Axially-offset differential interference contrast microscopy via polarization wavefront shaping

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Abstract: Sample-scan phase contrast imaging was demonstrated by producing and coherently recombining light from a pair of axially offset focal planes. Placing a homogeneous medium in one of the two focal planes enables quantitative phase imaging using only common-path optics, recovering absolute phase without halo or oblique-illumination artifacts. Axially offset foci separated by 70 μm with a 10x objective were produced through polarization wavefront shaping using a matched pair of custom-designed micromachined arrays, compatible with retrofitting into conventional commercial microscopes. Quantitative phase imaging was achieved by two complementary approaches: i) rotation of a half wave plate, and ii) 50 kHz polarization modulation with lock-in amplification for detection.

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1. Introduction

Phase-sensitive microscopy, including Zernike phase contrast and Nomarski differential interference contrast (DIC), allows visualization of weakly scattered samples with low contrast in conventional bright field microscopy [1–3]. Zernike phase contrast produces intensity contrast dependent on spatial interference between light patterned with annular rings, effectively highlighting phase differences between relatively tightly focused and relatively gently focused beams. Nomarski DIC instead produces contrast from interference between laterally offset locations within the field of view. In DIC, a Nomarski prism splits linearly polarized incident light to two orthogonally polarized components with a slight angle offset. By recombining the two components using a matched Nomarski prism, the resulting interferogram at a particular position within the field of view scales with the phase difference between that location and an adjacent spatially offset position. DIC microscopy has been actively used in biological research since the 1970s, including observing neurons in unstained tissue slices [4], studying kinesin-driven movement [5], visualizing microtubule-related motility in cells [6], etc. In both Nomarski and Zernike phase contrast imaging, artifacts related to the interference between locations within the field of view complicate image interpretation. Specifically, Zernike images generally produce orientation-independent “halo” artifacts and Nomarski orientation-dependent “side lighting” artifacts. Furthermore, both phase contrast microscopy and DIC microscopy lack the capability to quantitatively retrieve the absolute phase information from the recorded images without careful calibration [7].

To achieve artifact-free quantitative phase imaging (QPI), Gabor suggested the use of interferometry to quantitatively recover the complex optical field [8], in which a beam is split and recombined at an angle such that the focal plane array records an interferogram [9–12]. Based on the same physical principles, many other interferometric QPI approaches were developed recently [7,13,14]. Although the abovementioned QPI methods can provide
quantitative phase information with high precision, these dual-path interferometric approaches face the common problem of being vulnerable to environmental perturbations such as mechanical vibrations and temperature changes since the reference beam does not pass through the same optical path as the light coming from the object. In addition, the requirement of a long coherence-length reference beam increases the complexity of these QPI systems making them incompatible with retrofitting into existing microscopy systems. Finally, phase retrieval in dual-path QPI is performed by image analysis relative to a background, which can be challenging in complex samples for which a background reference is not trivially available.

Recent developments have helped address the limitations of dual-path interferometry for QPI. In work by Popescu and associates, a diffraction grating and spatial filter were used to produce interference from the 0th and 1st order of diffraction from the same microscope output, suppressing the phase instability in dual-path QPI microscopy [15–17]. However, these interference approaches are still inherently underdetermined; both phase and intensity must be inferred by image reconstruction rather than directly and independently measured at each pixel. As an alternative, Fourier ptychography [18], phase retrieval with designed periphery [19], using patterned illumination [20] or spatial light modulation for image shifting [21], and coherent diffraction imaging [22] recover the absolute relative phase information from adjacent objects without a reference beam. However, these methods are based on assumptions on the beam passing through the object and mathematically inferred a reference wavefront, complicating both the image acquisition and the post-processing. Therefore, an approach capable of independently recovering absolute phase at every pixel using common path optics may have distinct advantages in phase recovery for complex objects with low spatial correlation. Additionally, architectures that are compatible with retrofitting into existing microscopes rather than requiring custom designs have the potential to greatly expand the broader use and access to artifact-free QPI.

