the lens), and

di represents the image distance (distance of the image from the lens).

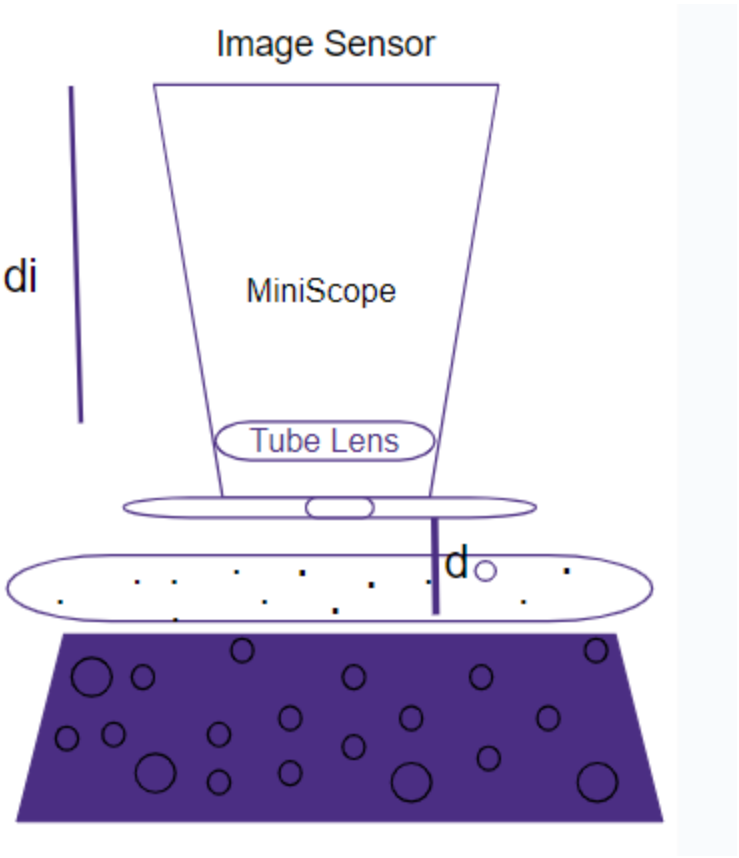


Fig: 7.0 Hyperboloid Experimental Demonstration

do = FL = Object Distance

di = Image Distance = 10mm

Image Sensor, Array Size=750um × 500um, ~ 1 um per pixel, 0.375MPixel.

1. Magnification of Microscope = Focal length of the tube lens/focal length of the objective lens
2. Magnification of lens = Size of image/Size of object

Using equation 1.

Expected magnification for the 1mm = 10/1 = 10

Expected magnification for the 2mm = 10/2 = 5

Expected magnification for the 4mm = 10/4 = 2.5

7.1 Hyperboloid Imaging of 2um beads

As discussed in the previous chapter hyperboloid lens is designed to achieve higher resolution at a narrow field of view.

7.1.1 Hyperboloid of focal length 1mm

Below set of pictures shows the magnification test of 2um single bead with lens of 1 mm of focal length.

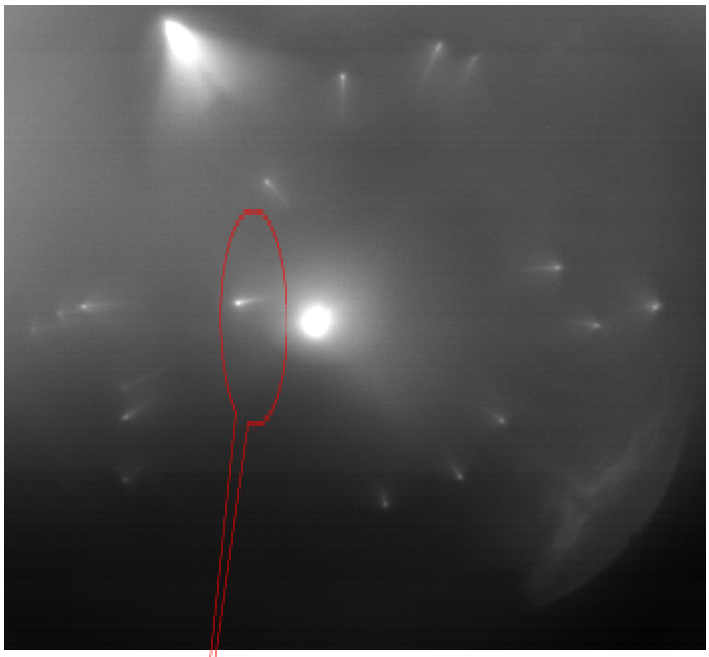


Fig: 7.1(a) Raw data of 1mm Hyperboloid

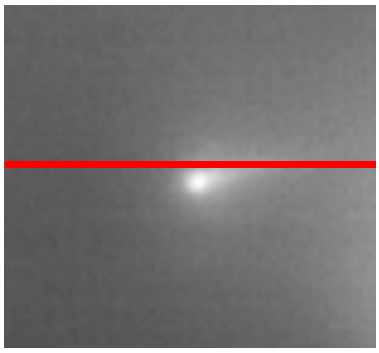


Fig: 7.1(b) Single Bead Analysis

Take a line cut along the x-axis to measure the diameter of the bead or FWHM.

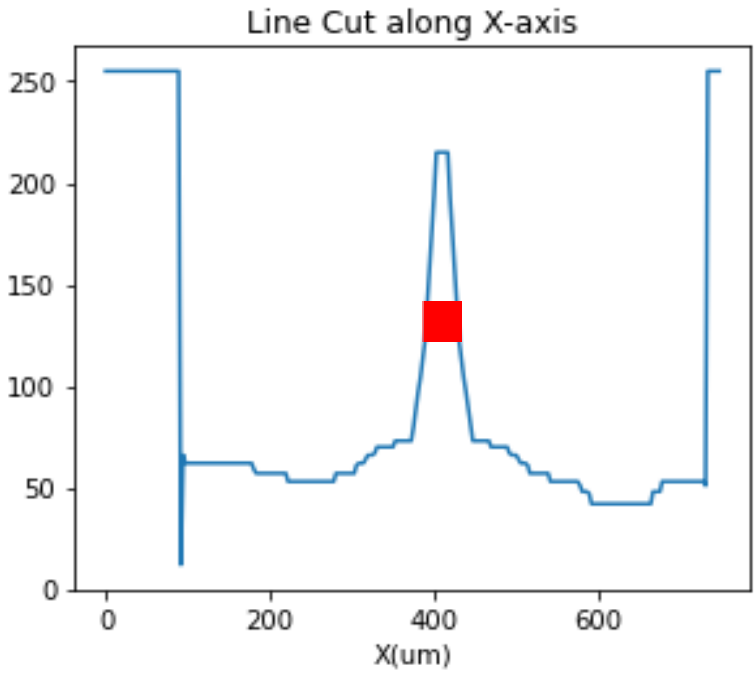


Fig: 7.1(c) FWHM for Single bead, X-axis shows the array size of the image sensor in um. Red line shows the approximate width of the FWHM of a single bead of um in the image plane.

Analysis: For this experiment I used 2um beads as a sample on the object plane. Focal length of the hyperboloid lens is 1mm. I focus the single bead (shown fig:7.1(b)) to measure the approximate diameter of the bead using FWHM, so I plotted the single bead from the image plane and measured the size of a single bead by line cutting on the bead (shown fig:7.1(c)).

Experimental Result

FWHM = 50 um = diameter of the bead.
(415 um - 389 um) = 26um

Image size of the bead or the diameter of the bead in the image is 50 um.

Actual size of the bead in the sample is 2um
Magnification = 26 um/2 um = 13

Theoretical Magnification or Expected Magnification = 10

From the above results I can conclude that experimental magnification (10) is very close to the theoretical value (13).

Observation: It can be observed in the Fig:7.1(a) that hyperboloid can be focused for a narrow field of view so only one bead can be seen focused and all other beads get distorted when it's a bit out of focus, that is what we expected from the design.

7.1.2 Hyperboloid of focal length 2mm

Above procedure has been repeated for 2mm of lens. Below set of pictures shows the magnification test of 2um beads with focal length of lens 2mm.

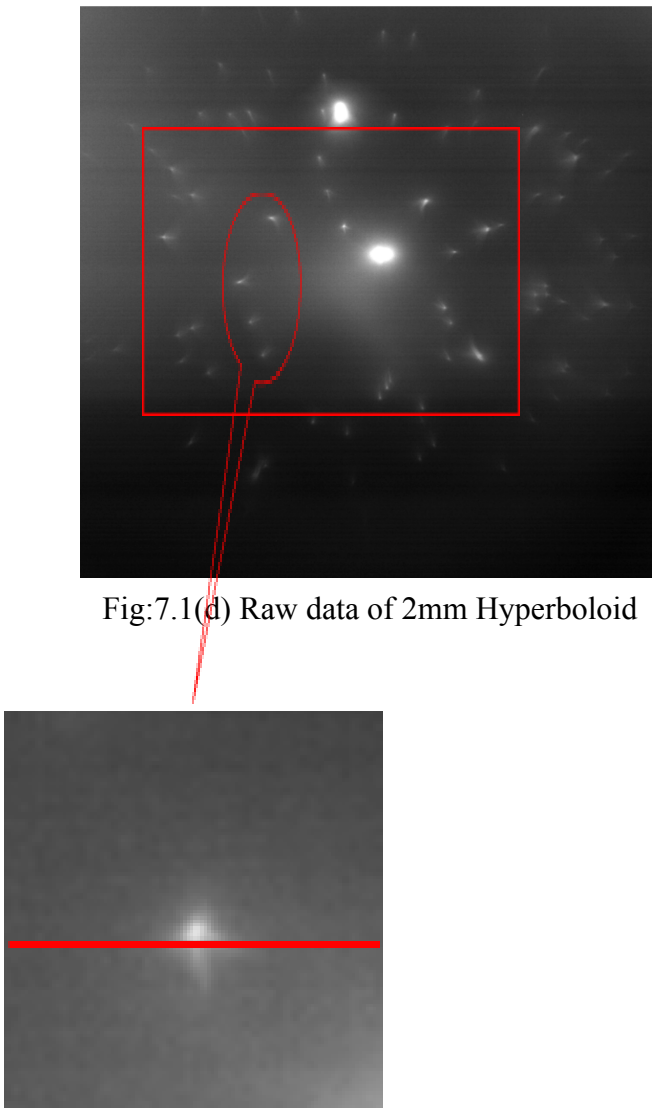


Fig:7.1(d) Raw data of 2mm Hyperboloid

Fig: 7.1(e) Single Bead Analysis

Take a line cut along the x-axis to measure the diameter of the bead or FWHM.

