Improved digital holographic reconstruction algorithm for depth error reduction and elimination of out-of-focus particles

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Abstract: The digital holography has immense potential related to many applications of science and engineering. It has many advantages compared to conventional holography i.e. characterization of online dynamic phenomena and imaging in small scale microscopic systems. However, its primary limitation is the size of sensors and number of sensors compared to the conventional holographic plate. The critical issue of digital holography is the numerical reconstruction procedure. The present study proposes a new reconstruction algorithm known as ‘Within Depth Intensity Averaging (WDIA)’. The effectiveness of the WDIA algorithm is demonstrated using both experiment and simulation for single particle, 2D and 3D distribution of particles. The 3D distribution of particles is experimentally simulated by using gelatin film on the glass slide. The particle images from digital holography compare well with that of microscopic images demonstrating the success of the proposed algorithm compared to the existing reconstruction procedure. The depth error significantly reduces (maximum 100%) and particles of any size can be characterized by the WDIA reconstruction algorithm contrary to the existing reconstruction algorithm available in literature. The effect of particle number density, particle size and sample volume depth on reconstruction effectiveness using the WDIA algorithm has been investigated and compared with the literature demonstrating its superiority in performance.

References and links

Nomenclature

\[ N = \text{number of pixels in each direction in a CCD array.} \]
\[ N_o = \text{number of object particles.} \]
\[ N_r = \text{number of reconstructed particles.} \]
\[ N_s = \text{density of particles.} \]
\[ \Delta \xi = \text{width of a square pixel.} \]
\[ d_p = \text{particle size.} \]
\[ x_o, y_o = \text{cartesian coordinates in object plane.} \]
\[ \xi, \eta = \text{cartesian coordinates in hologram plane.} \]
\[ x_o, y_i = \text{cartesian coordinates in reconstruction image plane.} \]
\[ z_o = \text{distance of object plane from hologram plane, i.e. recording distance.} \]
\[ z_r = \text{distance of image plane from hologram plane, i.e. reconstruction distance.} \]
\[ z_c = \text{critical recording distance.} \]
\[ k = \frac{2\pi}{\lambda} = \text{wave number.} \]
\[ \lambda = \text{wavelength of light.} \]
\[ z_{rms} = \text{rms depth error of reconstructed particle.} \]
\[ I(\xi, \eta) = \text{intensity distribution in hologram plane.} \]
\[ E_r = \text{complex amplitude of reference wave in object plane.} \]
\[ E_o(\xi, \eta) = \text{complex amplitude of object wave in hologram plane.} \]
\[ E_i(x_i, y_i) = \text{complex amplitude of real image wave in reconstruction plane.} \]
\[ L = \text{sample volume depth.} \]
\[ \Delta z_i = \text{volume depth of slice in reconstruction volume.} \]
\[ \delta_c = \text{the distance between two successive reconstruction planes.} \]

1. Introduction

Holography is an optical technique based on combined interference and diffraction phenomenon, which provides three-dimensional information about the object field of interest. It is carried out in two steps i.e. hologram recording and object field reconstruction. When both recording and reconstruction is carried out optically, the technique is known as optical
holography. When the reconstruction is carried out digitally, the technique is known as digital holography. The rapid development of computer and video technology has significantly enhanced the capability of digital holography technique.

The digital holography has been implemented in number of applications such as biological applications [1], micro-metrology [2], spray analysis [3] and microscopy [4, 5] etc. The potential of digital holography in particle field measurement has been examined and the error in particle location and size using intensity field information has been presented [6]. The characterization i.e. determination of size, number density and spatial \((x, y, \text{ and } z)\) location of particle field using digital in-line holography technique is carried out mainly two ways i.e. the intensity based method [6,8] and the complex amplitude based approach [9–11]. In the former case, the peak or dip of axial intensity profile inside the reconstruction volume is considered as the location of the particle. In the later case, the dip in variance of the imaginary part of the complex amplitude is used to determine the particle location.

The digital in-line holography for particle field measurements suffers from two major drawbacks i.e. depth resolution and speckle noise. In in-line digital holography with plane wave illumination, the far field scattering of object fields are recorded by a CCD camera, which is subsequently reconstructed numerically. The angle between the object i.e. the particle field and reference wave is restricted to a few degrees due to low resolution of the available CCD detectors, which decreases the numerical aperture (NA). The depth of focus becomes large during reconstruction because of small NA. This limits the depth resolution capability of digital holography. The speckle noise originates from coherent recording similar to that of the optical holography and from finite sizes of the sampling devices i.e. the finite size of the pixels in the CCD camera. The temperature variation in the media and visible blemishes on any window that light passes through can also cause diffraction, reflection & interference effects in the coherent optical field. In addition to above noise sources, the speckle noise in particle field holography is also generated because of superposition of virtual image wave, transmitted wave and real image wave of out-of-focus particles. The presence of speckle noise makes particle recognition difficult, where the intensity level of speckle noise is comparable with the average intensity of particle images and hence deteriorates the reconstruction effectiveness. Several approaches have been presented in the literature to encounter these problems, which are reviewed in the following paragraphs.

A method has been proposed [7], for inline arrangement with divergent spherical reference wave, by which the virtual image can be eliminated by iterative numerical reconstruction procedure. For inline arrangement with plane reference wave the influence of various recording parameters, such as recording distance, particle size, wavelength, and particle concentration on reconstruction effectiveness has been investigated and presented [8]. It is observed that when the recording distance is near the critical value and size of particles is about 1 pixel (i.e. equal to one pixel size of the sensor), the reconstruction is most effective. Therefore, intensity based particle field reconstruction is restricted for particles of smaller size (about 1 pixel) and large depth of error is encountered for three dimensional particle field distribution due to the appearance of out-of-focus and false particles.