In the present work, axially-offset differential interference contrast (ADIC) microscopy for QPI was developed via polarization wavefront shaping using a matched pair of micro-retarder arrays (μRAs). The μRA is a custom optic enabling patterning of the retardance and fast axis orientation of a liquid crystal on a per-pixel basis. Polarization wavefront shaping using the μRA to produce a polarization-dependent pattern expected from the combination of orthogonally polarized divergent and convergent beams. For linearly polarized incident light and half-wave retardance in every pixel in the μRA, the polarization wavefront is identical to that produced by the interference between a slightly diverging right circularly polarized (RCP) plane wave and a slightly converging left circularly polarized (LCP) plane wave. After passing through a 10x objective, each of the orthogonal polarization components focus to separate focal planes separated by 70 μm (~5.6 times the depth of field), serving as sample and reference planes. Two strategies, including half wave plate (HWP) rotation and lock-in amplified (LIA) detection, were adopted in ADIC microscopy for simultaneous retrieval of transmittance (real component of the image) and quantitative phase (imaginary component) images. The recovered quantitative phase (QP) images agreed well between the two strategies with a phase range from $-\pi$ to $\pi$ and a detection limit of 0.033 radian. Silica microbeads were used to investigate the refractive index with an agreement between the measurement and the refractive index of amorphous bulk silica. QP images in tissue section samples were measured by using ADIC microscopy.

2. Methods

2.1 Sample preparation

Two separate samples were prepared for analysis by ADIC: silica beads and mouse tail sections. Silica beads sized in diameter of 8 μm were donated by Prof. Mary Wirth (Purdue University, West Lafayette, IN). For quantitative phase imaging, the silica beads were
dispersed by ultrasonication in a commercially available nitrocellulose matrix with a high vapor pressure solvent plasticizer (Sally Hanson nail polish, Hardener) before sealing between a cover slip and a glass slide, followed by solvent evaporation. Mouse tail sections were provided by Prof. Philip Low (Purdue University, West Lafayette, IN). Mouse tails were first decalcified in the solution of 23% formic acid, 4% formalin, and 1% methanol for 2 hours, followed by sectioning longitudinally to ensure that sections were retrieved from the central region of the tail. The mouse tail section was then fixed in 10% formalin and embedded in paraffin prior to microtoming into 4 μm thick slides. After sectioning, the mouse tail was stained by hematoxylin and eosin.

2.2 Principles of polarization wavefront shaping by μRA

A linearly polarized incident beam can be viewed as a coherent combination of two orthogonally polarized components. In standard DIC, linearly polarized incident light is split into horizontally polarized and vertically polarized light using Nomarski prism [23]. In the more general case, the polarization state of light at any position on the Poincaré sphere can also be defined by a coherent combination of two orthogonally polarized components, such as RCP and LCP light.

While most conventional optics affect the polarization state of the entire beam identically, waveplate arrays, such as liquid crystal polymer waveplates, allow the tailoring of the polarization state of light on a per pixel basis. The geometrical phase modulation can be achieved by orientation modulation of liquid crystal polymers on thin polymer substrates. Waveplate arrays have been developed for adaptable lenses [24,25], grating prisms [26,27], spiral phase retarders [28–30], etc. Wavefront shaping has been used to split beam into beamlets of orthogonally polarized light focused or defocused depending on the handedness of polarization of the beam [31]. In the present work, a retardance pattern of concentric rings with a quadratic spacing was designed to produce an orthogonally polarized pair of converging and diverging beams.

The pattern necessary to produce this beam pair is most intuitively understood by first considering the anticipated polarization-dependent pattern produced by the interference of orthogonally polarized converging and diverging plane waves. The overlay of converging right circular and diverging left circularly polarized beams produces linearly polarized light with the axis of polarization rotated in a radial pattern as shown in Fig. 1(A), which has similarities to a Fresnel zone plate. Designing a μRA to produce this pattern for a linearly polarized incident beam therefore generates diverging and converging components, which can be recombined using a paired μRA in transmission. For linearly polarized incident light with the plane of polarization given by the angle γ, decomposition into the RCP and LCP contributions yields the expression shown in Eq. (1).