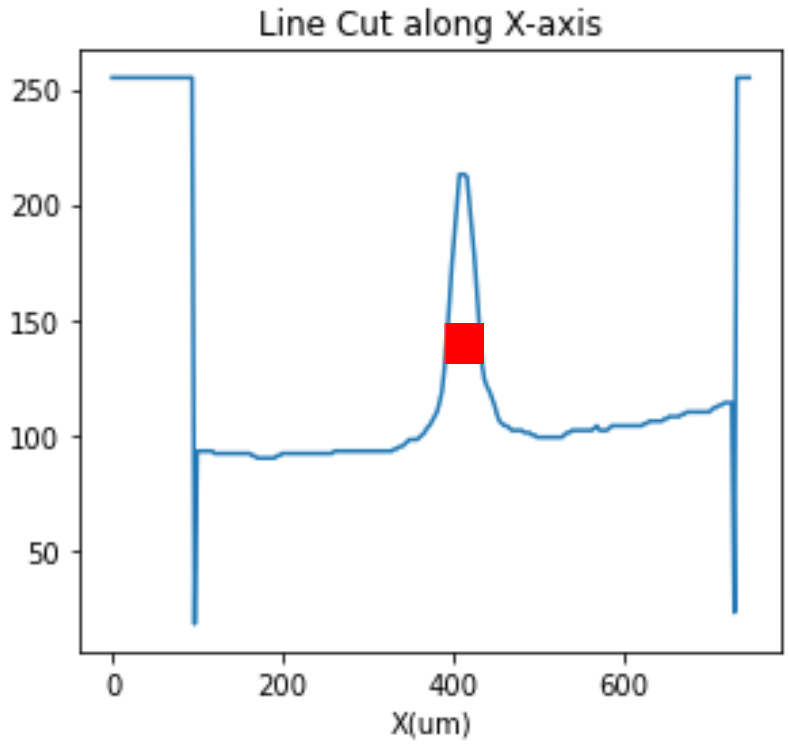


Fig: 7.1(f) FWHM for Single bead, X-axis shows the array size of the image sensor in um. Red line shows the approximate width of the FWHM of a single bead of um in the image plane.

Analysis: For this experiment I used 2um beads as a sample on the object plane. Focal length of the hyperboloid lens is 2mm. I focus the single bead (shown fig:7.1(e)) to measure the approximate diameter of the bead using FWHM, so I plotted the single bead from the image plane and measured the size of a single bead by line cutting on the bead (shown fig:7.1(f)).

Experimental Result

FWHM = 16 um = diameter of the bead.

(410 um - 394 um) = 16um

Image size of the bead or the diameter of the bead in the image is 16 um.

Actual size of the bead in the sample is 2um

Magnification = 16 um/2 um = 8

Theoretical Magnification or Expected Magnification = 5

From above calculations I can conclude that experimental magnification (5) which is little far to the theoretical value (8).

Observation: It can be observed in the Fig:7.1(d) that hyperboloid with focal length 2mm can focus more number of beads in a single plane with less distortion, above calculation shows the magnification is decreasing while increasing the focal length.

7.1.3 Hyperboloid of focal length 4mm

Same procedure has been repeated for 4mm lenses. Below set of pictures shows the magnification test of 2um single bead with focal length of lens 4mm.

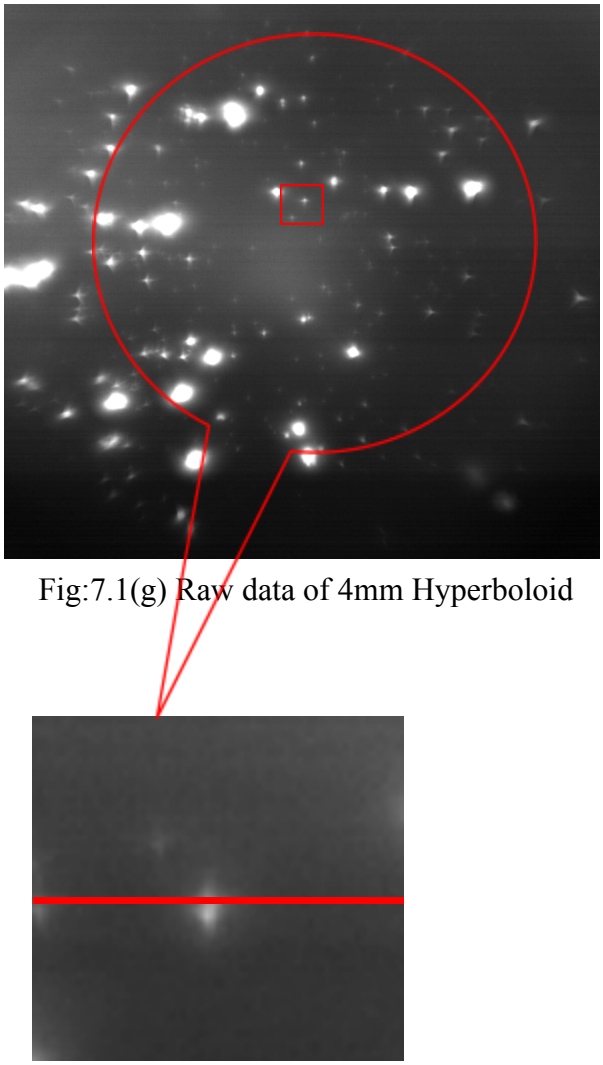


Fig:7.1(g) Raw data of 4mm Hyperboloid

Fig: 7.1(h) Single Bead Analysis

Take a line cut along the x-axis to measure the diameter of the bead or FWHM.

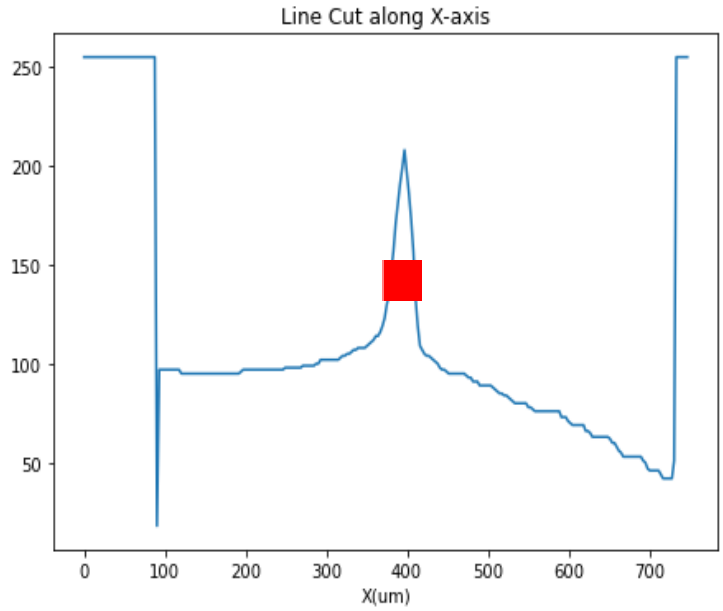


Fig: 7.1(i) FWHM for Single bead, X-axis shows the array size of the image sensor in um. Red line shows the approximate width of the FWHM of a single bead of um in the image plane.

Analysis: For this experiment I used 2um beads as a sample on the object plane. Focal length of the hyperboloid lens is 4mm. I focus the single bead (shown fig:7.1(g)) to measure the approximate diameter of the bead using FWHM, so I plotted the single bead from the image plane and measured the size of a single bead by line cutting on the bead (shown fig:7.1(i)).

Experimental Result

FWHM = 14 um = diameter of the bead.
(404 um - 390 um) = 14um

Image size of the bead or the diameter of the bead in the image is 14 um.

Actual size of the bead in the sample is 2um
Magnification = 14 um/2 um = 7

Theoretical Magnification or Expected Magnification = 2.5

From above calculations I can conclude that experimental magnification (7) which is very very close to the theoretical value (2.5).

Observation: It can be observed in the Fig:7.1(g) that hyperboloid with focal length 4mm can focus more number of beads in a single plane with less distortion, above calculation shows the magnification is decreasing while increasing the focal length and experimental & the expected magnification results are very very close.

Finally, from the above observation and analysis we can conclude that hyperboloid can be used to achieve best performance for normally incident light for a single wavelength with 1 mm of focal length.

7.2 EDoF: Extended depth of focus

Experimental Demonstration for EDoF [19]

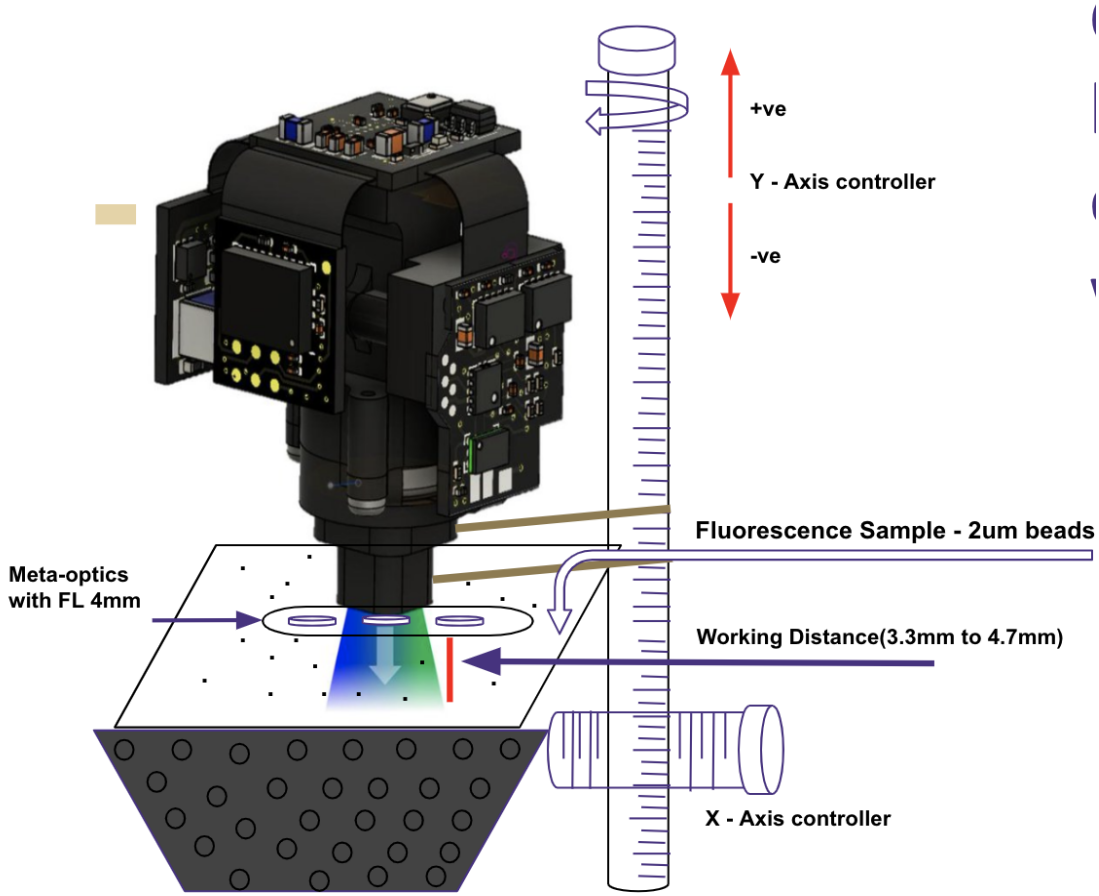


Fig: 7.2 - Schematic shows the experimental set up for EDoF with different working distance, precision control in Y-direction (positive & negative) is 5um.

