HOLOGRAPHIC FAST GRADIENT LIGHT INTERFERENCE MICROSCOPY (HF-GLIM)

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Abstract

Quantitative phase imaging has been an emerging technique in the field of biomedical imaging. As one type, gradient light interference microscopy (GLIM) is used to retrieve a quantitative gradient mapping of the phase delay that is produced by a biological sample. The method involves collecting four phase-shifted frames for a single field of view, which ultimately combines arithmetically into phase gradient information. The phase-shifting is done by a space light modulator (SLM) which intakes an appropriate voltage that converts into a corresponding phase retardation in the image field. Despite GLIM’s ability to generate a quantitative phase gradient of a sample, such module depends heavily on the operational speed of the SLM.

The holographic fast gradient light interference microscopy (HF-GLIM) aims to retrieve a field of view with only a single image from the conventional differential interference contrast microscope (DIC), and is sent to a tilted Sagnac interferometer to create a holographic output at the camera. Through digital processing of the Hologram using Fourier filtering, the phase gradient that is given by the sample can be retrieved only through a single recording instance from the HF-GLIM.

The publication aims to derive the functionality of HF-GLIM utilizing a mathematical derivation, geometrical analysis, and the simulation results.

Subject Keywords: Holography; Quantitative phase imaging; Sagnac interferometer; Gradient light interference microscopy; Differential interference contrast microscopy
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Contents

1 Introduction .............................................................................................................................. 1
  1.1 Evolution of biomedical imaging from phase contrast to QPI ............................................. 1
  1.2 Traditional vs common-path interferometry ..................................................................... 1
  1.3 Off-axis and phase-shifting reconstruction of phase .......................................................... 3
  1.4 Proposed research ............................................................................................................. 5

2 Literature Review ................................................................................................................... 6
  2.1 Gradient Light Interference Microscopy (GLIM) ............................................................... 6
  2.2 Digital holography for phase retrieval ............................................................................... 8
    2.2.1 General introduction to off-axis holography ................................................................. 8
    2.2.2 Digital holographic microscope (DHM) ..................................................................... 12
    2.2.3 Diffraction phase microscopy (DPM) ......................................................................... 14

3 Research Method .................................................................................................................... 17
  3.1 Testing of reduced Sagnac interferometer ....................................................................... 17
  3.2 Mathematical derivation of HF-GLIM’s function ............................................................... 19
  3.3 Configuration of HF-GLIM ............................................................................................... 21
    3.3.1 Differential interference contrast (DIC) microscopy ............................................... 21
    3.3.2 Polarization-dependent Sagnac interferometer ......................................................... 23
    3.3.3 Holography Correction System ............................................................................... 24

4 Research Results and Future Implications ........................................................................... 25
  4.1 Simulated result of HF-GLIM ........................................................................................... 25
  4.2 Digital filter as the noise source ....................................................................................... 27
  4.3 Run-time for creating gradient frame .............................................................................. 28

5 Conclusion ............................................................................................................................. 30
1 Introduction

1.1 Evolution of biomedical imaging from phase contrast to QPI

Microscopy has been the most sought-after tool for visualizing small and rapid biological activities with precision. Nevertheless, certain biological samples including cells and tissues appear to have a very low intensity contrast in a regular bright-field microscope due to their low absorption [1, 2]. Moreover, the scattering profile, as the dominant source of spatial contrast in biological samples, does not come from the amplitude, but rather from the phase, [3] which is not detected from an intensity profile.

With dark-field microscopy as the pioneer, a myriad of phase contrast microscopy techniques capitalized on the fact that the phase shift due to the property of the cells of interest is a source of contrast that does not rely on the exogenous staining [4, 5]. Phase contrast methods thereby overcome the challenges that are faced by fluorescent imaging, such as photobleaching and phototoxicity [6-8]. The two major techniques of phase contrast techniques, Zernike’s phase contrast microscopy [9] and differential interference contrast microscopy, yield outputs that are relevant to physical property of the sample [2, 10]. Despite a significant progress, the primitive phase contrast methods were rather qualitative than quantitative and thus only gives a qualitative intensity image only related to the phase shift induced [1, 3, 4, 11]. In response to previous concerns, QPI, short for quantitative phase imaging, has emerged in recent decades as a core imaging technique for quantitatively analyzing samples with low absorption and refraction from precisely measured phase shift induced by optical path difference [4, 12, 13]. Retrieval of sample information can not only help making numeral assessment but also enhance the qualitative in-vitro assessment of cells [14] and tissues as well [15].

Many intermediate products of the QPI technique such as the gradient field microscopy (GFM) [16], digital holographic microscopy (DHM) [17], Fourier phase microscopy (FPM) [18] and the diffraction phase microscopy (DPM) [19] have been used by various microscopy groups to retrieve phase delay by each point in a cell. Eventually, quantitative light imaging group in University of Illinois at Urbana-Champaign proposed spatial light interference microscopy (SLIM) [8] and gradient light interference microscopy (GLIM) [20] as a new QPI method for imaging biological samples. One significant advantage of QPI is that it does not require manufacturing a brand-new microscope for QPI. In other words, QPI is merely an optics add-on that follows the side-port of a conventional microscope [14].

1.2 Traditional vs common-path interferometry

Interferometry has been used in a plethora of fields to translate optical information into a mechanical change that is given to a system of interest. A difference in optical pathlength (either temporally or spatially) between two spatially isolated beams interfere back to create a certain interference fringe. The frequency of such fringe provides information about the spatial or temporal offset between two split beams depending on which geometry for interference is implemented. Figure 1.1 illustrates the setup of one type of an interferometer, Michaelson interferometer.
The Michaelson interferometer, as shown in Figure 1.1, involves two split beams that interact with two isolated optical paths while split. Such geometry creates a temporally unstable interference pattern due to a temporally stochastic fluctuation in space that may be different in two paths that cannot be canceled upon interference, thus leading to a low SNR (signal-to-noise ratio) [21].

On the other hand, common-path interferometry involves the interference of two split beams that travel in completely or almost identical geometrical paths, as the name suggests. This means that the temporal phase noise from the mechanical fluctuation and the CCD is reduced, leading to sensitive phase measurement [6, 8, 13]. One of the few implementations that involves a common-path geometry is a Sagnac interferometer. The ring geometry of a Sagnac interferometer, as shown below in Figure 1.2, that is rotated by an increasing angular velocity $\Omega_0$ introduces a corresponding increase in the phase difference between two counter-propagating beams that are split by the beam splitter [22].
Although a rotation-aided design of the Sagnac interferometer is practical, the exact same set up also introduces mechanical instability in the optical alignment that hinders its applicability in optical imaging. However, a variation of the Sagnac interferometry that is rotation-free can be used in various contexts in imaging, as will be introduced in future sections.

