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Pushing phase and amplitude sensitivity limits in interferometric microscopy

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Abstract

Sensitivity of the amplitude and phase measurements in interferometric microscopy is influenced by factors such as instrument design and environmental interferences. Through development of a theoretical framework followed by experimental validation, we show photon shot noise is often the limiting factor in interferometric microscopy measurements. Thereafter, we demonstrate how a state-of-the-art camera with million-level electrons full well capacity can significantly reduce shot noise contribution resulting in a stability of optical path length down to a few picometers even in a near-common-path interferometer.

Interferometric microscopy enables quantitative measurement of the phase and amplitude across the field of view. In both transmission and reflection geometries, the sample beam typically interferes with an off-axis reference beam to create interference fringes on the imaging plane [1–3]. The Mach–Zehnder interferometer is a classic example of an off-axis type of interferometer. Development of the near-common-path techniques greatly improved the temporal stability of the off-axis techniques since sample and reference beams travel nearly side by side [4]. However, the spatial sensitivity of these microscopes is still limited by factors such as speckle noise or interference effects due to multiple reflections between optics as a result of the long coherence length of the illumination source. Illumination with broadband sources has been suggested as a way of reducing the speckle noise inherent to laser sources [5–8]. These interferometric techniques have been used in studying the

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dynamics and morphology of a wide variety of specimens [9,10]. In all these applications, it is important to examine the sensitivity limits and their relation to the instrument design and environmental factors.

Instrumental parameters such as camera dark noise, read noise, quantum efficiency, and dynamic range of the cameras may all affect measurement sensitivity. Even with stable near-common-path designs, environmental factors such as mechanical vibrations and air density fluctuations may degrade sensitivity of interferometric measurements. However, when interference of environmental factors is minimized, the impact of shot noise is dominant at high imaging speeds or low photon flux [11]. Shot noise is inversely proportional to the square root of the number of photons captured by each camera pixel. Therefore, limited well capacity of the conventional CMOS and charge-coupled device (CCD) sets a bound to the best achievable sensitivity. There have been a few studies on the effect of shot noise on the sensitivity of the measurements in digital holographic microscopy [12–14]. Here, we initially lay a theoretical framework for how measurement sensitivity is ultimately limited by photon shot noise in interferometric microscopy and show this theoretical limit agrees well with the experimental measurements. Next, through use of a novel camera with superior million electrons level full well capacity, we show one can substantially push phase and amplitude sensitivities of interferometric microscopy and achieve an optical path stability of a few picometers.

In an interferometric imaging system, the signal beam that carries the sample information interferes with a plane wave reference, resulting in an intensity distribution on the camera plane as

$$I(x) = I_0 \{1 + \gamma(x) \cos[\Lambda x + \omega_0 \tau - \phi_s(x)]\}, \quad (1)$$

where $2\pi/\Lambda$ is the fringe period, ω_0 is the mean radial frequency, τ is the time delay, I_0 is the average intensity, $\gamma(x)$ is the amplitude modulation of the interference term, and $\phi_s(x)$ is the phase distribution of the sample. To obtain the phase one can either modulate the intensity in time by varying the time delay τ (with $\Lambda = 0$) or alternatively create a sinusoidal fringe pattern in space along x with a carrier frequency $2\pi/\Lambda$ (with $\tau = 0$). These two cases correspond to phase-shifting interferometry and off-axis interferometry, respectively. For an off-axis interferometer, one may sample one period of the interference pattern at the image plane with three or more camera pixels as shown in Fig. 1. I_1 , I_2 , I_3 , and I_4 are intensities interpreted at $x_1 = x$, $x_2 = x + \pi/2\Lambda$, $x_3 = x + \pi/\Lambda$, and $x_4 = x + 3\pi/2\Lambda$, respectively. Using four-step or four-bucket methods [15], the phase (ϕ_s) and amplitude (γ) of the interference term at any point can be retrieved as

$$\begin{aligned} \phi_s &= \tan^{-1} \left(\frac{I_1 - I_3}{I_2 - I_4} \right), \\ \gamma &= \sqrt{(I_2 - I_4)^2 + (I_1 - I_3)^2} / 2I_0. \end{aligned} \quad (2)$$

