Lensless Digital Holographic Microscopy

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Abstract -- Lensless Digital Holographic Microscopy (LDHM) is a novel, cost-effective, and portable solution for high-resolution imaging of microscopic objects. LDHM has emerged as a promising imaging technique for several biomedical applications due to its compact design, cost-effectiveness, and the ability to reconstruct both amplitude and phase information, which can then This innovative technique utilizes in-line holography, pixel super-resolution, backpropagation, Fourier and inverse Fourier transformations, phase retrieval, and twin image artifact removal to deliver high-quality images. The design incorporates a CMOS sensor, multiple light sources, and other software algorithms to reconstruct an image from its holograms and then enhance image resolution. We will present our study of a modular LDHM system utilizing an LED as the light source, a pinhole, and a CMOS sensor through a Raspberry Pi. The observed images will then use multi-frame pixel super-resolution to increase resolution. This project has the potential to increase interest and access to the field of microscopy and provide new opportunities for research in areas such as oil monitoring in ocean water, air health, and plant health.

I. INTRODUCTION

Digital holography is highly favored currently because of its non-contact nature, high resolution, high throughput, and ability to provide three-dimensional information. Current systems tend to be expensive, complex, and not portable. We present our own Modular Lensless Digital Holographic Microscope - simple, cost-effective, portable, and most importantly, relatively cheap. The principle of LDHM uses the interference of light waves originating from a mostly coherent light source and the object being imaged, forming a holograph that can be recorded on our CMOS sensor. Through digital back-propagation and reconstruction algorithms, both the amplitude and phase information of the object can be retrieved, allowing us to reconstruct 3D models of the subject. Moreover, LDHM can be further enhanced using pixel super-resolution techniques, allowing for a resolution beyond the limitations of the sensor's pixel size and pitch. Our system was focused on a 450 nm super bright LED as the light source and a pinhole to increase coherence and allow fresnel diffraction to occur, allowing our sensor to record the interference pattern. We calculate pinhole diameter, source-to-pinhole distance, and source-to-sensor distance.

II. SYSTEM COMPONENTS

The system is best presented in terms of system components; that is, the individual physical modules—whether purchased, designed, or printed—that are interfaced to create the final product. This section provides a semi-technical introduction to each of these components.

A. Microcontroller

This component really is a core piece of the Lensless Digital Holographic Microscope, it should be a more cost-effective and efficient piece over a standard computer. The tasks that it needs to accomplish on a basic level are as follows: store and send the images from the censor to another computer, and controller for the LED array. However, we would like to task the microcontroller with more intensive responsibilities so that the Lensless Digital Holographic Microscope is much more self-sufficient. The heart of the project is a Raspberry Pi 2B microcontroller. Raspberry PI 2B is chosen due to its qualities as an onboard computer, while we don't need to have the processing power of a modern PC for this project, being able to take a picture only would be too much work for a simple microcontroller. Rarely the choice of getting a better part is financially the right choice but here we needed something akin to the raspberry pi, and the 2B model is very cost-effective when compared to other, slightly newer models of other onboard computers. This allowed us to put as much work into the raspberry pi as we could, it has the potential to run all the code it needs to be fully autonomous, while still being modular enough to easily get the bare minimum to be a standalone part. Additionally, a full PCB design was made using the MSP430F5519, however, this was just used to offload some basic work off of the raspberry pi as well as being able to accommodate the number of LEDs required for successful image reconstruction.

B. CMOS Sensor

The backbone of this project is a good CMOS sensor. It is paramount that it has the correct pixel size, as well as an appropriate pixel resolution, otherwise either the images wouldn't come through correctly, or we wouldn't have the room to appreciate them. Additionally, it is one thing to have those specifications, it is a whole other problem to see if they are implementable into the design of the current electrical system. It seems to me that the specifics on how these parts work electrically is a closely guarded company secret, as to even get access to the pin diagram for the majority of parts we would have to go through the process of signing an NDA with the respective company via the university. This would only provide us with the pin map with zero ideas if there are other electrical specifications or implementations in the encoding and transfer of images to the raspberry pi, that would go beyond the scope of this project. We are aware that for this project we should not have to reinvent the wheel, however, implementation of any random CMOS sensor without outside help would be taking a shot in the dark randomly until something works. Therefore we have chosen the AR0234 evaluation board from Arducam, this is a CMOS sensor that meets all the optical requirements, with its 3 x 3 µm pixel size, and electrical requirements by being compatible with the raspberry pi 2B. These two elements are essential to be used in this project, and they are extremely rare qualities, with the only comparable part that we found costing nearly triple the market price of the AR0234.

