Unfocused images’ removal of z-axis overlapping Mie scattering particles by using three-dimensional nonlinear diffusion based on digital holography

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Abstract: We propose a three-dimensional nonlinear diffusion method to remove the unfocused images of certain sized Mie scattering particles which are overlapping along z-axis. It is simultaneously applied to all of the reconstruction slices that are generated from the captured hologram after each back propagation. For certain small sized particles, the maxima of maximum gradient magnitude of each reconstruction slice appears at the ground truth z position when the reconstruction range along z-axis is sufficiently long and the reconstruction depth spacing is sufficiently fine after applying the proposed scheme, therefore, the reconstructed image at ground truth z position is remained, however, the unfocused images are diffused out. The results demonstrated that the proposed scheme can diffuse out the unfocused images which are 20μm away from the ground truth z position in spite of that several Mie scattering particles were completely overlapping along z-axis with a distance 800μm when the diameter is 15μm and the hologram pixel pitch is 2μm. It also demonstrates that the sparsity of the ground truth z slice cannot be affected by the sparsity of corresponding unfocused images when the particle is small enough as well when reconstruction depth spacing is higher than 20μm.

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1. Introduction
Unlike traditional microscopy that merely focuses on one plane, digital holography can capture a volume, and reconstruct every plane of this volume. Nowadays, the sizing, counting, and locating issues of in situ micro-objects (bubbles, particles, or microorganisms) gains a wide variety of attentions, especially when in-line digital holography is developed and becomes a promising microscopic alternative [1-2]. The four papers [3-6] employed spherical wave as the reference wave to illuminate opaque particles, rod-shaped particles, and transparent particles suspended in water. Then the authors located the positions and measured the size of each particle from the captured hologram. Its experimental configuration is very compact and provided a magnification on the target objects. However, it reduced the field of view (FOV), in addition, since the traditional Fresnel diffraction is utilized, the accuracy of particle amount and the location along z-axis are significantly affected by the chosen depth spacing. Tian et al. employed plane wave as reference beam to illuminate bubbles [7], wherein the minimum intensity is used as a focus metric to detect edges of the bubbles, thereby locating each bubble’s location, especially z position. Despite it maintained large FOV and the processing time is faster than traditional Fresnel transform, the location accuracy is not accurate, especially when the bubble size is less than 10μm. Moreover, when several bubbles were clustering together, this scheme recognized them as one bubble. Overall, the traditional Fresnel transform can cause severe unfocused-image issue under the condition
that the ground truth $z$ position of each micro-object is unknown. It directly leads to the inaccuracy of micro-objects on the mount and locations.

On the other hand, constructing a compressive model with sparsity shows a good performance when encountering noise and ghost images by converting hologram reconstruction problem to a regularized nonlinear optimization. Brady et al. introduced compressive sensing algorithm into digital holography, it demonstrated that decompressive inference can infer multidimensional objects from a 2D hologram [8]. Liu et al. applied compressive holography to object localization [9-10], which has shown orders of magnitude improvement on lateral localization accuracy as long as the solution is sparse in its derivative. Chen et al. used plane wave to illuminate bubbles [11], and they suggested the compressive holographic method to locate the $z$ position of each bubble. It cannot distinguish the bubbles which are overlapping along $z$-axis completely or partially. In addition, if the amount of bubbles is higher 256 with the reconstruction depth spacing equals 250µm, the results were as same as the traditional Fresnel transform. Total variation (TV) regularizer was employed in all of the aforementioned papers. Tian et al. [12] suggested a 2D nonlinear diffusion scheme in the research of transport of intensity equation (TIE) to remove the low frequency artifacts on an image effectively. Zhang et al. [13] proposed a method including both simultaneous measurement and reconstruction tailoring for quantitative phase imaging. A mild sparsity promoter regularizer was utilized to minimize the expected end-to-end error and to yield optimal design parameters for both the measurement and reconstruction processes of the thin phase object.

