IMPLEMENTATION AND ANALYSIS OF FRESNEL BIPRISM-BASED DIGITAL HOLOGRAPHIC MICROSCOPES

Charity Hayes Rounds

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IMPLEMENTATION AND ANALYSIS OF FRESNEL BIPRISM-BASED DIGITAL HOLOGRAPHIC MICROSCOPES

by

Charity Hayes Rounds

A Thesis

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

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The University of Memphis

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Acknowledgements

This document is dedicated to my Lord and Savior. Thank you for allowing me to come this far in the program and life. I appreciate the green pastures and more importantly, the valley, where you have shaped and molded me into being more like you. Thank you for forgiving me of my sins and sending the Holy Spirit to help me throughout this journey called life. Thank you for sending me a patient husband to walk this collegiate path with and many more paths together. Thank you for motivating me through my beautiful nieces, keeping me grounded, constantly reminding me of the next generation that will come after me. Thank you for sending me a caring and supportive advisor, Dr. Ana Doblas, who has helped step by step, day by day, and I appreciate you allowing her to give me a chance where most would not have. Lord, I appreciate you allowing me to see this through. Amen.
Abstract

Digital holographic microscopy (DHM) provides Quantitative phase images (QPI) significant when imaging transparent (e.g., biological) samples. This method of imagining requires no damage to the sample due to toxic, chemical staining, leading to a non-invasive and label-free technique. Common-path DHM systems, which are based on self interference, are usually more robust than double-path DHM systems (based on Mach-Zehnder and Michelson configurations), being less exposed to external fluctuations. Common-path DHM systems usually require fewer optical elements which reduce the cost of the system. In this work, a 3D-printed common-path DHM system using a Fresnel biprism has been design and evaluated using both a star and USAF target samples from Benchmark Technologies. A common issue in these DHM systems is that the self-interference causes an overlay between the two sample’s images. Therefore, common-path DHM systems are restricted for dense biological and material science samples, limiting their use for only imaging sparse samples. To resolve the overlay issue in common-path systems, one can reduce the sample’s field of view using half of the imaging area or insert a spatial filter. In this work, we have also implemented two DHM systems that employ an optical pinhole to spatial filter one of the samples’ image replicas. The optimal pinhole size is evaluated by analyzing the frequency content of the reconstructed phase images of a transmissive star target.
**Table of Contents**

Abbreviations ......................................................................................................................... v

List of Figures .......................................................................................................................... vi

Chapter 1: Introduction ............................................................................................................ 1
  1.1: Overview ......................................................................................................................... 3

Chapter 2: Digital Holographic Microscopy (DHM) ................................................................. 5
  2.1: Optical Recording ........................................................................................................... 5
  2.2: Numerical Reconstruction ............................................................................................... 8

Chapter 3: 3D-printed Simplified FB-based DHM .................................................................... 13
  3.1: Fresnel biprism and the Simplified FB-based DHM ......................................................... 14
  3.2: Design of 3D-printed Parts ............................................................................................. 15
  3.3: Implementation and testing of the 3D-printed prototype ................................................ 18

Chapter 4: Pinhole-based DHM without spatial overlay ......................................................... 20
  4.1: Background of the problem ......................................................................................... 20
  4.2: Description of a Pinhole-based DHM using a Fresnel biprism ..................................... 22
  4.2.1: Implementation of the Pinhole-based DHM using a Fresnel biprism ....................... 23
  4.2.2: Testing of the Pinhole-based DHM using a Fresnel biprism .................................... 25
  4.2.3: Implementation and testing of the Pinhole-based DHM using a Fresnel biprism for reflective microscopic samples ................................................................................. 26

5. Conclusions ......................................................................................................................... 31

Appendix .................................................................................................................................. 35

Appendix A: MATLAB code to reconstruct DHM images with comments ............................. 36

Appendix B: Alignment Protocol to build a simplified FB-based DHM ................................. 40
Appendix C: Alignment Protocol to build a FB-based DHM system for transmissive and reflective microscopic samples.......................................................... 47
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCD</td>
<td>Charge-couple device</td>
</tr>
<tr>
<td>CMOS</td>
<td>Complementary metal-oxide semiconductor</td>
</tr>
<tr>
<td>DIC</td>
<td>Differential Interference Contrast</td>
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<td>DHM</td>
<td>Digital holographic microscopy</td>
</tr>
<tr>
<td>FB</td>
<td>Fresnel biprism</td>
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<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>IP</td>
<td>Image plane</td>
</tr>
<tr>
<td>MO</td>
<td>Microscope objective</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
</tr>
<tr>
<td>QPI</td>
<td>Quantitative phase image</td>
</tr>
<tr>
<td>TL</td>
<td>Tube lens</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1: Interference between the reference r(x) and object wave o(x).................................5
Figure 2: Interference angle between the object (O) and the reference waves (R)...............7
Figure 3: The MATLAB code to apply the Fourier transform to the hologram ..................8
Figure 4: The MATLAB code to spatially filter frequencies..............................................9
Figure 5: The MATLAB code to spatially filter frequencies continued.............................10
Figure 6: The MATLAB code to find the maximum peak value.......................................11
Figure 7: The MATLAB code to generate the reconstructed phase image..........................11
Figure 8: Reconstructed phase image of red blood cells..................................................12
Figure 9: Simplified FB-based DHM system/interference pattern created by a Fresnel biprism...14
Figure 10: 3D printed simplified common-path FB-based DHM system..............................17
Figure 11: Evaluation of the 3D-printed FB-based common-path system using phase target.....19
Figure 12: Illustration of spatial overlay between the replicas of the object images...............21
Figure 13: Common-path DHM using a Fresnel biprism and a spatial filtering system.........22
Figure 14: Evaluation of the pinhole size in the FB-based DHM system............................25
Figure 15: The Pinhole-based DHM using a FB for reflective microscopic samples.............27
Figure 16: Evaluation of the reflection-based digital holography microscopy system..........29
Chapter 1: Introduction

Quantitative phase imaging (QPI) techniques are extensively used to analyze micro-sized living cells and tissues and nonbiological specimens, advancing many areas of research and industry. QPI methods quantify changes in a sample’s phase, which contains information about its refractive index, thickness, and surface topography. Because QPI exposes features about a specimen, it enables a wide collection of applications for imaging such as live-cell imaging, improving the manufacturing process, and disease screening and diagnosis. Among the different QPI methods, we can highlight white light methods [1], Hilbert phase microscopy [2–4], Difference Interference Contrast microscopy (DIC) [5–9] and Digital Holography Microscopy (DHM) [10–15]. The latter one is an optical interferometer technique that measures both the amplitude and phase of a sample through the recorded intensity interferogram. DHM provides a non-invasive approach, beneficial when imaging unstained, transparent, biological samples, requiring minimum preparation and less possibility of damage due to staining. DHM systems operating in transmission mode have been widely used in imaging live cells, enabling analysis of cell quantity, cellular morphology, and intracellular refractive index [16] from the measured phase information. In contrast, reflection-based DHM systems are commonly used in academic and industry labs for research specifically in areas of material science. Reflection-based DHM systems provide rapid analysis of possible surface topography, Micro-Electro-Mechanical Systems (MEMS) measurement [17], and defect inspections [18].

In DHM systems, a digital camera records the interference pattern between two coherent waves. Traditional DHM systems follow a Mach-Zehnder [19] or Michelson [20] configuration based on the sample type. For instance, unstained biological samples present low optical scattering and reflectivity, making them almost transparent in bright-field imaging. Therefore,
imaging unstained biological samples require the use of DHM systems operating in transmission mode (e.g., Mach-Zender setup). In contrast, reflective samples like MEMS and other semiconductor components require the use of Michelson-based DHM systems (e.g., reflection-based DHM systems). Regardless of their optical configuration, both traditional DHM systems rely on the fact that the two interfering waves travel different optical paths, making them more sensitive to temporal fluctuations. Common-path DHM systems rely on the principle that both interfering waves travel nearly the same optical path, being the most stable, and robust DHM systems. The principle of common-path DHM systems is the use of an optical element such as a diffraction grating [21], a Wollaston prism [22], a lateral shear plate [23], or a Fresnel biprism [24–28] to generate a self-interference pattern. The hallmarks of common-path systems are their temporal stability and compactness, making them suitable for implementation as an external module in current commercial microscopes. However, despite these advantages, common-path DHM systems can only provide quantitative phase images of microscopic samples with low spatial density. This limitation is related to the fact that the self-interference pattern is generated by two replicas of the same object image. Consequently, common-path DHM systems have only been reported for biological sparse samples. To avoid any undesired superposition between these two object replicas, some researchers have restricted the usable image field of view by inserting the microscopic sample within half of the illuminated field of view [24,29]. Another approach to removing the overlay related to the self-interference pattern without restricting the image field of view is the insertion of an optical spatial filtering in one of the interference beams [24,30]. In this work, a compact common-path DHM system for reconstructing the 3D topography of transmissive and reflective samples (e.g., dual-mode DHM system) has been implemented. The proposed common-path DHM system is based on the use of
a telecentric microscopic imaging system, a Fresnel biprism, and a 4f imaging system with a spatial filter (e.g., pinhole) located at its Fourier plane.

1.1: Overview

A major advantage of implementing common-path systems over double-path interferometers (e.g., Mach-Zehnder and Michelson configuration) is their high temporal stability, being less prone to external fluctuations due to the fact that both interfering beams travel nearly the same optical path. In addition, common-path DHM systems are known by their condensed setup, requiring fewer optical elements (e.g., no multiple mirrors and/or beam splitters like in dual-path DHM systems). Although the cost of DHM systems depend on their configuration, the cost of common-path DHM systems can be greatly reduced considering fewer needed optical elements in their implementation.

For the Master’s thesis: 1. A 3D-printed DHM system is designed, 3D printed, evaluated, and compared to another non-3D printed system, and 2. Two implementations of a DHM system based on the insertion of a pinhole are presented to eliminate the issue of overlay in common-path interferometry. The 3D-printed DHM system is a reproduction of the simplified FB-based DHM system using 3D printed parts, producing a more economical option. Each part of the 3D printed system was tested for corrections and slight adjustments and then configured. After implementing the 3D-printed system, the imaging performance was evaluated and compared to the non-printed simplified FB-based DHM system. In the first implementation of a pinhole-based DHM system, holograms are recorded to analyze the fringes contrast of the interference pattern based on the dimensions of the pinhole. The reconstructed phase images from the recorded holograms are used to measure the system’s accuracy and resolution. The second
implementation of the pinhole-based DHM system presented is a compact dual-mode DHM system. The system’s accuracy is quantified and verified in its ability to image reflective-based samples.
Chapter 2: Digital Holography Microscopy (DHM)

Digital holography microscopy is a three-dimensional imaging technique that allows for the recovery of phase and amplitude information about a microscopic sample. The process of DHM involves a combination of optically recording a hologram and its numerical reconstruction. DHM systems can operate in transmission or reflection mode. Transmission-based DHM systems analyze the difference in the beam of light that passes through the sample suitable for imaging transparent biological samples. However, reflection-mode DHM produces an image formed by the sample’s reflection useful for material science applications that can analyze the finish of a surface or material defects. Regardless of the type of DHM implemented, the process of optical recording and numerical reconstruction remains necessarily fundamental to recovering amplitude and phase information about the sample.