### 1.3 Off-axis and phase-shifting reconstruction of phase

Any optical field can be represented as a complex function, associated with its amplitude and phase. However, any optical detector is only capable of detecting the intensity, which is directly proportional to the amplitude squared of the incoming field. From a single intensity image, it is difficult to infer the corresponding phase information of a field. Phase-shifting and off-axis holography each takes advantage of temporally and spatially offset interferometry to retrieve phase information from recorded intensity [6].

The first method that is primarily employed by GLIM and SLIM involves phase-shifting. The phase-shifting method involves performing a simple series of arithmetic to four replicas of an intensity image, each of which involves common-path interferometry between two incoming fields that are phase-shifted by $\frac{n\pi}{2}$ $\forall n \in [0,1,2,3]$ [1, 4, 7, 8, 11, 20, 23, 24]. The four images are then combined arithmetically as shown in Equation 1.1:

$$
\phi(r) = \arg \left( \frac{I\left(r; \frac{3\pi}{2}\right) - I\left(r; \frac{\pi}{2}\right)}{I(r; \pi) - I(r; 0)} \right) 
$$

where $\phi(r)$ is the mathematical expression for the reconstructed phase image and $I(r; \Delta\psi)$ is the given intensity image from the phase-shifting by $\Delta\psi$. The artificial phase shift $\Delta\psi$ is given by the space light modulator (SLM) [1, 23]. $\Delta\psi$ is quantitatively varied by applying a variable voltage by a driver [1]. Although the phase-shifting method is computationally quite simple, it requires four images to be taken.
per phase image reconstruction, each shift being controlled by the SLM. That is, the operational speed is limited not only by the exposure time and the frame rate but also by the operational speed of the SLM [25].

The second phase reconstruction method is the off-axis holography, aided by the Hilbert transform [4] [26]. This method is the core foundation of many traditional QPI techniques such as DPM and DHM [4]. Off-axis holography is performed by the interference of two different beams at the detector: an un-sheared sample beam that contains complex information about the scattered beam from the sample and a sheared reference beam with an almost uniform background [4, 17, 19].

Figure 1.3 illustrates the optical set up of DPM, a particular application that involves off-axis holography. A complex field containing sample scattering information is sent to a grating, from which many diffraction orders are sent out toward a 4f lens system with a spatially-filtering pinhole [5, 19, 27]. The pinhole is used to fully pass the 1st order diffracted beam to create a sample beam that travels straight along the optical axis of the CCD and to low-pass filter the un-diffracted beam, which becomes the reference beam that is sheared at some angle \( \theta \) respect to the optical axis into the CCD, where an intensity image is captured [26].

Further details regarding the method that is used to reconstruct a phase map digitally using the captured intensity will be discussed in the methods section. The core advantage of the off-axis method in phase reconstruction is that it only requires a single intensity image, whereas the previous method requires four replicas of the corresponding field of view [29-32]. When properly aligned, off-axis holography is capable of preserving both the amplitude and the phase information of the scattered field from the sample.
1.4 Proposed research

Holographic fast gradient light interference microscopy (HF-GLIM) combines a conventional DIC microscope with a different add-on from the traditional GLIM. The proposed renovation of GLIM involves the rotation-free Sagnac interferometer with a mechanically tunable tilt-angle between the cross-polarized components from the DIC to perform an off-axis holography to retrieve the phase gradient of the sample. HF-GLIM’s single-shot imaging aims to give the traditional GLIM a boost in its performance speed. The subsequent sections of the research will discuss a list of previous works around which the research is centered, and a simulation of fundamental concepts to buttress the plausibility of the declared modification to GLIM.

Following the introduction section, chapter two of the thesis will consist of literature review of past critical works which served as the building blocks to this publication. Chapter three will give mathematical derivation of HF-GLIM, as well as the optical circuit needed for its construction. Chapter four will present the simulated result from the HF-GLIM and discuss its strengths and still ongoing challenges. Lastly, chapter five concludes the thesis by giving overall summary of the presented work in the thesis.
2 Literature Review

2.1 Gradient Light Interference Microscopy (GLIM)
Gradient light interference microscopy is a relatively new technique in which a low coherence source is used to image thick biological samples in 2D or 3D and even in real-time. The GLIM module is connected to the side-port of an inverted DIC microscope. The authors of the GLIM publication used conventional microscopes from Olympus and Zeiss for their demonstration as shown below in Figure 2.1[1]. The left side of the image portrays a regular inverted microscope. The right side of Figure 2.1 is the GLIM module consisting of a 4f lens system with SLM installed in the Fourier plane right at the focal point between the two lenses. The authors removed the final analyzing polarizer at the end of the DIC optical configuration, and instead placed it before the camera to interfere the two sheared beams [1].

![Figure 2.1: Interface between conventional DIC microscope (left) and GLIM module (right) [1]](image)

The total cross-polarized complex field output from the DIC side port, and thus the input to the GLIM module is

$$U_T(r) = U(r)\hat{s} + U(r + \delta r)\hat{p} \quad (2.1)$$

where $U(r)$ and $U(r + \delta r)$ are two laterally sheared beams that are separated by $\delta r$ which is intrinsic to the microscope components that are responsible for giving a spatial offset between two orthogonal beams with $\hat{s}$ and $\hat{p}$ polarization [1, 33]. The beam with the total field, as shown in Equation 2.1, is first Fourier-transformed by the first lens and then phase-shifted by the SLM. The SLM in the GLIM module phase-shifts one polarization of two cross-polarized beams by $\frac{\pi n}{2}$ for $n \in [0, 1, 2, 3]$, as mentioned earlier. The result is then again Fourier-transformed back by the last lens before hitting the analyzer, and, finally, the camera. For each phase shift, the total field (not the intensity) that is detected by the camera is

$$I_T(r; n) = \left| U(r) + U(r + \delta r)e^{i\left(\frac{\pi n}{2}\right)} \right|^2 \quad (2.2)$$
where the complex exponential term $e^{i(n\pi/2)}$ denotes a phase shift by $\pi/2$. Note that the polarization of two image fields are now co-polarized and undergo coherent interference [1]. Further expansion of Equation 2.2 gives rise to Equation 2.3 which provides:

$$I_T(r) = |U(r)|^2 + |U(r + \delta r)e^{i(n\pi/2)} + U(r)U^*(r + \delta r)e^{-i(n\pi/2)} + U^*(r)U(r + \delta r)e^{i(n\pi/2)}$$

where the asterisk represents a complex conjugation of a field. Here, we further define two spatially separated beams as $U(r) \equiv A_1(r)e^{i\phi_1(r)}$ where $\phi_1(r) = k(r) \cdot r + \phi_1$ and $U(r + \delta r) \equiv A_2(r)e^{i\phi_2(r)}$ where $\phi_2(r) = k(r + \delta r) \cdot (r + \delta r) + \phi_2 + \frac{n\pi}{2}$. It is important to note that $A$ is an amplitude function which implies real and positive. By taking the two fields’ definitions into account, we arrive at the following expression after substitution:

$$I_T(r) = |A_1(r)|^2 + |A_2(r)|^2 + 2|A_1(r)||A_2(r)|\cos(\Delta \phi(r))$$

$$I_T(r) = I_1(r) + I_2(r) + 2|\sqrt{I_1(r)}I_2(r)} \cos(\Delta \phi(r))$$

where $\Delta \phi(r)$ is defined as $\phi_2(r) - \phi_1(r)$.