A method was proposed [9] for inline arrangement with plane reference wave using complex amplitude method known as PECA, where the dc term was removed by filtering out the zero-frequency component in Fourier domain during reconstruction. Therefore, the contribution of virtual image wave to the speck noise can be effectively suppressed as the directed transmitted wave (the dc term) is not present during the reconstruction so that the interference between the two waves is avoided. For far field particle holography, the magnitude of virtual image wave is very weak in the region where particles are extracted [10]. As a result, the speckle noise level is decreased, and the real image wave of out-of-focus particles becomes the dominant source of the speckle noise. It has also been proposed [9–11] that the depth error during reconstruction is reduced significantly and also the problem of
speckle noise can be handled when dip of variance distribution of imaginary part of complex amplitude is used to determine the particle location.

The present work proposes a novel approach for accurate determination of size and depth location of particle field using intensity distribution in the reconstruction volume. This approach alleviates the problem of out-of-focus particles and there is no restriction on size of object particles as observed by [8]. The size and spatial location of particles in each reconstruction plane are determined by using digital image morphological algorithm. This approach overcomes the difficulty of recognizing true particle among out-of-focus particles and the coordinates of all particles inside the reconstruction volume can be matched and segmented. The proposed reconstruction approach is evaluated using both numerically and experimentally simulated particle fields. Comparison has been made with the results from existing literature in terms of rms depth error, presence of out-of-focus particles and reconstruction effectiveness. The effectiveness of proposed algorithm is verified from using particle field of different sizes with different number density and sample volume size recorded at different recording distances. The implementation procedure and performance details of the proposed algorithm are discussed in the following sections.

2. Theoretical background

Brief theoretical background for generation of hologram and reconstruction of particle field using digital in-line holographic technique with plane wave illumination is presented in this section. Figure 1(a) shows the hologram recording of three-dimensional particle field. The particle field is illuminated by parallel plane monochromatic light \( E_r = A_r \exp(ikz) \). The part of the incident light which is obstructed by particles is diffracted and forms object wave at the recording plane and un-obstructed part of light serves as reference wave. These object and reference waves interfere and the resulting pattern is recorded on the digital recording media. This interference pattern is called hologram. The amplitude transmission function of object particle field is expressed by \( t(x_o, y_o) \). The intensity distribution of hologram is represented by \( IH(\xi, \eta) \). The recorded hologram is stored in computer and 3D image of the object field is generated by 3D numerical reconstruction (i.e. reconstruction is carried out on many transverse planes) of the hologram [see Fig. 1(b)].

The complex amplitude of object wave on the recording plane is obtained using Fresnel-Kirchhoff’s formulation as [14, 15]:

\[
E_o(\xi, \eta) = \frac{\exp(ikz_o)}{i\lambda z_o} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} t(x_o, y_o) \exp\left(i \frac{k}{2z_o} \left[(\xi - x_o)^2 + (\eta - y_o)^2\right]\right) dx_o dy_o
\]  

(1)

where, \( t(x_o, y_o) \) is the amplitude transmission function of the particle and \( z_o \) is its distance from the recording plane. It is assumed here that for plane monochromatic wave, \( E_r = 1 \). The interference pattern between this object wave and reference wave is recorded in form of intensity \( I_H(\xi, \eta) \) in the recording plane as:

\[
I_H(\xi, \eta) = |E_r + E_o(\xi, \eta)|^2 = A_r^2 + |E_o(\xi, \eta)|^2 + A_r E_r^* E_o(\xi, \eta) + A_r E_r E_o^*(\xi, \eta)
\]

(2)

where the reference wave amplitude, \( A_r^2 = |E_r|^2 = 1 \). The reconstruction of the real and virtual images require the illumination of the hologram by the reference wave. This process is numerically carried out by multiplication of the digital hologram \( I_H(\xi, \eta) \) with reference wave. The digital hologram is multiplied with the conjugate \( E_r^* \) of the reference wave to obtain the real image [14]. For plane monochromatic wave, \( E_r^* = 1 \). Thus, the complex amplitude of real image in the \((x_i, y_i)\) plane at a distance \( z_r \) from hologram plane is determined by the diffraction formula as:


\[ E_{i}(x, y) = \frac{\exp(ikz_{o})}{i\lambda z_{r}} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I_{H}(\xi, \eta) \exp\left[i\frac{k}{2z_{r}}((x_{i} - \xi)^{2} + (y_{i} - \eta)^{2})\right]d\xi d\eta \]  

(3)

For sharp focused image \( z_{r} = z_{o} \). The intensity distribution of reconstructed particle field can be obtained as:

\[ I_{r}(x, y) = |E(x, y)|^{2} \]  

(4)

The complex amplitude of object wave and real image wave has been determined using convolution approach. In this approach, the size of the reconstructed image is invariant with respect to the reconstruction distance i.e. \( \Delta x_{i} = \Delta \xi \) and \( \Delta y_{i} = \Delta \eta \) [12].

3. Reconstruction algorithm

The characterization of particle field is carried out using intensity field information inside the reconstruction volume. The size of the particle (\( d_{p} \)) is represented in terms of pixel size (\( \Delta \xi \)) of the sensor. The focal plane of the particle is defined as the plane located at a distance equal to its recording distance from the hologram plane inside the reconstruction volume. The plane of focus is defined as the plane inside the reconstruction volume, where the particle shows peak/dip value of intensity. The proposed approach uses the average intensity value of particle, where the averaging is carried out using selected pixel elements based on the proposed algorithm. Size of a particle and its \( x, y \) coordinates are determined by using the morphological technique explained in later sections. The depth position (\( z \)-coordinate) of a particle in reconstruction volume is determined by normalized intensity profile along axial direction. The reconstruction procedure of the proposed algorithm can be broadly described in the following steps.

