

# 4D tracking of biological samples using lens-free on-chip in-line holography

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**Abstract:** We propose an auto-refocused phase retrieval approach that combines refocusing and phase retrieving properties based on lens-free on-chip in-line holography. We demonstrate a 4D tracking application of imaging/monitoring *in vivo* biomedical scenes, e.g. Blepharisma.

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## 1. Introduction

Digital holography (DH) is a well-known coherent imaging method that is capable of refocusing and phase retrieving about a scene of interest. Refocusing [1, 2] and sectioning [3, 4] enables recovering 3D information from 2D images. Various applications have been found, such as particle imaging, tracking in biomedical microscopy [5, 8] and physical process profiling and measuring [6, 7]. In particular, the lens-free on-chip in-line holography (LOIH) has become an emerging imaging technology with high resolution, wide field-of-view and simple realization [8, 9]. Besides the 3D reconstruction capability, LOIH is also capable of revealing phase information. The phase retrieval task is commonly addressed in the literature of coherent diffraction imaging (CDI), where a diffraction pattern of the object signal is sampled instead of an interference pattern with a reference beam. In view of this, the LOIH setup aligns with CDI by enforcing the illumination arm to serve both as the reference and object beam. Several algorithmic schemes [9, 12] have been proposed for solving phase retrieval problem based on CDI/LOIH setup. This type of algorithms can be categorized as alternating projection methods, originally proposed by [10, 11], which aim to recover the complex field by iteratively imposing real-plane and Fourier-plane constraints, such as non-negativity and/or object boundary/support in real-plane and Fourier-plane magnitude, respectively.

In this paper, we combine the refocusing and phase retrieving capabilities of LOIH and propose an auto-refocused phase retrieval (APR) method. The auto-refocusing scheme automatically determines the propagation distance which was previously done visually [9]. After APR, we show that the recovered support, i.e., the shape of the object, improves depth estimation of object boundary. Our algorithm is validated by estimating the length of Blepharisma microorganisms. We further perform 4D (3D locations with time) tracking results with detailed orientations.

## 2. Method

A simple on-chip imaging setup is shown in Fig. 1(a). The system consists of a light-emitting diode (LED) and a CMOS sensor. The LED (quasi-monochromatic with wavelength  $\lambda = 625\mu m$ ) is positioned at approximately 40 cm above the sensor so that the spatial coherence of the source, defined by its distance from the sensor and the width of its active area, is sufficient to produce high-contrast diffraction fringes at the sensor plane. The samples for imaging are prepared on a transparent glass. The glass is closely placed and adjusted parallel to the sensor plane. The field propagation process is modeled as,

$$H(x, y; z) = \mathfrak{F}^{-1} \left\{ \mathfrak{F} \{ E(x, y) \} [k_x, k_y] \exp \left[ i z \sqrt{k^2 - k_x^2 - k_y^2} \right] \right\} [x, y], \quad (1)$$

where  $H(x, y; z)$  is the propagated field at axial distance  $z$  from the object field  $E(x, y)$ .  $\mathfrak{F}$  and  $\mathfrak{F}^{-1}$  denote the Fourier transform and its inverse transform.  $k_x$  and  $k_y$  are the corresponding spatial frequencies of  $x$  and  $y$ .  $k = 2\pi/\lambda$ . Inversely, a specific depth layer can be reconstructed by back-propagating  $H$  to  $E$ , which is equivalent of changing the sign of  $z$  in (1).  $H$  can be approximated by the intensity of the captured hologram [6]. Thus, a 3D volume  $U$  can be reconstructed by taking the absolute value of each back-propagated layer. Here, we design a refocusing scheme

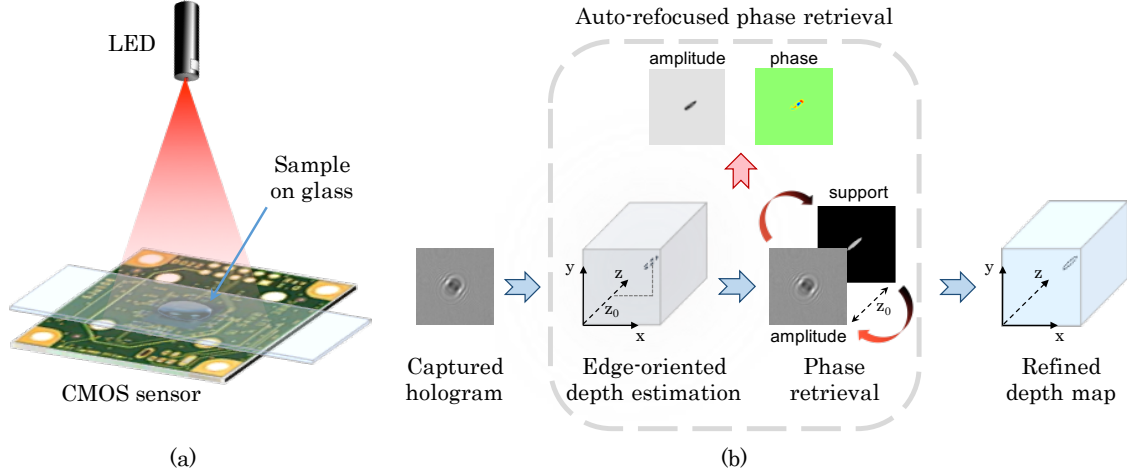


Fig. 1. (a) Experimental setup. LED: light-emitting diode. (b) Auto-refocused phase retrieval method for depth recovery of the sample.

similar to [1]. The first step is to construct a variance cube  $V$ , which is computed by scanning a square window of size  $(2n + 1) \times (2n + 1)$  (e.g.,  $n = 2$  pixels) in each depth layer and repeating this scanning procedure over all layers.  $V[x, y, z] = \sum_{i=x-n}^{x+n} \sum_{j=y-n}^{y+n} |U[i, j, z] - \bar{U}[x, y, z]|^2$ , where  $\bar{U}[x, y, z] = \frac{1}{(2n+1)^2} \sum_{i=x-n}^{x+n} \sum_{j=y-n}^{y+n} U[i, j, z]$ . The maximum projection along  $z$ -axis is recorded as  $D(x, y) = \max_z \{V(x, y, z)\}$ .