To perform the EDoF (extended depth of focus) imaging with V4 MiniScope, the same setup has been used which was used for Hyperboloid. MiniScope was installed to have control in positive and negative Y-direction with precision of 5um.

As discussed in the previous chapter 6 EDoF is supposed to be designed to have better broadband performance than hyperboloid metalens profile. The EDoF lens is designed to extend the focal spot into a line so that the lens is less sensitive to changes in wavelength.

7.2.1 EDoF of focal length 1mm

For this experiment the object plane was fixed with 2um fluorescence beads. I captured images with different working distances in the interval of 10um in positive Y-direction. To show the potential of EDoF meta-optics I focused on the same bead throughout the process. This procedure has been repeated several times to ensure the productivity of the result.

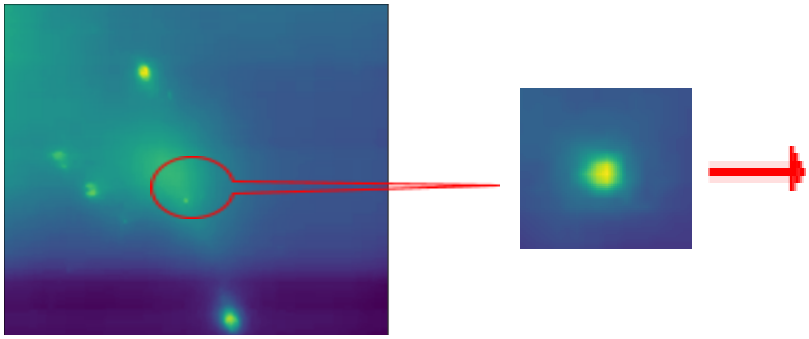


Fig: 7.2 (a), WD - 1mm

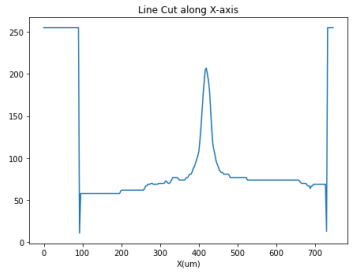


Fig: 7.2 (f)

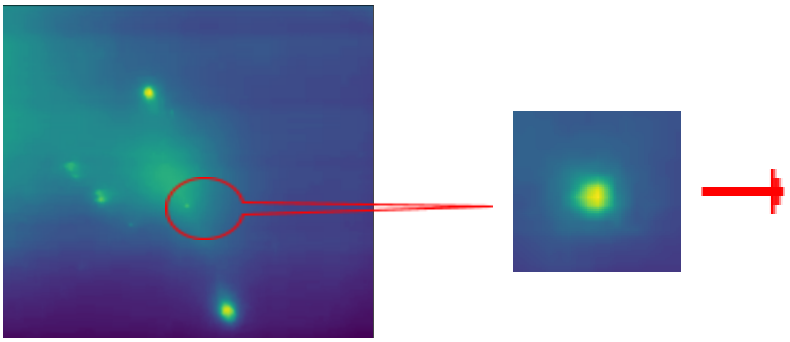


Fig: 7.2 (b), WD - 1.01mm

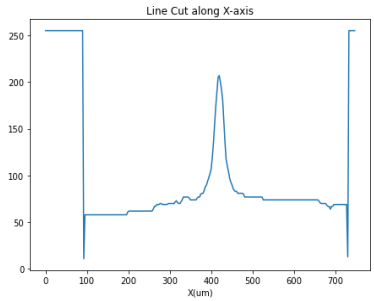


Fig: 7.2 (g)

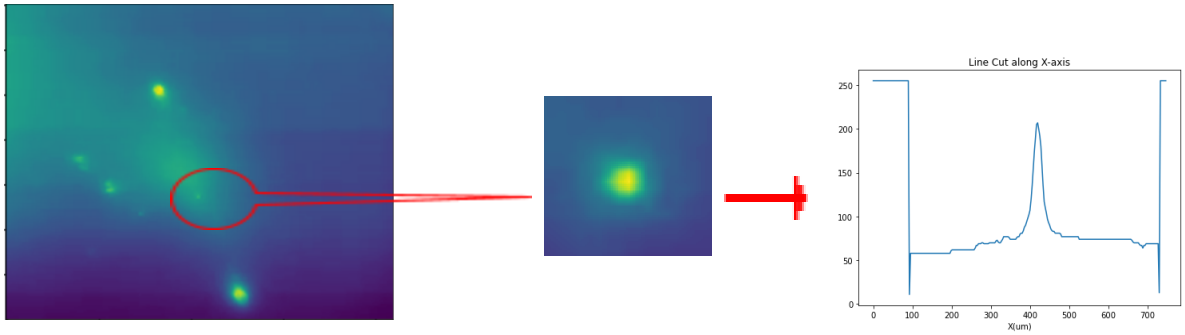


Fig: 7.2 (c), WD - 1.02mm

Fig: 7.2 (h)

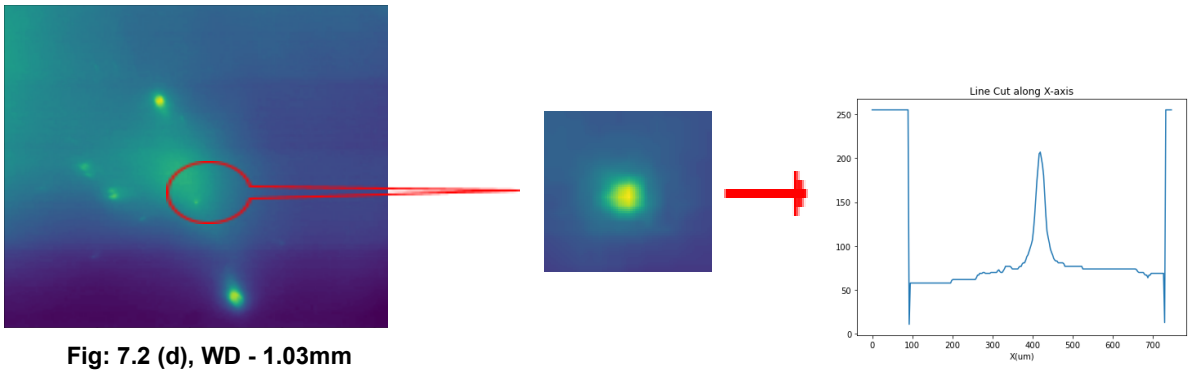


Fig: 7.2 (d), WD - 1.03mm

Fig: 7.2 (i)

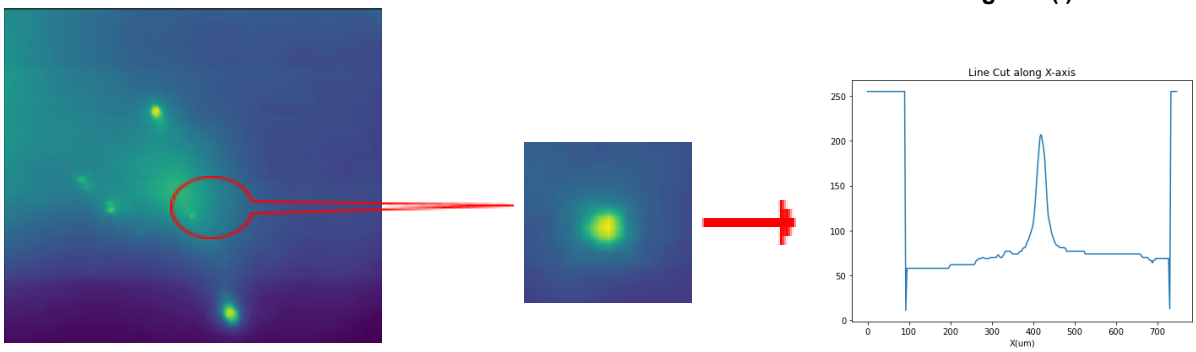


Fig: 7.2 (e), WD - 1.04mm

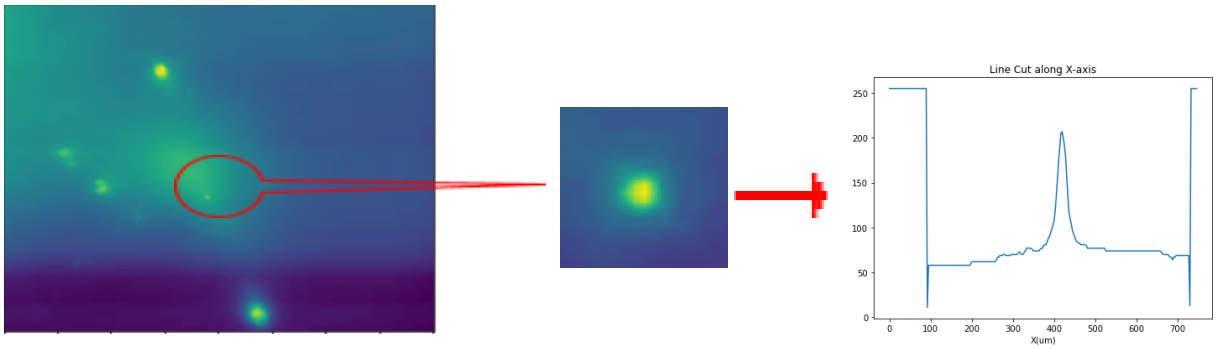
Fig: 7.2 (j)

Analysis: To characterize EDoF 1mm, I changed the working distance of the imaging plane (shown images in the Fig: 7.2 (a) to Fig: 7.2 (e)) in positive Y-direction with the interval of 10 um and captured images of the same bead. To analyze the single bead at different working distances, I plotted **the bead in the array of the sensor size (750 um × 500 um & ~1um per pixel)**. Afterward line cut has been taken along the x-axis to optimize HWFM for all the beads.