C. LEDs

We decided to go with LEDs for their low-cost, high output-price ratio, and high temporal coherence. Having monochromatic light is an absolute necessity for the system because this is what allows the Gabor hologram to be measured. The phase interference with the same frequency yields the information we seek. If there were other wavelengths tossed about in the mix we would have interference on multiple aspects of our project and why the LEDs are the right components for our project.

D. Battery Pack

Affordability and portability are one of the key things we looked to tackle with this project and having a battery pack became a crucial system component. Battery packs can be rechargeable and contain temperature sensors which help the batteries indicate when the end of the charging cycle is complete. A well-balanced pack lasts longer and delivers better performance. Battery packs are essential for this system as it contains many different electronic components that require power and it helps them with the necessary energy to do the product's features. As for the exact decision, it really is a simple choice, the raspberry pi needs 5V at 2A to run without error and can route power to other devices of equal, or lesser power requirements seamlessly, therefore any battery pack with those voltage requirements is a feasible option, from there it is only a choice of affordability, availability, and battery life. For our purposes, for all testing, demonstrations, and development, we are using an RP-PB19, and the A1271, interchangeably, which isn't a cost-effective choice but enables us to work on the electrical system comfortably, and for a very long time compared to similar products. We did test other options including the recommended Raspberry Pi dedicated power source, and found it effective but lacked luster. There is a good tech comparison table with other options, however in reality use what you have with the correct voltage requirements, and everything will work out. However, for the current moment, the RP-PB19 is more precisely what the design needs and gives a longer battery life, and while the A1271 works for this project this isn't what that part is meant for.

III. SYSTEM CONCEPT

To be understood as a complete system, a flowchart is helpful.

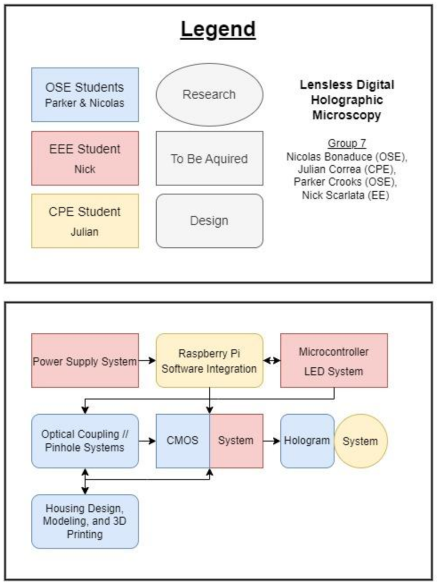


Figure 1: Block Diagram

A. Housing Unit (Chassis Design)

The Chassis was designed to be as light and portable as possible, while housing the necessary arrays for our optical design. A Prusa i3 printer along with PLA and PETG filament was used to complete our final prints.

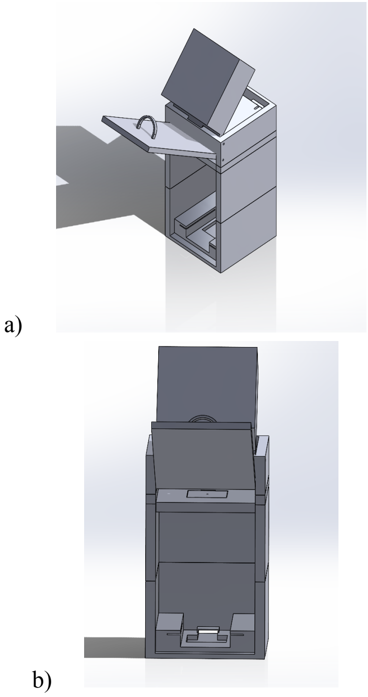


Figure 2: High-Powered LED - Pinhole Chassis Design ; a) Isometric View ; b) Front View

B. System Comparison with a Brightfield Microscope

Lensless Digital Holographic Microscope (LDHM) uses quantitative phase imaging for measuring cell properties, enabling applications in remote diagnostics and point-of-care devices. In contrast, Brightfield Microscopy (BM) struggles with low contrast, limited resolution, and difficulties imaging thick specimens.