2.1: Optical Recording

During the optical recording stage, a digital camera captures the interference pattern produced by the reference r(x) and object wave o(x), Fig. 1. The method of optical recording is

Fig. 1 Illustration interference between the reference r(x) and object wave o(x)
the same for DHM systems operating in transmission or reflective mode, not influenced by the configuration type. The complex amplitude distribution of the reference and object is given by

\[
\begin{align*}
    r(x) &= |r(x)| e^{j\phi_r(x)} \\
    o(x) &= |o(x)| e^{j\phi_o(x)}
\end{align*}
\]

where \( |r(x)| \) and \( |o(x)| \) is the amplitude distribution of the reference and object wave, respectfully and \( \phi_i \) is the phase distribution of each wave. The object information is magnified and expanded using an infinity-corrected microscopic objective lens (MO) and a tube lens (TL) to produce the object wave that interferes with the reference wave at the system’s image plane. The complex distribution of the object wave at the system’s image plane, \( u_{IP}(x) \), is

\[
    u_{IP}(x) \propto \left\{ o \left( \frac{x}{M} \right) \otimes_2 P \left( \frac{x}{\lambda f_{TL}} \right) \right\}
\]

In Eq. 3, \( u_{IP}(x) \) is the complex amplitude distribution of the object information at the image plane, which is located at the back focal plane of the tube lens, \( \otimes_2 \) represents the mathematical operation used for 2D convolution, \( x = (x, y) \) are transverse spatial coordinates, \( \lambda \) is the wavelength of the system’s illumination source, and \( M \) is the lateral magnification is given by the ratio between the focal lengths of the TL and MO (e.g., \( M = f_{TL}/f_{MO} \)). \( P(\cdot) \) is the 2D Fourier transform of the amplitude transmittance of the pupil distribution, \( p(\cdot) \). The interference between the object and reference wave recorded by the camera, known as the hologram, is

\[
    h(x) = |u_{IP}(x)|^2 + |r(x)|^2 + u_{IP}(x)r^*(x) + u_{IP}^*(x)r(x),
\]

where \( |\cdot|^2 \) represents the square modules and \(*\) is the complex conjugate operator. In Eq. 4, one can see that the hologram consists of four terms, where the first two terms do not carry information about the phase because they both produce intensities since the phase is
compensated. For example, assume that $u_{IP}$ can be written as a complex number with amplitude $A$ and phase $\phi$ (e.g., $u_{IP} = A \exp(i\phi)$), then

$$|u_{IP}|^2 = A \exp(i\phi) [A \exp(i\phi)]^*$$

$$= A \exp(i\phi) A \exp(-i\phi)$$

$$= A^2 \exp(i\phi - i\phi)$$

$$= A^2. \tag{5}$$

Depending on the positioning of the three orders in the Fourier spectrum, one can determine if DHM systems operate as either an off-axis, slightly off-axis, or on-axis configuration observed in Fig. 2. If the interference angle between the reference and object wave generates three

![Fig. 2 Illustration of the interference angle between the object (O) and the reference waves (R). The arrangement of the Fourier transform orders is positioned due to the angle between them.](image)

orders where there is no overlap between them, the DHM system operates as an off-axis configuration. The recorded hologram is captured using a digital camera (e.g., CMOS or CCD).
2.2: Numerical Reconstruction

Once the hologram is optically recorded, the next operation in the numerical reconstruction stage. The numerical reconstruction allows for the retrieval of complex object information that entails amplitude and phase information about the sample. The composition of the hologram spectral is key in the numerical reconstruction stage. For example, the reconstruction method for off-axis DHM systems are mainly based on the spatial filtering of the object spectrum in the Fourier domain, enabling the reconstruction of phase images using a single image due to the separation of the three orders in the Fourier space. MATLAB is the programming language used to compute the reconstruction phase images of the holograms shown in detail in the lines of codes below. The steps involved in the numerical reconstruction are understood using the Fourier domain based on a reconstruction method in Ref [31]. Therefore, we compute the Fourier transform of the hologram $H(u) = \text{FT}[h(x)]$

$$H(u) = \text{DC}(u) + U_{IP}(u - \frac{\sin \theta}{\lambda}) + U_{IP}^*(u + \frac{\sin \theta}{\lambda})$$


% Fourier transform of the hologram
fft_holo = fftshift(fft2(fftshift(holo)));

Fig. 3. The MATLAB code to apply the Fourier transform to the hologram

In the Fourier domain, the hologram spectrum is composed of three components: DC term, +1, and -1 terms. The DC term does not carry information about the phase of the object and the angle of the reference wave, centrally positioning the term in the spectrum. The +1 and -1 orders contain the entire sample information: amplitude and phase. In fact, these terms are related to
the Fourier transform of the object complex distribution at the image plane, $U_{IP} = \text{FT}[u_{IP}]$.

Equation (6) also highlights that whereas the DC term are always placed at the center of the spectrum of the hologram, the frequencies of the $\pm 1$ terms place these components at symmetric locations around the center with their positions, $(\sin \theta)/\lambda$, being proportional to the interference angle $\theta = (\theta_x, \theta_y)$. In other words, the degree of overlap between the different components of the hologram spectrum is dependent upon the interference angle between both waves of the DHM recording system. If the angle between both object and reference waves is such as there is no overlap between the different terms, then the DHM system operates in the off-axis regime (see Fig. 2). Specifically, for off-axis DHM systems, since there is no overlapping between the three orders, the next stage in the reconstruction process is to spatially filter the $+1$ term using a

$$H_F(u) = U_{IP}\left(u - \frac{\sin \theta}{\lambda}\right)$$

(7)

```matlab
% Filter the DC term
maskDC = ones(M,N);
% Filtering using a circular aperture
rsc=50; % radius to filter the DC order.
for r=1:N
    for p=1:M
        if sqrt(((r-(N/2+1))^2+(p-(M/2+1))^2)<rsc
            maskDC(r,p)=0;
        end
    end
end
fft_holo = fft_holo.*maskDC;
% Select order of diffraction for find the best compensation
% Max value first quadrant
maxValue_1 = max(max(abs(fft_holo(1:M/2,1:N/2))));
[fy_max_1 fx_max_1] = find(abs(fft_holo(1:M/2,1:N/2)) == maxValue_1);
```

Fig. 4. The MATLAB code to spatially filter frequencies
Fig. 5. The MATLAB code to spatially filter frequencies continued

circular mask, which is automatically set in the code. The dimensions of the circular mask filter
depend on the magnification and numerical aperture of the DHM system. Without knowing this
information, the radius of the circular mask (Fig. 5) can be assumed to be equal to

\[
1/3 \sqrt{u_0 - u_{\text{max}}}^2 + |v_0 - v_{\text{max}}|^2
\]

being \((u_0, v_0)\) the integer pixel position of the DC term and \((u_{\text{max}}, v_{\text{max}})\)
the integer pixel position of the maximum peak of the +1 term in the hologram spectrum. Note
that the interference angle (Fig. 6) is related to the maximum peak of the +1 term via
\[ \theta_x = \sin^{-1}\left(\frac{|u_0 - u_{\text{max}}| \lambda}{M \Delta_{xy}}\right) \quad \text{and} \quad \theta_y = \sin^{-1}\left(\frac{|v_0 - v_{\text{max}}| \lambda}{N \Delta_{xy}}\right) \] (8)

Fig. 6. The MATLAB code to estimate the interference angle

\[
C=\text{round}(N/2); \quad \% \text{pos DC horizontal} \\
R=\text{round}(N/2); \quad \% \text{vertical pos DC} \\
\text{ThetaYM}=\text{asin}((C-C_{\text{max}})*\text{lambda}/(N_{y}*dxy)); \quad \text{ThetaXM}=\text{asin}((R-R_{\text{max}})*\text{lambda}/(N_{x}*dxy));
\]

where \((M, N)\) is the size of the reconstructed image, and \(\Delta_{xy}\) is the square pixel size.

Afterward, one must compensate for the angle of the reference wave using a digital reference wave in the Fourier spectrum. To achieve this, the filtered spectrum is centered on a new matrix, finding the center of the filtered hologram spectrum, \((u_{\text{max}}, v_{\text{max}})\). In the space domain, the reference angle is compensated by multiplying the inverse Fourier transform of Eq. 9, \(h_r(x)\), and the digital reference wave \(r_g(x)\). Once the reference wave is compensated, the reconstructed phase image is generated by the angle of

\[
\hat{u}_{ip}(x) = r_{D}(x) \overline{h_{r}}(x).
\] (9)

\[
\text{Rhat}=\exp(1i*k*(\text{sin(ThetaYM)})*X*dxy+\text{sin(ThetaXM)})*Y*dxy)); \\
\text{Uobj} = \text{Rhat}.*\text{holo\_filter};
\]

Fig. 7. The MATLAB code to generate the reconstructed phase image

Since the sensor plane is positioned at the back focal plane of the TL (e.g., the image plane) and the sample is placed at the working distance of the MO lens, the camera sensor records the in-focus sample information. Therefore, there is no need to apply refocusing algorithms to retrieve
complex images containing amplitude, $a(x)$ and phase-contrast images $\hat{\phi}(x)$, as shown in Fig. 8.

Fig. 8 Reconstructed phase image of red blood cells. Illustration of (a) amplitude-contrast and (b) phase-contrast images retrieved from the optical DHM system.
Chapter 3: 3D-printed Simplified FB-based DHM

The simplified FB-based DHM system is digitally fabricated using a 3D printer also referred to as additive manufacturing. In the first stage, one drafts a 3D model of the part or element in development using a drafting software (e.g., Solidworks and AutoCAD). Afterward, stage two consists of importing the drawing as a 3D printed plot which will allow the file to be opened in a Slicer software used to digitally divide the model into layers. Within this software, the 3D printer settings can be adjusted for the necessary settings that can differ from one model to another. Once the printer’s settings are set, the model is sliced, and then it is saved as a file to print from the 3D printer. Lastly, in the third stage of the additive manufacturing process, using the file from the Slicer software and its applied settings, one must transport the file to a digital storage device that can be inserted into the 3D printer for printing. Power on the 3D printer and print.