\[
\begin{align*}
\cos(\gamma) &= \frac{1}{2} e^{-i\gamma} \begin{pmatrix} 1 \\ i \end{pmatrix} + \frac{1}{2} e^{i\gamma} \begin{pmatrix} 1 \\ -i \end{pmatrix} \\
\sin(\gamma) &= \frac{1}{2} e^{-i\gamma} \begin{pmatrix} 1 \\ -i \end{pmatrix} - \frac{1}{2} e^{i\gamma} \begin{pmatrix} 1 \\ i \end{pmatrix}
\end{align*}
\]

(1)

In this work, μRAs were designed with the pattern of half-wave retardance of 532 nm light at every position varying spatially in the azimuthal orientation of the fast-axis with a 60 μm × 60 μm pixel size per entry and an active radius of 12 mm. The azimuthal orientation θ as a function of x, y position agrees to the relationship \( \theta(x, y) = 2\pi[(x - r)^2 + (y - r)^2]/4f\lambda \), in which r is the active radius, f is focal length designed as 6.28 m and \( \lambda \) is the targeting wavelength as 532 nm. The azimuthal orientation was then wrapped in the range of [0, π].

The designed μRA was then custom produced by Thorlabs. No artifacts from the periodicity inherent in the μRA design were detectable in either the measured intensity patterns shown in Fig. 1(A) or in the resulting QP images. Unlike Nomarski DIC, in which the split beams are offset laterally within the sample plane [Fig. 1(B)], the decomposed converging LCP and diverging RCP light after the μRA were offset axially after the same objective, such that the
reference beam was defocused within the sample plane [Fig. 1(C)]. Axially offsetting the two focal planes allowed the use of a homogeneous medium (e.g., glass, solvent, air) as a reference. In the absence of a sample, a paired identical μRA in transmittance coherently recombined the two orthogonal components to recover a plane wave with the original polarization state of the incident light. Phase contrast imaging can be performed by placing a polarizer in the quadrature position, with shifts in phase between the sample and reference planes resulting in changes in polarization states, and correspondingly, changes in detected intensity. Modulating the polarization states of the incident light allows for signal to noise enhancement through LIA detection.

Fig. 1. (A) The design of μRA as half-wave retardance with spatially varied azimuthal orientation of the fast-axis targeted for 532 nm light. Scale bar: 500 µm. Bottom: part of the measured different intensity distribution with horizontal (H) and vertical (V) polarization detection when horizontally polarized light passing through the μRA. (B) The working principle of traditional Nomarski phase contrast microscope. (C) The working principle of ADIC microscope. L1 and L2: lens; RP: reference plane; SP: sample plane.

2.3 Instrumentation for ADIC microscopy

ADIC microscope was constructed based on a bright field microscope frame with the addition of several polarizing optics (Fig. 2). In brief, a 532 nm continuous laser (Millenia Vs J) was used for illumination with initial horizontal polarization, followed with a half wave plate (HWP) inserted in a rotation stage for linearly polarization modulation of the incident light. The beam was expanded to 15 mm in diameter to fill approximately half the area of the μRA and the full aperture of a 10x objective (0.3 NA, Nikon). The average laser power on the sample was around 5 mW. An identical 10x 0.3 NA objective was used as a condenser in transmittance, followed by passage through a paired μRA, positioned and oriented to recover an identical polarized plane wave in the absence of a sample. A sample scanning stage (Mad city labs Nano-Bio300) was driven by two phase-locked function generators (Tektronix AFG2021 and Agilent 33220A) for image acquirement with a frame rate of 20 s with a field of view (FoV) of 250 µm × 250 µm. Horizontally polarized transmittance was detected by passing the beam through a polarizer (LPNI100-B, Thorlabs) and a photodiode (DET-10A, Thorlabs). Signals were digitized at 20 kHz using a PCI-E digitizer oscilloscope cards (AlazarTech ATS-9462) and remapped into 200 × 200 images via custom software (MATLAB), giving a pixel size of 1.25 µm/pixel. Polarization modulation measurements were conducted via mechanical rotation of the HWP from 0 to 90 degrees with 3-degree intervals. For the fast polarization modulation coupled with LIA (Stanford Research Systems SR810) detection, a photoelastic modulator (PEM, Hinds instrument PEM-90M) was inserted between HWP and a quarter waveplate (QWP). PEM is made of isotropic optical materials and introduce retardance \( \Delta(\tau) \) to the incident light as a function of time when driven at a resonant frequency. It has two electric TTL outputs with one has the modulation frequency \( (f) \) and the other has the doubled frequency \( (2f) \) of the sinusoidal driven electric field. The
fast axis of the HWP, PEM and QWP were rotated to 22.5°, 0° and 45°, respectively, for modulation of the linearly polarized light before entering the µRA, as detailed in Eqs. (7) and (8) (Section 2.4). The PEM was operating at its resonance frequency of 50 kHz, with both 1f (50 kHz) and 2f (100 kHz) outputs delivered as the reference signals to the LIA. Both the quadrature and in-phase components of the LIA output were acquired simultaneously. The per-pixel integration time of the LIA was 30 μs. The same setup was used for both the investigation experiment of the dual-foci and the later QPI experiments.

