Three-dimensional measurement of a particle field using phase retrieval digital holography

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Digital inline holography (DIH) has long been used to measure the three-dimensional (3D) distribution of micrometer particles in suspensions. However, DIH experiences a virtual image problem that limits the particle density and the placement of the hologram plane relative to the sample volume. Here, we apply virtual-image-free phase retrieval digital holography (PRDH) to detect opaque particles in 3D volumes that exceed 2000 particles/mm³. PRDH is based on recording two holograms whose planes are displaced along the optical axis, and then reconstructing the complete optical waves estimated by the iterative phase retrieval algorithm. Both numerical and experimental tests are performed, and results show that PRDH recovers the original 3D particle distributions even when the hologram planes are within the particle suspensions. Moreover, compared to single-hologram-based DIH, PRDH is proved to have better particle detection qualities. The uncertainty in the localization of particle centers is reduced to less than one particle diameter.

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1. INTRODUCTION

Precise detection of the three-dimensional (3D) spatial locations of micrometer particles is crucial in many areas, from measurements of fluid velocity [1–5] to characterizations of particle transportation [6–12] and analysis of animal locomotion [13–16]. In recent years, digital inline holography (DIH) has been proved to be a simple yet powerful tool for accomplishing this task [17–20]. Using a coherent light, DIH first encodes the 3D information on a hologram, and then digitally reconstructs the hologram to obtain the 3D optical field [21]. The particles can then be detected and localized through a range of refocusing criteria, for example, by minimal intensity [22,23], largest intensity gradient [24–26], and minimal phase variation [27]. However, inherent to the inline setup, DIH experiences the virtual (or twin) image problem [19]. Reconstructing inline holograms creates the real image, in-focus virtual image, and out-of-focus virtual image. The in-focus virtual image and the real image are placed symmetrically on two sides of the hologram plane, and are identical to each other. The out-of-focus virtual image, located at the plane of the real image, reduces the signal-to-noise ratio of the real image. For an optical setup in which no imaging lenses are inserted before the camera, particles are always located on one side of the hologram plane. Reconstructing such a hologram to one side of the hologram plane involves only the real image and out-of-focus virtual image. In contrast, for an optical setup where an imaging lens is used and the focal plane of the imaging lens is placed within the particle suspensions, the hologram reconstruction generates an in-focus virtual image, in addition to the real image and out-of-focus virtual image. The in-focus virtual image prevents the detection of the original 3D particle distributions [28]. Past researchers [5,22] who used digital holographic microscopy for particle measurements had to place the focal plane of the microscope objective outside of the particle suspensions. Therefore, for applications where the particle density is high and objects appear on both sides of the hologram plane—for example, when measuring a 3D velocity field with a hologram plane intentionally placed in the particle suspensions to improve spatial resolution [2,29,30]—efficiently removing the in-focus and out-of-focus virtual images is highly desirable.

The key to eliminating virtual images from inline holograms is to recover the missing phase distribution at the hologram plane, such that the complete optical wave is known. Reconstructing this complete wave (or complex amplitude) generates a 3D optical field containing real images only. Various approaches have been developed in past years to estimate the phase distribution. One method is the so-called phase-shift digital holography (PSDH) [31], where multiple holograms are recorded by varying the phase of the reference wave while keeping the object wave constant. The complete object wave is calculated with a simple algebraic equation consisting of these phase-shifted holograms [19]. Since the phase shift applies only to the reference wave, PSDH commonly adopts a Mach–Zehnder interferometry setup instead of a simple

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Gabor configuration [32]. The phase-shifted holograms can be acquired either at different times, by using uniform phase-shift devices (e.g., wave plates) [33–36], or in a single shot, using pixelated phase-shifting devices (e.g., spatial light modulator) at the cost of spatial resolution [37–39]. The “single-shot” (also called “parallel”) PSDH is suitable for dynamic systems, and has been applied to the measurements of living cells [40] and density variations of high-speed gas flows [41]. However, since it requires a precise shifting device and a vibration isolation table, using it to measure particle suspensions is relatively difficult.