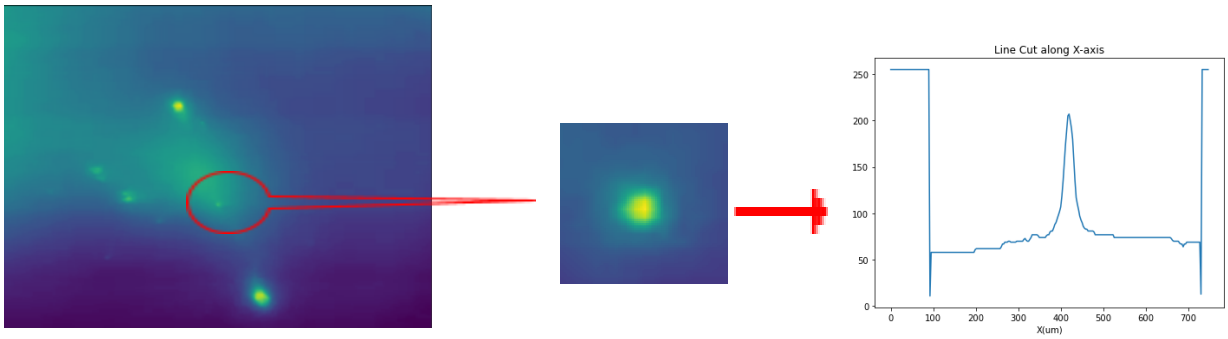
Observation: HWFM plot remains same (shown Fig: 7.2 (f) to Fig: 7.2 (j)) while changing the working distance in positive Y-direction.

From the analysis and observation I can conclude that while changing the working distance, beads remain the focus for different working distances i.e, 1mm to 1.04mm. So, I can infer the bead is focussed for around ~ 40um in positive Y-direction.

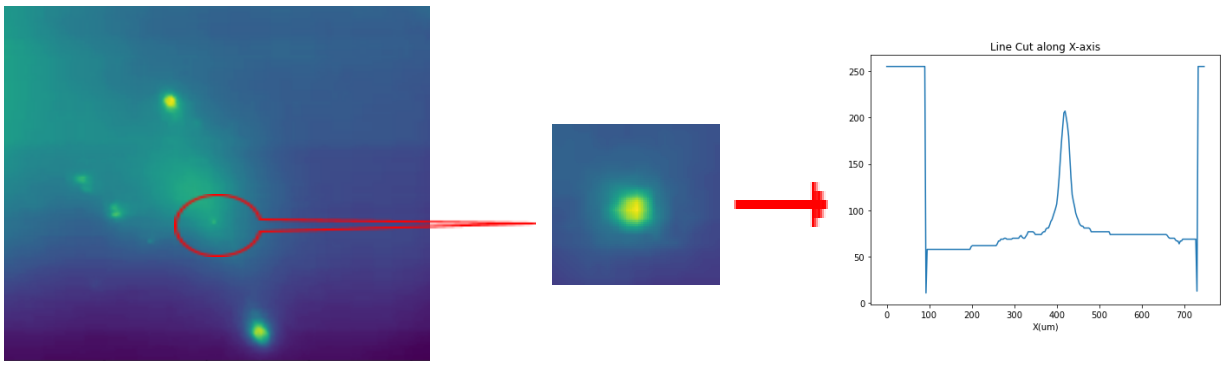
Above procedure has been followed for negative Y-direction. I captured images with different working distances in the interval of 10um in negative Y-direction. Again, To show the potential of EDoF meta-optics I focused on the same bead throughout the process. This procedure has been repeated several times to ensure the productivity of the result.



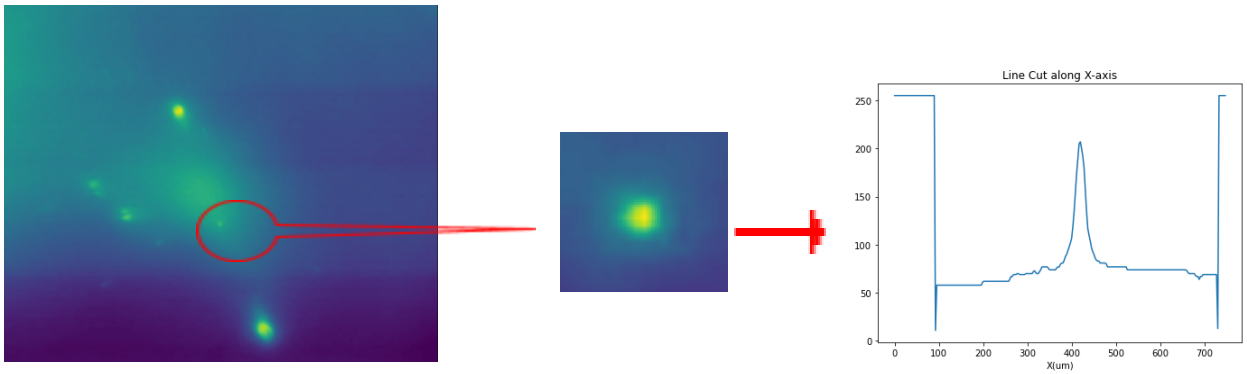
1. WD - 1mm



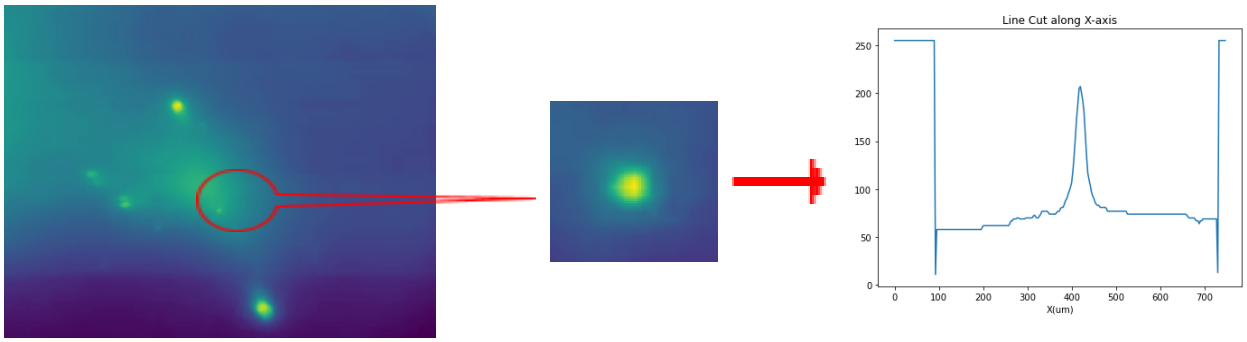
2. WD - 0.99mm



3. WD - 0.98mm



4. WD - 0.97mm



5. WD - 0.96mm

Analysis: Again, To characterize EDoF 1mm in negative Y-direction, I changed the working distance of the imaging plane (shown images in the picture **1. WD - 1mm to 5. WD - 0.96mm**) in negative Y-direction with the interval of 10 um and captured images of the same bead. To analyze the single bead at different working distances, I plotted **the bead in the array of the sensor size (750 um × 500 um & ~1um per pixel)**. Afterward line cut has been taken along the x-axis to optimize HWFM for all the beads.

Observation: Again, HWFM plot remains same while changing the working distance in negative Y-direction.

From the analysis and observation I can conclude that while changing the working distance, beads remain the focus for different working distances i.e, 1mm to 0.96mm. So, I can infer the bead is focussed for around ~ 40um in negative Y-direction.

Final conclusion of the 1mm EDoF lens is that it can be used to focus in a line at ~ 80 um depth i.e, ~ 40um in positive y-direction and ~ 40um in negative y-direction.

7.2.2 EDoF of focal length 4mm

To perform imaging with EDoF 4mm, the entire procedure has been repeated the same as EDoF 1mm to show the potential of the lens with the 2um fluorescence bead sample. For this experiment the object plane was fixed with 2um fluorescence beads. I captured images with different working distances in the interval of 10um in positive Y-direction starting from 4.0mm. To show the potential of EDoF meta-optics I focused on the same bead unless I get the distorted bead. This procedure has been repeated several times to ensure the productivity of the result.

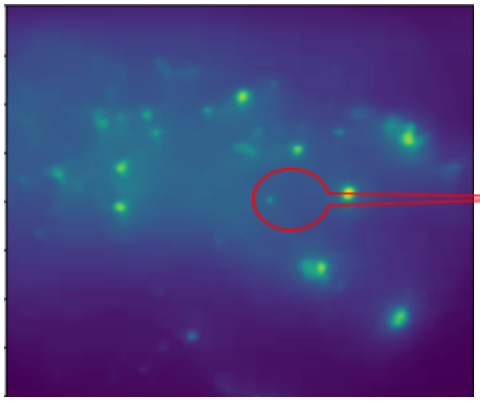


Fig: 7.2.2 (a) WD - 4mm

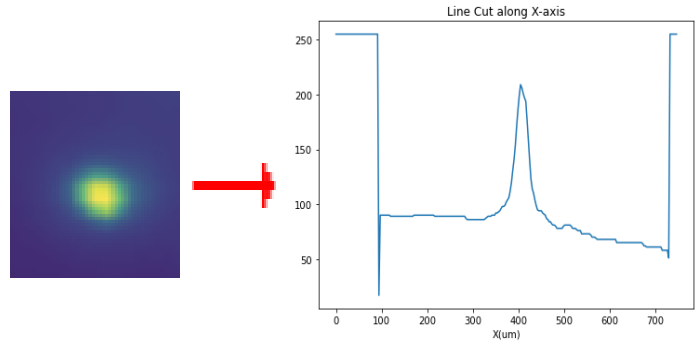


Fig: 7.2.2 (i)

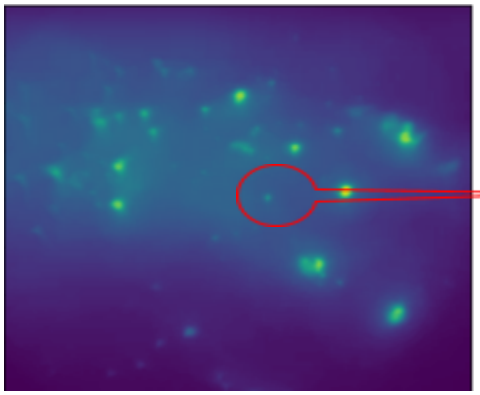


Fig: 7.2.2 (b) WD - 4.01mm

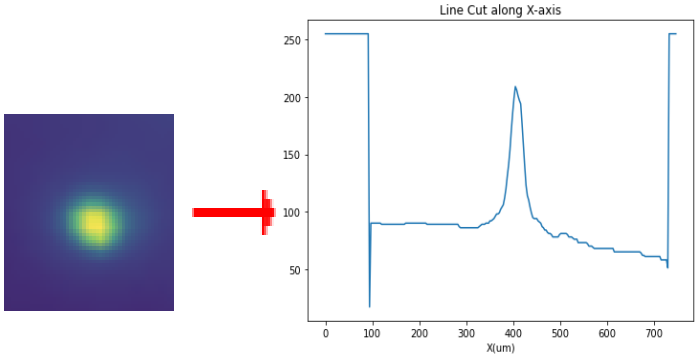


Fig: 7.2.2 (j)