We propose a three-dimensional nonlinear diffusion method to remove the unfocused images of certain sized Mie scattering particles which are overlapping along $z$-axis. It is applied to all of the reconstructed images that are generated from the captured hologram after each back propagation. For certain small sized particles, the maxima of the gradient magnitude curve of all of the reconstruction slices appears at the ground truth $z$ position when the reconstruction range along $z$-axis is sufficiently long and the reconstruction depth spacing is sufficiently fine after applying the proposed scheme, therefore, the reconstructed image at the ground truth $z$ position is remained, however, the unfocused images are diffused out. The numerical results demonstrated that the proposed scheme can diffuse out the unfocused images which are 20µm away from the ground truth $z$ position of the particle with diameter equals 15µm and the hologram pixel pitch is 2µm, and demonstrated that completely $z$-axis overlapping particles can be resolved as well.

The remainder of the paper is organized as follows. Three-dimensional Hybrid-Weikert diffusion regularizer is introduced in Section 2. In Section 3, the demonstrations of unfocused image removal are presented, which includes single-particle case and multiple-particle case. Finally, the conclusions are summarized in Section 4.

2. Three dimensional hybrid-Weikert diffusion regularizer

We investigated the impact of three-dimensional hybrid-Weikert nonlinear diffusion (3D HWNL) scheme on the Mie scattering particles suspended in the water. In the optical setup, a plane wave illuminates a cuvette in which plenty of particles suspended in the milli-Q water. Subsequently, charge-coupled device (CCD) camera captures the hologram of the whole volume. The schematics is shown below in Fig. (1). Assume there are $m$ particles in the captured volume $V$, and each particle $\text{particle}(_{\xi,\eta})$ corresponds to a distance $z_i$ away from CCD, whose Fresnel transform $H_{\textit{particle}}(x, y, z_i)$ is shown in Eq.(1) [14], and the summation $H(x, y)$ of the Fresnel transforms of all the $m$ particles is shown in Eq. (2). Since the plane wave is utilized, assume its intensity is 1 for the simplification. Hence the mathematic expression of the final hologram $I$ captured by CCD is shown in Eq. (3), in which the plane wave is omitted.
Fig. 1. Schematics of the optical setup, SF: spatial filter, L: collimating lens.

\[ H_i(x, y, z_i) = \text{FT}^{-1} \left\{ \exp(jkz_i) \times \text{FT} \{ \text{particle}_i(\xi, \eta) \} \times \exp(-j\pi\lambda z_i(f_x^2 + f_y^2)) \right\}, \quad (1) \]

\[ H(x, y) = \sum_i^m H_i(x, y, z_i) \quad (2) \]

\[ I(x, y) = H^2(x, y) + H(x, y) + H^*(x, y), \quad (3) \]

where \((\xi, \eta), (f_x, f_y), \) and \((x, y)\) represent the centroid plane of one Mie scattering particle, spatial frequency domain, and hologram plane, respectively. \(\text{FT} \{ \} \) and \(\text{FT}^{-1} \{ \} \) denote traditional Fresnel transform and inverse Fresnel transform, respectively [14]. \(H^*(x, y)\) stands for the complex conjugate of \(H(x, y)\). Since the particle is very small, reconstruction performance of \(H(x, y)\) is rarely affected by \(H(x, y)^2\) and \(H^*(x, y)\).