Lastly, the four intensity images go through simple arithmetic operations, as shown in the introduction section, to remove any dependence in intensity values $I_1(r)$ and $I_2(r)$. For a very small $\delta r = |\delta r|$ that is less than the size of the diffraction spot, the approximation $\Delta \phi(r) \approx V\phi(r)\delta r$ is reasonable for our calculation [1]. Using special trigonometric identities with shifts in phase, we can reconstruct the gradient of the phase, as shown in Equation 2.4:

$$V\phi(r) = \frac{\Delta \phi(r)}{\delta r} = \atan \left( \frac{I_T\left( r; \frac{3\pi}{2} \right) - I_T\left( r; \frac{\pi}{2} \right)}{I_T\left( r; \pi \right) - I_T\left( r; 0 \right)} \right)$$

where the second argument in the intensity that is followed after position vector $r$ is the SLM phase modulation between the two beams $U(r)$ and $U(r + \delta r)$. It is true that DIC output also resembles a gradient effect that depends on the difference in the phase between two adjacent spatial points [33], but the output is merely qualitative, unlike that of GLIM which precisely resembles the quantitative phase gradient.
From an optics point of view, GLIM’s usage of an incoherent light source limits the range of the optical path difference between the two interfering fields by less than a few microns [1]. Another way to frame this feature is that GLIM is capable of removing a significant amount of scattering that occurs due to the imaging environment. Due to its simple compact common-path geometry, GLIM reduces the temporal phase noise over the imaging time. GLIM’s capability to incorporate open-aperture imaging creates a high range of k-vectors that are incident on the sample, thus enhancing both the resolution and optical sectioning through a thick specimen. Spatial incoherence of the source reduces incoherent scattering noises from a thick sample such as tissues [1, 23].

![Figure 2.2: Time-lapse 3D tomography of He-la cell culture recorded by GLIM with time lapsing from t = 0 min (e) to t = 264 min (k) [1]](image)

A GLIM’s ability to be commercialized arises from its replicability and full automation. QPI group has rendered the GLIM software to automate stage movement, the SLM, and the camera which gives GLIM the ability to be used for real-time imaging over a long period of time without the presence of the user [1, 23]. Currently, GLIM is reported to be capable of imaging 10 images per second which corresponds to 40 frames per second for real-time imaging [1]. Despite its fast acquisition time owing to the low exposure time that is needed per frame, GLIM’s run-time remains limited by the phase shifting process by the SLM, which hinders a rapidly sampled real-time imaging [34]. Through the proposal of a single-shot GLIM, this problem is to be taken care of [35].

### 2.2 Digital holography for phase retrieval

#### 2.2.1 General introduction to off-axis holography

Holography was first discovered by Dennis Gabor [36]. Several years later, came up with off-axis holography (on which this publication will primarily focus) to isolate the virtual and real replicas upon
A very concise objective of holography is to store and project not only the amplitude but also the phase information of an object [4]. In other words, through holography, one can record the hologram coming from an object of interest by using a certain optical set-up and by reconstructing the complex field (both amplitude and phase) that was originally incident on the camera when recording. An optical configuration that is used to “write” a holography is shown in Figure 2.3 below. In the schematics, the sample is illuminated with a plane wave $U_0$ with a wave vector $k_0z$, where $z$ represents a unit vector across the horizontal direction. Simultaneously, another plane wave that is used as a reference beam is incident on the film with a wave vector $k_r$.

We will define $U_s(r) = U_0 e^{ik_0z \cdot t(x, y)}$ as the image field that is carrying both the attenuation and scattering information of the sample, and $U_r(r)e^{ik_r \cdot r}$ as the sheared reference beam. The tilted orientation of the reference beam directly translates to a phase modulation $e^{ik_r \cdot r}$ which in Fourier domain is a frequency shift [38]. If we take account of the schematics that are displayed in Figure 2.3, the total complex field that is incident on the film (or for our era, the camera) is defined as:

$$U_T(r) = U_r(r)e^{ik_r \cdot r} + U_s(r) \tag{2.5}$$

where the first term involving $U_r$ represents a modulated reference beam and the second term represents the sample beam that is incident along the optical axis of the CCD. The intensity that is detected by the film, however, will have more terms involving cross-conjugated products as shown in Equation 2.6

$$I_T(r) = I_r + I_s + U_re^{ik_x \cdot x} \cdot U_s^* + U_r^*e^{-ik_x \cdot x} \cdot U_s \tag{2.6}$$

where $I_r$ and $I_s$ are the DC (zero frequency) terms representing the intensity of the individual beams, and the last two terms involve the product of the cross-conjugate terms [4]. It is important to note that the k-vector must be projected onto the camera sensor, which means that only the transverse (x and y) projections of k are considered. For simplicity, we shear the reference only in respect to the x-z plane for projecting the hologram [37].

![Figure 2.3: Off-axis configuration used to record a hologram [4]](image-url)
a propagation in the z direction as shown in $k_x$. Here, we further define a completely uniform, un-scattered reference field as $U_r = |U_0|$, where $U_0$ is constant in space. Taking the necessary information into account, we arrive at:

$$I_p(r) = I_0 + |U_0|U_s^*e^{ik_xx} + |U_0|U_s e^{-ik_xx}$$

(2.7)

where $I_0 = I_r + I_s$ is a DC (low frequency) image, the second term $U_s^*|U_0|e^{ik_xx}$ is the virtual image, apparent from a backward propagation indicated by a complex conjugation [4], and the last term $|U_0|U_s e^{-ik_xx}$ is a real image that we are interested in retrieving. From a more qualitative standpoint, the image that is captured by the CCD is none other than a superposition of the DC term and a spatial sinusoid pattern with the spatial frequency $k_x$, as shown on the left side of Figure 2.4. After recording the hologram either by projecting onto a film or saving onto a computer, one can retrieve the complex sample image field by “reading” the hologram. One can retrieve the complex image field through either an analogue or digital method.