Phase retrieval digital holography (PRDH) [42,43] is another technique used to estimate the phase distribution at the hologram plane. PRDH is based on propagating light back and forth between two planes with constraints superimposed on these planes, the so-called Gerchberg–Saxton algorithm [44]. When imaging a single two-dimensional (2D) object, PRDH only requires a single hologram [45–48]. By propagating light between the hologram and object planes, and setting constraints at the object plane (e.g., the object’s absorption must be positive [45]), PRDH can effectively clear up the out-of-focus virtual images. In contrast, when measuring volume samples such as particle suspensions, PRDH has to record two or more holograms whose planes are separated by short distances along the optical axis. Then the phase can be approximated by propagating light between the hologram planes [28,50] or using simple algebraic equations [51–53]. In PRDH, a simple Gabor setup can be used, and two axially displaced holograms can be recorded simultaneously by using two cameras [28]. Therefore, PRDH is suitable for measuring the 3D spatial distribution of particles in suspensions.

Researchers [42,43,50] have applied PRDH with two holograms to detect particles, including both opaque and transparent ones [42]. They placed both hologram planes outside of the sample volume and showed that PRDH removed the out-of-focus virtual images and improved the quality of the reconstructed particle images. However, when an imaging lens is inserted before the camera and the focal plane of the imaging lens is placed within the particle suspension, an in-focus virtual image is generated during the reconstruction. To our knowledge, no one has tested whether PRDH can remove the in-focus virtual image and therefore allow the placement of the focal plane of the imaging lens within the particle suspensions. Furthermore, the improvement in the accuracy of particle detection and particle center localization in suspensions by PRDH has not been fully quantified.

In this paper, we test the performance of PRDH with two holograms in the detection of particles in suspensions using both simulations and experiments. In particular, we place the hologram planes in the middle of the sample volume such that objects present on both sides of the holograms. We will show that PRDH can effectively remove the virtual images and recover the original particle distribution. In addition, we will prove that PRDH has better particle detection qualities (e.g., less false detection, better 3D localization accuracy) than single-hologram-based DIH. The principle of PRDH will be presented in Section 2, followed by numerical and experimental tests in Sections 3 and 4, respectively, and conclusions in Section 5.

2. PRINCIPLE

The optical setup of PRDH with two holograms is shown in Fig. 1(a), similar to those shown in previous studies [28,50]. We use a Cartesian coordinate system (x, y, z), where x and y denote the two directions perpendicular to the optical axis and z the direction of the light beam. A collimated laser beam is used to illuminate the sample volume. The light scattered by objects (object wave) and the undisturbed light (reference wave) form interference patterns, i.e., the holograms. Two cameras are used to record two holograms simultaneously. A microscope objective is inserted before the cameras to magnify the interference pattern. The focal planes (i.e., object planes) of the microscope objective are placed intentionally within the sample volume. The hologram planes coincide with the focal planes of the microscope objective [22,54,55]. We define the positions of hologram planes 1 and 2 as z = 0 and z = D, respectively. Here, D is the distance between the two focal planes. The particle position is denoted as (x_p, y_p, z_p) and the particle diameter as d_p. Thus, z_p can be either positive or negative. As illustrated in Fig. 1(b), regardless of the sign of z_p, particles create diffraction patterns on the holograms due to the effect of the imaging lens. Effectively, for hologram 1 at plane z = 0, the forward scattering light from particles at z_p < 0 propagates in the +z direction, and that from particles at z_p > 0 propagates in the −z direction. Similarly, for hologram 2 at plane z = D, the forward scattering light from particles at z_p < D propagates in the +z direction, and that from particles at z_p > D propagates in the −z direction. The coordinate system defined in Fig. 1(a) applies to the entire paper.

