Phase imaging provides intensity contrast to visualize transparent samples such as found in biology without any staining. Among them, digital holographic microscopy (DHM) is a well-known quantitative phase method. Lensfree implementations of DHMs offer the added advantage to provide large field of views (several mm² compared to several hundred μm²) and more compact setups that traditional DHM which have high quality microscope objectives. In this article, a lensfree DHM is presented using a side illumination technique in order to further reduce the device size. Its practical use is described and results on a transparent (phase only) sample are shown.

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obtain a minimum spatial coherence. Hence, by placing the cell plane close the camera plane (small $z_2$), the fringe magnification is close to 1 and the FOV is the illuminated area (chip). Effectively, the coherent point source is collimated by the analog hologram which creates a secondary illumination making the distance $z_1$ and $z_2$ small. Inline holography requires, in most cases, more than one hologram to retrieve the quantitative phase information of the sample (in the previous described concepts for off-axis DHM, only one hologram was required). However, if a phase image is wanted, one image is sufficient (but the quantitative phase is lost).

In the present paper, we present a lensfree DHM where the novelty resides in the side illumination which reduces the height of a lensfree DHM by one order of magnitude. The compact in-line lensfree digital holographic microscope is described in Section 2. Then more practical details are given in Section 3. Results on biological cells are shown in Section 4. Section 5 contains discussion and conclusion.

2. Compact in-line digital lensfree holographic microscope description

2.1. Working principle

The proposed device is based on in-line digital holography. This technique consists in illuminating the sample with a coherent or partially coherent light, such as from a laser for example. After the sample, the light is decomposed in two parts: one disturbed part and one undisturbed part as shown in Fig. 1. The disturbed part (also known as scattered part) is the part of the light that has been scattered by the transparent sample and the undisturbed part is the part of the light that goes through without “seeing” the sample. Both parts are coherent with each other and interfere at the camera plane. This interference pattern is sampled and digitized by the chip to gives the so-called in-line hologram. The 3D information of the object is contained in this intensity image.

In order to retrieve the quantitative amplitude and phase of the sample, phase retrieval algorithms [25,26] require more than one hologram. This is due to the well-known twin-image problem. Indeed, the real, virtual and zero order images are superimposed in the spectrum, which implies that the image is disturbed by the out-of-focus twin-image [25] and the phase cannot be retrieved correctly. This twin-image problem arises from the intensity-only measurements and the subsequent loss of the phase. Fig. 2 shows a simulation of inline holography. The ground truth consists of human epithelial cells on a microscope slide have been imaged using a commercial DHM from LynceeTec. The reconstructed amplitude and phase of the cells measured with the DHM are then used to simulate inline holography. Fig. 2(a) is the original amplitude and Fig. 2(b) is the original phase. These two are combined to make a simulated hologram. Fig. 2(c) (resp. Fig. 2(d)) shows the reconstructed amplitude (resp. phase) using only back-propagation of one hologram. The twin image is clearly visible creating ringing structures around objects.

This twin-image problem is characteristic of in-line holography. One way to avoid it is to use off-axis holography has been primarily developed to avoid this problem. This technique consists in illuminating the sample with one beam that interferes coherently with a second beam that does not go through the sample and makes an angle with the first beam. The real image, virtual image and zero order are then separated in the spectrum, which allows retrieving a twin-image free image and a correct phase with simple spectrum filtering and backpropagation algorithm. Compact off-axis DHM have already been presented, either as a device to be inserted in a classical microscope [27], or as a portable device that uses a grating to redirect part of the incident light to create a reference beam with a specific angle [28–30]. This technique still limits the compactness and/or the FOV due to the necessary angle between the two beams to have interference fringes resolved by a camera with specific pixel size and the fact that the reference beam should not go through the sample part.

In inline holography, this twin image problem is solved numerically by the mean of phase retrieval algorithms. They estimate both phase and intensity from intensity only holograms using additional information. This can be prior knowledge about the sample as support constraints [31–35]. The required information can also be gathered by recording several holograms of the same sample with for instance different distances between the sample and the camera [36,37], different light source wavelengths [38], or by illuminating the sample with different illumination directions [24,39]. The last method is used in the proposed device.

2.2. Device

The proposed device is made of 3 elements: a vertical-cavity surface-emitting lasers (VCSELs) array, a prism and a camera. Fig. 3 shows a schematic of the device with all the components. The total height of the device is ~10 mm for a FOV of ~17 mm².

The array of sources is placed in front of the entrance surface of the prism. Each VCSEL is single-mode with a wavelength of 673 nm and a linewidth of 100 MHz (Vixar 680S). They consume low power (~1 mW) which makes them suitable for a battery operated device.

On the longest surface of the K9 prism, a photopolymer BAYFOL®HX is laminated. This material is sensitive to light: it changes its refractive index according to the amount of light it receives. Thanks to this property phase (transparent) volume hologram gratings can be recorded inside. A grating is used to redirect the light from one direction to another direction. Several gratings are recorded in the photopolymer. The sequential recording process is described in Ref. [24]. For each VCSEL corresponds one hologram grating which diffracts its light out of the prism with a specific angle. The diffracted light goes through the sample and the inline hologram is recorded by the camera. The angular scanning is performed in 2D.