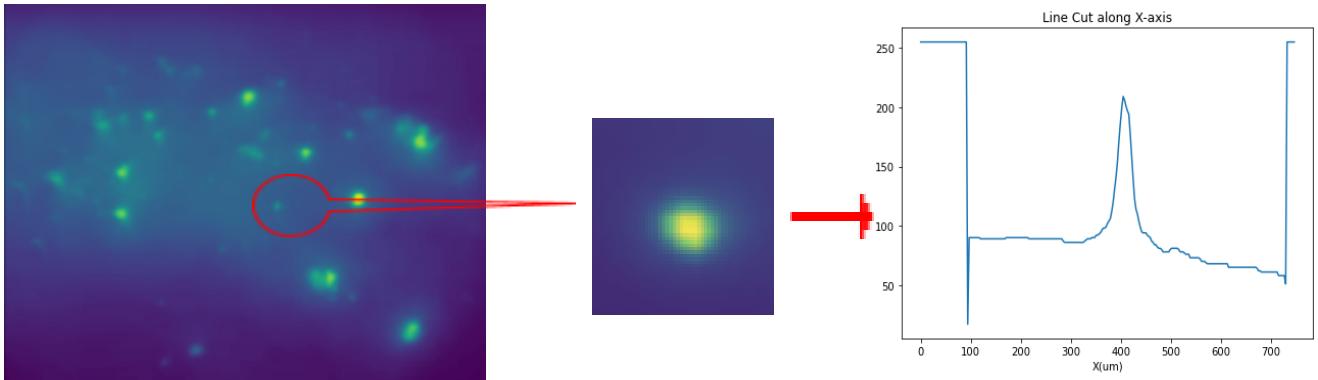


Fig: 7.2.2 (c) WD - 4.02mm

Fig: 7.2.2 (k)

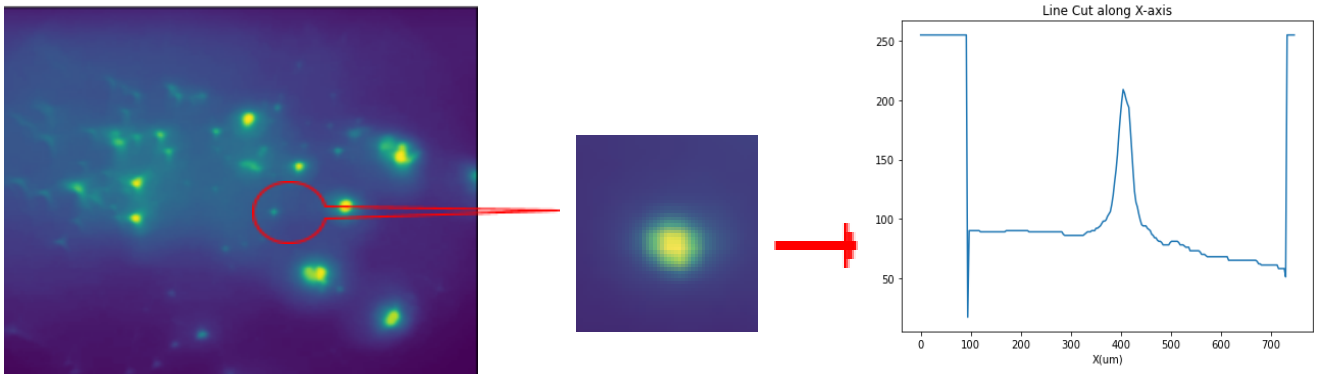


Fig: 7.2.2 (d) WD - 4.03mm

Fig: 7.2.2 (l)

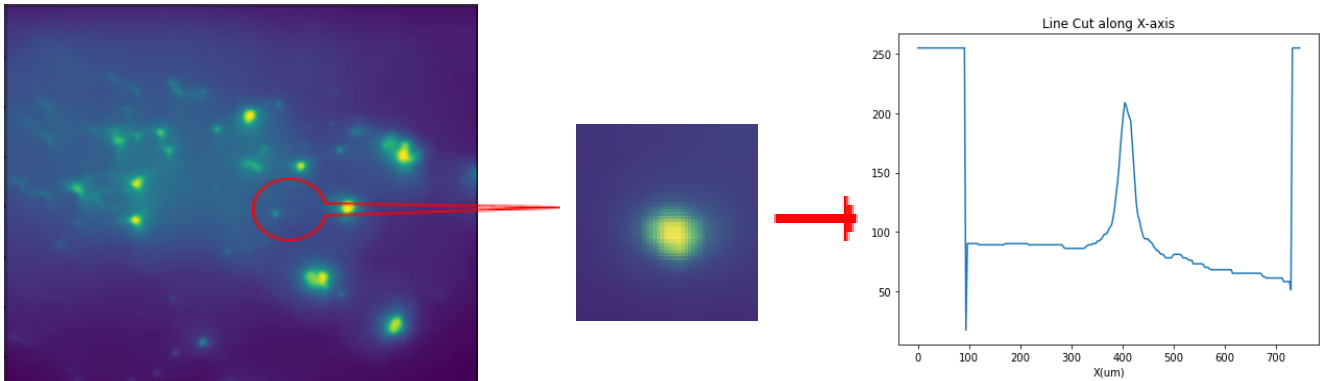


Fig: 7.2.2 (e) WD - 4.04mm

Fig: 7.2.2 (m)

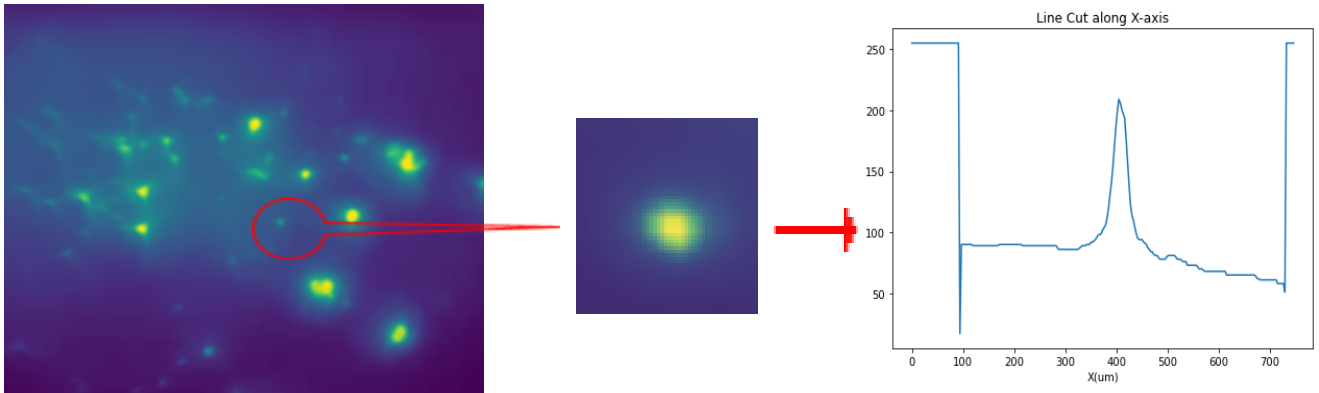


Fig: 7.2.2 (f) WD - 4.05mm

Fig: 7.2.2 (n)

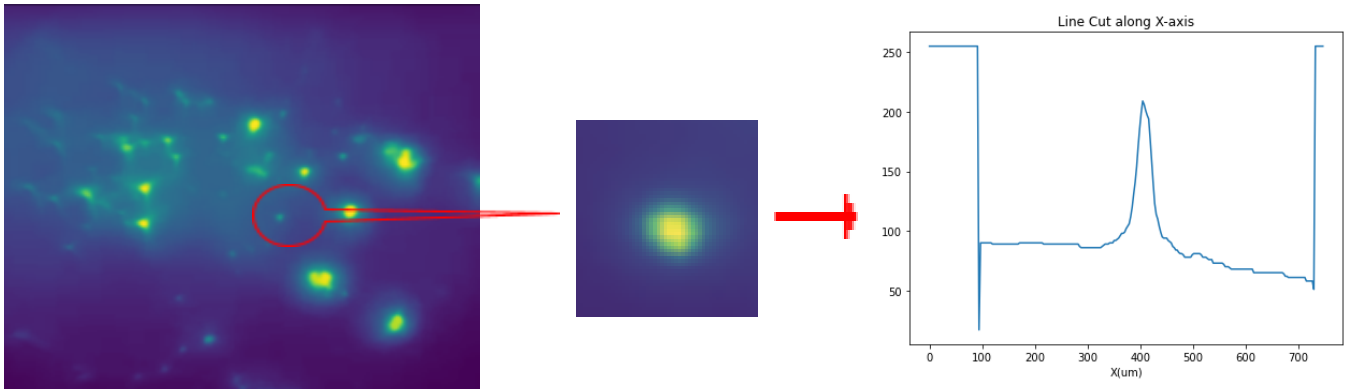


Fig: 7.2.2 (g) WD - 4.06mm

Fig: 7.2.2 (o)