![Experiment setup](image)

**Fig. 2.** Experiment set-up for QPI with a 10x objective to recover both bright field images and QP images. Blue circled optics: add-in parts for LIA detection.

### 2.4 QP image recovery

In the absence of a sample, the identity matrix produced by the sequential combination of the matched ADIC optics can be decomposed as a linear combination of Hermitian Pauli matrices as shown in Eq. (2). The decomposed matrices can describe the Jones matrices corresponding to the two foci with orthogonal polarized components.

\[
\overrightarrow{e} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}, \quad \overrightarrow{q} = \frac{1}{2} \left[ \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} + i \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix} + i \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} - i \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \right] \cdot \overrightarrow{e}
\] (2)

When a sample-induced phase shift is introduced in either focal plane, the Jones vector describing the signal electric field (\(\overrightarrow{e}_{\text{det}}\)) after the sample can be expressed by Eq. (3). The complex-valued amplitude transmittances \(t^+\) and \(t^-\) describe the field detected following interaction in the two foci separately. The phase change \(\delta\) induced by the sample at a given location is defined to be the phase shift between the two orthogonally polarized focal planes (sample and reference planes). In the absence of a sample, \(|t^+| = |t^-| = 1\) and \(\delta = 0\), resulting in \(e_{\text{det}} = e\) that the detecting signals are identical to the incident light.

\[
\overrightarrow{e}_{\text{det}} = \frac{1}{2} \left[ |t^+|^2 \begin{pmatrix} 1 & i \\ -i & 1 \end{pmatrix} \cdot e^\delta + |t^-|^2 \begin{pmatrix} 1 & -i \\ i & 1 \end{pmatrix} \cdot e^{-\delta} \right] \cdot \overrightarrow{e}
\] (3)

The (+) and (-) focal planes produced by the µRA are orthogonally polarized relative to each other and 90 degrees phase-shifted relative to the incident polarization state (e.g., for linearly polarized light, the polarization states of the (+) and (-) focal planes are RCP and LCP, respectively, while for RCP incident light, the (+) and (-) focal planes are horizontally and vertically polarized light, respectively. In the case of half wave plate rotation strategy...
describing horizontally polarized light passing through a half wave plate with fast axis rotated to angle \( \gamma \), the Jones vector describing the incident light is given by the following expression:

\[
\mathbf{e}^{\text{(in)}}(\gamma) = \begin{bmatrix}
\cos 2\gamma & \sin 2\gamma \\
\sin 2\gamma & -\cos 2\gamma
\end{bmatrix}
\begin{bmatrix}
1 \\
0
\end{bmatrix}
\]  

(4)

The detected intensity through a polarizer rotated to angle \( \phi_{\text{pol}} \) is given by Eq. (5).

\[
\mathbf{e}^{\text{det}}(\gamma) = \begin{bmatrix}
\cos \phi_{\text{pol}} & -\sin \phi_{\text{pol}} \\
\sin \phi_{\text{pol}} & \cos \phi_{\text{pol}}
\end{bmatrix}
\begin{bmatrix}
1 & 0 \\
0 & 0
\end{bmatrix}
\begin{bmatrix}
\cos \phi_{\text{pol}} & \sin \phi_{\text{pol}} \\
-\sin \phi_{\text{pol}} & \cos \phi_{\text{pol}}
\end{bmatrix}
\mathbf{e}^{\text{(in)}}(\gamma)
\]