![Fig. 1.](image-url)

- **Fig. 1.** (a) Optical setup for recording two holograms in phase retrieval digital holography by two cameras, (b) light propagation for particles located on two sides of the hologram plane, (c) Gerchberg–Saxton iterative algorithm, where h_D and h_D denote h(x, y, z = D) and h(x, y, z = −D), respectively.
Note that the current optical setup is slightly different from the one presented in Ref. [50]. In Ref. [50], no imaging lens is inserted before the cameras. The hologram planes coincide with the planes of digital sensors and are located outside of the particle suspensions. As a consequence, in Ref. [50], the hologram reconstruction only involves the out-of-focus virtual images, which are subsequently removed by the phase retrieval method. However, in current study, by inserting the microscope objective before the cameras, the hologram planes are placed within the particle suspensions. Thus, both out-of-focus and in-focus virtual images are generated in the current 3D reconstruction fields, as will be confirmed in the following sections. We will prove that the phase retrieval method removes both out-of-focus and in-focus virtual images.

The intensity distributions of the two holograms can be written as

\[ I_{H1}(x, y; z = 0) = |R_1|^2 + |O_1|^2 + R_1 O_1^* + R_1^* O_1, \]

\[ I_{H2}(x, y; z = D) = |R_2|^2 + |O_2|^2 + R_2 O_2^* + R_2^* O_2, \]

where \( R_1 \) and \( O_1 \) represent the complex amplitudes of the reference and object waves at plane \( z = 0 \), respectively, \( R_2 \) and \( O_2 \) are the complex amplitudes at \( z = D \), and the superscript \( * \) denotes a conjugate. As discussed, due the imaging lens, \( O_1 \) and \( O_2 \) contain the forward scattering light from particles on both sides of the hologram planes. Effectively, \( O_1(z < 0) \) and \( O_2(z < D) \) propagate in the \( +z \) direction, and \( O_1(z > 0) \) and \( O_2(z > D) \) propagate in the \( -z \) direction. The same applies to \( R_1 \) and \( R_2 \).

The 3D intensity fields reconstructed from holograms \( I_{H1} \) and \( I_{H2} \) can be expressed as

\[ I_{1DH}(x, y, z) = |I_{H1} \otimes h(x, y, z)|^2, \]

\[ I_{2DH}(x, y, z) = |I_{H2} \otimes h(x, y, z - D)|^2, \]

where the superscript “DIH” denotes the results in single-hologram-based DIH, the symbol \( \otimes \) represents 2D convolution, and \( h(x, y, z) \) is a diffraction kernel. We choose the Rayleigh–Sommerfeld diffraction formula,

\[ h(z) = \exp(ik\sqrt{x^2 + y^2 + z^2})/[i\lambda(x^2 + y^2 + z^2)] \]

\[ (6) \]

since the distance between the particle and the hologram plane can be as small as zero in our study. Here, \( k = 2\pi/\lambda \) is the wavenumber and \( \lambda \) is the wavelength of the laser beam. When \( D \) is not known, \( I_{H2} \) is reconstructed by using Eq. (3), i.e., without shifting the diffraction kernel, to obtain a shifted 3D intensity field \( I_{1DH} - \text{Shift} \).

The phase distributions on the hologram planes can be estimated by the Gerchberg–Saxton iterative algorithm, which was described and demonstrated in previous studies [42,43,50]. As illustrated in Fig. 1(c), each iteration consists of the following four steps: (i) propagating the complex amplitude in the first hologram plane \( E_{H1} \) to the second one: \( E_{H2} = E_{H1} \otimes h(x, y, z = D) \); (ii) applying a constraint by setting the amplitude of \( E_{H2} \) with the square root of the second hologram: \( |E_{H2}| = |I_{H2}| \); (iii) backpropagating this new complex amplitude to the first hologram plane: \( E_{H1} = E_{H2} \otimes h(x, y, z = -D) \); and (iv) applying a constraint by setting the amplitude of \( E_{H1} \) with the square root of the first hologram: \( |E_{H1}| = |I_{H1}| \). The iteration starts with an initial phase of 0 on both planes. The total number of iterations is denoted as \( n \).