The 3D manufactured simplified FB-based DHM consist of three basic printed components: the rails, optical plates, and mounting plates. It is composed of two 12” inch structural rails that optical plates are attached to utilizing a clamping method, four optical plates that hold each optical element using a screw thread method, and four vertical and horizontal mounting plates to connect the rails to the optical table. For each 3D printed part, 2-D AutoCAD drawings were retrieved from the Thorlabs website and used as a basic source of scale and dimension. The basic 2-D drawings are drafted and reworked into 3-D drawings to allow for the second stage in the additive manufacturing process.

The 3D printed system is a replica of non-3D manufactured simplified FB-based DHM but the rails, optical plates that hold each optical element, and mounting plates are all 3D-printed.
for a more economical option. The 3D printed configuration uses the same optical elements such as the laser, MO, TL, and FB specified in the Simplified FB-based DHM section below.

3.1: Fresnel Biprisms and the Simplified FB-based DHMS

The common-path DHM system, shown in Fig 9a, inserts a Fresnel biperism to generate the self-interference between two replicas of the object wave. Note that one of these replicas now becomes the reference wave. The microscopic sample in the FB-based DHM system is illuminated by a plane wave generated by a low-powered laser (Thorlabs CPS53, wavelength $\lambda$

---

Fig.9 (a) Illustration of the simplified common-path FB-based DHM system. (b) The interference pattern created by a Fresnel biperism (FB). The fringes are located inside the dark shaded area outlined in white

$\lambda = 532$ nm). The sample is imaged by an infinity-corrected 40X/ 0.65 NA Olympus MO lens coupled with an achromatic TL lens. Given the manufacturer’s specifications, the focal length of MO is 4.5 mm and TL is 200 mm, which produces a system lateral magnification of $M =$ -
The system is telecentric-based because the aperture stop of the MO is positioned at the front focal plane of the TL, allowing for accurate phase images. The CMOS sensor (Basler acA5472-17um, 5472x3648 pixels with size 2.4 µm) is inserted at the back focal plane of the TL at the imaging plane.

A Fresnel biprism is an optical element composed of two thin prisms connected at their base [32]. In common-path DHM systems, FB is used to create self-interference among the two beams. If the Fresnel biprism is inserted after the TL, the FB generates two coherent plane waves with amplitude distributions equal to \( u_{ip}(x - s \tan \delta, y) \) and \( u_{ip}(x + s \tan \delta, y) \). Note that the interference pattern is only observed within the intersection of these two beams, shown in the white-outline diamond area in Fig. 9b). Therefore, the interference fringes are confined. The separation between the two replica beams is dependent on the distance \( s \) and the greater distance of the FB from the camera, the more separation between the two replica beams. The maximum field of view (FOV) of the fringes is when the biprism is positioned at \( s_{\text{max}} \) which is equal to half of the lateral extension (L) of the FB (e.g. \( L/2 = \text{FOV}(s_{\text{max}}) \)). In all of the common-path DHM systems mentioned in this dissertation, the biprism is positioned to provide the maximum FOV, highly important because one can only reconstruct phase information if the sample information is within the area of the fringes. However, no fringes are found when the FB location is \( 2s_{\text{max}} \). The single-shot, off-axis FB-based DHM system provides high-speed, high accuracy, high resolution, high temporal stability, and polarization sensitivity capabilities.

### 3.2: Design of 3D-printed parts

The 3D printed simplified FB-based DHM consists of three basic printed components: the rails, optical plates, and mounting plates shown in Fig. 10. The development of the optical
rail was heavily influenced by the ability to mount it horizontally and/or vertically. Thus, the item was formed to adapt to a flexible mounting position, it is significantly dense to provide a durable foundation necessary for the optical system. The height of the rail was limited to the printable area of the 3D printer’s bed and allowed for the height of the tallest rail to be 12 inches. Overall, the design of the rail is practical and provides a sturdy base to carry the weight of the optical arrangement vertically or horizontally. During the process of 3D printing, due to the size of the part, the amount of time to print rail became a major challenge, with an estimated print time of about 3 days. Adjusting the 3D printer to the appropriate settings bed was important here to reduce print time. Leveling the print bed and adjusting the nozzle extrusion rate of filament to ease common issues of warped edges or rough exterior surfaces can destroy a final product and result in a loss the time of reprinting.

The original concept of the optical plate is only composed of segments that are necessary: a piece needed to connect or mount it to the rail and another piece that would allow for the attachment of optical elements. A major complication was designing a working screw thread to attach optical elements such as a mounted lens or insert a camera. The design of the screw thread was a long and tedious process considering that one must print, test the part to see if it works, if not make subtle changes to the drawings, and reprint again until the screw thread works. Another necessary part of the optical plate was the connection to the rail which was designed using abstract initials. For this piece, a smaller screw thread size is drafted in to allow for a screw to fasten the optical plate to the rail. To reduce the size of the optical plate, holes were inserted at each corner to allow for the insertion of rods. The goal was to make the cage plate as small as possible and depending on the number of required optical elements, this part is
printed many times (e.g. especially in the testing and reprinting stage of correcting the screw threads). So, to keep down the 3d printing time, one needs to reduce the size of the part significantly.

The goal in developing the vertical and horizontal mounting plates is to reduce the size of the object as much as possible and incorporate the screw thread to adhere the mounting plates together. A vertical mount is a square-shaped object with four holes embedded in each corner to secure the plate to the optical table. Also, two screw threads were inserted into the center of the vertical mount to attach it to the horizontal plate. The horizontal mount plate is a sleigh-shaped part with a center cutout. The opening allows for two screws to be inserted attaching them to the

Fig.10 3D printed simplified common-path FB-based DHM system. Illustration of parts optical rail, optical plate, horizontal and vertical mounts.
two screw threads on the vertical mount plate. Two screw threads are inserted on the side of the horizontal plate to fasten the mounting plate to the optical rail.

3.3: Implementation and testing of the 3D-printed prototype

To align the 3D-printed simplified FB-based DHM (Fig. 10), start by inserting a laser and adjust the tilt until the of center the beam is centered on an alignment target. Position the alignment target closest to the laser and further away when checking. Next, insert the tube lens (TL) with a focal length of 200mm allowing space (e.g. position the TL roughly 40cm from the laser) for other optical elements. Position the alignment target before and after the TL to center it. Afterward, insert the camera after the TL. To locate the camera’s position, adjust the sensor until the smallest point is focused and found at the imaging plane using the camera’s software.

Next, insert the infinity-corrected microscopic objective (MO) lens before the TL. To determine the position of the MO lens from the TL, one can estimate the distance between the MO and the TL to be the sum of their focal lengths (e.g. \( f_{MO} + f_{TL} \)) given by the manufactures specifications.

Insert a shearing plate after the TL and adjust the MO until observing parallel lines. To verify the alignment of the MO, insert an alignment target after the TL and check to see if the beam is centered. Adjust the position of the MO lens if the beam is not centered on the target. Afterward, insert the Fresnel biprism (FB) between the TL and the camera. The FB is mounted in a 3D printed holder that allows the flexibility of rotating it to the preferred position. The FB is rotated 45 degrees to allow for the use of the maximum camera bandwidth. Using the camera’s software, adjust the FB until one can observe the maximum fringes field of view, estimated to be \( \text{FOV}_{\text{max}} = 10 \text{ mm} \) (e.g. lateral extension (L)of the Fb = 20 mm, L/2). Next, determine if the system operates in an off-axis regime by recording a hologram and taking the Fourier transform.
the image. If there is no overlap between the three components, the DC term which is centrally positioned in the spectrum, and +1 and -1 term, are symmetrically arranged around the DC, therefore the system is off-axis. Lastly, insert a sample between the MO and the laser to allow for imaged samples.

The 3D-printed simplified FB-based DHM system (Fig. 10) was evaluated by testing the system’s resolution and comparing its results to the non-3D printed DHM system. The Benchmark Star target, Fig. 11 (a), demonstrates the major drawback in common-path systems where there is an overlay of object information producing two replicas. In the reconstructed phase image, one can observe the two replicas, one star is white, and the other replica is black due to each replica having a different phase shift. The USAF target is evaluated to determine the system’s resolution limit shown in Fig. 11 (b). The estimate of the shortest resolvable distance is found in the 9-3 element marked by the red arrow, equal to 775 nm. It was determined that the non-3D printed system’s resolution was the same as the Fb-based DHM system in Ref [27], verifying the resolution of the 3D-printed Simplified FB-based DHM.

![Fig. 11. Evaluation of the 3D-printed FB-based common path DHM system using phase targets:](image)

(a) Star and (b) USAF.
Chapter 4: Pinhole-based DHM without spatial overlay

The Pinhole-based DHM system addresses the issue of spatial overlay in common-path DHM systems shown in Fig. 8(a). The optical system inserts a FB after the TL that generates self-interference of the two waves. The Pinhole-based DHM system includes a 4f spatially filtering system where lens L1 and lens L2 are positioned in an afocal arrangement. A significant element is the utilization of a pinhole implemented to remove the high and medium spatial frequencies of one of the sample replicas, generating a uniform beam. The pinhole is positioned in the back focal plane of L1 and the front focal plane of L2, where the beams converge to the smallest point. Specifically, a pinhole wheel is employed, allowing for a simpler way of aligning the pinhole when rotating it to smaller pinhole diameters. The pinhole wheel includes 16 filtering sizes ranging from the smallest size of 25μm to the largest, 2mm. The overlay between the two replica images is visible in the large pinhole diameters but reducing the pinhole to a smaller diameter, eliminates the overlay of the two images.

4.1: Background of the problem

Common-path DHM systems are self-interference imaging systems that generate a duplicate replica of object information. Therefore, common-path interferometry is restricted to imaging sparse samples, characterized as a low-density sample with dispersed features. Figure 12 illustrates the overlay problem, where the two replicas become separated from the center of replica one to the center of the second replica. At this distance between the two replicas, the size of the microscopic sample Δx is half of the lateral extension of the Fresnel biprism, L/2, and at this position is where the maximum fringes field of view (FOV) is found. The separation distance between the two replicas is determined by the position of the FB. If the magnified
sample size, $M \cdot \Delta x$ is smaller than the separation between the two replicas, then there is no overlay, $M \cdot \Delta x \leq L/2$. However, there are cases when there is an overlay. Considering that the position of the FB is fixed (e.g., to provide the maximum fringes FOV at position $L/2$) which affects the separation between the replicas and the size of the sample, $\Delta x$, is fixed, to solve this issue of overlay, one can reduce the system’s magnification. The reduction in lateral magnification produces less overlay between the replicas but this is not a suitable solution for high-resolution quantitative phase imaging. Since the lateral magnification is determined by the ratio between the focal length of the TL and the MO, it is possible to reduce the lateral magnification by reducing the focal length of the TL. However, this is not a viable option for implementing an adaptable QPI module to a commercial microscopic due to not having access to its TL lens. Therefore, the lateral magnification is reduced by changing the MO lens. This

Fig. 12: (a) Illustration of no spatial overlay between the replicas of the object images. (b) Illustration of spatial overlay outlined in the shaded region
change more likely also decreases the numerical aperture of the systems, providing images with less resolution power (e.g., a drop in the system’s resolution).