Quantitative phase imaging (QPI) in LDHM measures the phase shift of light passing through a transparent or semi-transparent specimen. This phase shift relates to the specimen's thickness, refractive index, and opacity,

LDHM's limitations include lower spatial resolution and sensitivity to vibrations. BM is compatible with most staining stabilizing techniques and is compact and portable. Both techniques have possible future improvements, such as advanced illumination systems, improved sensor technologies, and integration with other imaging modalities.

The coherence length, an important parameter for LDHM, determines the maximum axial distance over which interference effects can be observed. In BM, the resolution is determined by the objective lens's numerical aperture (NA) and the illumination wavelength. In LDHM, the resolution depends on the sensor pixel size, the wavelength of the illumination, and the distance between the sample and the sensor. Throughput and field of view (FOV) in both techniques are influenced by factors such as imaging speed, sample handling system, and data processing capabilities. Upgrades are available for both techniques depending on the application's specific requirements.

Combining LDHM with other imaging modalities, such as fluorescence or Raman microscopy, could yield even more information

IV. OPTICAL COMPONENTS DETAIL

A. LED and Fiber Coupling Design

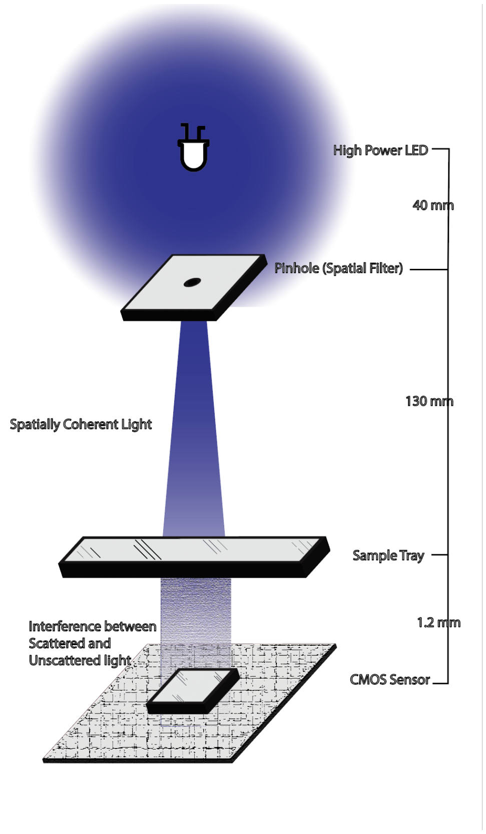


Figure 3: The basis for our microscope. A partially coherent light source ("Super Bright" LED) passes through a pinhole to increase spatial coherence. The distance from the pinhole to the sensor is just enough to completely illuminate the sensor and nothing more. The light that scatters off the subject interferes with the unscattered light and that interference pattern is recorded digitally through the CMOS sensor.

V. ELECTRONIC DETAIL

Due to the modular nature of this project, the electrical design is self-contained, for this project, this group only had one electrical engineer and given the goal of providing the optical system with a consistent way to operate an LED array at its' maximum brightness and give our computer engineer a way to perform image capturing and analysis, in a self-contained system. To accomplish these goals, we decided to keep the design simple where we can and increase the complexity when the need arises. This methodology would go on to make a system of strong independent parts that mostly came out of the box working as intended. This enabled a straightforward power system since we had planned for the rest of the parts to just have a USB connection, or a similar option, which made the entire system able to be powered by a rechargeable battery with the specifications of the most demanding part, and the remainder would be safe to plug into.