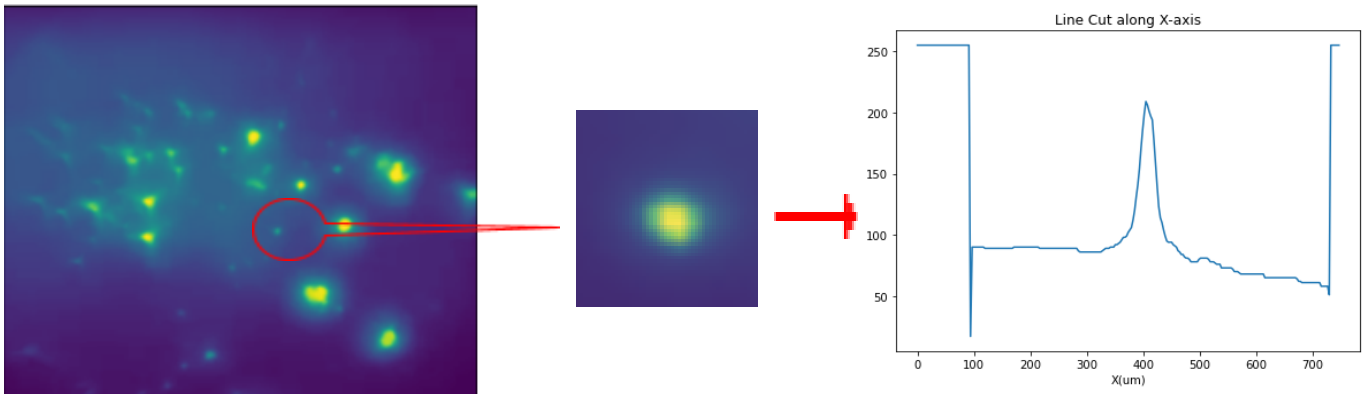


Fig: 7.2.2 (h) WD - 4.07mm

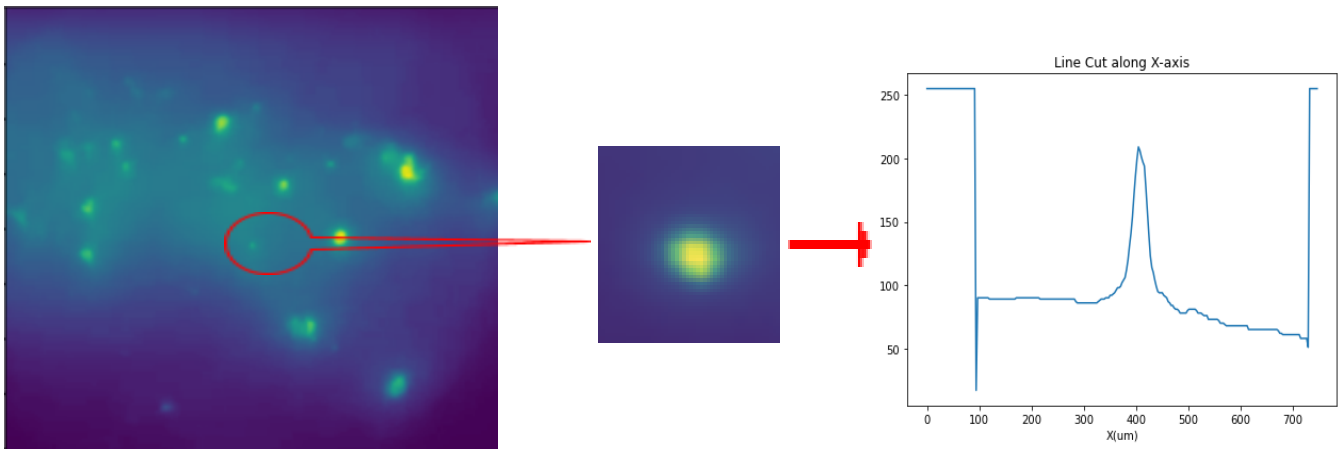
Fig: 7.2.2 (p)

Analysis: To characterize EDoF 4mm, I changed the working distance of the imaging plane (shown images in the Fig: 7.2.2 (a) to Fig: 7.2.2 (h)) in positive Y-direction with the interval of 10 um and captured images of the same bead. To analyze the single bead at different working

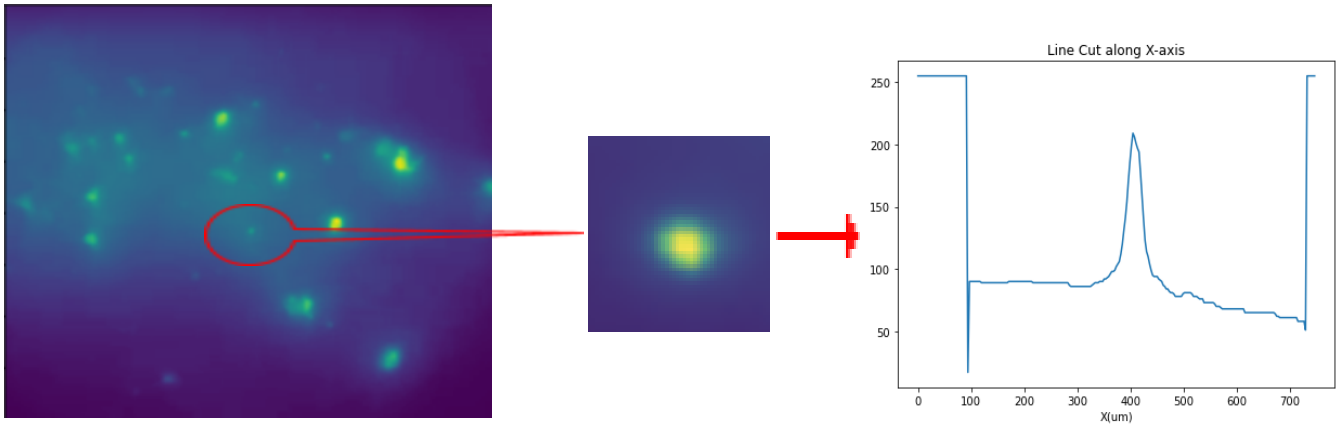
distances, I plotted **the bead in the array of the sensor size (750 um × 500 um & ~1um per pixel)**. Afterward line cut has been taken along the x-axis to optimize HWFM for all the beads.

Observation: HWFM plot remains same (shown Fig: 7.2.2 (i) to Fig: 7.2.2 (p)) while changing the working distance in positive Y-direction.
From the analysis and observation I can conclude that while changing the working distance, beads remain the focus for different working distances i.e, 4.0mm to 4.07mm. So, I can infer the bead is focussed for around ~ 70um in positive Y-direction.

Above procedure has been followed for negative Y-direction. I captured images with different working distances in the interval of 10um in negative Y-direction. Again, To show the potential of EDoF 4mm meta-optics I focused on the same bead starting from 4.0mm to 3.93mm. This procedure has been repeated several times to ensure the productivity of the result in negative Y-direction.