Once the phases and complete optical waves at the hologram planes are known, the virtual-image-free intensity fields can be reconstructed using

\[ I_{1PRDH}(x, y, z) = |E_{H1} \otimes h(x, y, z)|^2, \]

\[ I_{2PRDH}(x, y, z) = |E_{H2} \otimes h(x, y, z - D)|^2. \]

The superscript “PRDH” denotes the results in dual-hologram-based PRDH.

3. NUMERICAL TESTS

A. Generation of Synthetic Holograms

Following similar procedures to those in Ref. [56], we generate synthetic holograms of opaque particles based on Rayleigh–Sommerfeld diffraction theory. The coordinate systems in the simulations follows the one defined in Fig. 1(a). That is, two hologram planes are located at \( z = 0 \) and \( z = D \), and particle axial position \( z_p \) is either positive or negative. To account for the effect of the imaging lens, the simulated holograms capture the forward scattering light of particles located on both sides of the hologram planes. That is, when simulating hologram 1 at plane \( z = 0 \), we set the light to propagate in the \( +z \) direction for \( z_p < 0 \) and in the \( -z \) direction for \( z_p > 0 \). Similarly, when generating hologram 2 at plane \( z = D \), we set the light to propagate in the \( +z \) direction for \( z_p < D \) and in the \( -z \) direction for \( z_p > D \). We assume each particle to be a 2D aperture that has infinite absorption and zero phase shift. Thus, the particle has the following transmission function:

\[ t(x, y) = \begin{cases} 
0 & \text{for } (x - x_p)^2 + (y - y_p)^2 \leq d^2/4 \\
1 & \text{for } (x - x_p)^2 + (y - y_p)^2 > d^2/4
\end{cases}. \]

The holograms generated by the particle are simulated by 2D convolutions as

\[ I_{H1}(x, y; z = 0) = |t \otimes h(x, y, z = -z_p)|^2, \]

\[ I_{H2}(x, y; z = D) = |t \otimes h(x, y, z = -z_p + D)|^2. \]

Using these two equations, particles located at \( -z_p \) and \( +z_p \) generate identical fringe patterns at plane \( z = 0 \) (i.e., on \( I_{H1} \)), but different ones at plane \( z = D \) (i.e., \( I_{H2} \)). When simulating particle suspensions, the holograms are obtained by adding up the interference patterns by individual particles. For the highest particle density in our simulations, the fraction of the hologram area (i.e., image size) directly occluded by the particles is less than 0.02. Considering the relatively low particle seeding density, we ignore the multi-scattering between particles. As shown later, though the multi-scattering is ignored, more than 96% of all particles in the suspensions can be successfully detected in all simulations. Nevertheless, one can improve the accuracy of particle detection by accounting for the multi-scattering [57].
**B. Numerical Tests with a Single Particle**

When implementing PRDH for particle suspensions, the distance between the hologram planes $D$ is fixed, while the distance between the particle and the first hologram $|z_p|$ varies. Thus, we study the effect of $z_p/D$ on the effectiveness of the virtual image removal. We run simulations for a single particle with $z_p$ ranging from $-2000$ to $-20$ µm and $D = 50$ µm. Other parameters in the simulations are $d_p = 4$ µm, $λ = 532$ nm, pixel size is 1.0 µm, and image size is 300 µm. A sample case for $z_p = -100$ µm is shown in Figs. 2(a)–2(c). Clearly, in single-hologram-based DIH, a virtual image (located at $-z_p$) persists [Fig. 2(b)]. In contrast, in dual-hologram-based PRDH with $n = 50$, only the real image presents [Fig. 2(c)]. The out-of-focus virtual image noise at the real image plane is cleaned.

To quantify the effectiveness of virtual image removal, we define a particle focal parameter $F_p(z)$ for each plane $z$

$$F_p(z) = I_{x,y}^z(z) - I_{x,y}^z(z_p),$$

where $I(z)$ is the intensity value at plane $z$ spatially averaged over an $(x, y)$ area of interest defined by $P$, a 2D region that covers the particle, and $S$, a particle neighbor region that is a fifth as large as that of $P$. When a particle comes into focus during the reconstruction, it becomes darker compared to the background and reaches a peak $F_p$. Therefore, the signal ratio of the virtual to the real image can be approximated by $F_p(z = -z_p)/F_p(z = z_p)$. As shown in Fig. 2(d), for all $z_p$ except $z_p ≈ 0$, the value of $F_p(z = -z_p)/F_p(z = z_p)$ is less than 10% when iteration number $n ≥ 50$, indicating that the virtual image is effectively removed. The reason that $F_p(z = -z_p)/F_p(z = z_p)$ increases for $z_p ≈ 0$ is the elongation of reconstructed particle traces along the optical axis [18].