The second step is to filter out out-of-interest pixels. A preliminary filtering process is used by setting a threshold on the maximum values of the variance so that the background pixels are excluded. This is similar to [1]. Here we use k-mean clustering method ( $k = 3$ ) to further cluster the pixels of the depth map. The cluster with medium centroid value is shown in Fig. 2(b). The centroid value is also assigned as the overall depth estimation of the object. In [6], it is shown that the edge of object provides more accurate information in terms of depth estimation. Based on this insight, we employed a phase retrieval scheme [9, 11] for edge/shape recovery. This is because this types of alternating projection algorithm enables recovering the "support", i.e., the shape of the object. (A detailed description can be found in [9].) However, a pre-requisite for [9] is a coarse estimation of propagation distance between hologram plane and object plane. As described in Fig. 1(b), this coarse distance can be estimated from the filtered depth map. Using the phase retrieval support as a third filter, a refined depth map is obtained, as shown in Fig. 2(d).

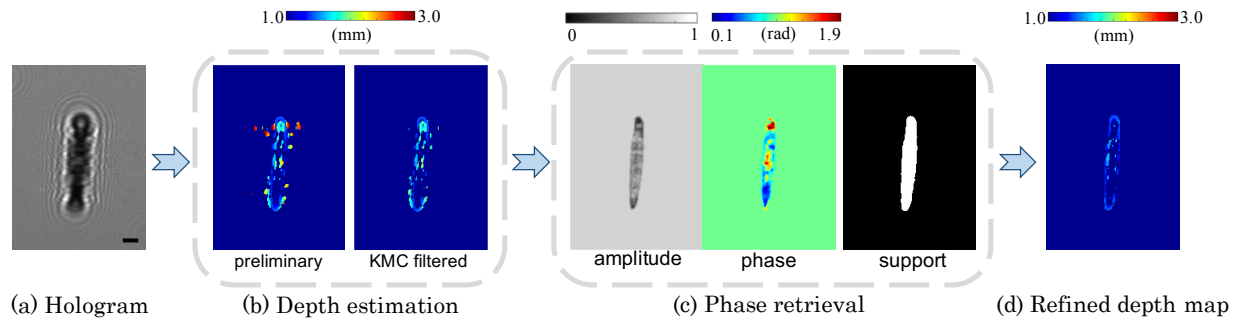


Fig. 2. Recovering depth for Blepharisma. Scale bar in (a) is  $40\mu\text{m}$ . KMC: k-mean clustering.

### 3. Results and conclusion

Figure 3 shows a localization result with a detailed orientation and length estimation of Blepharisma. In Fig. 3(c), near-boundary pixels are extracted from the depth maps in Fig. 3(b). For the convenience of representation, we fit the

extracted pixels (3D location) with a linear model and the maximum projection length are used as the estimated length of *Blepharisma*. Based on this representation approach, a 4D tracking result is shown in Fig. 3(d). A  $z$ -axial rotational behavior of *Blepharisma* is recovered.

Our proposed method serves as a useful tool for 3D localization of biological samples. Monitoring *in vivo* scenes also provides insights for behavior studies of microorganism.

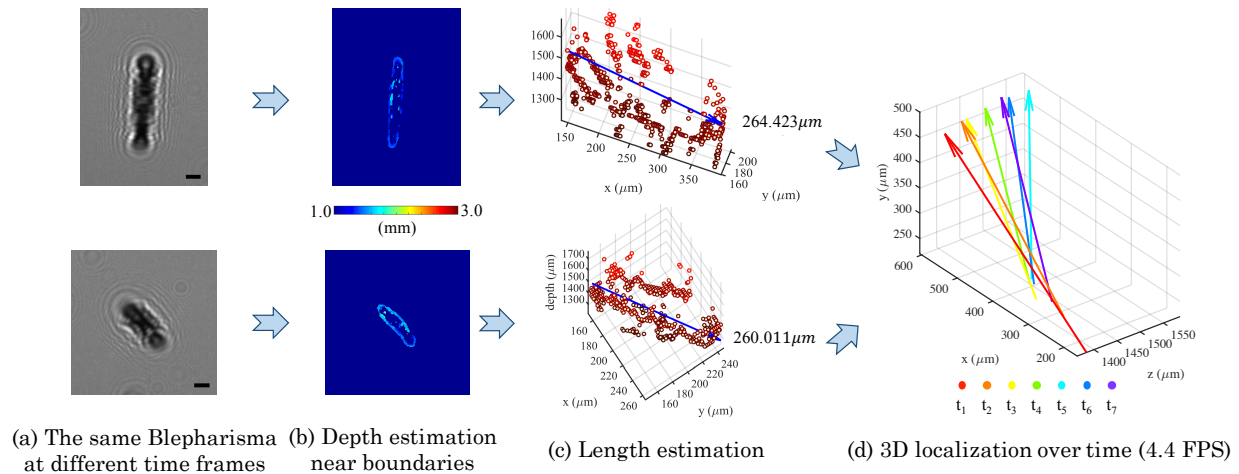


Fig. 3. (a) Experimental setup. LED: light-emitting diode. (b) Auto-refocused phase retrieval method for depth recovery of the sample. Scale bar in (a) is  $40\mu\text{m}$ .

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