For the analogue reconstruction method, the hologram that is created by a sample field and sheared reference field, expressed by Equation 2.7, is projected onto the image plane as shown in Figure 2.5. Illuminating the image film with the same reference field that is used in the recording process results in a product between the reference field and the hologram as shown in Equation 2.8 [4]. Performing the product results in Equation 2.9.

$$U(r) = [I_0 + |U_0|U_s^*e^{ik_xx} + |U_0|U_s e^{-ik_xx} \cdot e^{ik_xx}$$

(2.8)

$$U(r) = I_0e^{ik_xx} + |U_0|U_s^*e^{ik_xx} + |U_0|U_s$$

(2.9)

This process is analogous to propagating a plane wave into a sample with a transfer function $t(x, y) = I_0 + |U_0|U_s^*e^{ik_xx} + |U_0|U_s e^{-ik_xx}$ [4]. What we can conclude from Equation 2.9 is that an observer who has no direct view of an object can perceive not only the color and the intensity of an image but also the depth information. For the interest of imaging, this indicates that one can extract phase information from an intensity image that is recorded by a microscope.
Figure 2.4: Intensity hologram captured by CCD (Left) and its Fourier transform (Right) [17]

Figure 2.5: Off-axis intensity consisting of a virtual image, a real image, and a direct image all demodulated by a complex exponential to bring real image to the z-axis [4]

Digital reconstruction of complex fields involves taking advantage of Hilbert transform [4, 31]. First, taking a Fourier transform of Equation 2.7 representing an intensity hologram recorded at the CCD yields the following expression:

\[
\tilde{I}_r(\kappa) = \tilde{I}_0(\kappa) + |U_0| \tilde{U}_s^*(-\kappa + k_x) + |U_0| \tilde{U}_s(\kappa - k_x)
\]  

(2.10)

where tilde accent represents the Fourier transform of the original function with \( \kappa = [k_x \quad k_y]^T \) representing the lateral k-space. The resulting Fourier spectrum is shown on the right side of Figure 2.5. The three distinct regions that are located in the FT image each correspond to the three different terms that are shown in Equation 2.10. A series of simple filtering processes in the Fourier space, as shown in the following paragraphs, is used to reconstruct the complex field from the sample [32].

By first applying the high-pass filter to remove the zero-frequency term \( \tilde{I}_0(\kappa) \), we arrive at Equation 2.11. Furthermore, by applying a frequency shift of Equation 2.11 by \(-k_x\), which is equivalent to circular shifting the whole image by that amount, we arrive at Equation 2.12. Now, we have the desired FT corresponding to the complex sample field at the DC [4].

\[
\tilde{I}_r(\kappa) = |U_0| \tilde{U}_s^*(-\kappa + k_x) + |U_0| \tilde{U}_s(\kappa - k_x)
\]  

(2.11)

\[
\tilde{I}_r(\kappa) = |U_0| \tilde{U}_s^*(-\kappa + 2k_x) + |U_0| \tilde{U}_s(\kappa)
\]  

(2.12)

Equation 2.13 sets forth the result of applying a low-pass filter to remove the first term from 2.12 corresponding to an unwanted virtual image. Notice that the decoupling of a virtual and real image is
quite simple as the initial recording process involves an off-axis, not in-line holography [18]. Finally, taking an inverse Fourier transform gives us the desired sample field \( U_s(r) \) with a constant factor in front as shown in Equation 2.14. Taking a two-dimensional inverse tangent of Equation 2.14 yields the phase that is associated with the complex field \( U_s(r) \), which fulfills our initial objective as shown in Equation 2.15.

\[
\tilde{I}_\tau(k_\perp) = |U_0|\tilde{U}_s(k_\perp)
\]

\[
\mathcal{F}^{-1}\{I_\tau(k_\perp)\} = |U_0|U_s(r)
\]

\[
\phi_s(r) = \tan^{-1}(|U_0|U_s(r))
\]

In Equation 2.15, \( \phi_s(r) \) contains the phase information of the complex sample field \( U_s(r) = |U_s(r)|e^{i\phi_s(r)} \). Inverse tangent functions can be performed on an image by using “angle” command followed by “unwrap” functions in MATLAB, for instance. Phase unwrapping is crucial to take the phase values beyond the \( 2\pi \) in to account [34].

### 2.2.2 Digital holographic microscopy (DHM)

Digital holographic microscopy (DHM) is an interferometric imaging technique that combines a standard magnifying optics configuration with an off-axis interferometry technique to retrieve the phase of the sample. Digital holographic microscopy takes advantage of either a Mach-Zehnder interferometer [39] or a Michaelson interferometer [17] depending on which mode of imaging the user desires: transmission or reflection, respectively [40]. The term “digital,” when referring to a digital holographic microscope, derives its meaning from the fact that the sample phase information is retrieved through a two-dimensional digital FFT (fast Fourier transform) as shown in the previous section.

The configuration in Figure 2.6 incorporates Mach-Zehnder geometry to capture the forward-scattering light [41]. The sample beam, labeled \( \mathbf{O} \) in Figure 2.6, that is transmitting out of the beam splitter is used to illuminate the sample through an objective system before arriving at the CCD. Contrarily, the reference beam, labeled \( \mathbf{R} \) in the same figure, does not hit any optical component except that the mirror in the path is slightly shifted upwards and is given more tilt in respect to the mirror in the other path. Slight modification to the reference mirror allows one to provide the sample beam and reference beam to arrive at the CCD with an off-axis geometry.
On the other hand, one may also record reflection (backward scattering) from a very thick specimen as shown in Figure 2.7 [17]. Figure 2.7 makes use of the Michaelson geometry to interfere a tilted reference image and an un-tilted sample image. In Figure 2.7, a laser source splits into two components upon exiting the beam-splitter. The reflected sample field that is labeled in red comes from the reflection from the reflective sample (i.e. distorted mirror) M1 and is magnified before hitting the CCD. The transmitted reference beam reflects off from a tilted mirror M2 and travels toward the CCD.

Aided by digital imaging processing, as mentioned in section 2.2.1, DHM is capable of reconstructing a high-resolution phase map from a recorded hologram by the CCD. Figure 2.8 exhibits a phase reconstruction process by using a single-shot hologram (a) to reconstruct the phase map (c) of paramecium [17]. The output phase image (c) shows a vivid structural contrast compared to the intensity image (b) that is recorded without any interference.
DHM, as the founding technique of digital holography, emphasizes the structural detail of a transparent sample with a desired magnification. Nonetheless, the uncommon-path geometry of DHM in both its reflection and transmission mode inevitably suffers from the field noise arising from different optical components that each arm from the beam splitter encounters. DHM’s high resolution phase imaging capability enables the imaging of both biological and non-biological samples for material manufacturing [40].

2.2.3 Diffraction phase microscopy (DPM)
Diffraction phase microscopy (DPM), fundamentally speaking, achieves a similar holographic result as DHM. The key benefit of using the DPM technique lies in its common-path geometry [19]. As mentioned in the introduction, common-path geometry allows for temporal stability in phase reconstruction.