The image processing portion of this project necessitated the use of an onboard computer, due to the project having a lower standard to be considered a success. An onboard computer with less processing power than the current best computer on the market is acceptable since we are aiming to be optional, not necessarily optimal. Therefore, we went with an old Raspberry PI 2B to lower the price by a considerable margin, while still being accessible, and having a large amount of USB ports to make development and implementation a non-issue. The Raspberry PI 2B features 4 USB ports allowing it to have the CMOS sensor, microcontroller, WIFI adaptor, and Bluetooth adaptor, all with a USB connection and having a lower requirement for voltage, and current allowing us to just find a power source that is compatible with the Raspberry PI 2B. After a bit of testing of rechargeable batteries, we can now say for certain that using the Raspberry PI's companies power banks is just overspending for a brand, any 5V 2A power source works just fine, with either a pin-to-pin connection or a micro-USB port works just as well.

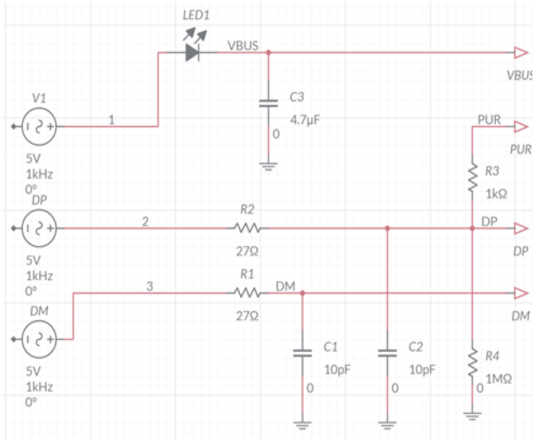


Figure 4: Multisim live recreation of micro-USB to microcontroller setup

The figure above shows the implementation of a micro-USB port for the chosen microcontroller, in this case, a PCB containing the MSP430F5519IPN. The figure features about a third of the complexity that is on the PCB that we have created for this project, and this feature is like the model shown in the separate datasheet for a group of Texas instrument microcontrollers namely the MSP430F55XX series (Texas Instruments). There were many problems with the PCB for this project, firstly the PCB was initially built for the MSP430FR6989, however when the PCB came in the microcontroller was not on the board along with a few other parts, making it basically useless since the microcontroller was out of stock for the perceivable future, this was a massive delay however it did encourage us to look into other similar microcontrollers. This led us to be even more confident in this current design using the MSP430F5519 which has more content that directly references a micro-USB port, which was a relief since otherwise we would be taking a shot in the dark again. Unfortunately, this new PCB came with a hefty shipping delay as well as multiple manufacturing errors, basically, the new PCB is salvageable we have all the parts and just need to solder them onto the board myself, however, because of the delays to see if the current PCB works we would forgo the ability to reiterate in time for the deadline.

However, we are confident that if there is an error in the PCB it is because of either an error from the manufacturer, or there was an additional complexity to the microcontroller that wasn't explicitly mentioned in the datasheet that we have read cover to cover multiple times. Additionally, as a last resort, we can still use the MSP430FR6989 development board which can accomplish the same tasks as the PCB that we designed, with certainty, and only costs about seven dollars more, when comparing the price of parts and labor to the wholesale of the development board, not counting shipping of course because that has been a whole separate different mess. In conclusion, the electrical situation of this project is entirely stable and has allowed the rest of the team to continue their work unimpeded, except for a small issue with the CMOS sensor that was resolved within our means, without having to change parts. Therefore we are confident in the electrical system of the project, which allows us to focus our efforts elsewhere.

VI. PHOTONIC METHODS

Before being able to understand how the software works we have to understand the different photonic methods and how they are implemented into the software.

A. Digital in-line holography

This is a technique used to record holograms of microscopic objects using a digital camera. This involves shining a laser beam through the object and capturing the interference pattern created by the scattered light with a camera. This interference pattern can be used to reconstruct a 3D image of the object. Digital In-line holographic microscopy is very different from other microscopy methods because, with the digital inline method, there is no recording of the projected image of the object. Instead, the light wavefront information originating from the object is digitally recorded as a hologram, from which a computer calculates the object image by using a numerical reconstruction algorithm. This calculation is done through programs such as MATLAB or already-created products. The image-forming lens in traditional microscopy is thus replaced by a computer algorithm.

