A. Adinda-Ougba*, N. Koukourakis, N. C. Gerhardt, and M. R. Hofmann

Simple concept for a wide-field lensless digital holographic microscope using a laser diode

Abstract: Wide field, lensless digital holographic microscopy is a new microscopic imaging technique for telemedicine and for resource limited setting [1].
In this contribution we propose a very simple wide-field lensless digital holographic microscope using a laser diode. It is based on in-line digital holography which is capable to provide amplitude and phase images of a sample resulting from numerical reconstruction. The numerical reconstruction consists of the angular spectrum propagation method together with a phase retrieval algorithm. Amplitude and phase images of the sample with a resolution of ~2 μm and with ~24 mm^2 field of view are obtained. We evaluate our setup by imaging first the 1951 USAF resolution test chart to verify the resolution. Second, we record holograms of blood smear and diatoms. The individual specimen can be easily identified after the numerical reconstruction. Our system is a very simple, compact and low-cost possibility of realizing a microscope capable of imaging biological samples. The availability of the phase provide topographic information of the sample extending the application of this system to be not only for biological sample but also for transparent microstructure. It is suitable for fault detection, shape and roughness measurements of these structures.

Keywords: lensless microscopy; digital holography; quantitative phase imaging

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1 Introduction

Holography invented 1948 by Dennis Gabor is a method of recording and reconstructing amplitude and phase of a wave field providing a three-dimensional image of a sample [2]. Digital holography introduced by Schnars and Juptner consists of recording the hologram on a digital camera in contrast to optical holography where the recording medium is a photorefractive crystal or a photographic film [3].The spatial resolution of a digital camera, commonly used in digital holography, is low in contrast to conventional analogue holography due to the limited pixel size. Therefore digital holographic imaging of microstructures requires a certain magnification, which is usually achieved by the use of microscope objectives. However, this lens-based magnification suffers from aberrations, which have to be compensated. Lensless digital holography is a well-established microscopic method providing the diffraction limited resolution in the order of the wavelength of the used light source [4–8]. It is based on inline holography. The distance between the source and the sample is smaller than the distance between the sample and the digital camera to achieve sufficient magnification. This leads to a small field of view. In addition a laser is focused on a small pinhole to achieve a high NA. This requires a microscope objective and mechanical alignment components. The group of Aydogan Ozcan proposed a new lensless digital holographic microscope which is capable of imaging samples with a field of view corresponding to the entire sensor active area with a resolution in the order of the pixel size of the sensor [1, 9–12].The main key concepts of this setup are the use of an LED with a pinhole of size ~50–100 μm as point source to achieve a high throughput and the placing of the sensor chip closer to the sample.

We propose in this paper the use of a laser diode without any need of a microscope objective and a pinhole as light source for a wide-field lensless digital holographic microscope. This approach allows imaging of samples with reduced number of components in the setup achieving resolution in order of the pixel size of the used sensor chip and a wide field of view corresponding to the active sensor area.

2 Experimental method

The experimental setup is shown in Figure 1. A divergent beam of a laser diode illuminates a sample which is placed about 5 to 15 cm away on a cover glass. The diffracted light by the sample interferes with the undiffracted part on a CMOS-sensor chip placed directly behind the sample (1–2 mm). This interference pattern called hologram is

***Corresponding Author: A. Adinda-Ougba:** Ruhr-University Bochum - Photonics and Terahertz Technology, 44801 Bochum, Germany, E-mail: adamou.adinda-ougba@rub.de; phone: +492343229353
N. Koukourakis: TU Dresden- Laboratory for Measurement and Testing Techniques, 01062 Dresden, Germany
N. C. Gerhardt, M. R. Hofmann: Ruhr-University Bochum - Photonics and Terahertz Technology, 44801 Bochum, Germany

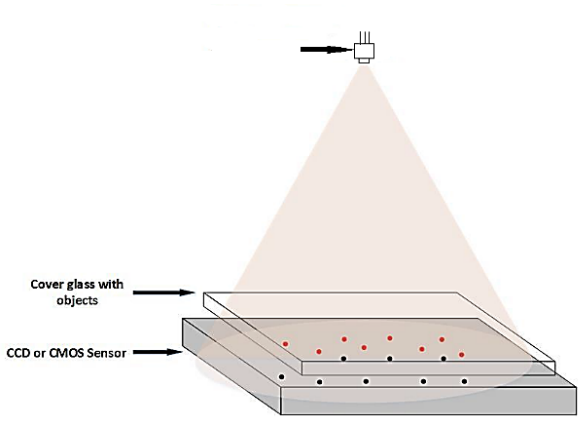

Figure 1: Experimental setup.

recorded by the CMOS-sensor. In order to achieve interference spatial coherence of the light source is required. In the case of LEDs, this is achieved by the use of a pinhole. In our case the spatial coherence is already achieved by the output aperture of the laser diode which is typically 1×10 µm so that a pinhole is not necessary. We operate the laser diode below lasing threshold to emit low coherence light to avoid speckle noise due to the temporal coherence of the laser source. The used laser diode has a central wavelength of 405 nm. The CMOS-sensor chip has a 2560 × 1920 pixel active area with 2.2 µm pixel size.