The camera is a complementary metal-oxide-semiconductor (CMOS) sensor with 5.2 μm pixel size (Thorlabs DCC1545M).

3. Compact in-line DHM in practice

Image acquisition and reconstruction steps are depicted in Fig. 4.

3.1. Image acquisition

In the presented results only one VCSEL on a translation stage was used. The system can be made more practical, instead of trans-
Fig. 2. Simulated backpropagation with an in-line hologram. (a) Original object amplitude. (b) Original object phase. (c) Reconstructed amplitude using backpropagation algorithm with only one simulated hologram. (d) Reconstructed phase using backpropagation algorithm with only one simulated hologram.

Fig. 3. Scheme of the compact in-line lensfree digital holographic microscope. The distance between the sample and the lower edge of the prism is less than 1 mm, as well as between the sample and the camera. The prism is 2 cm × 1.7 cm × 1 cm. The green inset shows the diffraction of two different illumination angles with two different gratings. The wavefront shaping is also depicted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
lating a single VCSEL, an array of individually addressable VCSELs would perform the same task but without any moving parts. A user-friendly Labview interface has been implemented to automate the recording procedure. The program starts by switching on the VCSEL, which is moved to the first position at the entrance surface of the prism. This first position creates a first beam by diffraction off the volume hologram. A first digital hologram is recorded. Then the VCSEL is moved to the second position at the entrance surface of the prism, which creates a second beam by diffraction off the volume hologram. A second digital hologram is recorded. This sequence is repeated for the 9 positions of the VCSEL which corresponds to 9 illumination directions of the sample.

3.2. Image reconstruction

The stack of holograms is then inserted in a registration algorithm \[40\] in order to extract the 2D shifts of each hologram at the camera plane with respect to the hologram taken with normal incidence. These shifts are then converted in illumination angles using the following equations

\[
\varphi = \tan^{-1} \frac{x \times p}{z}; \quad \theta = \tan^{-1} \frac{y \times p}{z}
\]

where \(x\) (resp.\(y\)) is the shift in pixels in one direction (resp. the other one), \(p\) is the pixel size and \(z\) is the distance between the sample and the camera. Fig. 5 shows the case of one illumination direction.

The algorithm takes the stack of holograms recorded with different illumination directions and iteratively estimates the object phase and amplitude with the help of appropriate proximity operators \[41\]. The reconstructed object \(o^* \in \mathbb{C}^N\) (where \(N\) is the number of pixels) is estimated in a variational framework by minimizing a cost function which is a sum of the likelihood term \(L\) and a regularization term \(R\):

\[
o^* = \arg \min_{o \in \mathbb{C}^N} L(o) + \mu R(o)
\]

where \(D = \{x \in \mathbb{C}; |x| = 1\}\) is the subspace of \(\mathbb{C}\) of phase only objects. \(\mu\) is a regularization parameter that tunes the balance between the information given by the measurements and the priors. In this approach known as penalized maximum likelihood, the data term is defined according to the forward model and the statistics of the noise, whereas the regularization function is designed to enforce some prior knowledge about the object (such as support, non-negativity, smoothness, \ldots). In the presented work,
Fig. 6. (a) Reconstructed phase with the proposed device and algorithm full FOV. (b) Reconstructed phase with the proposed device and algorithm (crop from a. of 0.0064 mm²). (c) Reconstructed phase with a Digital Holographic Microscope (DHM) using a 5× objective (crop 0.0064 mm²). (d) Reconstructed phase with the proposed device and algorithm (crop from a. of 0.0033 mm²). (e) Reconstructed phase with a Digital Holographic Microscope (DHM) using a 10× objective (crop 0.0033 mm²). (f) Reconstructed phase with the proposed device and algorithm (crop from a. of 0.004 mm²). (g) Reconstructed phase with a Digital Holographic Microscope (DHM) using a 10x objective (crop 0.004 mm²).
we use the well-known total variation regularization function [42].

The equation is solved by the mean of the alternating direction method of multipliers (ADMM). It uses a closed form solution for proximity operator of each function [41]. Such an iterative projection method is a Total Variation regularized evolution of the seminal algorithms of Gerschberg Saxon [43] and Fienup [44].

The outputs of the algorithm are the reconstructed amplitude and phase of the sample. In order to obtain quantitative phase results, a calibration of the reconstruction is made using commercial DHM images as references.

All the beams overlap on a ~17 mm² FOV, corresponding to ~50% of the camera chip size. This is in the same order of magnitude of FOV demonstrated in [22,23]. However it is larger than classical off-axis scheme since no objective is used.

5. Discussion and conclusions

A compact lensfree in-line digital holographic microscope was presented in this paper. The device is ~10 mm height and easy to use thanks to the user interface. It allows visualizing and measuring the thickness of transparent objects, which can be used to extract different parameters for cells study for example. The device has been tested with a phase only sample and showed good quantitative phase retrieval. We are currently working on the reconstruction algorithm to reduce the artefacts and increase the phase accuracy.

The digital holographic capacity can be combined with another microscopy modality such as fluorescence since the sample is visually accessible from the top of the device. Further work on fabricating a hand-held version of the device is ongoing.

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