B. Pixel super-resolution

Pixel super-resolution is a technique used to increase the resolution of digital images by using algorithms to predict and fill in missing pixel information. The easiest method of pixel super-resolution is a shift and add pixel super-resolution technique where multiple images taken from slightly different locations are layered over one another with the subject lined up perfectly. This allows the image processing software to see the distinct edges and detail that each individual image provides. This is also known as a sub-pixel resolution because some of the detail that spreads over multiple pixels can be brought back out and seen on the final image. Current leading-edge technology uses artificial intelligence and deep learning algorithms to deduce what the image should look like and introduce detail that would not have been there before. This is possible because any blur that occurs on the image would look similar to a human eye but in reality, the blurs are unique and contain information in them themselves. The implementation of Pixel Super-Resolution allows for sharper and more detailed images to be produced when an image has its resolution artificially increased using computer programs.

C. Backpropagation

Backpropagation is a technique used in artificial neural networks to adjust the weights of the connections between neurons during training. This involves propagating the error back through the network and adjusting the weights to minimize the error between the predicted output and the actual output. Backpropagation sequentially calculates and stores the gradients of intermediate variables and parameters within the neural network in reversed order. In our case dealing with fibers and optical components, digital backpropagation is an important method since it is a nonlinearity compensation method. We are able to use this technique in order to compensate for all fiber impairments in the optical transmission systems we may have.

D. Fourier & Inverse Fourier transformations

Fourier and Inverse Fourier transformations are mathematical operations used to analyze and manipulate signals, such as digital images. Fourier transformations break down a signal into its component frequencies, while inverse Fourier transformations reconstruct the signal from its frequency components. This method is very important for our software's ability to reconstruct holograms because the digitally recorded hologram we capture by the CMOS sensor is the interference pattern between the object wave and a reference wave. Meaning that the interference pattern is recorded in the detector plane, and in order to retrieve information about the object, it must be transformed into the object plane this process can only be done with the use of Fourier and Inverse Fourier Transformations and this is shown within the software with the Angular Spectrum Method.

E. Phase Retrieval

When we capture an image the information we would like to record is contained within the phase pattern and interference projected on the sensor. But all that is recorded is the intensity at a certain pixel. So we are left with an intensity pattern and no phase pattern. The Phase retrieval technique aims to recover the lost phase information from the measured intensity by making certain assumptions about the object or the measurement system. This is why we will be using multiple light point sources each fired off sequentially. This will allow us to trace back the phase information using only intensity patterns measured at different locations. The goal of phase retrieval is to obtain a reconstructed image, which in our case will be a reconstructed hologram, that contains both the amplitude and phase information of the object, enabling a more accurate and complete representation of the object being imaged.

F. Twin image artifact

When we do reconstruction we have to deal with twin image artifacts. Twin image artifact removal is a technique used to remove artifacts that are produced during holographic imaging. These artifacts are caused by the reconstruction of two images, a real image, and a twin image, from the same hologram. Twin image artifact removal involves using computational algorithms to separate the real image from the twin image, allowing for a clearer and more accurate image to be produced. This allows for the image to then be a higher quality one through the use of pixel super-resolution.

VII. SOFTWARE DETAIL

Before the software portion of the system can be explained in detail, a full understanding of the preferential nature of this project must be understood. The success of this project is dependent on the ability to use a CMOS sensor for all of the image capturing for the images used throughout the rest of the project. To capture images with a CMOS sensor that is integrated with a microcontroller, in this case, it is a Raspberry Pi, needs to be able to store the captured images later on for data transferring into the programs for Hologram Reconstruction and Multi-Frame Pixel Super Resolution.