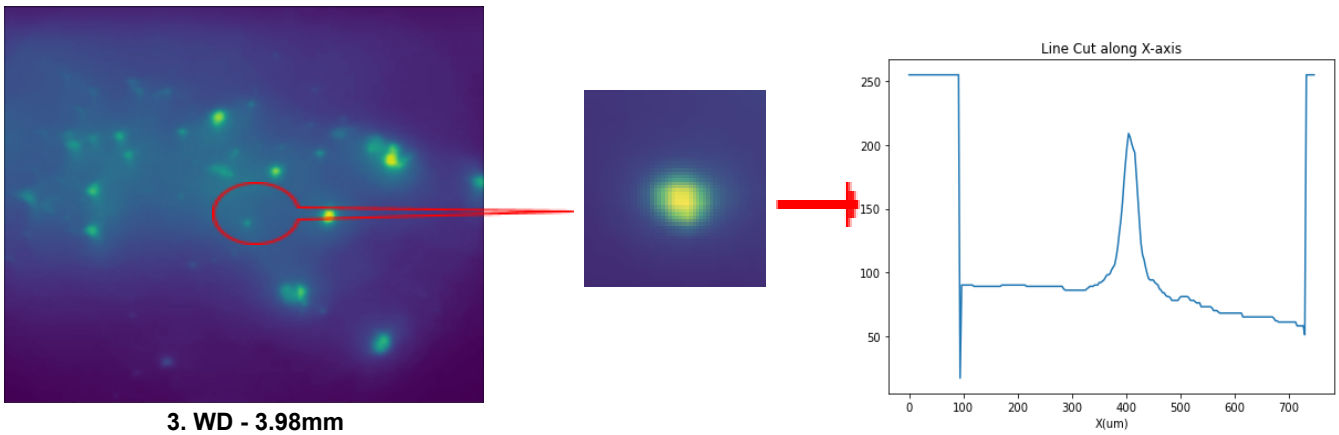


1. WD - 4.0mm

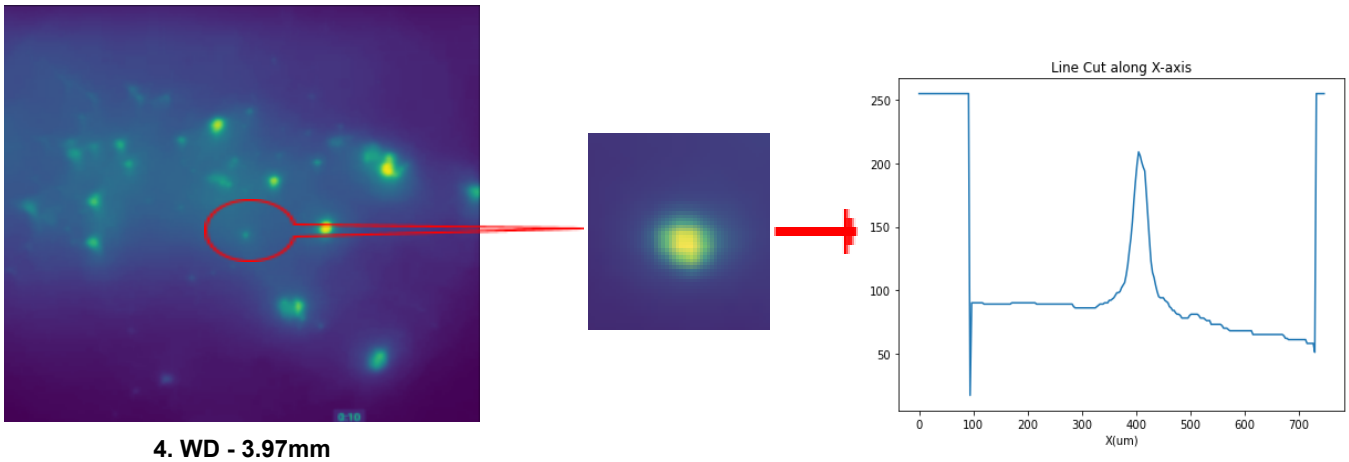


2. WD - 3.99mm

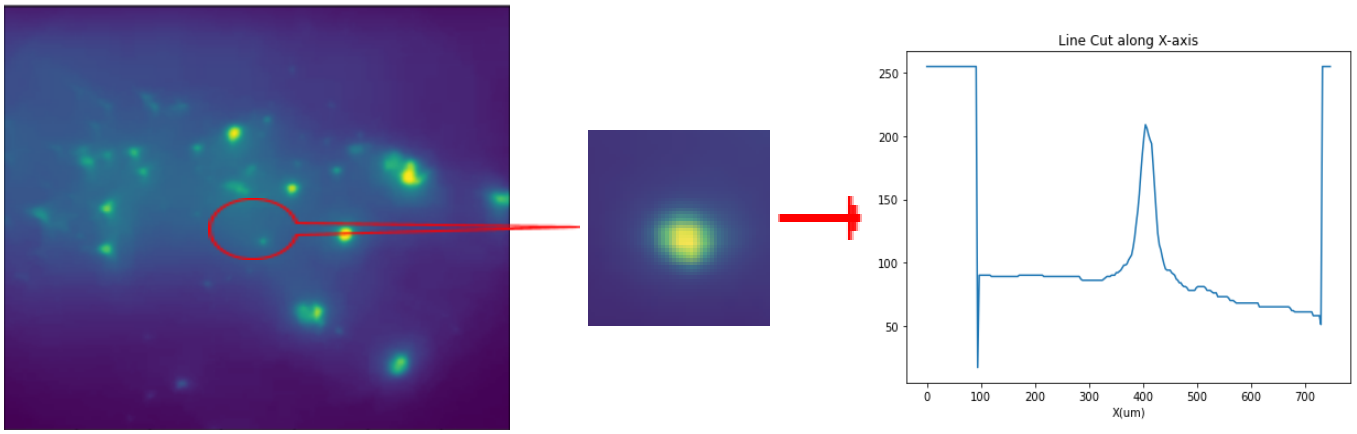
\



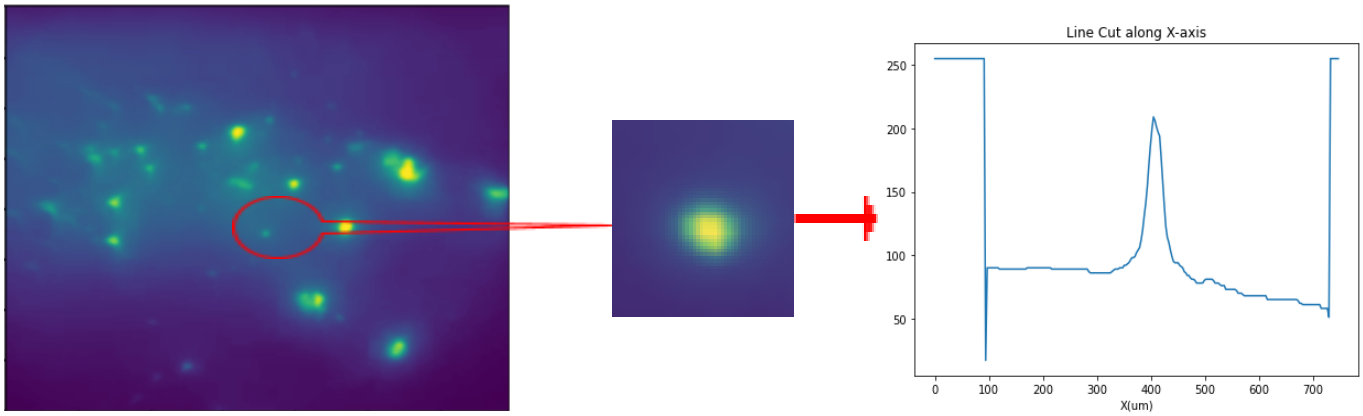
3. WD - 3.98mm



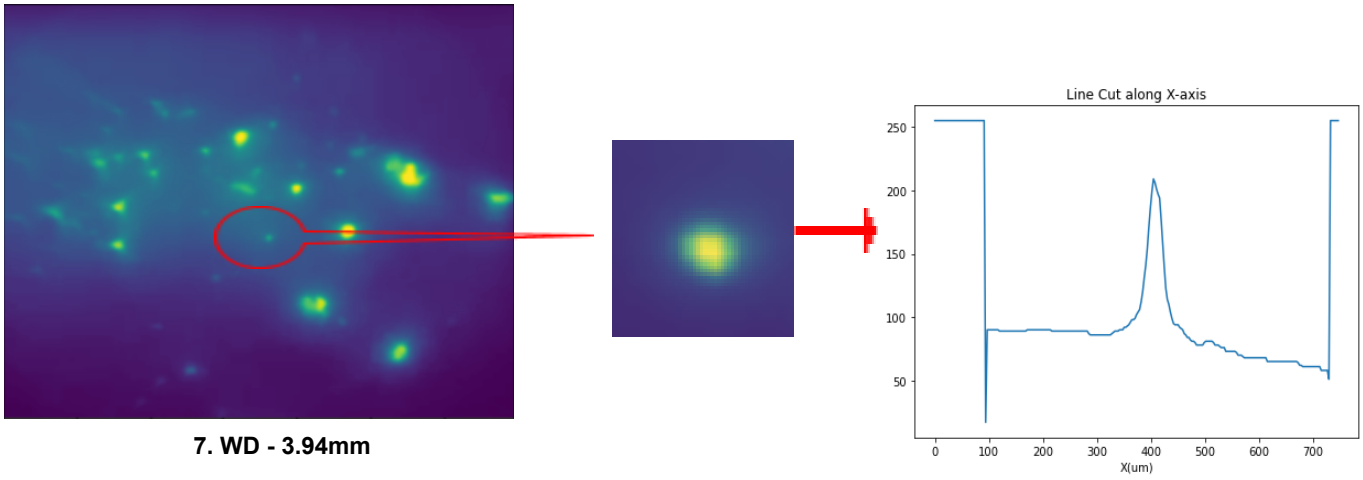
4. WD - 3.97mm



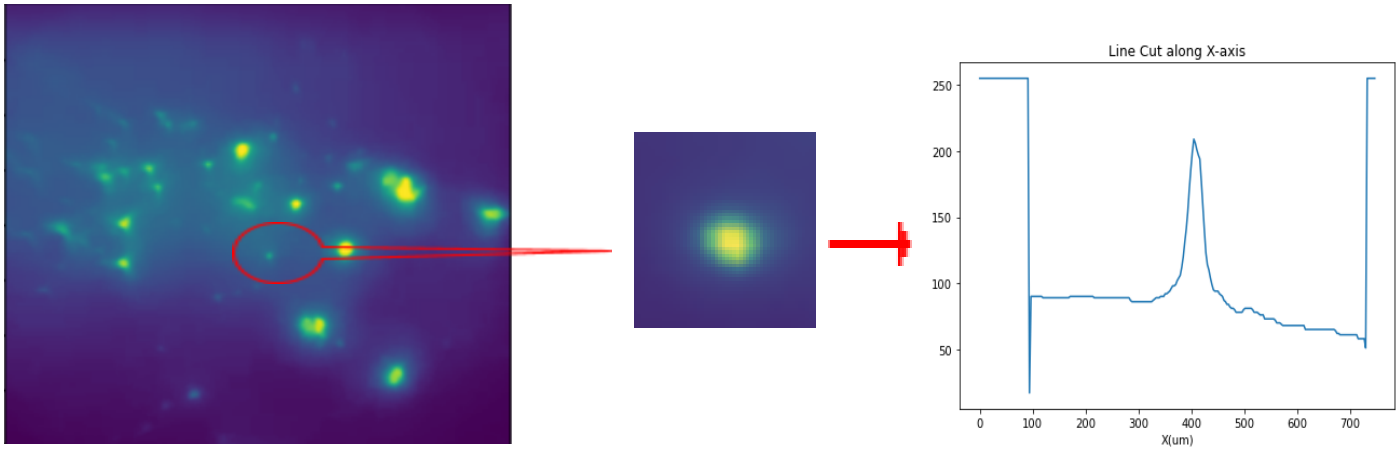
5. WD -3.96mm



6. WD - 3.95mm



7. WD - 3.94mm



8. WD - 3.93mm

Analysis: To characterize EDoF 4mm in negative Y-direction, I changed the working distance of the imaging plane (shown images in the images **1. WD - 4.0mm to 8. WD - 3.93mm**) in negative Y-direction with the interval of 10 um and captured images of the same bead. To analyze the single bead at different working distances, I plotted **the bead in the array of the sensor size (750 um × 500 um & ~1um per pixel)**. Afterward line cut has been taken along the x-axis to optimize HWFM for all the beads.

Observation: Again, HWFM plot remains same while changing the working distance in negative Y-direction.

From the analysis and observation I can conclude that while changing the working distance, beads remain the focus for different working distances i.e, 4.0mm to 3.93mm. So, I can infer the bead is focussed for around ~ 70um in negative Y-direction.

Final conclusion, EDoF 4mm can be used to focus ~ **140um** depth of field in a line, i.e, ~ 70 um in both positive and negative direction.

7.3 Preliminary Imaging Test with V3 MiniScope

Apart from characterizing Hyperboloid and EDoF meta-optics of different focal lengths, I also worked on the preliminary optical microscopy imaging test with V3 MiniScope to observe the pattern and to make sure of the analysis of fluorescence beads. To perform imaging with V3 MiniScope I used illumination techniques to generate contrast in the sample of tissue paper with

different thickness. Compared the results of GRIN lens and Meta-optics of fluorescence on tissue papers of different thickness.

To process the images I applied some of the microscopy imaging algorithms.

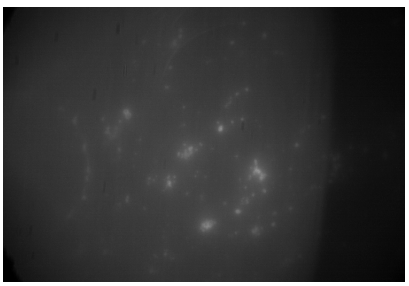


Fig:7.31 Original Image

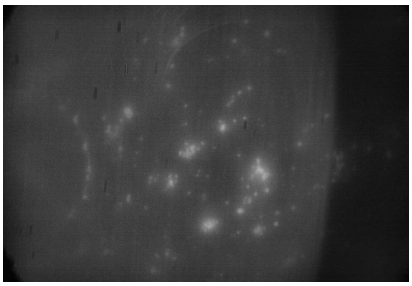


Fig:7.32 CLAHE Image

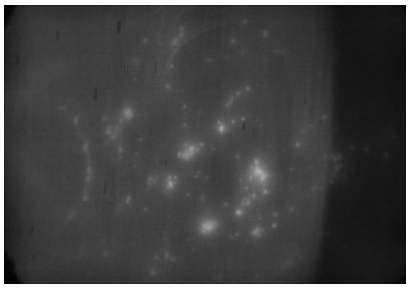


Fig:7.33 Denoised Image

Fig: 7.31 shows the raw data from 4mm of meta-optics directly with the image sensor of the V3 MiniScope.

Chapter8 : Conclusion and Outlook

8.1 Conclusion

The aim of this thesis was to expand the meta-optics research for biomedical imaging applications in the terms minimally non-invasive imaging. The research focuses on enhancing the functionality of metasurface for resolution and extended depth of focus.