4.2: Description of a Pinhole-based DHM using a Fresnel biprism

Figure 13 shows the experimental setup of the pinhole-based DHM using a Fresnel biprism. The system is illuminated by a low-powered collimated laser with a wavelength of 532nm (Thorlabs CPS532). The light transmitted by the sample is imaged by infinity-corrected Nikon 40X/0.75 NA a microscope objective (MO) coupled with an achromatic tube lens (TL) (e.g., 200mm focal length). The system operates in a telecentric regime because the aperture stop of the MO is positioned at the front focal plane of the TL, generating a plane wave after the TL lens. The Fresnel Biprism (FB) divides the beam into two and the interference caused by the two beams presents

Fig. 13: The optical configuration of the common-path digital holographic microscopy using a Fresnel biprism and a spatial filtering system

the issue of an overlay. Both beams travel through lens L1 (e.g, 125 mm focal length), where the plane waves converge to a focus spot. To prevent the overlapping, use a pinhole to spatially
filter one of the two beams in the Fourier spectrum, removing the high and medium frequencies of one of the beams and generating a uniform beam. Since the focal length of L1 and L2 are the same (e.g. \( f_{L1}/f_{L2} = 1 \)) so the lateral magnification of the system (e.g. \( M = -f_{TL}/f_{MO} \)), remains unchanged.

**4.2.1: Implementation of the Pinhole-based DHM using a Fresnel biprism**

To assemble the Pinhole-based DHM system using a Fresnel biprism, one must insert a laser and align it by adjusting the tilt until the center of the beam is centered on an alignment target. To check the alignment of the laser, move the target closest to the laser and then further away from the laser for examination. Insert the L2 lens with 125-mm focal length approximately 130 cm away from the laser allowing for enough space for other optical elements and at least 50 cm after L2. Align L2 by positioning the alignment target after L2 to check. Afterward, insert the camera after L2 and find the camera’s position by using the camera’s software. Reduce the saturation of the camera by decreasing the camera exposure time and/or by inserting a neutral density filter after the laser. Move the camera axially until the smallest point is focused through L2. Next, insert the L1 lens with a 125-mm focal length in the front focal plane of L2. To find the position of lens L1, insert a shearing plate after L2 and move L1 until one observes parallel lines in the interferometry. Once the position of lens L1 is determined, place the target in the back focal plane of L1 and then L2 to align lens L1 until the beam is centered on the alignment target. Afterward, insert the TL with a 200-mm focal length in the front focal plane of L1. To find the position of the TL, insert a shearing plate interferometry, after lens L1, and adjust TL until straight, parallel lines are observed in the shearing plate. Insert the alignment target in the back focal plane of TL and align it. Next, insert the infinity-corrected MO lens in the front focal
plane of the TL. To set up a telecentric MO-TL system, insert the shearing plate after the TL, and adjust the position of the MO until one notices parallel lines in the interferometer. Insert the alignment target in the back focal plane of the MO and align it accordingly. Afterward, insert the FB between the TL and L1 lens. Using the camera’s software, adjust the FB until one can observe the maximum fringes field of view, estimated to be \( \text{FOV}_{\text{max}} = 10 \text{ mm} \) (e.g. lateral extension (L) of the Fb = 20 mm, L/2). Next, insert the pinhole wheel between L1 and L2 lenses. Set the pinhole wheel axially where the beam converges to the smallest point. Rotate the pinhole to the largest pinhole diameter of 2000 um and move the pinhole until one of the beams passes through this pinhole. The FB generates two beams: Beam 1 should be filtered through the 2000-um diameter pinhole and Beam 2 is travelling without any distortion of the pinhole. Rotate the FB 45 degrees allowing both beams to travel to the camera. To align the pinhole, block the beam that is not filtered through the pinhole. Move the pinhole laterally until the filtered beam creates a uniform, consistent intensity of light and not a spot of light. Start by rotating the pinhole to the largest diameter of 2000-um. To align, move the x- and y-axis of the pinhole until the beam is centered when filtered through the pinhole. Check both beams for a uniform consistency of light. Rotate the pinhole to 1000-um diameter, align the beam by centering it and check for uniformity of the light. Repeat the following steps for each pinhole diameter and disregard the pinholes with a ring. Lastly, insert a sample between the MO and the laser to allow for imaged samples. A more detailed description on how to align the Pinhole-based DHM system using a Fresnel biprism can be found in Appendix B. Two YouTube videos have been creating to show how to align the pinhole [33,34].
4.2.2: Testing of the Pinhole-based DHM using a Fresnel biprism

The Pinhole-based DHM system using a FB was evaluated using a star target from the QPM target (Benchmark Technologies) shown in Fig. 14. Holograms of the star pattern were imaged for various pinhole sizes ranging from 30 μm to 2 mm (Fig. 14a). Whereas one can observe an overlay between the two replicas for the larger pinhole sizes (90 μm, 200 μm and 2 mm), no overlay is observed for the smaller pinholes sizes (30 μm and 50 μm). We have evaluated the fringes’ contrast in the hologram for each pinhole size along the diagonal line displayed in the 30-μm hologram, marked by the red line. The minimum and maximum values were taken from the intensity profile along the red line. The fringes contrast is determined as the difference of the mean max and mean min divided by the sum of the mean max and mean min (e.g., \( C = \frac{\text{max} - \text{min}}{\text{max} + \text{min}} \)). The mean and standard deviation of the fringes contrast values is reported for each hologram. Experimentally, the fringes’ contrast was not reduced by

![Fig. 14 Evaluation of the pinhole size in the FB-based DHM system: (a)-(b) Experimental holograms and reconstructed phase images of the star pattern for different pinhole sizes. The insets in panels (b) show the 2D Fourier transform of the reconstructed phase images for the pinhole 30 and 50μm. The value of the fringes’ contrast estimated along the red direction (red font) is shown in panel (a).](image-url)
changing the pinhole size due to the increase of the camera exposure time. To assess the system, we reconstructed the phase images from the star pattern displayed, see Fig. 14b. Again, the reconstructed phase images for the larger pinholes (e.g., 90 μm, 200 μm, and 2 mm) display the overlap between the two replicas, introducing unwanted phases issues emphasized in yellow. These complications are due to the overlay between the replicas which limits the usable FOV.

For pinhole sizes equal to 30 μm and 50μm, we found similar reconstructed phase image in terms of the accuracy and without overlay. For both pinhole diameters, we have measured the thickness of the start by converting the phase values (ϕ) into thickness, t (e.g. t = [ϕ λ] / [2π(n_g - n_m)]), where λ = 532 nm, n_g = 1.52, n_m = 1. The thickness value (mean ± standard deviation) of the reconstructed star target was equal to 398 ± 41 nm for the 30-μm pinhole, and 355 ± 9 nm for the 50-μm pinhole. These values agree with the manufacturer’s specification of t= 350 nm, within the experimental error.

4.2.3: Implementation and testing of the Pinhole-based DHM using a Fresnel biprism for reflective microscopic samples

Figure 15 shows the setup of the Pinhole-based DHM system using a Fresnel biprism for reflective sample. To align such system, insert the laser of the transmission imaging path. Position the alignment target closest to the laser and towards the end of the construction rail to align the laser. Insert the L2 lens of 75-mm focal length lens approximately 85 cm away from the beginning of the mounting rail (e.g., summing 2f_{TL}, 3 f_{L2}, and giving 22.5 cm to insert the objective lens and the sample stage). Align L2 lens by positioning the alignment target after L2 lens to check. Afterward, insert the camera after L2 and find the camera’s position by using the camera’s software. Insert a neutral density filter after the laser to avoid saturating the camera.
Move the camera axially until the smallest point is focused through L2. Next, insert L1 lens of 75-mm focal length before L2 lens. To find the position of lens L1, insert a shearing plate after L2 and move L1 until noticing parallel lines in the interferometer. Once the position of lens L1 is determined, align lens L1 until the beam is centered on the alignment target and adjust the alignment of the beam is not centered. Afterward, insert the TL with a 200-mm focal length in the front focal plane of L1. To find the position of the TL, insert a shearing plate interferometry, after lens L1, and adjust TL until straight, parallel lines are observed in the shearing plate. Insert the alignment target in the back focal plane of TL and align it. Next, insert the infinity-corrected MO lens in the front focal plane of the TL. To set up a telecentric MO-TL system, insert the shearing plate after the TL, and adjust the position of the MO until one notices parallel lines in

Fig. 15 The Pinhole-based DHM using a Fresnel biprism for reflective microscopic samples. Microscope objective (MO), tube lens (TB), Fresnel biprism (FB), Image plane (IP1 and IP2), lens (L1 and L2).
the interferometer. Insert the alignment target in the back focal plane of the MO and align it accordingly. Afterward, insert the FB between the TL and L1 lens. Using the camera’s software, adjust the FB until one can observe the maximum fringes field of view, estimated to be $\text{FOV}_{\text{max}} = 10 \text{ mm}$ (e.g., lateral extension ($L$) of the Fb = 20 mm, $L/2$). Next, insert a 30-µm pinhole between L1 and L2 lenses closest to the smallest focus spot in the back focal plane of lens L1. Manually, drill a large enough hole in the outer part of the pinhole allowing the object beam not to be clipped or filtered, seamlessly passing to the camera and filtering the reference beam using the 30-µm pinhole. To align the pinhole, block the object beam that is not filtered through the pinhole. Move the pinhole laterally until the filtered beam creates a uniform, consistent intensity of light and not a spot of light. Connect L1, pinhole, L2, and the camera using rods. Afterward, verify the transmission-based DHM system by imaging a target from the QPT Benchmark Technologies microscopic slide, evaluating the accuracy and resolution of the system. Next, mount the optical rail for the reflection-based path system, perpendicular to the transmission-based system, between the TL and FB. Afterward, insert another laser on the reflection-based path, on the end of the rail, farthest away from the transmission-based system. Position the alignment target closest to the laser and farthest away from the laser to align. Next, insert the beamsplitter (BS) between the TL and FB. Confirm that the reflected beam passes through the TL and MO lenses. Adjust the height and horizontal position of the beamsplitter making sure the beam is not clipped. Position one alignment target closest to the BS (e.g., in front of the TL lens), and position another alignment target close to the laser source of the transmission-based system. Ensure that the BS is set at a 45degree angle and adjust the height of the BS confirming that the beam from the reflected path is centered on the alignment target. Move the tilt of the BS to center the reflected beam on the alignment target closest to the transmission-based source then
adjust the lateral position of the BS, centering the beam on the alignment target closest to the BS. Move the BS to center the reflective beam, horizontally, on the target closest to the BS. Adjust the horizontal tilt of the BS to center the beam on the target closest to the transmission-based source. Continue this process until the reflected beam is centered in both alignment targets. Next, insert a 10X beam expander (BE) between the laser and BS. Position the alignment targets between the BS and MO, closest to the BS and closest to the transmission-based system’s source. Move the BE until the beams is centered on both alignment targets. Lastly, insert a sample after the MO allowing for imaged samples. See Appendix C, for an in-depth description on how to align the Pinhole-based DHM system using a Fresnel biprism for reflective sample.