(5)

\[
I(\phi_{\text{pol}}, \gamma) \propto |\mathbf{e}^{\text{det}}(\gamma)|^2
\]

(6)

when detecting the horizontally polarized component (\( \phi_{\text{pol}} = 0 \)), combining the expressions in Eqs. (3), (4), (5) and (6) followed by simplification yields Eq. (7), in which \( \gamma \) is the rotated angle of the incident HWP.

\[
I(\gamma) \propto \left| I^+ + I^- + 2|I^-| \right| \cos(\delta + 4\gamma)
\]

(7)

For the LIA detection, the PEM and the QWP were placed between the HWP and pair of lenses for beam expansion, and the fast axis of HWP and QWP were rotated to 22.5° and 45°, respectively, as shown in Fig. 2. The Jones vector describing the incident light is given by the following Eq. (8), in which \( \Delta(\tau) \) is the retardance modulation introduced by the PEM as a function of time (\( \tau \)) with a modulation amplitude of 2\( A \), as defined in Eq. (9). It can be seen that with a horizontally polarized incident light, a linearly polarized light was generated after HWP, PEM and QWP to pass through the \( \mu \)RA. The retardance modulation frequency for the PEM was \( f = 50 \) kHz.

\[
\mathbf{e}^{\text{(in)}}(\tau) = \begin{bmatrix}
1 & -i \\
-i & 1
\end{bmatrix}
\begin{bmatrix}
\cos(A\phi) & -\sin(A\phi) \\
\sin(A\phi) & \cos(A\phi)
\end{bmatrix}
\begin{bmatrix}
1 & 0 \\
0 & 0
\end{bmatrix}
\begin{bmatrix}
1 & 1 \\
1 & -1
\end{bmatrix}
\begin{bmatrix}
\sin \left( \frac{\Delta(\tau) + \pi}{2} \right) \\
\cos \left( \frac{\Delta(\tau) + \pi}{2} \right)
\end{bmatrix}
\]

\[
\Delta(\tau) = 2A \cdot \sin(2\pi f \tau)
\]

(8)

The time-dependent detected signal intensity of the horizontally polarized component measured in transmission is given by combining Eqs. (3), (5), (8) and (9).

\[
I(\tau) \propto \left| I^+ + I^- + 2|I^-| \right| \cos(\delta + 4\tau)
\]

when \( A \) is relatively small, a Taylor series expansion of Eq. (10) with respect to \( \tau \) shown in Eq. (11) converges rapidly.

\[
I(\tau) \propto \left| I^+ + I^- + 2|I^-| \right| \cos(\delta + 4\tau)
\]

(10)

\[
I(\tau) = 2|I^-| \sum \left| \frac{\Delta(\tau) + \pi}{2} \right| \cos(\delta + 4\tau)
\]

(11)

According to Eq. (11), the quadrature components (sine) only map to the odd harmonics of the Taylor series, while the in-phase components (cosine) terms are only present in the even
harmonics. The proportionality in Eq. (11) evaluated at seventh order will result in negligible errors for the PEM modulation for $A < \pi / 2$. In our experiments, the modulation amplitude of the PEM was set as $A = 0.3\pi$, consistent with the range indicated. Retaining the first seven terms of the expansion, the quadrature components (sine, $Y$) of the first harmonic ($1f$) and the in-phase components (cosine, $X$) of the second harmonic ($2f$) LIA detection are written as Eqs. (12) and (13), respectively.

\begin{align}
1f_y &= 2\left| r^* \right| \left| r \right| \left( 2A^2 - \frac{A^4}{6} + \frac{A^6}{72} \right) \cos \delta \\
2f_x &= -2\left| r^* \right| \left| r \right| \left( A^2 - \frac{A^4}{3} + \frac{A^6}{24} \right) \sin \delta
\end{align}

Combining Eqs. (12) and (13), one can recover values of $\cos \delta$ and $\sin \delta$ regarding $1f_y$, $2f_x$, and the PEM modulation $A$. Then $\delta$ can be achieved through Eq. (14) in a range of $[-\pi, \pi]$.

$$\delta = \text{Im}[\text{ln}(\cos \delta + i \sin \delta)]$$

Unlike the HWP rotation strategy, the transmittance image recovered from the LIA detection is defined as $|r^*| |r|$. It is noteworthy that both the HWP and PEM polarization rotation strategies recover phase values in the range of $[-\pi, \pi]$.