1. The intensity field of real image is reconstructed using Eq. (4) over the full volume i.e. at all reconstruction planes.
2. The intensity field images in each plane inside the reconstruction volume are converted into binary images using a threshold value based on the histogram of intensity values at that plane.
3. The size and \( x, y \) coordinates of particle images are determined at each plane by using the morphological technique as described below in Sec. 3.1.
4. After obtaining the particle coordinates at each plane, the group of pixel elements belonging to same particle on each plane are matched and segmented.
5. The particle size i.e. the number of pixel elements corresponding to a single particle is not same at all locations inside the reconstruction volume. The intensity values of all pixel elements corresponding to a particle are averaged in each reconstruction plane.
This averaged intensity ($I_{avg,p}(z)$) is normalized using minimum ($I_{min,p}$) and maximum ($I_{max,p}$) averaged value of intensity for the particle from all reconstruction planes:

$$I_{n,p}(z) = \frac{I_{avg,p}(z) - I_{min,p}}{I_{max,p} - I_{min,p}}$$

(5)

where, $I_{n,p}(z)$ is the normalized intensity. The location of peak/dip magnitude is the plane of focus of the particle. This step eliminates the problem of dual peak in the axial intensity distribution (see section 4.1.1).

6. The plane of focus of each particle may not coincide with its focal plane resulting in depth error of the particle. The following step is carried out to reduce this depth error. The intensity values of individual pixel elements of the particle located at plane of focus is now normalized using the following equation:

$$I_{n,pix}(z) = \frac{I_{pix}(z) - I_{min,pix}}{I_{max,pix} - I_{min,pix}}$$

(6)

Where, $I_{min,pix}$ and $I_{max,pix}$ are the minimum and maximum intensity values corresponding to that pixel element. This normalized axial intensity profile of each pixel may show peak/dip at different $z$-location from the plane of focus. The pixel elements of the particle showing peaks outside the depth interval of ±2$d_p$ about the plane of focus of the particle are excluded from group of pixel elements for that particle. This process is carried out for all particles.

7. The intensity values obtained after exclusion of pixel elements for a particle in previous step are retained and the average value of intensity for remaining pixel elements of the particle is calculated at all reconstruction planes. The averaged intensity distribution of the particle is normalized using Eq. (5) and the location of new peak/dip value provides the new plane of focus, which is the final depth ($z$ coordinate) of the particle.

The implementation of above reconstruction algorithm has been explained in section 4.1 using a sample example. It is expected that this example can better clarify the proposed algorithm.

3.1 Determination of particle size: morphological technique

The size and location i.e. $x$, $y$ coordinates of a particle are determined using the morphological technique [16]. Figure 2 shows the schematic sketch describing the implementation of this technique. Here, $Y$ represents a connected component with values $I$ (say, particle in an image) contained in a set $A$ (size $M \times N$ with elements having values $0$). Let’s assume that a point $p$, (say a pixel of particle) of $Y$ is known, as shown in Fig. 2(a). Then the following iterative expression yields all elements (pixels of a particle) of $Y$:

$$X_k = (X_{k-1} \bigoplus B) \cap A \quad k = 1, 2, 3, \ldots$$

where $\bigoplus$ denotes dilation, $\cap$ represents intersection and $B$ is a suitable structuring element of size $3 \times 3$ with values $I$, as shown in Fig. 2(b). The implementation detail of the above operation is described in the following paragraph.
Fig. 2. Morphological determination procedure of particle size and location: (a) Set A is the object in which all points/pixels of an element Y are to be determined. The initial point p of this element is known (shown as different hatching) from other element of Y indicating that they have not been found by the algorithm; (b) It shows the structuring element, B which convolves over p to determine the points of Y connected with p; (c) The results of first iterative step involving dilation and intersection after which the points of Y connected with p are found out; (d) The results after second iterative step; (e) The results of final iteration i.e. after determination of all the points of element Y.

First, matrix A is padded with zero at its edges so that the size of A becomes (M+1) × (N+1) and then a matrix $X_0$ with its entire elements having values 0 and of size same as that of A i.e. (M+1) × (N+1) is taken. This zero padding is essential for considering particles located at the edge of A. For $k = 1$, $X_0 = p$ i.e. the value of pixel of $X_0$ having same coordinate as that of p is made equal to 1. Now with this element at the center, dilation is carried out with structuring element B which makes values of pixels surrounding $X_0$ equal to 1. Then an intersection of this set is carried out with original set A resulting a new set $X_1$ as shown in Fig. 2(c). Similarly, the second iteration is carried out with set $X_1$ leading to a new set $X_2$ as shown in Fig. 2(d). After many iterations, $X_k = X_{k-1}$ and the algorithm has converged with $Y = X_k$ as shown in Fig. 2(e). Using this method, one can determine the size and x, y coordinates of each particle in the reconstruction plane.

4. Results and discussion

The following parameters have been used in the present study: wavelength, $\lambda = 0.6328$ µm, number of pixels, $N \times N = 512 \times 512$ and pixel size = 6.8 µm. The recording distance ($z_o$) i.e. the distance between the object particle and sensor plane, has been represented in terms of critical recording distance ($z_c$). The critical recording distance is calculated using the equation [8]:

$$z_c = \frac{\Delta z^2 \times N}{\lambda}$$  \hspace{1cm} (6)

The critical recording distance for the present study based on above equation is equal to 35996µm. The effectiveness of the proposed algorithm has been tested using both simulated and experimental data. In the numerical simulation, the amplitude transmission functions of the object particles are assumed to be equal to 0.2 in the bright background having amplitude transmission function of 0.8. Amplitude transmission function value of 0.8 and 0.2 respectively for the particle and background is also used for comparison with some of the literature data. The broad results from the present study are discussed in the following sequence: (a) single particle, (b) 2-dimensional particle field, and (c) 3-dimensional particle field characterization. The single particle results help in explaining the details of the proposed reconstruction algorithm. The 2-D and 3-D particle field results demonstrate the superiority of
the proposed reconstruction algorithm in comparison to the existing holographic reconstruction techniques. The reconstruction effectiveness is defined as the ratio of number of reconstructed particles and the actual number of object particles. The depth error is defined as the difference between the actual depth and the reconstructed depth location of particle. The rms depth error is calculated using depth error of all reconstructed particles.