For a given intensity field, the sensitivity is ultimately limited by the Poisson statistics of the photon shot noise that scales with the square root of the intensity $\sqrt{I}$. It is, however, less

obvious how phase and amplitude sensitivities are related to the intensity distribution over the fringes in interferometric imaging. We start our analysis by evaluating the uncertainty of the phase measurements as expressed by Eq. (2). The intensity of each pixel is proportional to the number of photons (n) multiplied by the quantum efficiency (η) of the imaging device ($I \propto \eta n$). Therefore, for small phase values ($\phi_s \ll 1$), we can write the phase at each pixel as

$$\varphi_s \approx \frac{n_1 - n_3}{n_2 - n_4}, \quad (3)$$

where n_1 , n_2 , n_3 , and n_4 are the number of photons for the corresponding intensities. Through propagating the uncertainty of the Eq. (3), the phase uncertainty can be calculated as

$$\delta^2 \varphi_s \approx \left(\frac{n_1 - n_3}{n_2 - n_4} \right)^2 \left[\frac{\delta^2(n_1 - n_3)}{(n_1 - n_3)^2} + \frac{\delta^2(n_2 - n_4)}{(n_2 - n_4)^2} \right]. \quad (4)$$

At the photon shot-noise limit $\delta^2 n_1 = n_1$, and $\delta^2 n_3 = n_3$. In the absence of any sample, $n_1 = n_3$, therefore,

$$\delta^2 \varphi_s \approx \frac{n_1 + n_3}{(n_2 - n_4)^2}. \quad (5)$$

In order to get the smallest phase noise, Eq. (5) needs to be minimized. This condition is satisfied when the bright fringe reaches the maximum photon count ($n_2 = N$) and the dark fringe is lying at zero ($n_4 = 0$), where N is the maximum electron well depth of the camera. As shown in Fig. 1, under such conditions, we have $n_1 = n_3 = N/2$. Therefore, for this optimum case,

$$\delta^2 \varphi_s \approx \frac{(N/2 + N/2)}{N^2} = \frac{1}{N}. \quad (6)$$

Similarly, one can obtain the amplitude sensitivity as well such that

$$\delta \varphi_s = \delta \gamma = \frac{1}{\sqrt{N}}. \quad (7)$$

Equation (7) therefore refers to the highest achievable theoretical shot noise limited sensitivity. This theoretical sensitivity limit may vary slightly depending on factors such as the method through which phase and amplitude are extracted from the raw interferograms, e.g., the use of discrete Fourier transform. Furthermore, it is often the case that the brightest fringe (n_2) does not match the full electron well depth and the dark fringe (n_4) is not exactly zero. For these cases, Eq. (5) provides the approximate sensitivity limit which yields similar results to Eq. (7) when the full electron well depth (N) is replaced by an effective electron well depth of $N_e = n_2 - n_4$. Figure 2(a) shows a sample-free two-dimensional interferogram

recorded using a diffraction phase microscope [4,16]. Using the Hilbert Transform, one can isolate the complex field of the spatially modulated order in the Fourier plane, see Fig. 2(b). The size of the cropping window is determined by the numerical aperture of the imaging objective. The inverse Fourier transform of the cropped information yields the complex amplitude of the sample beam from which the amplitude and phase of the field can be calculated, as shown in Figs. 3(a) and 3(b). Interferograms were captured using a Photron camera (model # FASTCAM 1024 PCI) at 500 frames per second (fps) with an illumination wavelength of $\lambda = 590 \pm 4$ nm (Fianium WL SC400-8). The aberrations in optics and fixed amplitude and phase patterns of each frame may be corrected by using the first interferogram as a calibration. Figures 3(c) and 3(d) show the corrected amplitude and phase, respectively.