Technically, it is difficult to obtain the exact \(z\) position of the object from one hologram especially while the objects are plenty (several hundred in one \(\text{cm}^3\)) with small size (in \(\mu\text{m}\)), which is caused by the limitation of the depth resolution of the hologram. The lateral resolution and depth resolution of the hologram are \(\Delta x = \Delta y = \frac{\lambda z}{2d} \) and \(\Delta z = \frac{2\lambda z^2}{d^2}\), respectively, where \(d\) is the height/width of the camera chip and \(z\) is the distance to the camera. Hence, 3D HWNLG regularizer is induced to defuse out the unfocused images and remain the focused images in the reconstruction stack of a hologram.

\[ x_{re} = \arg \min \frac{1}{2} \left\| y_{sum} - A x_{re} \right\|^2 + \tau \iiint \Psi(|\nabla x_{re}|)dx dy dz, \quad (4) \]

where \(y_{sum}\) denotes the captured hologram that is literally the \(I(x, y)\) in Eq. (3) and \(x_{re}\) denotes a stack of reconstructed images with certain depth spacing, respectively; \(A\) represents the forward propagation that is the Fresnel transform shown in Eq. (1), but with plenty distances simultaneously; \(\tau\) denotes an advanced settled parameter, and \(\Psi(|\nabla x_{re}|)\) represents the 3D HWNLG, in which \(\nabla\) stands for gradient operation on \(x, y, z\) axes, respectively; \(x_{re}\) is an estimate of \(x_{re}\). The implementation of this regularizer is shown in Eq. (5).

\[ \frac{\partial x_{re}}{\partial t} = \tau \nabla \cdot \left[ \Psi'(s) \frac{\nabla x_{re}}{|\nabla x_{re}|} \right], \quad (5) \]

where \(\Psi'(s) = F_H(s)\), and \(F_H(s)\) is a hybrid-Weikert function shown in Eq. (6), \(t\) is the time that is carried out by \(\tau\) multiplied by step-size that is controlled by the iterative shrinkage/thresholding (IST) algorithm. Usually, the initialization of step-size is 1, its value exponentially decreases when the program runs.

\[ F_H(s) = 1 - \exp\left(\frac{-3.86}{s^{12}}\right), \quad (6) \]
where \( s = \| \nabla x \| / k_0 \), and \( k_0 \) is a constant value. If the gradient magnitude of the voxel which is higher than \( k_0 \) is encountered, it is preserved; otherwise, it is diffused out fast. However, compared with total variation (TV) diffusion regularizer, in which \( \Psi'(x) = 1 \) and \( \Psi(x) = \| \nabla x \| \), HWNL D regularizer is more flexible but less powerful.

Before implementing 3D HWNL D, \( \tau \) is settled, and \( x_{re} \) is initialized by reconstructing the original hologram \( I(x, y) \). Thus, 3D HWNL D is applied on \( x_{re} \) to obtain a stack of estimated reconstructed images \( x_{re} \), the corresponding \( \Phi(\| \nabla x \|) = \iint \Psi(\| \nabla x \|)dx dy dz \) is calculated. If it is not less than a previously settled small value, \( x_{re} \) goes into next forward propagation to obtain a new hologram \( y_{sum} \). Then, the back propagation is carried out on the residual hologram \( y_{sum} - y_{sum} \) to obtain a new stack of reconstructed images \( resi_x_{re} \) multiplied by a step-size and \( x_{re} \) is replaced. Subsequently, apply 3D HWNL D on \( x_{re} \) and replace \( x_{re} \), then continue to carry out the loop until \( \Phi(\| \nabla x \|) \) is less than the settled small value and end this program. This process is implemented by IST algorithm, and the flowchart of the main program framework is shown in Fig. 2.

![Flowchart of the main program framework in IST.](image)

**3. The removal of unfocused images**

**3.1 Mie Scattering particles with different sizes**

When plane wave illuminates particles from water, several reflections and refractions occur before light leaving the rear surface of the particle. This physical phenomenon is named Mie scattering. Several applications of Mie scattering model is employed in digital holographic microscopy [15-17]. In this paper, the chosen physical model of the particle (refractive index = 1.58) is Mie scattering particle with diameters from 10\( \mu \)m to 60\( \mu \)m, several of them are shown in Fig. 3. It is noted that the edge is the lightest portion after subtracting the background noise, which implies the maximum magnitude of the gradient of the pixel is the edge of the particle.
3.2 Single-particle case