4.1 Single particle characterization

This section discusses single particle characterization as a function of its size and recording distance. Figure 3 presents the normalized intensity distribution along axial direction about focal plane for a single particle of size $d_p = 1$ pixel at different recording distances. Since, the particles are assumed bright in dark background the peak value of axial intensity profile gives the location of particle in reconstruction volume. This figure validates the present numerical procedure with that of [8]. It also indicates that at lower recording distances, intensity peak is sharp and as recording distance approaches towards the critical recording distance ($z_c$) and above, the intensity peak widens leading to increase in depth of focus and hence reduction in depth resolution.

Figure 4(a) shows a particle of size, $d_p = 3$ pixels in dark background. The hologram of this particle needs to be reconstructed using intensity distribution in $z$ direction. Figure 4(b) shows the normalized intensity distribution along axial direction about the focal plane for each pixel of the particle. From this figure it is clear that pixels 2, 4, 5, 6 and 8 show dual peaks whereas 1, 3, 7 and 9 show single peak. Thus, Fig. 4 shows the possible depth error due to dual peaks in the intensity distribution. If core pixel is assumed to be the representation of the particle, and its axial intensity profile is considered as the particle location inside the reconstruction volume, it will result in false depth detection due to the dual peaks (see Fig. 4(c)). Thus, to overcome the problem of dual peaks for particles of size more than 1 pixel, a new approach is proposed, implemented and discussed in the following sections.

![Fig. 3. The normalized intensity distribution along axial direction about the focal plane at different recording distances ($z_c/z_0 = 0.25, 1, 1.2$ and 7) for single particle of size $d_p = 1$ pixel. The lines and symbols correspond to the present data & that of Zhang et al. [8] respectively. The distance between the two successive planes during reconstruction is $\delta_z = d_p$.]

4.1.1 Intensity averaging: removal of dual peaks

Figure 4(c) shows the intensity of particle averaged over all its pixels in each reconstruction plane and normalized along axial direction. A single peak is obtained indicating the effect of intensity averaging and the removal of dual peaks. Thus averaging the intensity of particle eliminates the problem of dual peaks and hence false depth detection of particle during reconstruction is avoided.
Figure 5 demonstrates the benefits of intensity averaging for wider range of particle sizes and recording distances. It shows the axial intensity profile of core pixel of particle and averaged axial intensity profile as a function of particle sizes \(d_p = 2, 4, 6, \) and 8 pixels) and recording distances \(z_0/z_c = 0.65, 0.8, 1.0, 1.5 \) and 2.0). Figures 5(a), (c), (e) and (g) show the presence of dual peaks when using only core pixel of particle, which can result in false particle location. Figures 5(b), (d), (f) and (h) show the single peak for all particle sizes and at all recording distances when averaging the intensity over all pixels of the particle. Thus averaging the intensity of a particle over its pixels eliminates the problem of dual peaks and hence avoids false location of the particle.

After removal of dual peaks by averaging (see Fig. 5) the depth location of particle is analyzed in Fig. 6. Figure 6(a) shows the normalized intensity distribution along axial direction about focal plane after averaging the intensity over all pixels of particle of size \(d_p = 3\) pixels at recording distance \(z_0/z_c = 0.95\). The peak intensity of reconstructed particle i.e. plane of focus is observed in 5th plane left to the focal plane (see Fig. 6(a)). Hence, the depth error for this particle is \(1.25*d_p\), as the distance between two reconstruction planes, \(\delta_z = 0.25*d_p\). This depth error in locating the reconstructed particles restricts the application of digital in-line holography for PIV measurements as it results in wrong determination of \(z\)-component of velocity. Hence, it is necessary to reduce this depth error and a modification in the averaging procedure is proposed as discussed in the following section.

Fig. 4. The importance of dual peak removal: (a) the cartesian coordinate system with a bright particle (size, \(d_p = 3\) pixels) in dark background, whose hologram is reconstructed along \(z\)-direction; (b) the normalized intensity distribution about the focal plane for each pixel element of the particle, showing dual peaks for some pixel elements; (c) the normalized averaged intensity compared to the intensity distribution of the core pixel element.
4.1.2 Within depth intensity averaging (WDIA): reduction of depth error

This section shows that depth error can be reduced by averaging the intensity over selected pixels of a particle depending on the intensity peak location with respect to the plane of focus. Figure 6(a) shows the depth error of $1.25*d_p$ for a particle size, $d_p = 3$ pixels at recording distance $z_o/z_c = 0.95$ when using the averaged intensity over all pixels of the particle. Figure 6(b) shows the normalized axial intensity profiles of all 9 pixel elements contained in this particle. This figure indicates that pixels 1, 3, 7 and 9 show their intensity peaks close to the plane of focus (within $\pm 2d_p$) and pixels 2, 4, 5, 6, and 8 show peak outside. Figure 6(c) shows the intensity distribution of pixels after excluding the pixel elements with peaks located outside the plane of focus. Figure 6(d) shows normalized averaged intensity distribution along axial direction by using only pixels of Fig. 6(c). In Fig. 6(d), the peak is located at 1st plane to the left of focal plane and hence depth error is equal to $0.25*d_p$. Thus, there is 80% reduction in depth error from $1.25*d_p$ (see Fig. 6(a)). Hence, averaging the intensity and using the pixels located close to the plane of focus overcomes the problem of dual peaks and reduces the depth error considerably. The following section describes the implementation of the proposed reconstruction algorithm for 2D particle field.
Fig. 5. The normalized intensity distribution along axial direction about the focal plane for core pixel element of particle ((a), (c), (e) and (g)) and the corresponding averaged intensity ((b), (d), (f) and (h)) for particle of size, \(d_p = 2, 4, 6,\) and 8 pixels respectively as a function of recording distances (\(z_0/z_c = 0.65, 0.80, 1, 1.5\) and 2). This figure demonstrates that by averaging the intensity over all elements of the particle single peak during reconstruction is obtained for particles of different sizes and at different recording distances.
Fig. 6. Procedure explaining the depth error reduction: (a) The normalized intensity distribution about the focal plane, averaged over all elements of a particle of size $d_p = 3$ pixels, along axial direction for the recording distance, $z_o/z_c = 0.95$. The depth error is equal to $1.25*d_p$. (b) The intensity distribution for individual pixel elements of the same particle. The normalized intensity peak of elements 2, 4, 5, 6, and 8 lies outside $4*d_p$ of focus and that of 1, 3, 7 and 9 pixel elements lies inside (here we call these elements as ‘within depth’ elements); (c) The intensity distribution of ‘within depth’ elements of particle. (d) The intensity distribution averaged over ‘within depth’ elements (pixel elements 1, 3, 7, & 9) of particle. The depth error is equal to $0.25*d_p$ showing 80% reduction in comparison to $1.25*d_p$ depth error value in (a). i.e. without any correction