Figures 4(a) and 4(c) show variations in the phase and amplitude over time, for about 2 s. Temporal fluctuations over the mean phase and amplitude value could be due to factors such as illumination source 1/f noise. One can correct such power fluctuations simply by selecting a small area in the field of view and subtracting the average phase and amplitude of this area from the phase and amplitude measured over the whole field of view. Since certain environmental interferences like mechanical vibrations tend to happen at specific frequencies, the frequency spectrum of phase and amplitude fluctuations provides additional insight on possible causes of such interferences. Figures 4(b) and 4(d) show the frequency spectra of the variations in phase and amplitude, respectively. In the case of average phase, a strong interference is observed around 60 Hz that corresponds to the operating frequency of many electrical devices and potential sources of vibrations such as a room air-conditioning system. Additionally, it must be noted that higher phase or amplitude sensitivities can be achieved at certain frequency regimes such as 70–120 Hz that is relatively free of environmental interferences.

In the case of a Photron camera, the pixel full well capacity is about 60,000 electrons. Therefore, according to the theoretical limit of Eq. (7), the phase and amplitude sensitivities should be about 4.1×10^{-3} . However, due to a variety of reasons, actual interference pattern almost always deviates from the ideal case as shown in Fig. 1. Two such factors are: (1) dark level of the camera, which often makes it hard to make dark fringe hit zero count and (2) nonuniformity of the fringe pattern that makes saturation of all camera pixels difficult. In the case of interference pattern shown in Fig. 2(c), the effective electron well depth is approximately 22,300 electrons. The expected sensitivity is, therefore, about 6.7×10^{-3} . However, there are factors that can enhance the predicted sensitivity calculated through Eq. (7) as well. The number of camera pixels used in fringe sampling and the sampling of a diffraction-limited spot are two such factors. In our case, each fringe is sampled using approximately three camera pixels that is three-fourths of the number used in the theoretical framework. Additionally, each diffraction-limited spot is projected over approximately seven camera pixels. Therefore, one can apply an averaging filter to improve the sensitivity by approximately $\sqrt{7} \sim 2.6$ times. Considering these two ratios, the best theoretical sensitivity is about 3.4×10^{-3} . It is quite interesting that the average phase and amplitude sensitivities over the field of view are exactly the same, that is 3.3×10^{-3} , which is very close to the shot noise limited predicted value. Excellent match between theoretical prediction and

experimental values demonstrates that shot noise, rather than environmental disturbances, is often the primary factor in limiting most interferometric microscope designs today.

An alternate way of achieving higher sensitivity is recording more images and averaging the frames, which is effectively increasing the number of collected photons on each pixel of the image while sacrificing temporal resolution. As shown in Table 1, amplitude noise improves with an increase in the number of the averaged frames, i.e., with a 10 times increase in the number of frames, we observe $\sqrt{10} \sim 3.2$ improvement in amplitude sensitivity, as expected. Likewise, when the number of averaged frames increases from 10 to 100, we observe roughly another three-fold increase in the amplitude sensitivity. Next, we record interferograms using a novel Adimec camera (model # Q-2A750/CXP) with a superior full well depth of about 1,600,000 electrons. We record interferograms at 600 fps for 2 s with an illumination wavelength of $\lambda = 800 \pm 7$ nm (Verdi V18 pumped into a Ti:sapphire Mira 900, Coherent Inc.). Our analysis of the fringe pattern in this case shows an effective camera count of about 835,000 electrons that corresponds to a sensitivity of roughly 1.1×10^{-3} . Each diffraction-limited spot is again sampled over seven camera pixels and each interference fringe is sampled using four pixels in this case, which is consistent with the theoretical framework. Therefore, the theoretical limit for sensitivity is expected to be about 4.1×10^{-4} that matches well with experimental results. By increasing the number of averaged frames from 1 to 10, phase and amplitude sensitivities improve roughly three times. However, increasing the number of frames from 10 to 100 increases the phase and amplitude sensitivities by slightly above two times, instead of the expected three-fold increase.