It is observed that the centroid image of the Mie scattering particle is a ring pattern according to Fig. 3. Fig. 5 shows that the maximum intensity of each reconstruction slice in the reconstruction stack (101 reconstructed slices along z-axis, namely, the range of reconstruction distance is [4338\(\mu\)m, 6338\(\mu\)m]) of each sized particle (diameters equal 15\(\mu\)m, 25\(\mu\)m, 30\(\mu\)m, 35\(\mu\)m, 40\(\mu\)m, 45\(\mu\)m, and 55\(\mu\)m, respectively), and the depth spacing of reconstruction equals 20\(\mu\)m between two neighboring reconstruction slices. The intensity becomes higher and higher and reaches a peak at the two sides of the ground truth z position, moreover, the positions of the peak intensity appear further and further away from the ground truth z slice when the diameter becomes bigger and bigger. The hologram of Mie scattering particle with diameter 15\(\mu\)m is depicted in Fig. 4(a).

![Fig. 3. The centroid images of Mie scattering particles with different diameters, each image size: 41\(\times\)41.](image)

![Fig. 4. (a) Hologram of one single particle, (b) hologram of three overlapping particles along z-axis (particle diameter=15\(\mu\)m).](image)
Fig. 5. The maximum intensity of each reconstruction slice in the reconstruction stack of single Mie scattering particle hologram with different diameters.

It depicts the maximum gradient magnitude of each reconstruction slice in the reconstruction stack of each sized particle’s hologram in Fig. 6. The maxima of each curve do not appear at the ground truth z position, and the jamming of the unfocused images along z-axis becomes so severe that the sparsity of the edge in the ground truth z slice is affected when the particle size becomes bigger and bigger.

Assume the ground truth z position is at 5338µm away from CCD camera when a single particle with diameter equals 15µm are firstly investigated, the depth spacing equals 20µm. There are 50 reconstruction slices in each side of ground truth z position, hence overall 101 reconstruction slices. The utilized wavelength is 660µm, and pixel pitch equals 2µm in the 256 x 256 hologram. The maximum intensity curve and maximum gradient magnitude curve of the reconstruction stack utilizing Fresnel back propagation are depicted in Fig. 5(a) and Fig. 6(a), respectively. After 70th iteration of the 3D HWNLD, the reconstructed images turn into Fig. 7(a). Most of the unfocused images are diffused out; the reconstructed image at ground
truth \( z \) position is remained properly, including particle’s shape and intensity. The maximum intensity of each reconstructed image is plotted in Fig. 8(a), wherein the maxima of the curve appears at the ground truth \( z \) position, the maximum intensity of the other unfocused images are almost 0. However, the unfocused images in the neighboring slices of the ground truth \( z \) position are not completely diffused out. The maximum gradient magnitude is plotted in Fig. 8(b) where the maxima of the curve appears at the ground truth \( z \) position and the maximum gradient magnitude in neighboring slices are both much smaller than the maximum gradient magnitude in the ground truth \( z \) slice. It implies that more iteration can fully diffuse out the neighboring unfocused image.

Fig. 7. (a) Central 11 reconstructed image after traditional Fresnel transform, (b) Central 11 reconstructed images after applying the proposed scheme, GT: ground truth \( z \) slice, NBor, neighboring slice of GT.

Fig. 8. (a) Maximum intensity, and (b) maximum gradient magnitude of each reconstruction slice in the reconstruction stack after applying the proposed scheme (81 reconstruction slices are shown and particle diameter=15\( \mu \)m).

However when a single particle with diameter equals 40\( \mu \)m is investigated, in which the other parameters are as same as the investigation of the single particle with diameter equals 15\( \mu \)m except that \( r \) equals 3 rather than 1.5, the reconstructed images converge at two positions which located at two sides of the ground truth \( z \) position. After the 653\textsuperscript{th} iteration of 3D HWNLD, the maxima of the maximum intensity curve and the maxima of the maximum gradient magnitude curve do not appear at the ground truth \( z \) position, which are depicted in Fig. 9(a), and Fig. 9(b), respectively. Moreover, the \textit{step-size} is decreased to 1/524288, which is significantly small so that the trend of the curve in Fig. 8(a) cannot change too much even though the program runs forever.
Fig. 9. (a) Maximum intensity, and (b) maximum gradient magnitude of the reconstruction stack after applying the proposed scheme (diameter = 40 µm).