The journey of this thesis has underscored the importance of metasurface for minimally non-invasive imaging devices. Mostaly, focussed to replace objective module with meta-lens inorder to reduce the size of the optics to achieve imaging functionality for medical devices.
In conclusion, metasurfaces hold significant potential for biomedical imaging applications. Metasurfaces are artificially engineered structures that can manipulate the properties of light at subwavelength scales. By tailoring the geometry, composition, and arrangement of subwavelength elements, metasurfaces can control the phase, amplitude, and polarization of incident light with high precision.
Secondly, metasurfaces can be designed to manipulate the properties of light in ways that enhance contrast and sensitivity in imaging. For example, they can selectively enhance the scattering or absorption of light by specific tissue components or biomarkers, enabling the detection of subtle variations in tissue composition or molecular expression. This capability holds great promise for early disease diagnosis, as well as for monitoring disease progression and treatment response.

Furthermore, metasurfaces can be integrated into compact and flexible imaging devices, enabling the development of portable and wearable imaging systems. This portability opens up new possibilities for point-of-care diagnostics, remote monitoring, and personalized medicine, where imaging can be performed outside of traditional clinical settings.

Despite these promising advantages, there are still challenges that need to be addressed in the field of metasurface-based biomedical imaging. One such challenge is the integration of metasurfaces into existing imaging modalities and equipment, as well as their compatibility with biological systems. Ensuring biocompatibility, stability, and long-term functionality of

metasurfaces in physiological environments is crucial for their successful translation into practical biomedical imaging tools.

In summary, metasurfaces offer unique opportunities for advancing biomedical imaging by providing enhanced resolution, contrast, and sensitivity, as well as enabling portable and wearable imaging devices. Continued research and development in this field have the potential to revolutionize diagnostics, treatment monitoring, and ultimately improve patient outcomes in the field of healthcare.

8.2 Outlook

The integration of metasurfaces with MiniScope technology holds great promise for the future of biomedical imaging. MiniScopes are compact, miniaturized imaging devices that can be used for various applications, including endoscopy, intraoperative imaging, and point-of-care diagnostics. By combining the unique capabilities of metasurfaces with the portability and versatility of MiniScopes, we can expect significant advancements in the field of biomedical imaging

One of the key advantages of using metasurfaces in conjunction with MiniScopes is the potential for subwavelength imaging. Metasurfaces can manipulate light at subwavelength scales, enabling the MiniScope to visualize smaller features and structures with higher resolution. This enhanced resolution can greatly improve the accuracy of diagnoses and aid in the identification of early-stage diseases. Additionally, the subwavelength imaging capability of metasurfaces can also enable the detection of molecular-level details and biomarkers, further expanding the diagnostic capabilities of MiniScopes.

Another important aspect is the potential for multifunctionality. Metasurfaces can be designed to possess multiple functionalities simultaneously, such as polarization control, spectral filtering, and wavefront shaping. Integrating these multifunctional metasurfaces with MiniScopes can provide versatile imaging capabilities in a single device. For example, metasurfaces can selectively enhance the contrast of specific tissue components or biomarkers, allowing for targeted imaging and improved visualization of relevant structures. This could be particularly

beneficial in cancer imaging, where the ability to detect specific biomarkers can aid in early detection and accurate staging of tumors.

Furthermore, the combination of metasurfaces and MiniScopes can enable real-time imaging and image-guided interventions. Metasurfaces can dynamically manipulate light properties, such as focusing and steering, in response to changing imaging conditions or tissue properties. This adaptability can be leveraged to improve imaging quality and enable real-time adjustments during procedures. For instance, metasurfaces can dynamically compensate for tissue motion or aberrations, ensuring high-quality imaging even in challenging imaging scenarios.

While there are still technical challenges to overcome, such as integrating metasurfaces into the compact and flexible form factors of MiniScopes, the future outlook for this combination is promising. Continued research and development efforts are likely to lead to the commercialization of metasurface-enabled MiniScopes, revolutionizing various fields of medicine, including minimally invasive surgery, diagnostics, and personalized healthcare.

In conclusion, the integration of metasurfaces with MiniScopes opens up new possibilities for high-resolution, multifunctional, and real-time biomedical imaging. This convergence of technologies has the potential to enhance diagnostics, guide interventions, and improve patient outcomes. As research progresses and technological advancements are made, we can expect metasurface-enabled MiniScopes to become indispensable tools in the field of biomedical imaging.

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Source Codes

1. **Source code for bead analysis by taking line code along X-axis. This python script defines the exact Array size of the Image Sensor in X-direction.**

```
@author: khushboo
"""
import cv2
import numpy as np
import matplotlib.pyplot as plt

def line_cut(image, axis, position):

# Define the image matrix size in micrometers

    """
    Perform a line cut on an image matrix.

    Args:
        image (ndarray): Input image matrix.
        axis (str): Axis along which to perform the line cut ('x' or 'y').
        position (int): Position of the line cut along the specified axis.

    Returns:
        ndarray: The line cut data.
    """
    if axis == 'x':
        line = image[position, :]
    elif axis == 'y':
        line = image[:, position]
    else:
        raise ValueError("Axis must be 'x' or 'y'.")

    return line

# Generate some example image data
image=
cv2.imread(r"C:\Users\khush\OneDrive\Desktop\Analyzed_Miniscope_Data\Diffractive\1.png",
0)
```

```
# Perform a line cut along the x-axis at position 50
x_line_cut = line_cut(image, 'x', 118)
print(x_line_cut)
print(len(x_line_cut))
plt_x=np.arange(0,750.0,750/len(x_line_cut))
# Perform a line cut along the y-axis at position 30
#y_line_cut = line_cut(image, 'y', 143)
print(plt_x)
# Plot the line cuts
plt.figure(figsize=(12, 5))
plt.subplot(1, 2, 1)
plt.plot(plt_x,x_line_cut)
plt.title('Line Cut along X-axis')
plt.xlabel('X(um)')
#plt.ylabel('Intensity')

#plt.subplot(1, 2, 2)
#plt.plot(y_line_cut)
#plt.title('Line Cut along Y-axis')
#plt.xlabel('Pixel')
#plt.ylabel('Intensity')

plt.tight_layout()
plt.show()
```

2. **Source code to check the magnification of the image matrix based on the sensor size. This python script defines the exact Array size of the Image Sensor in X & Y direction.**

```
@author: khushboo
"""
import cv2
import numpy as np
import matplotlib.pyplot as plt

# Define the image matrix size in micrometers
image_width_um = 750
image_height_um = 500
```

```
# Convert the image size from micrometers to pixels (assuming a pixel size of 1 µm)
image_width_px = int(image_width_um)
image_height_px = int(image_height_um)

# Generate a random image matrix
image_matrix=
cv2.imread(r"C:\Users\khush\OneDrive\Desktop\Analyzed_Miniscope_Data\EDoF\4mm.png",
0)

# Create a figure and plot the image matrix
fig, ax = plt.subplots()
ax.imshow(image_matrix, cmap='gray')

# Set the x and y axis labels as micrometers
ax.set_xlabel('Width (µm)')
ax.set_ylabel('Height (µm)')

# Set the x and y tick labels to match the image size
x_ticks = np.linspace(0, image_width_um, num=5)
y_ticks = np.linspace(0, image_height_um, num=5)
ax.set_xticks(np.linspace(0, image_width_px, num=5))
ax.set_yticks(np.linspace(0, image_height_px, num=5))
ax.set_xticklabels(['{:.0f}'.format(tick) for tick in x_ticks])
ax.set_yticklabels(['{:.0f}'.format(tick) for tick in y_ticks])

# Show the plot
plt.show()

##################################################################

@author: khushboo
"""

import cv2
from skimage import io
from matplotlib import pyplot as plt

img = cv2.imread(r"Image_Path", 1)
```

```
lab_img = cv2.cvtColor(img, cv2.COLOR_BGR2LAB)

l, a, b = cv2.split(lab_img)

#plt.hist(l.flat, bins=100, range=(0,255))
#plt.hist(a.flat, bins=100, range=(0,255))
#plt.hist(b.flat, bins=100, range=(0,255))

equ = cv2.equalizeHist(l)

#plt.hist(equ.flat, bins=100, range=(0,255))

updated_lab_img1 = cv2.merge((equ,a,b))

hist_eq_img = cv2.cvtColor(updated_lab_img1, cv2.COLOR_LAB2BGR)

clahe = cv2.createCLAHE(clipLimit=40.0, tileGridSize=(8,8))
clahe_img = clahe.apply(l)
#plt.hist(clahe_img.flat, bins=100, range=(0,255))

updated_lab_img2 = cv2.merge((clahe_img,a,b))

CLAHE_img = cv2.cvtColor(updated_lab_img2, cv2.COLOR_LAB2BGR)
plt.hist(CLAHE_img.flat, bins=100, range=(0,255))

cv2.imshow("Original image", img)
cv2.imshow("Equalized image", hist_eq_img)
cv2.imshow('CLAHE Image', CLAHE_img)
cv2.imwrite("output_path",  hist_eq_img)
cv2.imwrite("output_path", CLAHE_img)
cv2.waitKey(0)
cv2.destroyAllWindows()

######################
import cv2

img = cv2.imread(r"Image_Path", 1)
gaussian_using_cv2=cv2.GaussianBlur(img, (5, 5), 0, borderType=cv2.BORDER_CONSTANT)

cv2.imshow("Original",img)
```

```
cv2.imshow("Using cv2 gaussian", gaussian_using_cv2)


cv2.imwrite(r"C:\Camera_Wafer\Using cv2 gaussian.jpg", gaussian_using_cv2)
cv2.waitKey(0)
cv2.destroyAllWindows()

############################################################
import cv2
import numpy as np

img = cv2.imread(r"Image_Path", 0)

# Canny
canny_edge = cv2.Canny(img, 50, 80)

# Autocanny
sigma = 0.8
median = np.median(img)

# apply automatic Canny edge detection using the computed median
lower = int(max(0, (1.0 - sigma)*median))
# Lower threshold is sigma % lower than median
# If the value is below 0 then take 0 as the value

upper = int(min(255, (1 + sigma)*median))
# Upper threshold is sigma % higher than median
# If the value is larger than 255 then take 255 as the value

auto_canny = cv2.Canny(img, lower, upper)

cv2.imshow("Original", img)
cv2.imshow("Canny", canny_edge)
cv2.imshow("Auto Canny", auto_canny)

cv2.imwrite(r"Final_Result_Folder", auto_canny)

cv2.waitKey(0)
cv2.destroyAllWindows()
```