The Pinhole-based DHM using a Fresnel biprism for reflective microscopic samples is evaluated using a USAF target from Edmund Optics (Fig. 16a) and a sample with an array of squares (Fig. 16b). Both samples were converted to reflective-based samples by a DC-Sputtering technique, where a 40-nm film layer is accumulated onto the sample, using an EMS 550 coater (Electron Microscopy Sciences Hatfield, PA, USA).

Fig. 16 Evaluation of the reflection-based digital holography microscopy system (a) The system is evaluated using a USAF target and determined the smallest resolvable distance 9-4 element (b) Reconstructed phase image of the array of squares.
The USAF target was imaged to evaluate the system’s spatial resolution demonstrated in Fig. 13(a). The smallest resolvable element was determined to be 9-4 element, corresponding to a distance equal to 691 nm, agreeing with the theoretical prediction of 709 nm (e.g. \( \lambda/NA = 532 \text{ nm} / 0.75 \approx 709 \text{ nm} \)). The sample with array of squares provides clear distinctive squares with high contrast spaced about 0.0035mm apart.
Chapter 5: Conclusions

Digital Holography Microscopy provides a full-field quantitative phase image if microscopic samples with high speed, accuracy, resolution and temporal stability. The specimen under investigation using DHM systems is not required to undergo an extensive staining process where the toxic dyes could alter or damage a sample (e.g., non-invasive and label-free). Since off-axis configurations provide no overlap between the three diffraction orders in the Fourier spectrum, object information can be reconstructed using a single shot. Self-interfering geometries such as common-path DHM setups are more compact in size, require fewer optical elements, and usually more robust and less prone to external fluctuations than double-path systems (e.g., Mach Zehnder and Michelson arrangements). However, a major drawback of common-path systems is due to their self-referencing composition that generates overlapping replicas of the object information restricting only imaging sparse samples. The issue of overlay is resolved by spatially filtering one of the beams using a pinhole.

The process of implementing each system requires an understanding and a working knowledge of optical hardware and how to assemble the system. The development of each DHM setup consists of hardware or optical components used to build it. The basic process of aligning each element consists of inserting an iris or alignment target after the optical element close to and farther away, to verify the vertical and horizontal positions and well as the tilt to center the beam. The position of the lens is determined by the focal length distance, where the object is formed, given by the manufacturer’s specifications. The position of the Fresnel biprism displaced until one observed the maximum fringes FOV which is important because only within this fridges’ FOV is where phase information can be reconstructed. The microscopic objective lens is also positioned at the focal length distance given by the specification. To provide a
telecentric system, the distance between the MO and the TL is the sum of the focal length. Specifically, the aperture stop of MO is located at the front focal plane of the TL which allows for accurate, high-resolution reconstructed phase images. For the pinhole, position it until a uniform beam is observed and not a spot of light. Once the position is set, the pinhole provides spatially filtering that eliminates overlay in self-interferometry such as common-path DHM systems. The beam splitter is positioned to direct the reflected toward the TL and MO which is an essential element for the reflective-based system.

This Master’s study is focused on investigating a 3D printed simplified FB-based DHM, Pinhole-based digital holographic microscopy, and Pinhole-based digital holographic microscopy for reflective samples. In summary, understanding the advantages of using a common-path DHM system than double-path systems was the underlying objective of the thesis. The simplified FB-based DHM provided a system with high speed, resolution, accuracy, and temporal stability in addition to a more economical 3D printed version of the system. A major limitation of this system is the overlay between the two replicas. To solve this, the implementation of the Pinhole-based DHM system was developed that inserted a pinhole to eliminate the object overlay in sparse samples. The performance of the Pinhole-based DHM was evaluated for transmissive and reflective samples.
References


Appendix
Appendix A: MATLAB code to reconstruct DHM images with comments

% Title: The Universal Digital Holographic Microscopy (tuDHM) Algorithm
%
% This algorithm allows to recover the complex object information for %
% single-shot digital holographic microscope (DHM) operating in the telecentric %
% regimen. The algorithm is an automatic method based on the minimization of a %
% cost function that finds the best numerical conjugated reference beam to %
% compensate the filtered object information, eliminating any undesired phase %
% perturbation due to the tilt between the reference and object waves.
%
% If using this code for publishing your results, please kindly cite us:
% R. Castaneda, and A. Doblas, "Fast and automatic algorithm to universal %
% recovery of the quantitative phase distribution in digital holographic %
% microscopy", IEEE Transactions on Imaging Processing xxx (2020)
%
% Authors: Raul Castaneda and Ana Doblas
% Department of Electrical and Computer Engineering
% The University of Memphis
% Memphis, TN 38152, USA.
%
% Email: rctstdqnt@memphis.edu and adoblas@memphis.
% version 1.0 (2020)
%
% Specifications
% Input:
% holo = Recorded Off-axis Hologram operating in telecentric regimen
% lambda = wavelength
% pixel size = dxy
%
% Output: phase = Reconstructed Phase Image
%
% Functions: The algorithm implement a set of functions
% - holo_read
% - holo_filter
% - reference_wave
% - cost_function
% - phase_reconstruction
% - unwrap_phase
% % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % % %
%% Clear Memory
clc% Clear command window
close all% close all windows
clear all% clear of memory all variable

%% Load and Crop Hologram
I=imread('Star_633Laser_Olympus 40X MO_200TL_160 FB_Basler 17um Camera_Exp1703_2.tif');
I=double(I);
holo = I(1001:2800,1801:3600);
[M,N] = size(holo);
[m,n] = meshgrid(-M/2:M/2-1,-N/2:N/2-1);
figure,imagesc(holo),colormap(gray),title('Hologram'),daspect([1 1 1])

%% Parameters of reconstruction (this parameters depend of the hologram record)
lambda = 0.532;% source's wavelength in microns
dxy = 2.4;% size of pixel in microns along the vertical and horizontal direction

%% Spatial Frequency Filter
% filter the +1 term from the Fourier transform of the hologram
[holo_filter,fx_max,fy_max] = holo_filter(holo,M,N,'yes');

%% Cost Function
% Cost function based on the Eq. (8)
seed_maxPeak = [fx_max, fy_max];
J = cost_function(seed_maxPeak,lambda,dxy,M,N,m,n,holo_filter);

%% Reconstruction, non optimal reference wave distribution
[ref_wave] = reference_wave(M,N,m,n,lambda,dxy,fx_max,fy_max);
[phase,amplitude] = phase_reconstruction(holo_filter,ref_wave,'not');%not
figure,subplot(2,2,1),imagesc(amplitude),colormap(gray),colorbar, title('amplitude reconstruction'),daspect([1 1 1])
subplot(2,2,2),imagesc(phase),colormap(gray),colorbar, title('Phase reconstruction'),daspect([1 1 1])
%% Minimization
options = optimset('Display','iter', 'MaxIter', 30,'TolX',1e-3);
[MaxPeaks, J] = ... 
    fminunc(@(t)(cost_function(t,lambda,dxy,M,N,m,n,holo_filter)), seed_maxPeak, options);

fx_max_best = MaxPeaks(1,1);
fy_max_best = MaxPeaks(1,2);

fprintf('Cost at peaks found by fminunc: %f
', J);
fprintf('fx: %f
', fx_max_best);
fprintf('fy: %f
', fy_max_best);

%% Reconstruction, best phase distribution
[ref_wave] = reference_wave(M,N,m,n,lambda,dxy,fx_max_best,fy_max_best);
[phase,amplitude] = phase_reconstruction(holo_filter,ref_wave,'not');%not

figure,subplot(2,2,1),imagesc(amplitude),colormap(gray),colorbar, title('amplitude reconstruction'),daspect([1 1 1])
subplot(2,2,2),imagesc(phase),colormap(gray),colorbar, title('Phase reconstruction'),daspect([1 1 1])

%% Manual compensation of the reference wave
close all
colsofMaxPk = fx_max_best;rowsofMaxPk=fy_max_best;
k=2*pi/lambda;

[Ny Nx]=size(holo);

for Cmax= colsofMaxPk-0.9: 0.10:colsofMaxPk-0.9; %Horizontal position of the peak of gplus (small to large)
for Rmax= rowsofMaxPk-2.3: 0.10:rowsofMaxPk-2.3; %Vertical position of the peak of gplus
C=round(Nx/2);%pos DC horizontal
R=round(Ny/2);%vertical pos.DC
ThetaYM=asin((C-Cmax)*lambda/(Ny*dxy));ThetaXM=asin((R-Rmax)*lambda/(Nx*dxy));
[X Y]=meshgrid(-Nx/2+1:Nx/2,-Ny/2+1:Ny/2); %Discrete transversal coordinates
Rhat=exp(1i*k*(sin(ThetaYM)*X*dxy+sin(ThetaXM)*Y*dxy));
%figure; colormap gray; imagesc(angle(Rhat));

Uobj = Rhat.*holo_filter;
```matlab
figure,imshow(angle(Uobj),[]); axis image;title([\'colmax',num2str(Cmax),\'rowmax',num2str(Rmax)])
% subplot(121);imshow(abs(Uobj),[]); axis image;
% subplot(122);imshow(angle(Uobj),[]); axis image;

end
end

%% Fast 2D phase unwrapping implementation in MATLAB
% Fast unwrapping 2D phase image using the algorithm given in:
% M. A. Herraez, D. R. Burton, M. J. Lalor, and M. A. Gdeisat,
% "Fast two-dimensional phase-unwrapping algorithm based on sorting by 
% reliability following a noncontinuous path", Applied Optics, Vol. 41,
% close all
unwrapped_phase = unwrap_phase(angle(Uobj));
%unwrapped_phase=unwrapped_phase(1:1100,:);
figure;imagesc(unwrapped_phase),colormap(gray),axis square,axis off,title('Unwrapped phase');
```
Appendix B: Alignment Protocol procedure for FB-based DHM system