**C. Numerical Tests with Particle Suspensions**

As shown in Fig. 3(b), the simulations include randomly distributed particles in a 3D volume of size $300$ µm × $300$ µm × $600$ µm ($x$ × $y$ × $z$). The synthetic hologram planes are located at $z = 0$ and 50 µm. The total number of particles $N_p$ varies from 60 to 540, and the number density $C_p$ ranges from 1100 to 10,000 particles/mm$^3$. Other parameters are $d_p = 2$ µm, $λ = 532$ nm, pixel size is 1.0 µm, and $n = 50$. A sample hologram $I_{HI}$ with $C_p ≈ 7400$ particles/mm$^3$ is shown in Fig. 3(a).

To test whether the PRDH recovers the original particle distributions, we identify and localize the particles in the reconstructed intensity fields (e.g., $I_{PRDH}$), by 3D segmentation, following procedures similar to those in [1,22]. First, we apply a global threshold to the entire 3D volume to differentiate between particle traces and background noise. The threshold is manually selected based on the intensity of in-focus particle images. The reason for using a global instead of local threshold is that in our simulations particles have the same size and very similar intensity at their re-focus planes. Then the particle centers are measured based on the centroids of individual particle traces. We also test the localization of particle centers based on the positions of minimal intensities, and find very similar results. Figure 3(b) shows a sample detected 3D particle distribution from $I_{PRDH}$ at $C_p ≈ 7400$ particles/mm$^3$. Clearly, most of the original and detected particles overlap, indicating that the PRDH allows the holograms to be located within the sample volumes.

To quantify the particle detection quality, we define $N_{seg}$ as the total number of “particles” identified from the 3D segmentation procedures, and $N_{seg}$ as the number of detected particles overlapping with the original ones ($N_{seg} ≥ N_p$). Then the quality of particle detection is evaluated via the particle detection rate $PDR = N_{seg}/N_p$ and the false detection rate $FDR = (N_{seg} - N_p)/N_{seg}$. In addition, we calculate the standard deviation of the particle localization error in the axial direction and denote it as $σ_z$. For comparison with the proposed PRDH method, we run additional simulations of single-hologram-based DIH. The hologram plane is located at $z = 400$ µm, i.e., outside of the sample volume, to avoid the in-focus virtual images. Figures 3(c)–3(e) compare the values of PDR, FDR, and $σ_z$, respectively, for the two methods. Each point in the figures is calculated by running and averaging the results of 20 simulations at a given $C_p$. Results show that both PRDH and DIH have high PDR > 96%, low FDR < 4%, and low $σ_z < 1.5d_p$, even at the highest $C_p = 10,000$ particles/mm$^3$. Moreover, as expected, for high values of $C_p$, PRDH has lower FDR and $σ_z$ compared to DIH, due to the removal of out-of-focus virtual image noise and the short distance between the hologram plane and sample volume. We anticipate the same trend holds for other situations, such as larger particle sizes or thicker sample volumes.
**4. EXPERIMENTAL TESTS**

**A. Hologram Recording**

The optical setup in our experiments follows Fig. 1(a). The light source is a Q-switched pulsed Nd:YLF laser ($\lambda = 532$ nm). The laser beam is first attenuated by a neutral density filter, spatially filtered by a 25 $\mu$m pinhole, and then collimated. The sample volume is located in the middle of a water-filled glass container with a cross section of 10 mm $\times$ 10 mm. It contains silver-coated glass spheres with a mean diameter of $d_p = 2$ $\mu$m (manufactured by Potters Industries) and a concentration exceeding $C_p = 2000$ particles/mm$^3$. The combined object and reference beams pass a $10 \times$ infinity-corrected objective (working distance of 34 mm), a tube lens (focal length of 200 mm), and a cube beam splitter before being recorded by two CCD cameras (Imperx ICL-B6640, 6600 $\times$ 4400 pixels, 5.5 $\mu$m/pixel).