4.2 Characterization of 2D particle field

The single particle simulation results presented in the previous section have demonstrated the reduction in depth error of a particle during holographic reconstruction. This section presents the simulation and experimental results using the proposed WDIA reconstruction algorithm for 2-dimensional particle field.

4.2.1 Simulation results

Figures 7(a) and (b) shows the reconstruction effectiveness as a function of depth distribution i.e. distance of particles from their focal plane for particles of size $d_p = 2$ pixels and 3 pixels respectively. The amplitude transmission function value of the particle and background is equal to 0.8 and 0.2 respectively. The results are compared between averaging the intensity without and with correction i.e. considering only within-depth pixels. The number of particles in object plane is equal to 200 and the recording distance, $z_o/z_c = 0.95$. The reconstruction has been carried out over the planes at interval of $\delta z = 0.25*d_p$ about the focal plane. Figure 7(a) shows that about 80% of total particles appear within the depth interval of $\Delta z/d_p = 1$ when averaging using the within-depth pixels only compared to 50% without this correction. This result indicates that for particles of size $d_p = 2$ pixels, correction during intensity averaging
leads to narrower distribution of particles about focal plane as compared to that without correction leading to reduction in depth error. Figure 7(b) shows that about 70% and 95% particles are reconstructed within depth interval of $\Delta z/d_p = 1$ about focal plane for without and with correction respectively for particles of size $d_p = 3$ pixels. Thus, Fig. 7 shows significant improvement in reconstruction effectiveness and reduction in depth error by the proposed intensity averaging. It is also evident that reconstruction effectiveness increases and depth error reduces as size of particles increases, which is a significant improvement in comparison to earlier results [8], where large size particles were considered to be more erroneous from depth error point of view.

Table 1 compares the percentage reduction in depth error during reconstruction by proposed intensity averaging at different recording distances for particles of size, $d_p = 2$ and 3 pixels. The number of particles in the object plane is equal to 200 and the distance between two successive reconstruction planes, $\delta_z = 0.25*d_p$ about the focal plane. It is clear from Table 1 that there is significant reduction in depth error (maximum 73.5%) when reconstruction is carried out by averaging the intensity of ‘within depth’ pixels at all recording distances for both 2 and 3 pixels diameter particles. This reduction is higher for particle of size $d_p = 3$ pixels in comparison to the particle of size, $d_p = 2$ pixels.

### Table 1. Percentage reduction in depth error due to the removal of off-focal plane intensity peaks as a function of recording distance ($z_o/z_c$) for particle of size, $d_p = 2$ and 3 pixels. Total number of particles ($N_o$) is equal to 200.

<table>
<thead>
<tr>
<th>Recording distance ($z_o/z_c$)</th>
<th>$d_p = 2$ pixels</th>
<th>$d_p = 3$ pixels</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.65</td>
<td>37.5</td>
<td>45.9</td>
</tr>
<tr>
<td>0.80</td>
<td>24.6</td>
<td>73.5</td>
</tr>
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<td>0.95</td>
<td>47.6</td>
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</tr>
<tr>
<td>1.5</td>
<td>36.6</td>
<td>60.0</td>
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</tbody>
</table>

![Fig. 7](image)

**Fig. 7.** The improvement in reconstruction effectiveness because of the correction of ‘outside-depth’ pixel elements influence for particle size, (a) $d_p = 2$ pixels and (b) $d_p = 3$ pixels. Here, total 200 number of particles are distributed over a 2D plane of size 512 x 512 pixels and the recording distance, $z_o/z_c$ is equal to 0.95. There is significant improvement in reconstruction effectiveness when axial intensity is averaged over only ‘within depth’ elements of each particle.

Figure 8 compares the reconstructed particle histogram about focal plane between present study and that of [8]. The object field consists of 310 inhomogeneous particles randomly distributed over a 2D plane of size 512 x 512 pixels i.e. 100 particles of size 1 pixel, 100 particles of size 2 pixels and 110 particles of size 3 pixels. The recording distances are taken as $z_o/z_c = 0.65, 0.75$ and 1.35. Figure 8 shows that particles are reconstructed within few planes about the focal plane when using the proposed WDIA algorithm in the present study. In contrast, a large number of particles show their depth position at far off location from the...
focal plane in the results of [8] indicating greater presence of false particles. The success of the proposed WDIA algorithm can be attributed to the removal of dual peaks by averaging and the reduction in depth error by restricting the pixels to ‘within depth’ pixel elements only.