The most sensitive measurement by the Adimec camera shows an optical path length stability of about 10 pm at 6 fps. Figure 5 shows the temporal standard deviation maps of the amplitude and phase at this speed for both cameras. As seen in Figs. 5(a) and 5(b), temporal fluctuations of the amplitude and phase is relatively flat over the field of view for the case of the Photron camera. However, as shown in Figs. 5(c) and 5(d), there is a certain pattern present for the phase and amplitude fluctuations in sensitive measurements done by the Adimec camera. This suggests that we may be facing another environmental limit at a frequency around 20 Hz. Since mechanical vibrations tend to happen at much higher frequencies, we speculate that this distribution could be due to a mismatch of the optical path length between the reference and the sample arms in the noncommon path part of the interferometer due to air-density fluctuations.

In this Letter, we initially lay out a theoretical framework to link the phase and amplitude sensitivity of the off-axis interferometric measurements to the intensity distribution of the fringe in the recorded interferogram at the shot noise limit. The conclusions, however, are readily extensible to the case of phase shifting interferometry where the intensity variations occur temporally for the same pixel rather than spatially over a few pixels. Following this theoretical analysis, we show experimental sensitivity values match very well with this predicted theoretical limit. This agreement means sensitivities of phase and amplitude measurements made with a conventional CMOS camera is mostly limited by photon shot noise measurement, particularly at high imaging speeds, e.g., hundreds of fps. Integration of the signal through averaging of the frames can improve measurement sensitivities until we

face environmental disturbances at approximately 20 Hz in our case. Therefore, when integration time is faster than approximately 50 ms, the measurement sensitivities improve by the square root of the number of the averaged frames, as expected. However, as noted earlier, integration in time will compromise the temporal resolution of the measurements and does not improve sensitivity when integration time is beyond 50 ms (i.e., 20 Hz environmental noise). Using a novel camera with more than a million full electron well capacity, we show that the contribution of the shot noise can be significantly reduced and sub-milliradian sensitivity can be achieved at high-speed single frame measurements. Through temporal averaging, sensitivity can be further improved achieving an optical path stability of a few picometers. Our conclusion is that sensitivity at this order is limited by the air-density fluctuations even for near-common-path interferometers. We are designing next generation interferometers that can even better reduce these residual noncommon path environmental effects.

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Fig. 1. Sketch of the raw interferogram and the intensity profile distribution relative to the full electron well depth of the camera.

Fig. 2.

(a) An experimentally measured interferogram. (b) The Fourier transform of the interferogram. (c) Histogram of the intensity distribution of the raw interferogram. The unit is electrons in all plots. Scale bar is $2.5 \mu\text{m}$.

Fig. 3.

(a) Amplitude and (b) phase of the raw interferograms in absence of any sample. Corresponding corrected (c) amplitude and (d) phase maps. Scale bar is 2.5 μm .

Fig. 4.

(a), (c) Variations in the average phase and amplitude over time, respectively. (b), (d) Frequency spectra of the phase and amplitude fluctuations. The vertical axes of the frequency spectra are normalized relative to the peak value.

Fig. 5.

(a), (b) Standard deviation maps of the temporal fluctuations of the amplitude and phase measured by the Photron camera. (c), (d) Standard deviation maps of the temporal fluctuations of the amplitude and phase measured by the Adimec camera. Scale bar is 2.5 μm .

Table 1

Phase and Amplitude Sensitivities as a Function of the Number of Averaged Frames

	Photron		Adimec	
	phase (rad)	amplitude	phase (rad)	amplitude
1 frame	3.3×10^{-3}	3.3×10^{-3}	5.9×10^{-4}	5.9×10^{-4}
10 frame	1.1×10^{-3}	1.0×10^{-3}	19.1×10^{-4}	18.7×10^{-4}
100 frame	3.4×10^{-4}	3.3×10^{-4}	8.3×10^{-5}	7.2×10^{-5}