3. Results

3.1 Experimental evidence of axially-offset dual-foci produced by µRA

Experimental measurements of a 1951 USAF resolution test chart were performed to assess the change in 3D point spread function induced by the µRA, the results of which are shown in Fig. 3. From the images, the radial extent of the point spread function was evaluated based on edge analysis, in which the derivative of the intensity profile was fit to a Gaussian function to recover the RMS (root mean square) beam width. Plots of the recovered Gaussian beam cross-section are shown in Fig. 3 for multiple z-positions of the test grid in order to map the beam profile in all three dimensions assuming cylindrical symmetry. For an object (e.g., the test grid) initially in the focal plane of the microscope, addition of the µRA resulted in a slight blurring. However, crisp images were recoverable upon axial translation of either ± 35 µm. Consistent with this observation, analysis of the images shown in Fig. 3(A) generated by the wavefront shaping of the µRA produced two foci separate by 70 µm symmetrically distributed about the original focal plane [Fig. 3(B)]. Due to analysis of the results summarized in Fig. 3, the cross-sections of the axially offset foci are statistically indistinguishable from the original focus, indicating no substantial perturbation to the point spread function upon addition of the µRA. Comparison of the point-spread functions between Figs. 3(A) and 3(B) indicates that the spatial resolution (~2 µm) is unchanged by the addition of the µRAs. The introduction of the large spatial offset between the two foci (roughly 1/4 of the 250 µm × 250 µm FoV) makes it possible to create a stable and uniform reference plane positioned within a homogeneous medium (e.g., glass, air, or solution) immediately adjacent to the sample plane, removing imaging artifacts arising from the use of sample and reference locations cohabitating in the focal plane as in Nomarski and Zernike phase contrast microscopy.
3.2 QPI through HWP rotation

To retrieve the QP images from ADIC imaging using the HWP rotation strategy, a whole set of images was collected with the HWP rotating through a 90-degree range, with the horizontally polarized transmitted beam detected using a photodiode. The whole set of images with HWP at different angles can be found in Visualization 1, Supplementary Materials. Then a pixel-by-pixel nonlinear fit was applied to the intensity trace as a function of the HWP rotation angle $\gamma$ referring to Eq. (3) to recover images of phase ($\delta$) and transmittance $\left([|f^*|+|f|]^2\right)$. Figures 4(A) and 4(B) show the recovered transmittance and QP image from a stained mouse tail section. The areas with larger magnitude phase shift [red and blue areas in Fig. 4(B)] highlight the detailed spatial distribution of the fibrils in mouse tail tissues, which produced low contrast in the retrieved transmittance image. The average intensities of the blank (no sample) areas in the raw ADIC image were used to calibrate the detector sensitivity and the HWP rotation angle used in Eq. (3) as shown in Fig. 4(C). The measured intensity trace and fitted results show excellent agreement, supporting the validity of this calibration strategy. The $\sim$6° phase shift in the HWP rotation angle corresponding to the minimum transmitted intensity of the blank is attributed to uncertainty in the $\mu$RA orientations, which were not in precision rotation stages. Fits for quantitative phase retrieval for two representative pixels in the image are shown in Fig. 4(D), with good agreement between the measurements (dots and stars) and fits (lines). The mean of the sum of squared errors of prediction (SSE) and the coefficient of determination ($R^2$) for the nonlinear fit for the whole FoV were 0.0018 and 0.9164, respectively. These results confirm the degree of statistical confidence with which the phase angle can be recovered by nonlinear fitting.
3.3 QPI through LIA detection