Our previous study [28] showed that $D$ has to be 2.5 times larger than $d_p$ in order to effectively remove the virtual images in PRDH. To achieve a suitable $D$, we fix one of the two cameras and move the other either closer to or farther away from the objective. As a consequence, the two holograms have slightly different magnifications $M_1$ and $M_2$, which are calibrated by recording and reconstructing holograms of a target with known size. Assuming that the objective and tube lens act as a thin lens, we have $D = D'/M_1/M_2$, where $D'$ is the distance between image planes. In our experiments, we move camera 2 about 2.7 mm farther away from the objective (i.e., $D' \approx 2.7$ mm), with $M_1 = 9.97$ and $M_2 = 10.04$. As a consequence, we achieve $D \approx 27$ $\mu$m, which will be confirmed in the next section by measuring displacements of particle images between two reconstructed 3D fields.

To remove the stationary background noise (e.g., fringe patterns originating from the stretches on lenses and windows), we record more than 100 holograms on each camera under the same conditions. Since the particles in the suspensions continuously move, a background image containing only background noise is estimated by averaging a large number of holograms. The noise-free holograms are calculated by subtracting this background image. In the following, a 1024 $\times$ 1024 pixel region is selected for analysis.

**B. Matching Holograms and Estimating $D$**

Due to the misalignment between two cameras (caused by, e.g., a rotation or a shift) and the difference in magnification, the images of any particular particle on $I_{H1}$ and $I_{H2}$ are located at different in-plane positions. Previous studies [28,50] showed that even one pixel shift of the particle image in one of the two holograms will significantly increase the signature of the virtual image. Thus, before we perform the phase retrieval algorithm, the mismatch between holograms must be corrected.

We first calculate the 2D minimal intensity maps $I_{DH1,\text{min}}$ and $I_{DH2,\text{min}}$ from $I_{DH1}$ and $I_{DH2}$ as

$$I_{DH1,\text{min}}(x, y) = \min_{z} I_{DH1}(x, y, z),$$

$$I_{DH2,\text{min}}(x, y) = \min_{z} I_{DH2}(x, y, z),$$

with $M_1 = 9.97$ and $M_2 = 10.04$. As a consequence, we achieve $D \approx 27$ $\mu$m, which will be confirmed in the next section by measuring displacements of particle images between two reconstructed 3D fields.
\[ I_{2,\text{min}}^{\text{DIH}}(x, y) = \min_z I_{2,\text{min}}^{\text{DIH-Shift}}(x, y, z), \]  

where the operator \( \min_z \) detects the minimum intensity over the entire depth. Thus, all particles in the 3D volume are projected onto one 2D plane. An image overlapping \( I_{1,\text{min}}^{\text{DIH}} \) and \( I_{2,\text{min}}^{\text{DIH}} \) shows pairs of particle images [Fig. 4(a)], indicating the mismatch. We denote the in-plane displacement of two particles within each pair as \((u, v)\). The spatial distribution of \((u, v)\) is calculated by 2D correlations between \( I_{1,\text{min}}^{\text{DIH}} \) and \( I_{2,\text{min}}^{\text{DIH}} \), similar to typical particle image velocimetry. The result is shown in Fig. 4(b). Final, the mismatch is corrected by registering a new hologram as \( I_{1}^{\text{H}} \) where the operator min detects the minimum intensity over each pixel and the intensity value at sub-pixel locations.