A. Image Capturing

The code that controls the imaging software is a scripting language that uses installed packages and applications from Arducam to allow for the images to be captured from the CMOS sensor and to be stored in the Raspberry Pi for later data transfer. For the image-capturing process, we implement scripts in the command line to have the CMOS sensor active for a duration of time and capture images at a given time interval. For example, to command line code will look like this:

```
libcamera-still -t 30000 --timelapse 2000 -o image%04d.jpg
```

This allows for the CMOS sensor to be on for a duration of time that we specify so that all the LEDs that are run are turned on in a sequential sequence and it allows for the image capturing to be timed with those LEDs being on so that we can capture the images. After we capture all the images we store them in folders and transfer them over to a flash drive in order for those images to later send to the MATLAB and Python programs for Hologram Reconstruction and Multi-Frame Pixel Super-Resolution of the captured holograms.

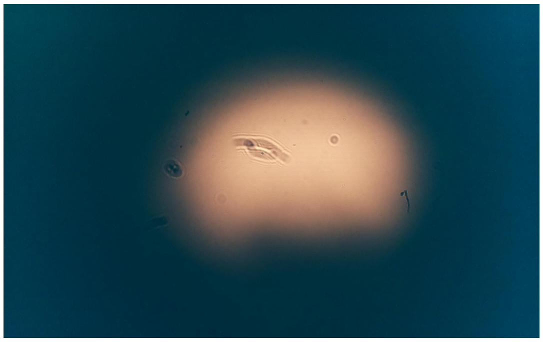


Figure 5: Captured hologram by the CMOS sensor and the input into the first Matlab code

B. Hologram Reconstruction

For the hologram reconstruction we took the route of using and creating a Matlab code that will handle the majority of the workload for the computational needs of the different photonic methods. The Matlab code is composed of two parts: the reconstruction and simulation of the hologram and the iterative twin image artifact removal for the final hologram. For both of the Matlab codes, you must hard code in the set parameters for the algorithms to work such as the wavelength of the light used to acquire the hologram, the number of pixels, the number of iterations we want the twin image code to undergo, the size of the object area and the object-to-detector distance. allows the user to select the file they want to put into the code. For the first program, the first thing the user must select is the captured holograms wherever the user saved the images captured to load into the code. The code then prompts the user to name the results of the first program so that the user will know how it is saved within the program's folder. With all the parameters already set in the code will run and output a monochromatic image of the original hologram padded with zeros and then a digitally simulated hologram while also saving the hologram needed for the twin image artifact removal code. The code is able to reconstruct the hologram by using the Angular Spectrum Method of propagation for the hologram that is input into the program. The output image and the hologram that is then used for the twin image artifact removal are created by taking the 2D-Fourier Transform of the pressure field of the hologram. What this allows for is the decomposition of the field into a 2D "angular spectrum" of component plane waves each traveling in a unique direction. Then by being able to multiply each point by the 2D-Fourier Transform we are able to account for the phase change that each plane wave will undergo on its journey to the prediction plane of the reconstructed hologram. Taking the 2D-Inverse Fourier Transform we are able to get the resulting data for the twin image artifact removal step and we repeat this same process for the given amount of iterations for the second Matlab Program. This allows for the results of the Angular Spectrum Method and propagation of the holograms to be seen below.