Fig. B1 Proposed common-path digital holographic microscopy based on an FB. (a) Schematic of the system. One of the object replicas is spatially filtered to remove all the object information.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Component</th>
<th>Thorlabs part number</th>
<th>Basler part number</th>
<th>Newlight Photonics</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Construction Rail, 1000mm</td>
<td>XT66-1000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>30mm Cage Plate to XT66 Rail Adapter</td>
<td>RCA1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Collimated laser-diode-pumped DPSS laser module with power supply, 532 nm, up to 4.5mW</td>
<td>CPS532</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Laser diode mount</td>
<td>KAD11F</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>f=125 mm, Ø1” Achromatic Doublet, SM1-Threaded Mount, ARC: 400-700 nm</td>
<td>AC254-125-A-ML</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Ace-IMX 183, 1inch, C-Mount, 5472x3648 px², 2.4µm² pixel size, 17fps, Mono, CMOS, USB 3.0, Rolling Shutter</td>
<td>-</td>
<td>acA5472-17um</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Adapter with External SM1 Threads and Internal RMS Threads</td>
<td>SM1A3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>f=200 mm, Ø1” Achromatic Doublet, ARC: 400-700 nm</td>
<td>AC254-200-A</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
To build the system, the following steps are needed:

**Step 1:** Insert a diode laser. Align the collimated beam emerging the laser using an alignment target.

**Step 2:** Insert the lens L2 of focal length 125 mm. Align the transverse coordinates of the lens using the alignment target. Leave enough space (approx. 1 meter before L2 to insert the other optical elements).
Step 3: Insert the camera after the L2. Although the camera position is not critical, to reduce the computational processes, the use of DHM systems operating at the image plane is recommended. This means that the camera must be placed at the back focal plane of the L2. To find this position, use the camera’s software. It is important to significantly reduce the intensity of the object beam to avoid pixel saturation on the camera, for instance, by inserting a neutral density filter in the optical path. The axial position of the camera can then be set by finding the narrower focus spot generated by the L2. After this position is found, the DHM system operates in the image-plane regime.

Step 4: Insert the lens L1 of focal length 125 mm. Align the transverse coordinates of the lens using the alignment target. The lenses L1 and L2 must form a telecentric system. Thus, the distance between them is the sum of their focal lengths. Align the axial position of the L1 so that the beam emerging from the L2 is a collimated plane wave. This means that the back focal plane of L1 (i.e., image focal plane) coincides with the front focal plane (i.e., object focal plane) of the L2. Insert the shearing interferometer after the L2, and axially displace the L1 until straight, parallel lines are observed in the shearing interferometer. Once the axial position of the L1 is determined, insert the alignment target after the L1 and verify its lateral alignment. Consider the re-alignment of the L1 lens if the beam is not centered on the alignment target.
Step 5: Insert the lens TL of focal length 200 mm. Align the transverse coordinates of the lens using the alignment target. The lenses TL and L1 must form a telecentric system. Thus, the distance between them is the sum of their focal lengths. Align the axial position of the TL so that the beam emerging from the L1 is a collimated plane wave. This means that the back focal plane of TL (i.e., image focal plane) coincides with the front focal plane (i.e., object focal plane) of the L1. Insert the shearing interferometer after the L1, and axially displace the TL until straight, parallel lines are observed in the shearing interferometer. Once the axial position of the TL is determined, insert the alignment target after the TL and L1 and verify its lateral alignment. Consider the re-alignment of the TL lens if the beam is not centered on the alignment target.

Step 6: Insert an infinity-corrected MO lens before the TL. To provide a linear shift-invariant DHM system, the MO and TL must form a telecentric system. Thus, the distance between them is the sum of their focal lengths. Note that the focal length of the MO can be estimated by $f_{MO} = \frac{f_{TL}}{M}$ where M is the lateral magnification displayed in the MO, and $f_{TL}$ is the focal length of the TL recommended by the manufacturer. For the most common MO manufacturers, $f_{TL} = 160$
mm for Zeiss, $f_{TLM} = 180$ mm for Olympus, and $f_{TLM} = 200$ mm for Nikon and Mitutoyo. Use the alignment target to align the MO lens. Align the axial position of the MO so that the beam emerging from the TL is a collimated plane wave. This means that the pupil plane of the MO coincides with the front focal plane (i.e., object focal plane) of the TL. Insert the shearing interferometer after the TL, and axially displace the MO until straight, parallel lines are observed in the shearing interferometer. Once the axial position of the MO is determined, insert the alignment target after the MO and verify its lateral alignment. Consider the re-alignment of the MO lens if the beam is not centered on the alignment target.

**Step 7:** Insert the Fresnel biprism between the TL and the camera. Find the axial position of the Fresnel biprism by observing the fringes’ field of view using the camera’s software. Displace axially the Fresnel biprism until the fringes’ field of view is maximum. The maximum fringes’ field of view should be equal to $L/2$ being $L$ the lateral extension of the Fresnel biprism. Assuming that $L = 20$ mm, the maximum fringes’ FOV is 10 mm. Fix the position of the Fresnel biprism.
**Step 8:** Although the interference angle between both plane waves emerging from the Fresnel biprism is fixed (i.e., period of the fringes cannot be tuned), we need to ensure that the biprism-based DHM system operates in an off-axis regime. The Fourier spectrum of the hologram, which is the interference pattern between the two replica plane beams, is composed of three terms: DC term and ±1 terms. For telecentric DHM systems, the compact support of these terms is related to the numerical aperture (NA) of the MO lens and the effective magnification of the telecentric MO-TL imaging system, $M_{\text{eff}} = f_{\text{TL}}/f_{\text{MO}}$, which can be expressed in terms of the objective specifications as $M_{\text{eff}} = (f_{\text{TL}} M)/f_{\text{TL},\text{M}}$. Particularly, the compact support of these terms is $(2\text{NA})/(\lambda M_{\text{eff}})$ for the DC term, and $\text{NA}/(\lambda M_{\text{eff}})$ for the ±1 terms. The DC term is always centered at the Fourier spectrum, while the position of the ±1 terms depends on the interference angle of the object and reference beams. The DHM system operates in an off-axis regime if there is no overlap between the different components of the hologram spectrum. Note that, in off-axis DHM, the better optimization of the finite space bandwidth of the sensor is achieved when the ±1 orders are placed along the diagonal of the camera’s space bandwidth, which is at 45 degrees, allowing their optimal allocation with no overlapping. Whenever possible, consider rotate the biprism to set the components in the hologram’s spectrum at 45 degrees.

![Angle Comparison](image)

**Step 9:** Fine adjustment of the telecentric MO-TL configuration. Knowing that the Fourier transform of a collimated (plane) beam is a Delta function, the telecentric configuration can be
verified by observing the Fourier spectrum of the interference between the object and reference waves. Visualizing the center of the ±1 term, the position of the MO can be finely adjusted. The experimental DHM system operates in the telecentric regime if and only if the center of the ±1 term is a maximum peak. This condition must be verified. If there is more than one maximum peak in the Fourier spectrum, the axial position of the MO must be adjusted. If such adjustment of the MO is needed, its alignment must be verified.

**Step 10:** Filtering one of the beam emerging from L1 lens. Insert a pinhole mounted onto a 3D stage between the L1 and the L2 (closer to the Fourier plane). Align the pinhole by observing the fringes’ contrast on the camera as well as the maximum intensity emerging from the pinhole. Align the pinhole by setting up a 4F system between the pinhole and the camera. Replace the L2 with 100mm lens, and thus image from pinhole should form on the camera. Remove L1 and the FB to illuminate the pinhole with a plane wave. Adjust the axial position of the pinhole till a clear image forms on the camera.
Step 11: Start by moving the mounted pinhole between L1 and L2 where the beam converges to a smallest point. Move the pinhole to where it overlaps the beam of the system. Turn the pinhole to the largest hole size, 2000um. Move the mounted pinhole until one of the beams passes through the large pinhole size. There should be two beams as a result of the FB; 1. that is filtered through the 2000um pinhole and 2. the other beam maybe blocked by another part of the pinhole or off a skew permeating the edge of the pinhole. Rotate the FB to 45 degrees direction until both beams reaches the camera. Align the pinhole by blocking one path (reference) and centering the other beam where it passes through to the camera using a uniform light. Move the pinhole position laterally to achieve a uniform light where the spot has a consistent intensity of light piercing the pinhole. A uniform light is needed and not a spot of light to achieve a correctly filtered image.

![Uniformity Light Beam](image_a) ![Spot of Light Beam](image_b)

Step 12: Start with rotating the pinhole back to the largest size of 2000um. To align this size of the pinhole, move the x and y axis to centralize the beam filtering through the pinhole. Check the
lateral axis to make sure that both beams have a uniform consistency of light filling similar in both beams shown in image and video. Repeat the same steps of rotating the pinhole from 2000um to 25um and move the x and y axis accordingly to align the beam through each of the pinhole. Skip the pinholes noted with a ring.

Step 13: Insert the sample stage between the laser and the MO lens. After the implementation of the FB-based DHM system, the system’s temporal stability, accuracy, and resolution limit must be evaluated by imaging a calibrated phase target. For the verification of the imaging capabilities, use of the phase target from Benchmark Technologies is recommended, since it is the first commercially-available quantitative phase target for the evaluation of phase imaging systems. This target has seven distinct feature heights from 50 nm to 350 nm containing familiar microscopy targets such as a focus star with a 400-nm pitch, and a USAF resolution target whose smallest resolvable elements are 274 nm. By reconstructing the phase image of the USAF target, the experimental resolution limit can be estimated as its smallest resolved element. Additionally, the phase image of the USAF target can be used to verify if the DHM system operates at the diffraction limit, comparing the agreement between the experimental and theoretical resolution limits. For coherent imaging systems, the theoretical resolution limit is λ/NA. Alternatively, the experimental resolution limit can also be estimated by imaging a star test from the phase target. Using the star target, the experimental resolution limit is determined by the minimum resolvable star pattern. For
this evaluation, since there is not a calibrated table as in the USAF target, the lateral magnification of the imaging system must be determined. The experimental measurement of the lateral magnification can be done by imaging a micrometer (R1L3S2P, Thorlabs). However, one can use the theoretical value of the magnification, $M_{\text{eff}} = (f_{\text{TL}} M)/f_{\text{TM}}$, since the error difference between the experimental and theoretical values, which is usually less than 5%, is negligible.