Figure 2.9 shows a standard schematic that is used for DPM involving an inverted microscope and a compact Mach-Zehnder interferometer, which is created by the diffraction from the grating [18]. The collimated magnified image from the microscope hits the grating, which spreads the light in many diffraction orders. Of all the diffraction orders, the spatial filter only selects the first two orders [42]. The 4f lens system involving two different lenses $L_1$ and $L_2$ spaced by sum of two focal lengths performs an analog Fourier transform twice [43]. Placing a pinhole spatial filter between the two lenses allows it to perform a spatial Fourier filtering of the incoming fields [19, 32]. For the pinhole design that is shown in Figure 2.9, the first diffracted order enters the smaller hole to be lowpass-filtered into a reference beam with an almost uniform intensity and phase. The purpose of the bigger hole in the spatial filter design is to pass all the frequency components in the un-scattered (zeroth order) field to utilize the output as the sample field [4, 25]. Consequently, the tilted, lowpass-filtered reference beam and direct unfiltered sample beam interfere in an off-axis geometry. The phase reconstruction process is no different from that of the DHM.
Figure 2.9: DPM schematic involving image from inverted microscope illuminating on a grating creating multi-order scattering. SF selects two diffraction orders in Fourier lens system comprised of $L_1$ and $L_2$ [42]

Figure 2.10: (a) Phase map reconstructed from DPM imaging for red blood cell and (b) corresponding path-length vs time for 1000 sampled acquisitions of background image (without any sample) for a single point (top) and overall field of view labeled with dashed border (bottom) – sampling period was 10.3ms [19]

As previously mentioned, DPM distinguishes itself from other holographic microscopy techniques with its common-path geometry in the interference [4, 19, 42]. That is, both the sample field and the reference field encounter the same optical components prior to their interference at the CCD which should further reduce the temporal phase noise. Figure 2.10a shows a reconstructed phase image of a group of red blood cells and 2.10b displays the corresponding plot of the path-length difference respect
to time. The top part of Figure 2.10b portrays the fluctuation in the path-length difference that is plotted for a single point over one thousand timesteps, and the bottom part shows the same kind of plot for the overall ROI. The acquired standard deviations for a single point and overall ROI are 0.7nm and 0.04nm, respectively [19].

Usage of common-path geometry involving a spatial filter may also have detrimental drawbacks. The first challenge that DPM faces is that there is a significant tradeoff between using a laser source and a broadband source. For a laser source, the image output suffers from speckle noises due to its high spatial coherence length [18, 44]. On the other hand, a low-coherence broadband source causes strong 3D scattering at the edge of the sample. The scatter angle exceeds the bandwidth of the sample filter and instead enters the reference pinhole [4]. This results in a distortion in the reference field which creates a halo effect as shown in Figure 2.11, which is characterized by edge phase values that are abnormally low [4, 44]. In order to avoid such effect, the pinhole must be aligned in the proper position, not only in x and y, but also in z, since the scattering effect for that portion of the sample is no longer in 2D, but in 3D [4, 45]. Another way is to force the DC pinhole to be much smaller so that a narrower range of k-vectors can create the reference field [20].

Figure 2.11: Simulated output due to halo artifact on an image of a 3-micron polystyrene beam. a) Ideal, halo-free phase map b) low-passed version of phase map. c) output DPM phase map with halo artifact. d) 1D projection along the center for all simulated images [44]
3 Research Method

3.1 Testing of reduced Sagnac interferometer

HF-GLIM aims to combine GLIM’s high optical sectioning, DHM’s off-axis interferometry, and Sagnac interferometer geometry to generate a temporally stable phase gradient from a biological sample with a fast acquisition time. In a publication on GLIM by QPI group, it was mentioned that the GLIM system is able to generate 10 phase images per second [1], meaning that this speed can be far more enhanced if the phase-shifting method is to be replaced by a single-shot off-axis method.

A traditional Sagnac configuration in many publications involves a quadrilateral geometry [21]. The holographic effect that is used in off-axis interferometry is created by retaining the sample beam to travel in a perfectly rectangular path while the sample beam’s trajectory is altered by the tilt in the polarizing beam splitter. The polarization-dependent bias that is mentioned in the previous sentence is due to the fact that a tilt in a beam splitter only affects the angle of reflection, but not the angle of transmission.

Figure 3.1 shows an implementation of an off-axis Sagnac interferometer that is used to retrieve sample phase information. The microscope optics on the left performs a simple magnification to sample image and is sent to a Sagnac loop with a tilted PBS. One of the beams is then modulated respect to each other at the CCD. The purpose of the two polarizers is first, to provide an even contribution by $\hat{s}$ and $\hat{p}$ polarization, and second, to force Sagnac output components to align in the sample polarization state for interference. Figure 3.2 exhibits two different paths that are created by the same PBS circuit as shown in Figure 3.1 due to a difference in the effect of the tilt in the PBS on the beam path in (a) the transmission and (b) the reflection [21].

![Figure 3.1: Quadrilateral off-axis Sagnac interferometer using a laser imaging system connected to a PBS circuit (offaxis-geo) [21].](image)
Nevertheless, the same geometrical effect can be elicited by taking one of the mirrors out of the schematics [25]. In other words, a reduced Sagnac setup instead with a triangular configuration can work in the same way as a trapezoidal setup. Figure 3.3 shows our initial approach to alter the original set-up as shown in Figure 3.1. The mirrors M2 and M3 were aligned to ensure that an incident transmitted beam should reflect at 45 degrees from its incident angle. Figure 3.4 gives a series of interference fringes that are recorded with our own implementation of the reduced off-axis setup. With less mirrors to consider for its alignment, a geometrical modification to the Sagnac loop indicates practicality for the HF-GLIM set up which will be discussed in the later sections.
3.2 Mathematical derivation of HF-GLIM’s function

The mathematical derivation of the output of HF-GLIM can easily be derived and predicted by combining the output of DIC with the calculation from the off-axis Sagnac interferometry. As with the implementation of the original GLIM model, the output polarizer for our implementation is removed from the DIC microscope to retain the cross-polarization in the two DIC output fields [1, 23]. Thus, the output from the DIC microscope for our implementation of HF-GLIM is defined as shown in Equation 2.1. By sending each of the cross-polarized components into the beam splitter, the beam-splitter would leave one component tilted in respect to the optical axis and the other tilted by angle $\theta'$, where $\theta'$ is the angle of modulation respect to the optical axis. The tilt angle $\theta'$ at the output can be derived geometrically from the tilt angle from the beam-splitter – let’s say $\theta_0'$, the incident angle respect to the normal vector of the mirror surface of PBS [21, 25] as shown in Figure 3.2. It is important to note that for a beam that only undergoes transmission at the PBS surface is not affected by the tilt by the PBS. Taking this information and Equation 2.1 into account, the output from the off-axis interferometer before hitting the final polarizer is defined as the following:

$$U_T(r) = U(r)e^{ikr \cdot \hat{s}} + U(r + \delta r)\hat{p}$$  \hspace{1cm} (3.1)

where the $k_r = k_0 \hat{r} = [k_0 \cos(\theta)  \hspace{0.5cm} k_0 \sin(\theta)]$ for a tilted reflected beam at some angle $\theta$. For now, we sheared the unshifted field (s-polarization) from the DIC [1, 16, 33]. Switching which polarization to shear ultimately inverts the GLIM’s gradient angle. After going through the final polarizer to co-polarize the two beams, the intensity that is recorded by the CCD is then:

$$I_T(r) = I(r) + I(r + \delta r) + U^*(r + \delta r) \cdot U(r)e^{ik_{r+} \cdot \hat{r}} + U(r + \delta r) \cdot U^*(r)e^{-ik_{r+} \cdot \hat{r}}$$  \hspace{1cm} (3.2)
where $\mathbf{k}_{r\perp}$ is the x-y projection of the tilted k-vector $\mathbf{k}_r$. By taking into account that $U(\mathbf{r}) = \lvert U(\mathbf{r}) \rvert e^{i\phi(\mathbf{r})}$ and $U(\mathbf{r} + \delta \mathbf{r}) = \lvert U(\mathbf{r} + \delta \mathbf{r}) \rvert e^{i\phi(\mathbf{r} + \delta \mathbf{r})}$, the expression in Equation 3.2 is reduced to the following:

$$I_T(\mathbf{r}) = I(\mathbf{r}) + I(\mathbf{r} + \delta \mathbf{r}) + I_m(\mathbf{r}, \delta \mathbf{r}) \left[ e^{i(\nabla \phi(\mathbf{r}) \delta \mathbf{r} + \mathbf{k}_r \mathbf{r})} + e^{-i(\nabla \phi(\mathbf{r}) \delta \mathbf{r} + \mathbf{k}_r \mathbf{r})} \right]$$  

(3.3)

where $\nabla \phi(\mathbf{r})$ is the phase gradient information that we are interested in for $\nabla \phi(\mathbf{r}) \delta \mathbf{r} \approx \phi(\mathbf{r} + \delta \mathbf{r}) - \phi(\mathbf{r})$ [1, 23] and $I_m(\mathbf{r}, \delta \mathbf{r}) = \lvert U(\mathbf{r}) \rvert \lvert U(\mathbf{r} + \delta \mathbf{r}) \rvert$ to represent the mutual intensity between the two fields, which must be real and positive. Thus, the recorded intensity from this modified GLIM is a holography, for which we have already derived a method to retrieve the phase information. For our ease of representing the Fourier transform of the detected intensity, we will utilize $T(\mathbf{r}, \delta \mathbf{r})$ to represent a product between $I_m(\mathbf{r}, \delta \mathbf{r})$ and the complex exponential $e^{i\nabla \phi(\mathbf{r}) \delta \mathbf{r}}$. By applying this change, we arrive at Equation 3.4.

$$I_T(\mathbf{r}) = I(\mathbf{r}) + I(\mathbf{r} + \delta \mathbf{r}) + T(\mathbf{r}, \delta \mathbf{r}) e^{ik_{r\perp} \mathbf{r}} + T^*(\mathbf{r}, \delta \mathbf{r}) e^{-ik_{r\perp} \mathbf{r}}$$  

(3.4)

Taking the Fourier transform of Equation 3.4 yields the following results, again, with three distinct frequency bands:

$$\tilde{I}_T(\mathbf{k}) = \tilde{I}(\mathbf{k}) + \tilde{I}(\mathbf{k}) e^{ik_{r\perp} \mathbf{r}} + \tilde{T}(\mathbf{k} - k_{r\perp}) + \tilde{T}^*(-(\mathbf{k} - k_{r\perp}))$$  

(3.5)

In order to avoid aliasing in the hologram, $|k_{r\perp}|$ needs to be sufficiently large such that $|k_{r\perp}| \geq \frac{B_0 + B_{\pm 1}}{2}$

where $B_0$ is the frequency bandwidth of the DC component (from the sum of two intensities) and $B_{\pm 1}$ is the frequency bandwidth of one of the shifted components (from T function) [18]. Assuming such condition is satisfied, the phase gradient that is embedded in the T function can be retrieved by the same filtering process that was presented in consecutive steps from 2.11 to 2.13. First, by removing the DC component through a high-pass filter to the expression that is shown in Equation 3.5, we arrive at Equation 3.6. Performing a circular shift by $k_{r\perp}$ to move the un-conjugated term (the real image) to be centered at the origin in k-space yields the following expression in Equation 3.7.

$$\tilde{I}_T(\mathbf{k}) = \tilde{T}(\mathbf{k} - k_{r\perp}) + \tilde{T}^*(-(\mathbf{k} - k_{r\perp}))$$  

(3.6)

$$\tilde{I}_T(\mathbf{k}) = \tilde{T}(\mathbf{k}) + \tilde{T}^*(-(\mathbf{k} - 2k_{r\perp}))$$  

(3.7)

Lastly, performing a lowpass filtering just enough to remove the virtual image term $\tilde{T}^*(-(\mathbf{k} - 2k_{r\perp}))$ followed by the inverse Fourier transform returns $T(\mathbf{r}, \delta \mathbf{r})$. Recalling that $T(\mathbf{r}) = I_m(\mathbf{r}, \delta \mathbf{r}) e^{i\nabla \phi(\mathbf{r}) \delta \mathbf{r}}$, performing a two-dimensional arctangent yields the phase gradient as follows [1, 23]:

$$\nabla \phi(\mathbf{r}) = \tan^{-1}(T(\mathbf{r}, \delta \mathbf{r}))$$  

(3.8)
This method diverges substantially from that of the original GLIM method that was presented in the literature review section, in which the retrieval of the phase gradient from a DIC image required four different samplings of a single field of view, each phase-shifted by multiples of $\pi/2$. By proper implementation of HF-GLIM, one can retrieve the phase gradient merely from one frame per field of view, subsequently saving a significant amount of time. The most profound benefit of using GLIM with holography is that it does not require the phase-unwrapping process, contrary to the mainstream off-axis methods. Since GLIM measures the phase gradient between adjacent points, instead of the phase value at a point, the measured GLIM output is usually guaranteed to be less than $2\pi$, rendering phase unwrapping unnecessary [1, 23]. This will further curtail the amount of time that is needed for phase reconstruction.

### 3.3 Configuration of HF-GLIM

The schematic of HF-GLIM can be divided into three essential components: DIC microscope, off-axis Sagnac interferometer, and holography correction lens system [43] as shown in Figure 3.5. By using the configuration shown in Figure 3.5, we aim to achieve a single-shot retrieval of the phase gradient. This section will entail a discussion of the functional details of a DIC, off-axis Sagnac interferometer, and holography correction.