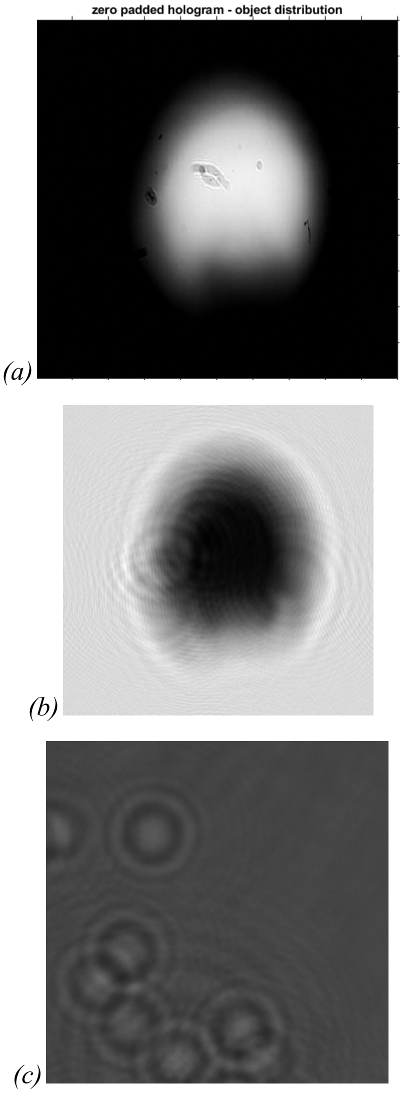
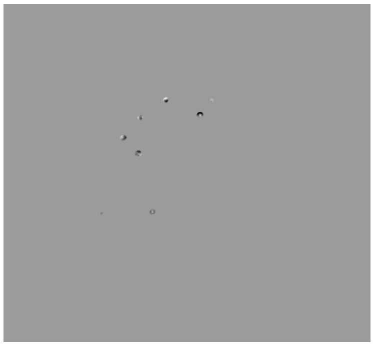
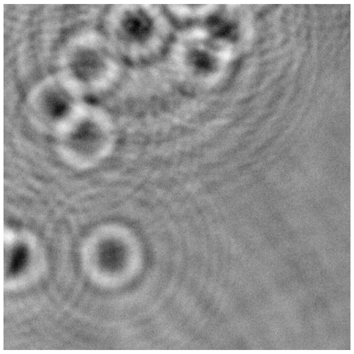


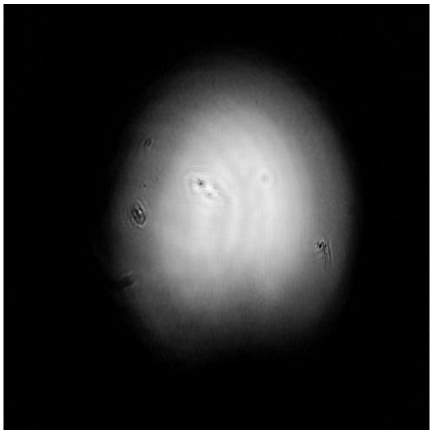
Figure 6: The results of the first Matlab code (a) being the hologram padded with zeros and (b) the simulated hologram; (d and e) displaying the reconstructed amplitude and phase showing depth for c; (f) displaying the residues of the infractions

e)

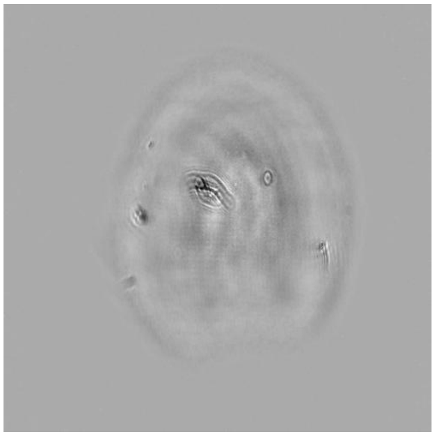



f)


So following the run and saving the output of the first program we then turn to run the iterative twin image artifact removal program. The idea behind this program is to take the hologram that was reconstructed with the parameters that are already hard coded in and iteratively run the twin image artifact removal algorithm in order to present the highest quality reconstructed image. Twin image removal allows for the accuracy and quality of the reconstructed holographic image to be significantly improved which leads to more reliable and precise data output. So we set aside the number of iterations we want the twin image to run for a while, selecting the file created by the first code and running the programming following the same steps of how the program will save the output. So after the code runs for the user-defined amount of iterations it now outputs the reconstructed hologram with its reconstructed amplitude and phase while showing the original amplitude before the code runs. This process is repeated for the number of holograms captured and the reconstructed images are put through the Multi-Frame Pixel Super Resolution program to generate a higher-resolution final image.



