Self-referenced digital holographic microscopy

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Abstract—By developing a self referenced digital holographic microscope it becomes possible to record holograms and numerically reconstruct volumetric images of low coherence fluorescent objects such as (auto)fluorescent biological samples (e.g. algae). Our goal was to develop and construct a simple, compact portable device. In contrast to the common holographic approaches where there is a conventional reference beam, a reference beam should be produced together with the object beam from the same fluorescent source via imaging it by two separate optical paths (with near zero path length differences) to get interferences fringes. These interference forms separate holograms of all the point sources. The waves coming from the separate sources are mutually incoherent but have an inherent short coherence length. Initially we have tested the self referenced digital holographic microscope setup with test objects illuminated by LED light source that has similar spectral bandwidth as the fluorescence sources like chlorophyll. Digital reconstructions of the measured holograms need considerable processing. To accelerate the hologram processing a parallel implementation of processing seems essential. Using GPU-s we were able to enhance the algorithm’s speed considerably, without the loss of the reconstruction accuracy.

I. INTRODUCTION

Digital holographic microscopy have recently become fields of increasing interest because of the ability to numerically reconstruct holograms, which are captured by digital detectors, creating high quality images with high lateral and sometimes extremely high axial resolutions. [1]–[4]. This technique makes it possible for example to produce lensless microscopes [5] or also to alter conventional microscopes to measure volumetric sample with a single exposure without scanning [6]. At the field of material science and biology fluorescence microscopic applications were needed for such advantages as selectivity and sensitivity. Because of the need of fluorescence and volume detection several 3D fluorescence microscopic applications have already been developed such as Fluorescent Coherence Tomography [7], Lens Free Fluorescent Imaging [8], Fresnel Incoherent Correlation Holography [9] and Three-Dimensional Holographic Microscopy [10]. In this article a Self-referenced Digital Holographic Microscope is presented. The final purpose of this setup is to detect freely floating, living microbiological organisms and to create their fluorescent images within a volume. This solution is similar to the combination of the FINCH method [5] with modified Hariharan-Sen method [11] which means that after the objective the reference and object bands are created on an asymmetric triangular optical path, avoiding the need of any spatial light modulator. The main advantage of this setup is that it uses only inexpensive passive optical elements. Design concepts, the applied test targets and the first measurement results are presented in this paper.

II. THE OPTICAL SETUP

A. Self-referenced holographic setup

The key idea of any digital holographic system is to create an interference pattern between two coherent beams, traditionally a reference beam and an information carrying object beam, and to capture this interference pattern with an image sensor. Reconstruction of these captured interference patterns are done numerically. The maximum optical path difference of an interferometer should be smaller than the coherence length of the used light in order to get the interference pattern. At self-referenced holographic setups the light source of the reference and the corresponding target beam is the same; and all the particles of the observed object that emit or reflect light are the light sources themselves. The self-referenced holographic setups divide the light coming from every object point into two parts to image them separately: generating the reference beam and focusing the target beam. The corresponding beams are reunified to create the hologram. Because of using fluorescent light, whose coherence length is usually only a few micrometers, the applied interferometer should have a nearly zero path length difference between the corresponding beams. In the presented optical setup an assymetric optical path triangular creates the reference and target beams. These two beams go around in this triangular in opposite directions taking the same optical path length. Because of the triangular’s asymmetry the curvarures of thewavefront of the two beams will be different. The curvature of the target beam’s wavefront will be higher than the reference beam’s curvature. Therefor the reference beam is assumed to be a plane wave.

B. Optical parameters

The light originating from the sample is collected with a triplet lens with a focal length of 40.3 mm that makes an intermediate image. Because of this long focal length and a near unity magnification short object distance change makes short image distance change as well. The entrance of the asymmetric triangular is a pellicle beam splitter that directs 67% of the incoming light clockwise to produce the reference
beam and 33% anticlockwise to produce the target beam. This rate of the beam separation is needed because of the divergence of the reference beam is higher and at the hologram plane the intensity density of the reference beam should be higher than that of the target beam. The triangular path contains a lens with focal length of 150 mm shifted out from the symmetrical center plane by 10 mm. The reference and the target beams reach this different sides of this lens after taking different pathes, and traveled different distances from the entrance of the asymmetric triangular path. As a result of this the lens creates two images from the intermediate image with different magnifications and image distances, so the radii of the wave-front curvature of the reference and target beams will be different. The pellicle beam splitter reunites these beams after they take the same length path in different directions and creates the interference pattern that is captured by a monochrome Lucam camera(). The self-referenced holographic optical setups photo can be seen in Fig. 2. In Fig. 1, we can compare two optical pathways that is emitted from one point.