![Figure 3.5: Desired schematic of HF-GLIM with analyzer-free DIC on the right side: WP defines Wollaston prism and CL and OL each represent condenser and objective lens. Holography correction lens system on the left side is used to converge two modulated beams to the CCD.](image)

#### 3.3.1 Differential interference contrast (DIC) microscopy

A traditional differential interference contrast (DIC) microscopy involves spatially shifting two beams by some finitely small spacing $\delta$ that is less than the size of the diffraction spot [1]. Intrinsically, DIC provides a phase contrast by creating an artificially slanted view of the sample; however, the contrast is not quantitative on its own [1, 33]. The schematic of the original DIC is shown below in Figure 3.6.
The output from a traditional DIC is evaluated by recombining two cross-polarized image fields, each phase shifted by the sample using an analyzing polarizer cross-polarized in respect to the input polarizer. DIC maps the qualitative information at each point \((x, y)\) based on the phase offset between one polarization at \((x, y)\) and another polarization at \((x + \delta_x, y + \delta_y)\) where \(\delta_x\) and \(\delta_y\) are spatial offset created between the two cross-polarized fields output the first Wollaston prism.

The phase delay that is given by the two spatially offset fields after the sample can result in three different types of polarization: linear, circular, or elliptical [46]. If no phase difference is introduced between the two beams, the output field is also linearly polarized in the same direction (±45 degrees) after which the analyzing polarizer results in a zero-signal due to the orthogonally directed polarizers. On the other hand, if the sample creates a phase offset between two beams that are equal to \(\frac{\pi}{2}\), the resulting polarization is circular for which the signal out from the DIC is maximized. Any phase shift values in between yield a circular polarization before the analyzer and provide a spectrum of intensity that is detected between zero and the maximum.

The HF-GLIM module requires the output from the DIC just before entering the analyzer [1, 23]. In other words, the analyzing polarizer must be removed prior to using the DIC system for the module, since the off-axis Sagnac interferometer splits the two beams based on the polarization of each one of them, and co-polarizing the two beams using the analyzer will hamper this process. The resulting modification of the DIC that is used for our implementation is shown in Figure 3.5.
3.3.2 Polarization-dependent Sagnac interferometer

The effect that a Sagnac interferometer has on each of the cross-polarized inputs is different. As previously discussed, the reflected polarization component reflects in a slightly distorted path whereas the transmitted polarization component is not affected by the angular tilt of the PBS. Here, we assume that the s-polarized beams are reflected, and that the p-polarized beams are transmitted. That is, by the end of the Sagnac loop, an s-polarized component would have undergone reflection twice, and a p-polarization would have undergone transmission twice with some tilt at an angle $\theta$. The behavior of the polarization that is dependent on the Sagnac interferometer can be explained with a Jones matrix formulation with a few approximations.

The Jones matrix is a two by two linear transformation matrix that is applied over a column vector with each of the two polarization components. Equation 3.1 is generated by going through two instances of Jones matrix multiplication. Firstly, the Jones matrix product from just the tilted Sagnac interferometer is defined as:

$$
U_{BS}(r) = \begin{bmatrix} R^2 e^{i k r \cdot r} & 0 \\ 0 & T^2 \end{bmatrix} \begin{bmatrix} U(r) \\ U(r + \delta r) \end{bmatrix}
$$

where $R$ and $T$ are each the reflectance and the transmittance from the beam splitter, and $e^{i k r \cdot r}$ is the shearing exponential that only applies to the reflected components [47]. The zeros in the diagonal of the matrix is attributed to the orthogonality between reflection and transmittance: one polarization reflects while the other does not. For the polarization vector $[U(r) \quad U(r + \delta r)]$, the left element corresponds to the reflected s-polarization component, whereas the right element corresponds to the transmitted p-polarization component from DIC, respectively.

The final analyzing polarizer from the DIC is placed instead at the output side of the PBS to co-polarize them. Furthermore, the Jones matrix that is employed to describe the behavior of a polarizer at 45 degrees is shown in Equation 3.10 [48]. Applying the linear transformation from the polarizer to the product in Equation 3.9 yields Equation 3.11:

$$
J_{45} = \frac{1}{2} \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix}
$$

$$
U_p = \frac{1}{2} \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} R^2 e^{i k r \cdot r} & 0 \\ 0 & T^2 \end{bmatrix} \begin{bmatrix} U(r) \\ U(r + \delta r) \end{bmatrix}
$$

With an ideal PBS with 50% reflection and 50%, $T = R = \frac{1}{2}$, we arrive at the following polarization vector:

$$
= \frac{1}{8} (U(r) e^{i k r \cdot r} + U(r + \delta r)) \begin{bmatrix} 1 \\ 1 \end{bmatrix}
$$
As two fields \( U(r) \) and \( U(r + \delta r) \) are successfully co-polarized with one component \( U(r) \) modulated in respect to the other, we face another challenge at the output from the analyzing polarizer: the tilt angle \( \theta = \tan^{-1}(k_r) \) makes \( U(r) \) diverge away from \( U(r + \delta r) \), not converge at the CCD for any distance \( z \). The solution is to send the beams through a system of lens that we call a holography correction system, which will be discussed in the upcoming section.

### 3.3.3 Holography Correction System

Holography correction system is used to converge the two beams about the same spatial position in order to ensure that the two beams are not spatially shifted more than what is signified by the Wollaston offset \( \delta r \). The lens correction system consists of a 4f lens configuration as shown in Figure 3.7 [43]. The two lenses are separated by the sum of the focal length of each of the lenses. The name 4f is derived from the fact that the illumination side and detector size span a total of four focal distances that add up to \( 2(f_1 + f_2) \) [4]. In general, if the two beams approximately come from the same spatial location, the distance to the left of the first lens can be flexible if the first lens has a sufficient numerical aperture to accept the diverging ray. Additionally, the two lenses may have different focal lengths if the proper spacing is applied between the lenses.

![Figure 3.7](image.png)

*Figure 3.7: Function of 4f system of converging a diverging set of beams at the CCD. \( f_1 = f_2 = 100\,mm \). The simulation done through [49].*
4 Research Results and Future Implications

4.1 Simulated result of HF-GLIM

A MATLAB simulation was conducted in order to validate the functionality of HF-GLIM. For our simulation, a phase map of a neuron culture imaged using spatial light interference microscope (SLIM), as shown in Figure 4.1, was used. Since a biological sample induces very little amplitude modulation [1, 4], we constructed a complex valued transfer function with a unit amplitude for HF-GLIM, defined as $U_i(x, y) = e^{i\phi(x, y)}$, where $\phi(x, y)$ represents the phase values from SLIM [16]. Following, two replicas of $U_i(x, y)$ were created, one unshifted and one circularly shifted. The shifted image was then multiplied by a complex plane wave expression $e^{i k_s x}$ where the $k_s$ was selected so that enough separation is given between the Fourier components. The sum of the two complex images ultimately resembles the expression that is shown in Equation 3.1.