For LIA detection, both the pure-tone $1f$ (50 kHz) and $2f$ (100 kHz) signals generated by the PEM were used as references for the LIA. The raw images (cosine components and sine components) collected from $1f$ and $2f$ LIA measurements were shown in Figs. 5(A)-5(D) for the same FoV of a mouse tail section. Figures 5(A) and 5(B) share the same image contrast settings, as did Figs. 5(C) and 5(D). Consistent with the predictions from Eq. (11), the cosine components of $1f$ and the sine components of $2f$ produced negligible contrast. The transmittance bright field image [Fig. 5(E)] and QP image [Fig. 5(F)] were calculated using Eqs. (12)-(14). Similarities were qualitatively clear between the transmittance bright field image and the sine components of $1f$ LIA detection, as well as between the QP image and cosine components of $2f$ LIA detection. According to Eqs. (12) and (13), the sine components from $1f$ and the cosine components from $2f$ converge to the bright field image and QP image, respectively, in the limit of low phase shifts $\delta$. It can also be seen that the QP image revealed more detailed structures and exhibited higher contrast for those areas with higher transmittance, such as the fibrils shown in the circle in Figs. 5(E) and 5(F).
The agreement between the two QPI strategies applied to the ADIC microscopy, HWP rotation and LIA detection were tested by imaging the same FoV of a mouse tail section as shown in Fig. 6. The transmittance bright field images [Fig. 6(A)] was recovered by nonlinear fitting through the HWP rotation strategy. To compare the retrieved QP images with different approaches [Fig. 6(B) and 6(C)], same image contrast settings were applied. Agreement between the retrieved results from the two strategies provides cross-validation of both approaches. The difference image in phase shift obtained from the two strategies was shown in Fig. 6(D). It can be seen that most differences between the two methods arise at the pixels with larger absolute phase shift values. These differences could be attributed to the phase wrapping issue that is sensitive to spherical structures and phase retrieval strategies.
4. Discussion

4.1 Separation distance between the dual-foci for QPI

The separation distance between the two foci depends on the designed periods of the centric polarization rotation pattern of our µRAs. With smaller periods of the centric modulation pattern in µRA, larger separation is expected. When the separation distance is much smaller, the setup converges to a result qualitatively analogous to Zernike phase contrast, in which only a single ring is included, and the reference plane is so close to the sample plane that the contrast is simply a halo (i.e., the negligible displacement limit). The upper limit for separating the two foci is ultimately dictated by the manufacturing precision of the µRAs; a higher density of fringes at the extrema of the array corresponds to a larger separation between foci. Manufacture with the maximum stated manufacturing resolution of 30 µm corresponds to a maximum fringe period of 60 µm (with Nyquist sampling), producing a theoretical separation distance of 360 µm for a 10x objective. In this work, a longer maximum period was used to reduce the potential for artifacts from the discretization effects at the extrema, giving a separation distance of 70 µm. By enabling such a large axial offset between the two focal planes, the reference beam is significantly defocused in the sample plane to a spot size of ~44 µm in diameter. As such, the phase shift induced at a given ~3 µm² location within the sample plane is interfered with the average phase within a ~1500 µm² area comprising the cross-sectional area of the reference beam within the sample plane. While not serving as an entirely independent reference as in dual-path QPI, the reference effectively spans the optical phase averaged over an area in the field of view ~500-fold larger than the focal volume using common-path optics. For sparse, well-separated objects such as recorded herein, the phase-bias in the reference path induced by the sample is negligible and two conditions converge.