4.2.2. Experimental validation

This section presents the reconstruction results using the proposed reconstruction algorithm i.e. ‘Within Depth Intensity Averaging (WDIA)’ on the experimental data. Figure 9(a) shows the image of copper particles of size, \( d_p = 3 \) to 5 pixels (20 to 30 µm) spread over a glass plate and captured by inverted microscope (Leica 5000/M). Figure 9(b) shows the experimental hologram generated using the object particles of Fig. 9(a) at recording distance \( z_o/z_c = 0.80 \) captured by PCO Sensicam CCD camera. Figure 9(c) shows the reconstructed image of particle field at focal plane \( z_r = z_o \). The size of object field, hologram, and reconstructed image is equal to 512 x 512 pixels. The distance between two reconstruction planes, \( \delta_z = 3 \) pixels.
Comparison of Fig. 9(c) with Fig. 9(a) indicates excellent match of number, size and shape of the reconstructed particles from the hologram with that of actual particles.

Figure 10 compares the particle distribution about focal plane i.e. histogram using experimental data and simulated data to illustrates the advantage in using the correct intensity averaging i.e. ‘within depth’ pixel element only. The simulated data are generated for same conditions as that of experiment. The reconstructed particle histogram is presented for two cases: (a) depth of particles detected by averaging the intensity of all pixels i.e. without correction, and (b) with correction i.e. including only ‘within depth’ pixels during averaging. The experimental data show reduction in depth error from 30.11µm to 18.26µm. The numerical data shows reduction in depth error from 17.89µm to 10.95µm. Overall, there is about 40% reduction in depth error when considering ‘within depth’ pixel for intensity averaging.

![Fig. 9. The comparison of the proposed holographic reconstruction algorithm with that of microscopic images: (a) The microscopic image of copper particles of size 20 to 30 µm distributed over a glass plate, (b) The experimental hologram of particle field in (a), (c) The reconstructed particle field using hologram of (b).](image-url)
Fig. 10. The histogram showing the depth error by (a) including all the pixel elements during average intensity calculation and (b) the average intensity calculation carried out by including only the ‘within-depth’ pixels of particles. The left hand side of figure corresponds to the experiment and the right hand side corresponds to the simulated data at similar condition as the experiment. The experiment uses copper particles of size $d_p = 20$ to $30 \mu m$ distributed over glass plate at recording distance, $z_o/z_c = 0.80$. There is appreciable reduction in rms depth error ($z_{rm}$) of both experiment and simulation. The distance between two successive planes during reconstruction, $\delta_z = 3$ pixels.

Table 2 presents the percentage reduction in depth error when depth of particle is detected by averaging intensity without correction and with correction i.e. including ‘within depth’ pixel at different recording distances ($z_o/z_c$) for both simulated and experimental data. There is significant reduction in depth error (maximum 100%) for both experiment and simulation at all recording distances when using the proposed WDIA reconstruction algorithm.

Table 2. Percentage reduction in depth error due to the removal of off-focal plane intensity peaks as function of recording distance ($z_o/z_c$) for experimentally generated hologram and numerically simulated hologram with same parameters as that of the experiment.

<table>
<thead>
<tr>
<th>Recording distance ($z_o/z_c$)</th>
<th>% reduction in depth error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental data</td>
</tr>
<tr>
<td>0.65</td>
<td>46</td>
</tr>
<tr>
<td>0.80</td>
<td>39.4</td>
</tr>
<tr>
<td>0.95</td>
<td>73.7</td>
</tr>
<tr>
<td>1.1</td>
<td>68.2</td>
</tr>
<tr>
<td>1.4</td>
<td>68.5</td>
</tr>
<tr>
<td>2.0</td>
<td>61.2</td>
</tr>
</tbody>
</table>

4.3 Characterization of 3D particle field

The previous sections have demonstrated the superior performance of the proposed algorithm for holographic reconstruction of 2D particle field. This section presents the performance of
this algorithm for characterization of 3D particle field using both simulated and experimental data. The performance of the proposed algorithm has also been compared with that of literature.

4.3.1 Simulation results

The distribution of particle field in the 3D sample volume is simulated by using random number generator. The distance of left and right most faces of sample volume from recording plane is equal to $z_o$ and $z_o + L$ respectively where, $L$ is the depth of the sample volume (see Fig. 1). The value of the amplitude transmission function $t(x_o, y_o)$ is equal to 0.2 for the particle and equal to 0.8 for the background. The hologram is generated on a 2D plane using Eq. (2). The numerical reconstruction of the digital hologram is carried out in 3D volume at number of transverse planes. The distance between two successive reconstruction planes, $\delta_z = 0.5*d_p$. The depth position of particle from reconstruction is compared with its known depth position and difference between the two yields the depth error. The reconstruction effectiveness has been examined with respect to particle density, particle size, and sample volume depth.

Figure 11 compares the percentage of extracted particles as a function of sample volume and particle density between present study with that of [13]. The particle field has been reconstructed on 314 and 1981 planes for sample depth $L = 3$ and 20 mm respectively and the distance between two successive reconstruction planes is equal to $\delta_z = 0.5*d_p$ in each case. Figure 11 indicates higher reconstruction effectiveness for smaller sample depth size. The reconstruction effectiveness reduces with increase in density of object particles. Hence, both sample depth and number density of object particles adversely affect the reconstruction effectiveness. Figure 11 also shows significant improvement in percentage of extracted particles in present study compared to that of Malek et al [13] where wavelet transform was used for reconstruction of the particle field. About 90% of particles are extracted at particle density of 30 (1/mm$^3$) and sample size of 3 mm using the present approach compared to about 50% reconstruction effectiveness observed in [13]. Superior reconstruction effectiveness is also observed by the present approach at higher sample volume depth, $L = 20$ mm in comparison to that of [13].