![Ground Truth Sample Image from SLIM](image)

Figure 4.1: Phase map (in radians) of a neuron culture imaged using SLIM [ECE398] [50]

Afterwards, the hologram can be obtained by taking an absolute value squared of the sum which would serve as the intensity of the HF-GLIM output that is recorded by the camera. The holograms in a spatial and frequency domain are presented in Figure 4.2a and b, respectively. Interestingly enough, the hologram has nearly zero DC components, apparent from the FT image that is shown in 4.2b. We therefore skipped the high-pass filter process, although a traditional hologram processing would first require a high-pass filter to remove the DC-associated FT component. By performing the needed Fourier processing that is mathematically equivalent to Equations 3.5 to 3.7, we arrive at the filtered hologram on the Fourier domain as shown in Figure 4.3. Finally, taking the inverse Fourier transform of Figure 4.3 yields a complex image with the phase gradient embedded in the phase term. Extracting the phase information from the complex-valued image yields the phase gradient as shown in Figure 4.4a and 4.4b.
Figure 4.2: Recorded hologram in (a) spatial domain and (b) frequency domain

Figure 4.3: Filtered hologram in FT Space
Figure 4.4: Constructed phase gradient map (in radians) (a) before and (b) after background correction

Figure 4.4a looks similar to 4.4b, only that it also carries a linearly darkening spectrum on top of the sample image. This artifact is not sample-dependent, but rather an artifact that is due to the shifting mechanism in the holography processing stage. Simply put, if a background (as in no sample) is imaged with the same environment, the linear phase change is equally as present. Thus, by performing a prior calibration of the system, one can simply subtract the background image from the image in Figure 4.4a to diminish such effect and finally retrieve a background-corrected image as shown in Figure 4.4b. It is important to notice that no sample information is either lost or distorted in the correction stage. The output of the HF-GLIM as shown in Figure 4.4b closely resembles that of the traditional GLIM output, if not, perfectly matches the appropriate gradient and range of values (-0.05~0.05 radians).

4.2 Digital filter as the noise source

For the simulation above, we have performed the signal-to-noise (SNR) ratio assessment for the performance of HF-GLIM. Since a sample part of the image contains high-contrast phase values that bestow texture upon the image, SNR is usually measured in a sampled portion of the output with no sample information [18, 51]. To make an accurate assessment of the sample-independent performance of the system, the definition of SNR must be modified so that the SNR of the input phase image is taken into account. Our definition of a modified SNR parameter $\tilde{\text{SNR}}$ is defined below as:

$$\tilde{\text{SNR}} = \frac{\text{SNR}_{\text{out}}}{\text{SNR}_{\text{in}}}$$  \hspace{1cm} (4.1)

where $\text{SNR}_{\text{out}}$ is the SNR of the output phase gradient from the HF-GLIM system, and $\text{SNR}_{\text{in}}$ is the SNR of the input phase map that is imaged using the SLIM system. In decibel (dB), Equation 4.1 transforms into:

$$\tilde{\text{SNR}}_{\text{dB}} = 20 \log_{10} \left( \frac{\text{SNR}_{\text{out}}}{\text{SNR}_{\text{in}}} \right)$$  \hspace{1cm} (4.2)
With the general image processing definition of SNR that is given by \( \text{SNR} = \frac{\mu}{\sigma} \), where \( \mu \) is the spatial average and \( \sigma \) is the spatial standard deviation of the image [4], the three different SNR values were measured and recorded in Table 4.1. Figure 4.5 presents sampled background from the phase images presented in Figure 4.4 that was used for noise analysis. Although the output from the HF-GLIM on the right side of Figure 4.5 shows the smoothened version of the input shown on the left side, The modified SNR is negative in decibel unit shown in Table 4.1 which suggests that background from HF-GLIM has become more noisy in space. Spatial filtering in the Fourier domain, which is a core step in performing the Hilbert transform of the image, is highly sensitive to the design of the digital filter [10], similar to how the size and location of each pinhole in DPM is critical [20, 44].

![Sampled background region for input and output](image)

**Figure 4.5:** Sampled background from: (left) input SLIM image, and (right) output HF-GLIM image

<table>
<thead>
<tr>
<th>Table 4.1</th>
<th>SNR Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( SNR_{in} )</td>
<td>0.0806</td>
</tr>
<tr>
<td>( SNR_{out} )</td>
<td>0.0520</td>
</tr>
<tr>
<td>( SNR )</td>
<td><strong>0.6446</strong></td>
</tr>
</tbody>
</table>

**Table 4.1:** Spatial SNR measurements to determine performance of HF-GLIM

### 4.3 Run-time for creating gradient frame

Similar to the GLIM [1, 23], the key advantage of HF-GLIM over other off-axis methods stems from its ability to retrieve an accurate phase gradient measurement without unwrapping the phase, which often results in runtime problems for many holography-based imaging modalities [18, 26, 34]. Using a similar simulation process, the average runtime for constructing a phase gradient both with and without the presence of a phase unwrapping process is shown in Figure 4.6. The runtime measurement only measures the reconstruction process under two assumptions: first, the time elapsed through the optical
path is considered to be negligible, and second, the camera-dependent integration time is not a dominant factor. We further verified the equivalence in the output between the two methods as shown in Figure 4.7.

Figure 4.6: Run-time measurement for phase gradient reconstruction over 100 trials for an identical image (left) with phase unwrapping and (right) without phase unwrapping.

Figure 4.6: Equivalence of HF-GLIM output between HF-GLIM output taken (left) with phase-unwrapping and (right) without phase-unwrapping.

Followed by a prior background calibration, the main reconstruction takes little time (~68ms) if phase-unwrapping is implemented. Unlike the traditional GLIM that requires four phase-shifted frames per field of view, HF-GLIM generates the phase gradient single-shot. That is, for HF-GLIM, the number of image frame that is generated per second is, in itself, the number of phase gradient generated per second.
5 Conclusion

To achieve a high-speed phase gradient extraction, HF-GLIM serves to optimize efficiency not only from the single-shot reconstruction, but also from GLIM’s capability of imaging with an open-aperture source. On a side note, HF-GLIM does not require any SLM or gratings for filtering, which enable the overall system to retain the output power from the microscope throughout [25]. Both the mathematical and simulation-based results, as delineated in this publication, serve to buttress HF-GLIM’s performance. Our work is yet ongoing to achieve a higher spatial SNR in the system and, ultimately, to implement this idea in reality by connecting the Sagnac loop to the DIC microscope, with the aim of further suggesting the applicability of our research in heavy-duty, real-time biological imaging.
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