5(a)



5(b)

Figure 5: The results of the second Matlab code (a) reconstructed amplitude and (b) the reconstructed phase

C. Multi-Frame Pixel Super-Resolution

For the Multi-Frame Pixel Super-Resolution we took the route of using and creating a python code that will handle the majority of the workload for the two image stacking algorithms found that will allow for the low-resolution images to be put into a folder and then read by the python program to output the high-resolution image. The process of the Python code is to align and average multiple frames to reduce noise and increase the signal-to-noise ratio, which leads to a higher-quality image. By using Python import libraries of NumPy and OpenCV for loading in the images and using OpenCV's imread function to read all the files that are in the folder corresponding to the low-quality images that are to be combined together. Below is a test run of the image files inputted into the Python folder.

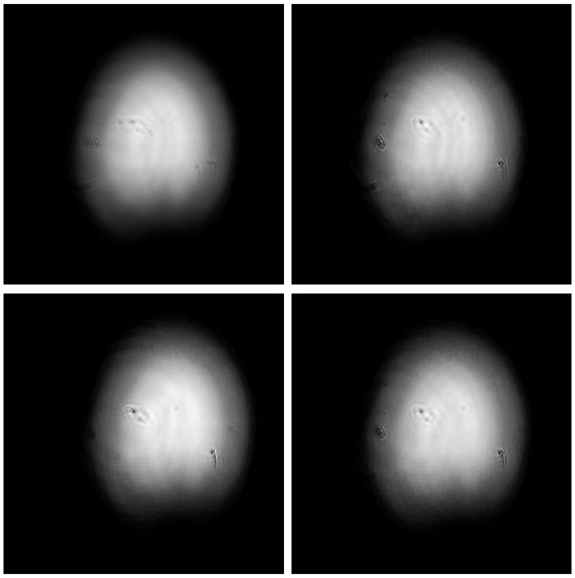


Figure 7: The inputted image files of the reconstructed holograms that are placed in the images folder for Multi-Frame Pixel Super-Resolution

We then created a function that will take the list of low-resolution images and resize them to upscale them by a special factor using the OpenCV library. After that is done we use another function called stack_images that takes the list of high-resolution images that were once low-quality images and stacks them on top of each other using NumPy's dstack function creating a single image with a higher resolution than the original images had. So the program takes a set of low-resolution images, upscales them, and then stacks them to create a single high-resolution image. Below you can see the images that were placed into the program's image folder and the outputted final high-quality image.

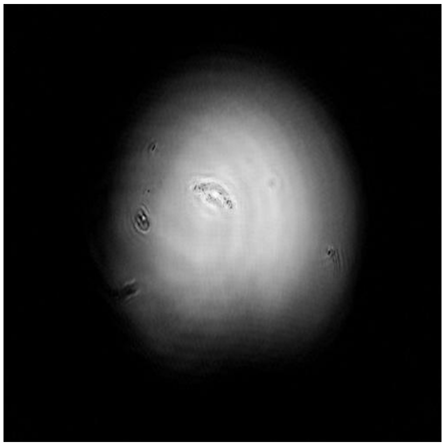


Figure 8: The final output high-resolution image file of the reconstructed holograms that are placed in the images folder for Multi-Frame Pixel Super-Resolution

VIII. CONCLUSION

By bringing together Electrical and Computer Engineering with Optics and Photonics the Lensless Digital Holographic Microscope is able to go from thought to reality. The Lensless Digital Holographic Microscope helps bring forth a challenge to the issues of current Digital Holographic Microscopes and makes a product that is simple, cost-effective, and portable that can be constructed at home or mass manufactured with smartphone compatibility (Bluetooth or direct lens imaging). Through deep research and extensive testing into the different fiber arrangements, and the different LEDs we use. With the spacing distances we settle on, the mat behind the holograms, pixel super-resolution, back-propagation, and all the computer software we will put into it, we have a perfect blend of our programs and our strengths. We will bring this technology to UCF and ensure we dive deeply into each concept and come out stronger and smarter than before.

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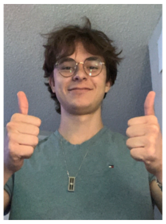
BIOGRAPHY/ THE ENGINEERS



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Nick Scarlata is a 24-year-old graduating Electrical Engineer student, he is going into the power industry, after completing the power track.

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