III. THE SAMPLE

There are many different biological objects whose measurement with fluorescent imaging setups, e.g. fluorescently marked or autofluorescent cells living cell samples, is useful. Our aim is to detect and make images of algae. These usually contain chlorophyll-A or chlorophyll-B. Cyanobacteria, which can be toxic, besides phycocyanin and phycoerytin (also autofluorescent proteins) contain Chlorophyll-A. Below in Fig. 3 the emission curve of a cyanobacteria can be seen when excited by 405 nm light. A maximum value of the emittance curve is at wavelength 657 nm.

The bandwidth is about 30 nm. Eq. 1 shows the calculation of the coherent length ($L_c$) from the middle wavelength ($\lambda_0$) and the bandwidth ($\Delta \lambda$).

$$L_c = \frac{\lambda_0^2}{\Delta \lambda} \quad (1)$$

The calculated coherence length of the fluorescently emitted light of the Cyanobacteria is about 14 $\mu$m. This coherence length can be increased with a laser line filter decreasing $\delta \lambda$. This way we can increase the contrast of the interference pattern, but the available light is reduced. We applied a test target in our setup, which was a black plate with six holes of 30 $\mu$m diameter. The distance between the holes were 0.36 mm. This was back-lighted with a LED light source emitting light with the approximately the same coherence length as the chlorophyll-A, but its color is amber. Using this target and illumination we are able to model the fluorescent objects.

IV. THE RECONSTRUCTION

A digital holographic microscopes consist of two basic units: optical setup that creates the interference pattern called hologram that carries the information from the observed volume and the numerical reconstruction that retrieves the visual information from the hologram. The quality of the reconstructed image depends on the aberrations of the target and reference beams, and on the numerical reconstruction method [12]. To get a correct image reconstruct of an object from a hologram, the reference and the beam used for the numerical reconstruction should be the same. Here, the angular spectrum method (plane-wave propagation) is used that is equivalent with the Rayleigh-Sommerfeld description of diffraction [13], [14]. This reconstruction method has been applied before in our in-line digital holographic setup [6], [15], where the numerical reconstruction beam is defined as
a plane wave that spreads along the optical axes. However, in the self-referenced case both the reference beam and the object beam are formed by the optical system, resulting in a reference beam with special curvature. We have to tune the optical setup in a proper way to produce a near-spherical reference beam, or some more complicated algorithms have to be used to compensate its deficiencies. The angular spectrum (plane-wave) propagation algorithm is described by the Eq. 2, where HOLO is the hologram from that we reconstruct the image of the object, REC is the numerical reconstructing wave, \( I_d \) is the reconstructed image, \( \mathcal{F} \) means Fourier and \( \mathcal{F}^{-1} \) inverse Fourier transform operators, \( H = e^{ik_zd} \) is the transfer function of spatial frequencies. All of these functions are matrix represented in the algorithm.

\[
I_d = \left| \mathcal{F}^{-1} \{ \mathcal{F} \{ \text{HOLO} \ast \text{REC} \} e^{ik_zd} \} \right|^2
\]  

(2)

The reference beam is nearly spherical, and its spreading angle is not parallel to the optical axes if the object is out of the optical axes. This kind of geometrical changes of the reference and numerical reconstructing beams makes the reconstructed image smaller. Because the reference beam’s angle depends on the object distance from the optical axis, and the numerical reconstructing beam is parallel to the optical axes a pincushion distortion appears on the image. The effects of the change of the reference beam angle will be detailed in a next paper, where its numerical compensation will also be described. In this setup higher aberration called coma occurs if the optical elements are not properly threaded on the optical axes.

V. RESULTS

The self-referenced holographic setup was built and tested with the target that was placed in different distances. The depth of the measured area was 1mm. The distances between of the reference (Fig. 1. gr) and the target images (Fig. 1. gt) changed between 112 and 117 mm. The magnifications of the target changed between 3 and 3.2 and the magnifications of the reference changed between 2.2 and 2.3. Fig. 4. shows the image of the target objects. Here the detector was put to the target image plane(gt)). When the detector is out of the image plane hologram can be saved. Fig. 5. shows, this self-referenced digital holographic setup is able to create self-referenced holograms with non-coherent light. Fig. 6. shows a reconstructed image. This finding corroborate that it is possible to measure auto fluorescent live cells with this kind of self-referenced holographic setup. To implement the measurements on fluorescent samples a large f-number lens should be applied.
because the auto fluorescent light provides much less light than LED illumination.

Fig. 4. This was captured when the detector was put to the image plane.

Fig. 5. The self-referenced hologram of 6 pinholes back-lighted with LED light colored amber

Fig. 6. This is a numerical reconstruction from the hologram.

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REFERENCES