4.2 Limit of detection (LoD) of ADIC-QPI

The images produced by ADIC are free of halo and side-lighting artifacts routinely encountered in Zernike and Nomarski phase contrast methods, respectively. The absence of these artifacts arises from the spherical symmetry of the interference condition coupled with the relatively large area within the field of view serving as a phase reference for the sample plane. The limits of detection for the phase shift calculation from the two strategies were investigated by analyzing the QP images obtained from ADIC microscopy in absence of samples. The measured standard deviation (σ) of each phase image retrieved by the HWP rotation and LIA detection strategies was 0.003 rad and 0.011 rad, respectively. Thus the LoD (3σ) of each QPI approach was deduced to be 0.009 rad and 0.033 rad. Considering a thin film of lipid (n = 1.50) [32–34] in an aqueous environment (n = 1.33) measured using ADIC microscopy, the smallest optical path length that could be determined with the two strategies is 4.5 nm and 16.4 nm, respectively. Note the self-calibration of the nonlinear fitting could reduce the 1/f noise arising from the long acquisition time in the HWP rotation strategy (about 10 min for a whole set of 90-degree rotation with 6-degree intervals, corresponding to integration time of 480 µs for each pixel). However, the pixel-by-pixel nonlinear fitting leads to the time-consuming data analysis process. Due to the limit capability of the LIA used in our experiments, the 1/f and 2/f ADIC raw images were acquired separately with different reference signals. In addition, the LIA integration time used in our experiments were limited to 30 µs (3 modulation periods). The ~3-fold improvement in the phase uncertainty using HWP rotation is attributed to the ~16-fold increase in per-pixel measurement time relative to the PEM measurement. Significant improvements are reasonable to expect using longer integration times with simultaneous LIA detection of the 1/f and 2/f signals.
4.3 Recovery of refractive index of microspheres

Silica beads 8 μm in diameter were used for quantitative phase imaging and to calculate the refractive index of the silica beads. Figure 7 shows the QP images for the same FoV of 8 μm silica beads retrieved from both HWP rotation and LIA strategies. Great agreement can be seen between the results of the two strategies [Figs. 7(A) and (B)], as mentioned in Section 3.3. The refractive index of silica microbeads was calculated based on the measured phase shift and bead size. The phase shift line profile of a single bead [insets of Figs. 7(A) and 7(B)] obtained from both strategies are plotted in Fig. 7(C). The phase shift (δ) in the center of the measured 7.3 μm silica bead was 2.53 radian. Statistical analysis of silica microspheres in Fig. 7 allows us to calculate the difference between the refractive indices of silica bead and nitrocellulose matrix: Δn = δλ/2πD = 0.0293 ± 0.0007. Given the refractive index of nitrocellulose is 1.505 at 543.5 nm [35], the refractive index of silica bead is calculated as 1.4757 ± 0.0007 at 532 nm. Although there is no report on the optical constants of silica microparticles, our result is consistent with the refractive index of amorphous bulk silica as 1.461 [36].

![Fig. 7. Quantitative phase contrast images of the same FoV of 8 μm silica beads recovered from both HWP rotation (A) and LIA detection (B) strategies. Color bar unit: phase shift in radian. Scale bar: 50 μm. Inserts: zoom-in for one single bead. (C) Phase shift line profiles of the cross line in the insets retrieved from the HWP rotation (blue dots) and LIA detection (orange squares) approach.](image)

5. Conclusion

A quantitative phase contrast microscope was developed by ADIC imaging through polarization wavefront shaping via a matched pair of μRAs. HWP rotation and LIA detection strategies enabled simultaneous recovery of both transmittance and QP images, with good agreement observed between the recovered QP images from both the strategies. The smallest detectable phase shift was determined to be 0.009 radian in HWP rotation strategy with integration time of 480 μs for each pixel and 0.033 radian in LIA detection strategy with an integration time of 30 μs for each pixel. Proof of concept studies with tissue samples and silica beads indicated excellent agreements between the quantitative phase shift analysis by both HWP rotation and LIA detection strategies, as well as with the theoretical predictions. The μRAs can be customized for particular wavelengths and axial offsets between foci, supporting design for application-specific imaging. ADIC was enabled by the addition of two fixed thin optics within an otherwise standard optical path, suggesting broad compatibility for retrofitting into existing commercial microscopes. In ongoing work, the ADIC is being extended into a wide field quantitative phase contrast imaging using a broadband and spatially incoherent LED illumination source. In addition, another autocorrelation method is also under development using ADIC principle for retrievable of quantitative phase information and the particle size distribution, which can be utilized to calculate the absolute refractive index of nanoparticles.
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Disclosures
The authors declare that there are no conflicts of interest related to this article.

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