The above matching procedure assumes that \((u, v)\) are constant at different \(z\) values, given that the two hologram planes are nearly parallel. To validate this, we model the displacement field as \( u = (M_2/M_1 - 1)(x - x_0) + u_0 \) and \( v = (M_2/M_1 - 1)(y - y_0) + v_0 \), where \( u_0 \) and \( v_0 \) are shifts in the \(x\) and \(y\) directions, respectively, and \((x_0, y_0)\) is the image center. Using \( u_0 = 4.6 \) pixels, \( v_0 = -5.6 \) pixels, and the measured magnifications \( M_1 = 9.97 \) and \( M_2 = 10.04 \), the modeled \((u, v)\) [Fig. 4(c)] agrees well with that obtained by 2D correlations, which suggests negligible tilt between hologram planes.

Next, we need to find the value of \(D\). One can estimate \(D\) by employing \( D = D' / M_1 / M_2 \) under the thin lens assumption, which may involve large measurement errors. Following our previous study [28], we calibrate \( D \) based on the axial displacement of particles in two reconstructed 3D intensity fields, \( I_1^{\text{DIH}} \) and \( I_2^{\text{DIH-Shift}} \), where the latter is the reconstruction of \( I_{H2} \) using Eq. (3). As demonstrated in Ref. [28], the particle images in \( I_1^{\text{DIH}} \) and \( I_2^{\text{DIH-Shift}} \) are axially displaced by either \(D\) or \(-D\), depending on which side of the hologram plane the particle is originally located on. By measuring more than 500 particles, we find \(D\) varies from 26.2 to 27.5 \(\mu\)m across the image plane, and has an average value of 27.0 \(\mu\)m. This small variation again confirms that the hologram planes are nearly parallel to each other (the tilt angle is about 0.2 deg).

### C. Reconstruction and 3D Particle Detection

Applying the phase retrieval algorithm to \( I_{H1} \) and \( I_{H2}^{\text{PRDH}} \) recovers \( E_{H1} \) and \( E_{H2} \), from which \( I_1^{\text{PRDH}} \) and \( I_2^{\text{PRDH}} \) are generated. Figure 5 compares results from single-hologram-based DIH (i.e., \( I_1^{\text{DIH}} \)) and from dual-hologram-based PRDH (i.e., \( I_2^{\text{PRDH}} \)). As shown in Figs. 5(b) and 5(c), \( I_{2,\text{min}}^{\text{DIH}} \) includes both real and virtual images located symmetrically on two sides of the hologram plane. In contrast, \( I_2^{\text{PRDH}} \) shows only real images [Figs. 5(e) and 5(f)]. A comparison of the minimal intensity maps [Figs. 5(a) and 5(d)] reveals that the reconstructed particle images by PRDH have better qualities (e.g., higher signal-to-noise ratio, more accurate shape) than those by DIH. Again, the reason is that PRDH removes out-of-focus virtual image noises.

To recover the 3D spatial distribution of particles in experiments, we apply the same 3D segmentation procedures as in the numerical tests. Figure 6(a) shows the distributions of particles detected from \( I_1^{\text{PRDH}} \) and \( I_2^{\text{PRDH}} \). As expected, particles detected from \( I_2^{\text{PRDH}} \) mostly overlap with these detected from \( I_2^{\text{PRDH}} \). The overlapping ones can be considered as correct detection, and the non-overlapping ones are considered as uncorrelated noise in two fields. As shown in Fig. 6(b), more than 90% of all detected particles can be considered as correct detection. We approximate the error of particle axial position as \( \varepsilon_z = \left| z_{\text{Seg1}} - z_{\text{Seg2}} \right| \), where \( z_{\text{Seg1}} \) and \( z_{\text{Seg2}} \) are axial locations of particles measured from \( I_1^{\text{PRDH}} \) and \( I_2^{\text{PRDH}} \), respectively. We only measure the error for these overlapping particles (i.e., the correct detection). Figure 6(c) shows that the standard derivation of \( \varepsilon_z \) is about 0.6 particle diameter.