**Step 14:** The FB-based DHM system without problems with the sparsity is ready to go!

**Note 1:** If one changes the MO lens, it is needed to change the distance of the MO and TL lenses and ensure that the new imaging system operates in the telecentric regime (Steps 4 & 7)

**Note 2:** If one wants to illuminate with linear polarization, insert a linear polarizer (LPVISE100-A, Thorlabs) mounted on a motorized cage rotator (K10CR1/M, Thorlabs) between the illumination source and the sample stage.

**Note 3:** Make sure to align the pinhole by scanning it back and forth along the optical axis till the filtered image fills the frame. i.e., the size of filtered image is the same as size of unfiltered image at L2. i.e., The filter has no spatial effect but only a Fourier effect.
Appendix C: Alignment Protocol to build a FB-based DHM system for transmissive and reflective microscopic samples

This appendix provides supplementary information to build a dual-mode telecentric-based digital holographic microscope. Here, we include the list of optical and optomechanical components from Thorlabs, one of the major vendors, and the optical schematics for each alignment step.

C1. Optical and Optomechanical Components

To ease the implementation of the dual-model common-path DHM system using a Fresnel biprism, Table C1 is the list of the components required to construct the system using products from Thorlabs, which is one of the major vendors of optical elements and optomechanical components. In the list, the camera and Fresnel biprism are purchased through Edmund Optics and Newlight Photonics, respectively.

Table C1. List of hardware to implement the dual-mode common-path DHM system.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Component</th>
<th>Part number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Construction Rail, 500 mm length</td>
<td>XT66-500</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>6</td>
<td>XT66 Horizontal Mounting plate</td>
<td>XT66P3</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>2</td>
<td>Collimated laser-diode-pumped DPSS laser module with power supply, 532 nm, up to 4.5mW</td>
<td>CPS532</td>
<td>Thorlabs</td>
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<tr>
<td>2</td>
<td>Laser diode mount</td>
<td>KAD11F</td>
<td>Thorlabs</td>
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<tr>
<td>9</td>
<td>SM1-Threaded 30 mm Cage Plate for 66 mm Rails</td>
<td>RCA1</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>2</td>
<td>Snap-On 30 mm Cage Mounting Bracket for 66 mm Rails</td>
<td>RCA2</td>
<td>Thorlabs</td>
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<tr>
<td>1</td>
<td>Adapter with External M25.5x0.5 Threads and Internal SM1 Threads for Nikon microscope objective lenses</td>
<td>SM1A25</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>1</td>
<td>40x Nikon Plan Fluorite Imaging Objective, 0.75 NA, 0.66 mm WD</td>
<td>N40X-PF</td>
<td>Thorlabs</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Part Number</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------</td>
<td>------------------</td>
</tr>
<tr>
<td>1</td>
<td>f=200 mm, Ø1” Achromatic Doublet, ARC: 400-700 nm</td>
<td>AC254-200-A-ML</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>2</td>
<td>f=75mm, Ø1” Achromatic Doublet, SM1-Threaded Mount, ARC: 400-700 nm</td>
<td>AC254-075-A-ML</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>1</td>
<td>Ø1” Mounted Pinhole, 30 ± 2 µm Pinhole Diameter, Stainless Steel</td>
<td>P30K</td>
<td>Thorlabs</td>
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<tr>
<td>1</td>
<td>XY Translator with Micrometer Drives, Metric, for aligning the lateral position of the pinhole</td>
<td>ST1XY-S/M</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>1</td>
<td>Ace-IMX 183, 1 inch, C-Mount, 5472x3648 px², 2.4µm² pixel size, 17fps, Mono, CMOS, USB 3.0, Rolling Shutter</td>
<td>11-502</td>
<td>Edmund Optics</td>
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<tr>
<td>1</td>
<td>Adapter with External C-Mount Threads and External SM1 Threads for mounting the camera</td>
<td>SM1A39</td>
<td>Thorlabs</td>
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<tr>
<td>1</td>
<td>BK7 Fresnel prism 20x20x1 mm apex angle 170 deg</td>
<td>FBP2020G-170</td>
<td>Newlight Photonics</td>
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<td>1</td>
<td>25x36 mm 50:50 UVFS Plate Beamsplitter, Coating: 400 - 700 nm, t = 1 mm</td>
<td>BSW10R</td>
<td>Thorlabs</td>
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<td>30 mm Cage Cube with Filter Mount (Metric)</td>
<td>CM1-DCH/M</td>
<td>Thorlabs</td>
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<tr>
<td>1</td>
<td>48.6 mmx48.6 mm Kinematic Platform Mount for mounting the plate beamsplitter</td>
<td>KM100B/M</td>
<td>Thorlabs</td>
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<tr>
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<td>Small Adjustable Clamping Arm, M4 x 0.7 Threaded Post</td>
<td>PM3/M</td>
<td>Thorlabs</td>
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<tr>
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<td>Extension Post for PM3/M Clamping Arm, M4 x 0.7 Threaded</td>
<td>PM3SP/M</td>
<td>Thorlabs</td>
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<tr>
<td>1</td>
<td>Universal Post Holder Adapter</td>
<td>UPHA</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>1</td>
<td>Ø12.7 mm Aluminum Post, M4 Setscrew, M6 Tap, L = 150 mm</td>
<td>TRA150/M</td>
<td>Thorlabs</td>
</tr>
<tr>
<td>1</td>
<td>10x Beam Expander, 400-650nm</td>
<td>GBE10-A</td>
<td>Thorlabs</td>
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<tr>
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<td>Adapter with External SM2 Threads and Internal M43 x 0.5 Threads</td>
<td>SM2A30</td>
<td>Thorlabs</td>
</tr>
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<td>60 mm Cage Plate, SM2 Threads, 0.5” Thick, M4 Tap (Two SM2RR Retaining Rings Included)</td>
<td>LCP01/M</td>
<td>Thorlabs</td>
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<tr>
<td>Quantity</td>
<td>Description</td>
<td>Code/Manufacturer</td>
<td></td>
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<td>----------</td>
<td>-------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cage Assembly Rod, 1” Long, Ø6 mm for mounting the beam expander</td>
<td>ER1 Thorlabs</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cage Assembly Rod, 6” Long, Ø6 mm, 4 Pack</td>
<td>ER6-P4 Thorlabs</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cage Assembly Rod, 3” Long, Ø6 mm, 4 Pack</td>
<td>ER3-P4 Thorlabs</td>
<td></td>
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<tr>
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<td>Cage Assembly Rod, 1.5” Long, Ø6 mm, 4 Pack (auxiliary for building the cage)</td>
<td>ER1.5-P4 Thorlabs</td>
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<tr>
<td>1</td>
<td>XYZ Translation Stage, 50 mm Travel, Metric – sample stage</td>
<td>LT3/M Thorlabs</td>
<td></td>
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<tr>
<td>1</td>
<td>Ø12.7 mm Aluminum Post, M4 Setscrew, M6 Tap, L = 100 mm – sample stage</td>
<td>TRA100/M Thorlabs</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Quick-Release Rectangular Filter Holder – sample stage</td>
<td>SFH2 Thorlabs</td>
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<tr>
<td>2</td>
<td>SM1-Threaded 30 mm Cage Plate for 66 mm Rails – for aligning the system</td>
<td>RCA1 Thorlabs</td>
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<tr>
<td>2</td>
<td>SM1 Series Alignment Disk – for aligning the system</td>
<td>SM1A7 Thorlabs</td>
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<td>Shear Plate, 2.5-5 mm Beam Diameter – for setting the lenses in afocal configuration</td>
<td>SI050P Thorlabs</td>
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<tr>
<td>1</td>
<td>Shearing Interferometer with a 10-25.4 mm Beam Diameter Shear Plate – for setting the lenses in afocal configuration</td>
<td>SI254 Thorlabs</td>
<td></td>
</tr>
</tbody>
</table>

**C2. Alignment Protocol of the Dual-mode Digital Holographic Microscopy using a Fresnel biprism**

For every optical element, the general alignment procedure consists of checking the lateral and vertical alignment as well as the tilt. For this verification, two alignment targets are used: one target is placed just behind the element to be aligned, and the second target is placed as far as possible of the beam path. After inserting the optical element, one must adjust the height of the element to ensure that the beam comes out vertically level on both alignment targets; this means that the beam is centered vertically on the optical element. Next, one must align laterally the optical element by
sliding the optical element laterally across the optical axis to align the beam on the first target, the closest one to the element, as well as adjusting the tilt of the optical element to align the beam on the second target, the furthest one to the element. The lateral alignment is an iterative process as changing the lateral position, and the tilt of the element affect the lateral position of the beam in the two targets. Once the beam is laterally centered on both targets, the optical element will be horizontally and vertically centered and orthogonal to the optical axis (i.e., no tilt). One can alternatively use irises instead of alignment targets. As one inserts new optical elements (e.g., mirrors and lenses), two irises should be placed. To build the system, the following steps are needed:

**Step 0:** Mount the optical rails of the transmission-based illumination path onto an optical table.

Leave around xxx mm between both rails.

**Step 1 – Illumination source of the transmission-based DHM system.** Insert a laser source.

The laser source should be mounted in a kinematic mount that allows the control of the tilt.

Set the two alignment targets along the optical rails to align the laser; one should be closer to the laser head, whereas the other should be placed at the end of the second construction rail. Ensure that the collimated beam emerging of the laser is straight and parallel to the optical axis defined by these two alignment targets.
**Step 2 – L2 lens from the 4f system.** Insert the lens L2 of focal length 75 mm. Align the transverse position of the lens using two alignment targets. Insert the L2 lens approximately 920 mm from the beginning of the construction rail.

![Diagram of Step 2](image)

**Step 3 – Camera.** Insert the camera after the L2 lens. Although the camera position is not critical, to reduce the computational processes, the use of DHM systems operating at the image plane is recommended. This means that the camera must be placed at the back focal plane of the L2 lens. To find this position, use the camera’s software. It is important to significantly reduce the intensity of the object beam to avoid pixel saturation on the camera, for instance, by inserting a neutral density filter in the optical path. The axial position of the camera can then be set by finding the narrower focus spot generated by the L2 lens. After this position is found, the DHM system operates in the image-plane regime.

![Diagram of Step 3](image)

**Step 4 – L1 lens from the 4f system.** Insert the lens L1 lens of focal length 75 mm. Align the transverse coordinates of the lens using the alignment targets. The lenses L1 and L2 must form an afocal system. Thus, the distance between them is the sum of their focal lengths. Align the axial position of the L1 so that the beam emerging from the L2 lens is a collimated plane wave. This
means that the back focal plane (i.e., image focal plane) of L1 lens coincides with the front focal plane (i.e., object focal plane) of the L2 lens. Insert the shearing interferometer (SI050P) after the L2 lens, and axially displace the L1 lens until straight, parallel lines are observed in the shearing interferometer. Once the axial position of the L1 lens is determined, insert the alignment target after the L1 and verify its lateral alignment. Consider the re-alignment of the L1 lens if the beam is not centered on the alignment target. Connect the components L1, L2 and sensor using rods.

