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## ► To cite this version:

Dylan Brault, Anthony Berdeu, Ferréol Soulez, Fabien Momey, Thomas Olivier, et al.. Reconstruct-to-refocus: joint reconstruction of a biological sample and of calibration objects for accurate auto-focusing in digital holography. *Digital Holography and Three-Dimensional Imaging*, 2021, Washington, United States. pp.DW4C.8, 10.1364/DH.2021.DW4C.8 . hal-03926250

**HAL Id: hal-03926250**

**<https://hal.science/hal-03926250v1>**

Submitted on 6 Jan 2023

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# Reconstruct-to-refocus: joint reconstruction of a biological sample and of calibration objects for accurate auto-focusing in digital holography

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**Abstract:** We propose to reconstruct biological samples using calibration beads to perform numerical autofocusing in in-line holographic microscopy. The approach is based on a joint inversion using both a parametric and a regularized inversion method. © 2021 The Author(s)

## 1. Joint estimation approach

Since most of biological samples are transparent, they are usually stained to improve the imaging contrast. Another way to characterize these objects is to consider their morphological properties and their refractive index. By being sensitive to the phase shift induced by the objects, holographic microscopy offers a simple and efficient way to access information about the refractive index distribution in the sample [1, 2]. Holographic microscopy records the intensity  $I_d$  of the out-of-focus diffraction pattern produced by the sample under a coherent illumination. Reconstruction algorithms require an accurate knowledge of the propagation distance  $z$  to perform the numerical reconstruction. Inserting calibration objects, like transparent beads, in the sample provides a means to accurately estimate this propagation distance. The recorded intensity hologram can be modeled as  $I_m(\theta, \underline{t}) = |\underline{U}^b(\theta) + \underline{U}^s(\underline{t})|^2$ , where :

- $\underline{U}^b(\theta)$  is the complex amplitude of the beads diffracted wave. It is parameterized by the set  $\theta = \{(x_d, y_d, z_d, r_d, n_d)\}_{d \in \llbracket 1, N \rrbracket}$ , and often referred to as a "parametric image formation model" given the limited number of parameters in  $\theta$ . To this aim, the Lorenz-Mie model [3] is well suited as it provides a physically accurate diffraction pattern of a perfect sphere with respect to the 3D position  $(x, y, z)$ , the radius of the beads  $r$  and the real part of the refractive index of the beads  $n$ .
- $\underline{U}^s(\underline{t})$  the complex amplitude of the object wave (produced by the biological sample), corresponding to a discretized diffraction model:  $\underline{U}^s(\underline{t}) = \underline{h}_z * \underline{t}$ , where  $\underline{h}_z$  represents the Rayleigh-Sommerfeld propagation kernel for propagation at distance  $z$  [4],  $*$  is the bidimensional convolution, and  $\underline{t}$  is the complex transmittance of the object. Note that  $z$  is the same parameter as for the beads since we assume that the beads lie in the same plane than the biological material.

We propose to reconstruct  $\underline{U}^b$  and  $\underline{U}^s$  using an inverse problem approach [5–7]. In this application, the estimation of the bead parameters may be disturbed by the sample of interest. Therefore, we suggest a reconstruction algorithm based on the minimisation of Cauchy's robust cost function for the parametric part (beads estimation) [8], followed by the minimization of a least squares cost function for the biological sample. The minimisation criteria are written as follows :

$$\hat{\theta} = \arg \min_{\theta} \frac{1}{2} \log \{1 + [I_d - I_m(\underline{U}^b(\theta), \underline{U}^s(\underline{t}))]^2\} \text{ (Problem 1)}$$

$$\hat{\underline{t}} = \arg \min_{\underline{t}} \frac{1}{2} \|I_d - I_m(\underline{U}^b(\theta), \underline{U}^s(\underline{t}))\|^2 + \mathcal{R}(\underline{t}) \text{ (Problem 2)}$$

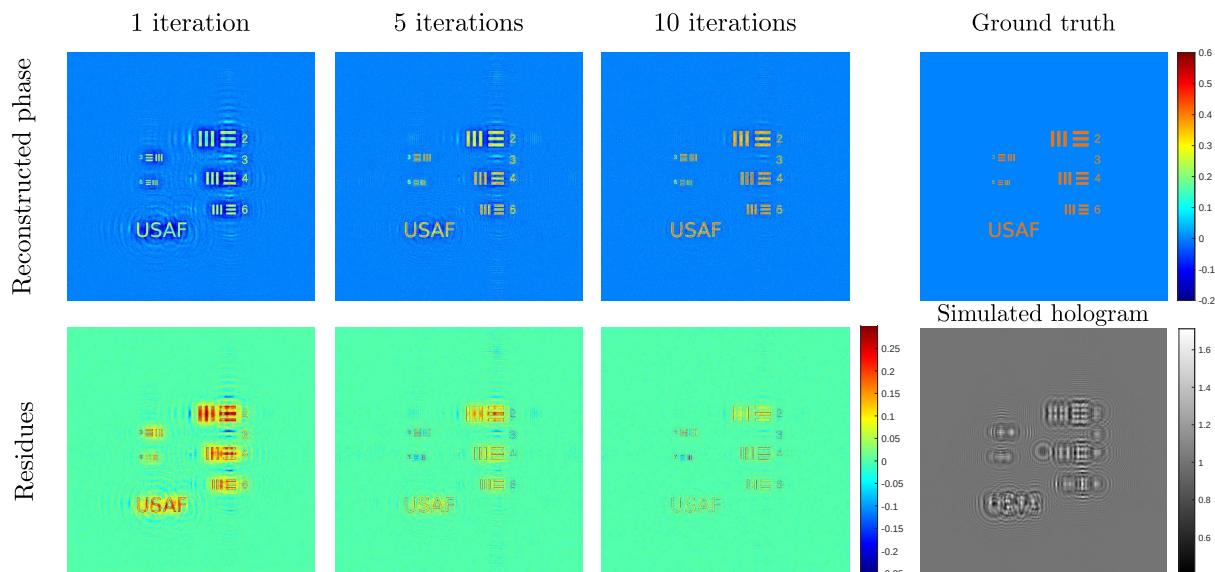


Fig. 1. Phase reconstruction of a simulated phase sample (first row) and residues with respect to the ground truth (second row). The hologram has been propagated at a distance  $z = 10 \mu m$

where  $\mathcal{R}$  is a regularization term penalizing non sparse solutions ( $\ell_1$  norm of the phase angle of  $\underline{t}$ ) [9].

We propose to alternatively solve Problem 1 and Problem 2 [7] so that the initial estimation of  $\theta$  allows a correct focus estimation that is then used in the forward model of  $\underline{U}^s$  to reconstruct the object  $\underline{t}$ , which in turn benefits from the refinement of parameters  $\theta$ .

## 2. Results

Figure 1 reports the phase reconstruction image using the proposed algorithm on a simulated sample perturbed by a white Gaussian noise (the ratio between the amplitude of the beads diffraction pattern and the standard deviation of the noise is 10). A single bead is simulated on the same plane as the extended object (a USAF target). The amplitude of the residues shown in Fig.1 decreases after each refinement of the focus. Twin-image artefacts also become less visible in the reconstructions thanks to this improved focusing.

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