Figure 12 compares the reconstruction effectiveness between the proposed WDIA algorithm of the present study with that of [11]. The average reconstruction effectiveness as function of depth distribution has been compared in this figure. The sample/hologram size is
3.48(H) × 3.48(W) mm² for both present study and that of [11]. Particle size ($d_p$), density of particles ($N_s$), and sample depth ($L$) are equal to 1.47 pixels, 18 (1/mm³), and 10 mm for [11]. Present study uses particle size, $d_p = 2$ pixels, density of particles, $N_s = 6$ and 18 (1/mm³) for sample depth size, $L = 10$ mm. Particle field has been reconstructed at 1521 transverse planes and the distance between two successive reconstruction planes is equal to $\delta z = 0.5d_p$. Figure 12 shows that about 70% particles are reconstructed within the depth interval of $2d_p$ for both present study and [11] at particle density of 18 (1/mm³). The present study also shows more than 90% of reconstructed particles to be present inside the depth interval of $2d_p$ with respect to the actual depth position for lower particle density of 6 (1/mm³) indicating higher reconstruction rate at lower particle density.

![Reconstruction Effectiveness](image)

**Fig. 12.** The comparison of reconstruction effectiveness between the ‘Present Study’ and that of Pan and Meng [11]. Both the studies have sample volume depth of 10 mm and the hologram/sample size is equal to 3.48 x 3.48 mm². The density of particles for present study is 6 and 18 (1/mm³) in comparison to 18 (1/mm³) for Pan and Meng [11].

Table 3 presents the depth error normalized by the size of particle ($z_{rms}/d_p$) for different sample depth ($L$) and density of object particles ($N_s$). The depth error is calculated using all reconstructed particles inside the full reconstruction volume. The results from the present study show increase in depth error of reconstructed particles with increase in sample depth ($L$) size. There is also an increase in depth error of reconstructed particles with increase in object particle density for the same sample depth size. The results of present study are also compared with that of [6] and [11]. Table 3 shows more than 100% reduction in depth error by the present approach compared to that of [6] i.e. the normalized depth error ($z_{rms}/d_p$) using the present WDIA algorithm is equal to 0.78 compared to 1.72 in the reference [6]. Similarly, the comparison of present study with that of [11] for sample depth, $L = 10$ mm and object particle density, $N_s = 6$ (1/mm³) shows drop in normalized depth error from 0.69 to 0.48.
Table 3. Comparison of normalized rms depth error ($z_{rms}/d_p$) from present study with that of literature (Murata and Yasuda [6], Pan and Meng [11]) as a function of sample volume depth ($L$) and particle density ($N_s$) for sample/hologram size of 3.48 x 3.48 mm$^2$. The pixel size is equal to 6.8 µm.

<table>
<thead>
<tr>
<th>Method</th>
<th>$L$ (mm)</th>
<th>$N_s$ (Particles/mm$^3$)</th>
<th>$d_p$ (pixels)</th>
<th>$z_{rms}/d_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0.17</td>
</tr>
<tr>
<td>Present</td>
<td>3</td>
<td>15</td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td>Present</td>
<td>3</td>
<td>30</td>
<td>3</td>
<td>0.54</td>
</tr>
<tr>
<td>Present</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>0.48</td>
</tr>
<tr>
<td>Present</td>
<td>10</td>
<td>18</td>
<td>2</td>
<td>1.60</td>
</tr>
<tr>
<td>Present</td>
<td>100</td>
<td>0.004</td>
<td>32</td>
<td>0.78</td>
</tr>
<tr>
<td>Ref [6].</td>
<td>100</td>
<td>0.004</td>
<td>32</td>
<td>1.72</td>
</tr>
<tr>
<td>Ref [11].</td>
<td>10</td>
<td>6</td>
<td>1.47</td>
<td>0.69</td>
</tr>
<tr>
<td>Ref [11].</td>
<td>10</td>
<td>18</td>
<td>1.47</td>
<td>1.28</td>
</tr>
</tbody>
</table>

4.3.2 Experimental Results

This section reports the performance of proposed WDIA algorithm using experimental data. Three different arrangements of particle distribution in 3D volume are explored. The hologram is generated using digital inline holographic arrangement with plane wave illumination (see Fig. 1(a)). The hologram subsequently undergoes pre-processing steps i.e. Fourier filtering and spatial domain filtering (mean filter of size 3x3). Both the steps are performed using ‘ImageJ’ software. The pre-processing step is followed by reconstruction using proposed WDIA algorithm. Copper particles of average size, $d_p = 6$ pixels ($\sim 40$ µm) are used for three different experimental data sets as discussed in the following paragraphs.

Figure 13(a) shows the schematic of particle field for the first experimental data set, where particles are deposited on both surfaces of the glass slide (see Fig. 13(a)). The thickness of the glass slide is equal to 1.2 mm. The recording distance is $z_o/z_c = 1.2$. Figure 13(b) shows the hologram of the particle field in Fig. 13(a). The reconstruction of the hologram is carried out for 101 planes at a depth interval, $\delta z = 20$ µm. Figures 13(c) and (d) show the microscopic images (using 10x microscope objective) of particles corresponding to two planes 1 & 2 respectively. Figures 13(e) and (f) show holographic reconstruction images at 21 and 81 planes in the reconstruction volume. These images show the presence of both in-focus and out-of-focus particles on the reconstruction planes. These in-focus and out-of-focus particles are subsequently identified using the proposed algorithm “within depth intensity averaging (WDIA)”. Figures 13(g) and (h) show the images of in-focus particles using WDIA algorithm on the images of Figs. 13(c) & (f) respectively. The comparison of Figs. 13(c) and (d) with Figs. 13(g) and (h) respectively show good match between object and reconstructed particles. Thus, Fig. 13 confirms the capability of WDIA algorithm in successfully differentiating between in-focus and out-of-focus particles contrary to the regular holographic reconstruction procedure.