**Step 5 – TL lens:** Insert the TL lens of focal length 200 mm. Insert the TL lens as close as possible of the end of the first construction rail. Align the transverse coordinates of the lens using the alignment targets. The lenses TL and L1 must form an afocal system. Thus, the back focal plane of TL lens coincides with the front focal plane of the L1 lens. Unfortunately, we cannot use the shearing interferometer after the L1 lens because the beam size is too small. The beam diameter after L1 lens is approximately equal to $3.5\text{mm}/(M_{\text{TL-L1}}) = 1.3125 \text{ mm}$ being $M_{\text{TL-L1}} = f_{\text{L1}}/f_{\text{TL}} = 75/200 = 0.375$. Therefore, an alternative approach is the insertion of a mirror such that the reflected beam is projected to the furthest wall. Then, we axially displace the TL lens until the smallest focus spot is observed on the wall. Once the axial position of the TL lens is determined, insert the alignment target after the TL and L1 lens to verify the lateral alignment of TL lens.
Step 6 – MO lens. Insert an infinity-corrected MO lens before the TL lens. To provide a linear shift-invariant DHM system, the MO and TL must form a telecentric system. Thus, the distance between them is the sum of their focal lengths. Note that the focal length of the MO can be estimated by \( f_{MO} = \frac{f_{TLM}}{M} \) where \( M \) is the lateral magnification displayed in the MO, and \( f_{TLM} \) is the focal length of the TL recommended by the manufacturer. For a Nikon MO lens, \( f_{TLM} = 200 \) mm. Use the alignment target to align the MO lens. Align the axial position of the MO so that the beam emerging from the TL is a collimated plane wave. This means that the pupil plane of the MO lens coincides with the front focal plane of the TL lens. Insert a shearing interferometer (SI254) after the TL lens, and axially displace the MO lens until straight, parallel lines are observed in the shearing interferometer. Once the axial position of the MO lens is determined, insert the alignment target after the MO and verify its lateral alignment.

Step 7 – Fresnel biprism. Insert the Fresnel biprism between the TL and L1 lenses. The Fresnel biprism is inserted within a 3D-printed mount which is screwed into a RCA1 mount. Find the axial position of the Fresnel biprism by observing the fringes’ field of view using the camera’s software. Displace axially the Fresnel biprism until the fringes’ field of view is maximum. The maximum fringes’ field of view should be equal to \( L/2 \) being \( L \) the lateral extension of the Fresnel biprism. If \( L = 20 \) mm, the maximum fringes’ FOV should be around 10 mm. Fix the position of the Fresnel biprism.
**Step 8 – Off-axis configuration.** Although the interference angle between both plane waves emerging from the Fresnel biprism is fixed (i.e., period of the fringes cannot be tuned), we need to ensure that the biprism-based DHM system operates in an off-axis regime. The DHM system operates in an off-axis regime if there is no overlap between the different components of the hologram spectrum. The Fourier spectrum of the hologram is composed of three terms: DC term and ±1 terms. For telecentric DHM systems, the compact support of these terms is related to the numerical aperture (NA) of the MO lens and the effective magnification of the telecentric MO-TL imaging system, $M_{\text{eff}} = f_{\text{TL}}/f_{\text{MO}}$, which can be expressed in terms of the objective specifications as $M_{\text{eff}} = (f_{\text{TL}} M)/f_{\text{TLM}}$. Particularly, the compact support of these terms is $(2\text{NA})/\lambda M_{\text{eff}}$ for the DC term, and $\text{NA}/(\lambda M_{\text{eff}})$ for the ±1 terms. The DC term is always centered at the Fourier spectrum, while the position of the ±1 terms depends on the interference angle of the object and reference beams. Note that, in off-axis DHM, the better optimization of the finite space bandwidth of the sensor is achieved when the ±1 orders are placed along the diagonal of the camera’s space bandwidth, which is at 45 degrees, allowing their optimal allocation with no overlapping. Whenever possible, consider rotate the biprism to set the components in the hologram’s spectrum at 45 degrees.
**Step 9 – Fine adjustment of the telecentric MO-TL configuration.** Knowing that the Fourier transform of a collimated (plane) beam is a Delta function, the telecentric configuration can be verified by observing the Fourier spectrum of the interference between the object and reference waves. Visualizing the center of the ±1 term, the position of the MO can be finely adjusted. The experimental DHM system operates in the telecentric regime if and only if the center of the ±1 term is a maximum peak. This condition must be verified. If there is more than one maximum peak in the Fourier spectrum, the axial position of the MO must be adjusted. If such adjustment of the MO is needed, its alignment must be verified.

![FT Hologram](image)

**Step 10 – Setting of the spatial filtering system (e.g., pinhole).** Remove the rods between the L1-L2-Camera components. Insert the 30-µm pinhole mounted onto a XY translation stage. Drill a manual hole to avoid the spatial filtering of one of the beams emerging from L1 lens (e.g., no filter of the object beam, only the reference beam). Connect the XY translational stage using rods to the RCA2 mounting brackets for 66-mm construction rails. Insert the pinhole between the L1 and the L2 lens as closest as possible to the Fourier plane (e.g., axial plane where the narrowest
focus spots are found onto the pinhole mount). Block the object beam. Course lateral alignment of the pinhole by the horizontal and vertical micrometers of the translational stage until the beam emerging the pinhole is laterally uniform (e.g., a uniform plane wave).

![Diagram of optical setup](image)

Note that a uniform beam is needed, not a spot of light. If you find a spot, please move the axial position of the pinhole mount. Verify that the lateral alignment of the pinhole. Allow the object beam to interference with the object beam. Make sure that the manual hole is big enough, so the object beam is not clipped. Connect L1-Pinhole-L2-Camera mounts using rods.

This is the two object replicas with correct spatial filtering. Note that the image of the star information has been filtered out in the reference replica. You can watch these videos to learn more about how to filter a beam: [https://www.youtube.com/watch?v=-fmMCMQfXLo](https://www.youtube.com/watch?v=-fmMCMQfXLo) and [https://www.youtube.com/watch?v=WaAkwVRc7Is](https://www.youtube.com/watch?v=WaAkwVRc7Is).
Step 13 – Verification of the performance of the transmission-based DHM system. Insert the sample stage between the laser and the MO lens. After the implementation of the FB-based DHM system, the system’s accuracy and resolution limit must be evaluated by imaging a calibrated phase target. For the verification of the imaging capabilities, use of the phase target from Benchmark Technologies is recommended, since it is the first commercially-available quantitative phase target for the evaluation of phase imaging systems. This target has seven distinct feature heights from 50 nm to 350 nm containing familiar microscopy targets such as a focus star with a 400-nm pitch, and a USAF resolution target whose smallest resolvable elements are 274 nm. By reconstructing the phase image of the USAF target, the experimental resolution limit can be estimated as its smallest resolved element. Additionally, the phase image of the USAF target can be used to verify if the DHM system operates at the diffraction limit, comparing the agreement between the experimental and theoretical resolution limits. For coherent imaging systems, the theoretical resolution limit is $\lambda/NA$. Alternatively, the experimental resolution limit can also be estimated by imaging a star test from the phase target. Using the star target, the experimental resolution limit is determined by the minimum resolvable star pattern. For this evaluation, since there is not a calibrated table as in the USAF target, the lateral magnification of the imaging system must be determined. The experimental measurement of the lateral magnification can be done by imaging a micrometer (R1L3S2P, Thorlabs). However, one can use the theoretical value of the magnification, $M_{\text{eff}} = (f_{\text{TL}}$.
$M/f_{TLM}$, since the error difference between the experimental and theoretical values, which is usually less than 5%, is negligible.

**Step 14:** Mount the optical rail of the reflection-based illumination path onto an optical table.

**Step 15 – Illumination source of the reflection-based DHM system.** Insert a laser source. The laser source should be mounted in a kinematic mount that allows the control of the tilt. Set the two alignment targets along the optical rails to align the laser; one should be closer to the laser head, whereas the other should be placed at the end of the second construction rail. Ensure that the collimated beam emerging of the laser is straight and parallel to the optical axis defined by these two alignment targets.
**Step 16 - Reflection of the illumination beam.** Insert the plate beamsplitter (BS) between the TL and FB. Ensure that the reflected beam is going through the TL and MO imaging system. Course adjustment of the beamsplitter ensuring that the reflected beam is not clipped by adjusting the height and horizontal position of the beamsplitter. Set an alignment target closer to the beamsplitter. The second alignment target should be located as closer as possible to the transmission-based illumination source. The beamsplitter must be set at a 45 degrees angle. Change the height of the beamsplitter, ensuring that the illumination beam from the reflection path impacts roughly in the center of it. Set beamsplitter’s tilt so that the reflected beam is centered in the furthest alignment target. Now align the lateral position of the beamsplitter centering the beam in the closest alignment target. Slide the beamsplitter to center horizontally the beam on the first target. Then, change the horizontal tilt of the beamsplitter to set the beam in the second target. Repeat this procedure until the reflected beam is aligned in both irises.
Step 16 – Increase the reflected beam diameter. Insert the 10× beam expander (BE) between the laser and beamsplitter. To align the beam, insert the alignment targets after the beam expander (same positions as in step 15). Adjust the beam expander until the beam is centered.

Step 17 – Fine tune of the collimation in the beam expander. Insert a plane mirror as the object. Adjust the sliding lens so that the beam reflected from the plane mirror and passing through the MO and TL lenses is collimated. Insert the shear plate after the TL lens (right before the Fresnel biprism) and ensure that we observe straight, parallel fringes. In the event that this is not possible, insert of an additional mirror such that the emerging beam from the MO lens is projected to the
furthest wall. Then, we axially displace the sliding lens until the smallest focus spot is observed on the wall.

**Step 18 – Verification of the performance of the reflection-based DHM system.** Insert a reflective sample at the working distance of the MO lens. Verify that the 30-µm pinhole mounted is aligned to the reflection-based imaging modality. If it is not, fine tune the position of the beamsplitter or the beam expander. Another alternative is aligning the pinhole for the reflection-based imaging modality. But, be aware that every time that you changes the imaging modality, there is need to adjust the lateral position of the pinhole. For the verification of the imaging capabilities, use of the high-resolution USAF target in which you can add a 40-nm reflective layer. Read the manuscript for more details.