For comparison, we also detect particles from \( I_1^{\text{DIH}} \) and \( I_2^{\text{DIH-Shift}} \) using the same 3D segmentation procedures. Since each intensity field is symmetric with \( z = 0 \), we apply 3D segmentation only to the fields at \( z > 0 \). Particles originally located at \( z < 0 \) will appear as virtual images at \( z > 0 \), mixing with the real images. As mentioned early, for a segmented trace to be a correct detection (i.e., either a real or virtual image), it has to satisfy the condition that \( \left| \Delta z^{\text{DIH}} \right| = D \), where \( \Delta z^{\text{DIH}} \) is the axial displacement of particle images between \( I_1^{\text{DIH}} \) and \( I_2^{\text{DIH-Shift}} \). Figure 6(b) shows that only 60% of all detected “particles” satisfy this condition and can be considered as correct detection, much

![Fig. 4](image-url)  
(a) Image combining two minimal-intensity maps \( I_{1,\text{min}}^{\text{DIH}} \) and \( I_{2,\text{min}}^{\text{DIH}} \). Arrows indicate the displacements of particle images from \( I_{H1} \) to \( I_{H2} \).  
(b) Spatial distribution of \((u, v)\) calculated by 2D correlations, (c) modeled \((u, v)\) by a translation and a magnification change. The contours show the particle displacement in pixels.
**Fig. 5.** Experimental demonstration of PRDH: (a)–(c) reconstruction results from single-hologram-based DIH; (d)–(f) reconstruction results from two-hologram-based PRDH. (a) and (d) are the minimal-intensity maps. (b), (c), (e), and (f) are intensity distributions in the $y-z$ plane for the selected particles $p_1$ and $p_2$. The hologram plane 1 is located at $z = 0$. Circles in (a) and (d) mark sample particle images whose qualities are improved by PRDH.

**Fig. 6.** Experimental demonstration of PRDH: (a) spatial distributions of particles detected from $I_{1}^{\text{PRDH}}$ and $I_{2}^{\text{PRDH}}$, (b) number of particles detected from $I_{1}^{\text{PRDH}}$, $I_{2}^{\text{PRDH}}$, $I_{1}^{\text{DIH}}$, and $I_{2}^{\text{DIH−Shift}}$, (c) error of the particle axial positions $e_z$. 
lower than for PRDH. We approximate the error of particle axial position as 
\[ \varepsilon_{\text{PRDH}}^z = |d_z^{\text{PRDH}}| - D. \]
Figure 6(c) shows that the standard derivation of \( \varepsilon_{\text{PRDH}}^z \) is about 2.3 particle diameters, much larger than that of PRDH. Thus, we prove that PRDH has better particle detection quality compared to DIH. We also try using local threshold [17] to segment all the 3D intensity fields, and find the same trend—that PRDH has a better particle detection quality than DIH.

5. CONCLUSIONS

Although DIH is a powerful tool for measuring particle distributions in 3D space, its application is limited by the longstanding virtual (or twin) image problem. To solve this issue, we applied virtual-image-free PRDH to measure the 3D spatial distribution of micrometer particles in suspensions. PRDH solved the virtual image problem by simultaneously recording two holograms whose planes are displaced along the optical axis, and subsequently reconstructing the complete optical waves estimated using the Gerchberg–Saxton iterative phase retrieval algorithm. We discussed the optical setup for the recording of the axially displaced holograms, as well as the data analysis procedures for the correction of camera misalignment and magnification variation.

Using both numerical and experimental tests, we showed two improvements by PRDH due to the removal of virtual images. First, we proved that PRDH recovered the original particle distributions even when the hologram planes were located in the middle of the particle suspensions. Thus, PRDH removed the restriction on the placement of the hologram plane with respect to the sample volume, and allowed objects to be presented on both sides of holograms. In situations with high particle seeding density, reducing the distance between the hologram plane and sample volume will improve the spatial resolution of the digital sensor. Second, we found that virtual-image-free PRDH had better particle detection qualities compared to virtual-image-contaminated DIH. PRDH had a low rate of false detection caused by the interplay of out-of-focus fringes, and it reduced the uncertainty in the localization of particle centers (to less than one \( d_p \)). We anticipate that PRDH has wide applications in fluid dynamics and environmental and biological sciences.

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