The second experiment uses one layer of particles distributed on the glass slide followed by deposition of a film of gelatin of concentration 10% w(gm)/v(ml) over this layer. The second layer of particles is then sandwiched between the gelatin film and cover slip. Here, the particle field in two planes is separated by a distance of 400 µm contrary to 1.2 mm of the first experiment. The schematic of this arrangement is shown in Fig. 14(a). Figure 14(b) shows the hologram generated from the particle distribution of Fig. 14(a). Figures 14(c) and (d) show the bright field microscopic images of particles corresponding to planes 1 & 2 respectively. The numerical reconstruction of the hologram is carried out on 101 planes at a depth interval $\delta z = 20$ µm. Figures 14(e) and (f) show holographic reconstruction images at 51 and 71 planes in the reconstruction volume. Figures 14(g) and (h) show the particle...
distribution images in plane-1 and plane-2 respectively after application of the WDIA algorithm.

The comparison of Fig. 14(c) with 14(g) and Figs. 14(d) with 14(h) show good match of object particles from microscopy with the reconstructed object particles from holography. The out-of-focus particles present after regular holographic reconstruction in Figs. 14(e) and 14(f) are successfully separated from the in-focus particles by WDIA algorithm as shown in Figs. 14(g) and (h).

Fig. 13. The comparison between microscopic and holographic particle field images: (a) The schematic showing particle field distribution on two opposite surfaces of glass slide of thickness 1.2 mm; (b) The hologram of particle field in (a); (c & d) The bright field microscopic images of the particle field in plane-1 and plane-2 respectively; (e & f) The holographic reconstructed images of particle field in plane-1 and plane-2 corresponding to 21 & 81 plane in the reconstruction volume respectively; (g & h) The particle field images after implementation of proposed WDIA algorithm on the particle field of (e) & (f) respectively.
Fig. 14. The comparison between microscopic and holographic particle field images: (a) The schematic showing copper particle distribution on a glass slide (plane-1) and cover slip (plane-2) with gelatin film sandwiched in between; (b) The hologram of particle field in field (a); (c & d) The bright field microscopic images of particle field in plane-1 and plane-2 respectively; (e & f) The holographic reconstructed images of particle field in plane-1 and plane-2 corresponding to 51 and 71 plane in the reconstruction volume respectively; (g & h) The particle field images after implementation of proposed WDIA algorithm on particle field of (e) & (f) respectively.

The last experiment uses an additional layer of particle i.e. total three planes for simulation of 3D particle field. Figure 15(a) explains the schematic of this distribution. In this arrangement, the particles are distributed on both planes of glass slide (planes 2 and 3). Subsequently, gelatin film of concentration 10% w(gm)/v(ml) is deposited over the glass slide. A third layer of particles is then deposited over the gelatin film of plane-2 side and a cover slip is placed over this layer (plane-1). The thickness of each gelatin film is equal to 400 µm. The hologram generated using the particle field in Fig. 15(a) is shown in Fig. 15(b). Figure 15(c), (d) & (e), show the bright field microscopic images corresponding to planes 1, 2 & 3. Some out-of-focus particles located in other planes inside the object volume are also visible in these microscopic images. The hologram in the Fig. 15(b) is numerically reconstructed on 201 planes at a depth interval, \( \delta_z = 20 \) µm. Figures 15(f), (g) & (h), show the numerically reconstructed images corresponding to planes 70, 91 & 151 in the reconstruction volume. Figures 15(i), (j) & (k) show output images after application of the proposed “within depth intensity averaging (WDIA)” algorithm. Comparison of final images in Figs. 15(i), (j) and (k) with the respective microscopic images in Figs. 15(c), (d) and (e) shows good match between the object particle field and that obtained from the holography after application of the
proposed algorithm. The disadvantage of presence of out-of-focus particles in Figs. 15(f), (g) and (h) by regular holographic reconstruction is alleviated by the proposed algorithm.

Fig. 15. Comparison between microscopic and holographic particle field images: (a) The schematic showing the particle distribution in plane-1 (cover slip), plane-2 (one side of the glass slide with gelatin film sandwiched in between), plane-3 (other side of the glass slide with gelatin film layer above it); (b) The hologram of particle field in (a); (c, d & e) The bright field microscopic image of particle field in plane-1, plane-2 and plane-3 respectively; (f, g & h) The holographic reconstructed images of particle field in plane-1, plane-2 & plane-3 corresponding to 70, 91, and 151 plane in the reconstruction volume respectively; (i, j, & k) The particle field images after implementing the proposed WDIA algorithm on particle field of (f), (g), & (h) respectively.

5. Conclusions

Primary limitation of digital holography for particle field characterization is the large depth error and low reconstruction effectiveness. The numerical reconstruction is the critical step for
successful application of digital holography. Therefore, various algorithms i.e. peak intensity, complex amplitude method (PECA) and wavelet transform have been proposed in the literature. The present work proposes a new reconstruction algorithm known as ‘Within Depth Intensity Averaging (WDIA)’ and compares its performance with the existing algorithms. The WDIA algorithm is based on intensity averaging of pixels within a particle after removal of ‘outside-depth’ pixel elements. Both experimental and simulation results are reported for single particle, 2D particle field and 3D particle field at different recording distances, particle size and sample volume depth.

The 3D particle field is experimentally simulated using gelatin film on the glass slide. The particle field images from holographic reconstruction at different planes inside the reconstruction volume successfully compares with that from microscopy. The proposed WDIA algorithm is successful in reducing the inherent problem of out-of-focus particles and works effectively for all particle sizes in contrast to the observation in literature. As high as 100% reduction in depth error is observed when using the proposed WDIA reconstruction algorithm compared to that of literature.

Acknowledgements

The authors are thankful to Department of Science and Technology (DST), Govt. of India, for the financial support and Mr. Harsh Mishra and Mr. Abhishek Nigam for help in conducting the experiments.