It is observed that the maxima of the maximum gradient magnitude curve does not appear at the ground truth z position when the reconstruction z-axis range is relatively long and depth spacing is fine, such as the particle with diameter equals 25 µm and reconstruction depth spacing equals 20 µm depicted in Fig. 6(b). However, the maximum magnitude gradient that appears at the unfocused image is too few to impact the distribution of gradient magnitude at the ground truth z position according to the red area upper the double-arrow line in Fig. 6, since the purpose of the proposed scheme is to remove the red area. Therefore, 3D HWNLD renders the reconstructed images to converge at the ground truth z position and renders the unfocused images diffuse when the particle size is relatively small (less than 40 µm in the case of this paper). It indicates that the sparsity of the ground truth z slice cannot be affected by the sparsity of corresponding unfocused images when the particle is small enough as well when reconstruction depth spacing equals 20 µm.

3.3 Multiple-particle case

When several particles are located at the same position in lateral plane but with different z positions, which indicates they are completely overlapping in the captured hologram, 3D HWNLD still can remove the unfocused images and remain the focused image at the ground truth z positions. Assume three particles with diameter equals 15 µm are settled at 4538 µm, 5338 µm, and 6138 µm away from CCD camera, respectively. The hologram is shown in Fig. 4(b). After applying the proposed scheme, the central 11 reconstructed images around the ground truth z slice of each particle are depicted in Fig. 10, and the maximum intensity and maximum gradient magnitude of each reconstructed image in the reconstruction stack is plotted in Fig. 11(a) and (b), respectively. Even though a fairly long distance between two neighboring particles (800 µm) is required between two particles, it demonstrates that completely overlapping particles along z-axis can be resolved with small reconstruction depth spacing (200 µm) and unfocused images can be completely removed. However, if any particle with diameter bigger than 15 µm, the unfocused images will be diffused out as long as much further distance is required between the two neighboring particles.
Fig. 10. (a) The central 11 reconstructed images around the ground truth \( z \) slice of the first particle, (b) the second particle, and (c) the third particle after 3D HWNL, GT\(_i\): ground truth \( z \) slice of each particle, NBor\(_i\), neighboring slice of GT\(_i\), \( i = 1, 2, 3 \).

Fig. 11. (a) Maximum intensity, and (b) maximum gradient magnitude of each reconstructed image in the reconstruction stack of the three particles after applying the proposed scheme.

4. Conclusions

In this paper, it demonstrated that 3D HWNL can remove the unfocused image of certain sized Mie scattering particles which are overlapping along \( z \)-axis. It is applied to all of the reconstructed images after each back propagation. Despite the maxima of maximum gradient magnitude of each reconstruction slice does not appear at the ground truth \( z \) position when the reconstruction range along \( z \)-axis is sufficiently long and the reconstruction depth spacing is sufficiently fine, the gradient magnitude of unfocused image cannot affect the gradient distribution of the reconstructed image at ground truth \( z \) position when the particle is sufficiently small. Therefore the sparsity of the ground truth \( z \) slice cannot be affected so that the reconstructed image at ground truth \( z \) position is remained and the unfocused image is diffused out after applying the proposed scheme. The numerical results also demonstrated that the proposed scheme can diffuse out the unfocused images which are 20 \( \mu \)m away from the ground truth \( z \) position in spite of that several Mie scattering particles were completely overlapping along \( z \)-axis when the diameter is 15 \( \mu \)m and the hologram pixel pitch is 2 \( \mu \)m.
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