We use the angular spectrum method to reconstruct the hologram [17]. The inline configuration does not allow the separation of the different parts of the hologram after the reconstruction, so that DC part, real and twin images overlap. The DC part has no disturbing effect as it consists of a constant background. The twin image in contrast provides a distortion which introduces additional noise to the reconstructed image. In many lensless microscope approaches this twin image noise effect is neglected as it appears defocused on the reconstruction plane of the real image [8]. In other approaches effort has been made to reduce the effect of the twin image [1, 13–16]. This will be necessary for many samples such as biological samples as the inner structure may be hidden by the twin image. We use the phase retrieval approach described in [1] to reduce the twin image noise effect.

3 Experimental results

In order to determine the resolution of our setup, we firstly record holograms of the 1951 USAF resolution test chart.

Figure 2 shows the hologram of the test chart corresponding to the entire sensor active area. After the reconstruction the 6th element of group 7 corresponding to a resolution of 2.19 µm is resolved. This corresponds exactly to the pixel size of our CMOS sensor chip. Thus, higher resolution can be achieved with sensors having smaller pixel size.

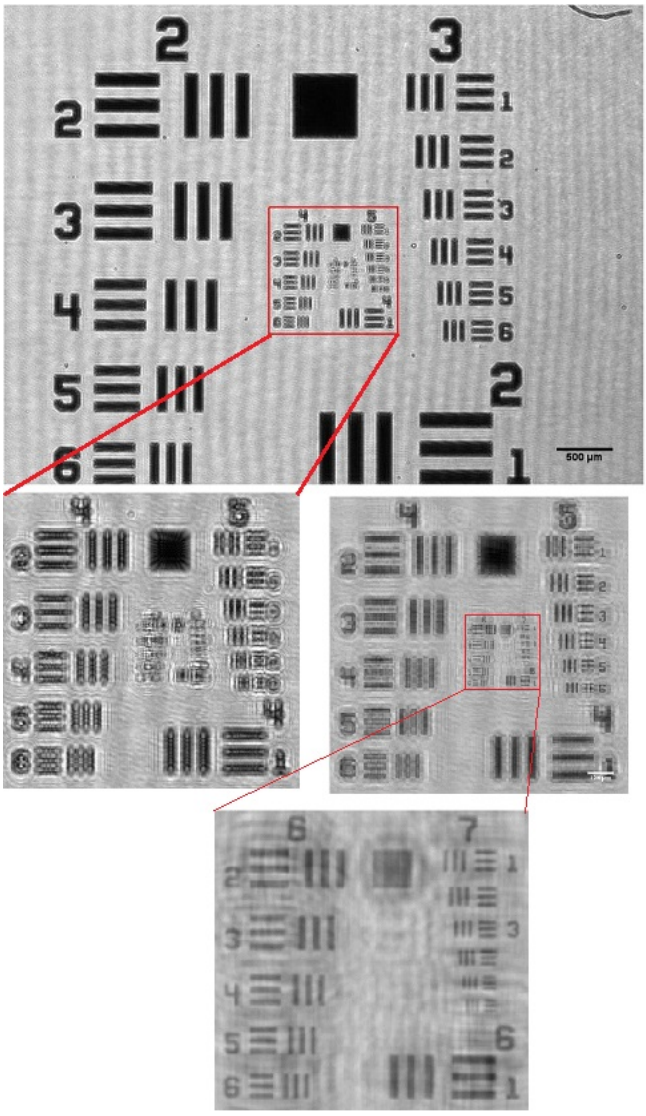

Figure 2: Hologram of USAF resolution test chart (top), the chosen region of interest (middle row left), the ROI after reconstruction (middle row right), and zoom of the red marked part of ROI (bottom).

We then follow the evaluation of our system by imaging blood smear and diatoms.

In the case of the blood smear the distortion by the twin image as discussed in the experimental methods can be seen in the Figure 3. The amplitude and phase images are better reconstructed using the phase retrieval algorithm. Blood cells can be well seen.

The upper image in the Figure 4 shows the hologram of the diatoms. We choose the red marked part for the evalu-

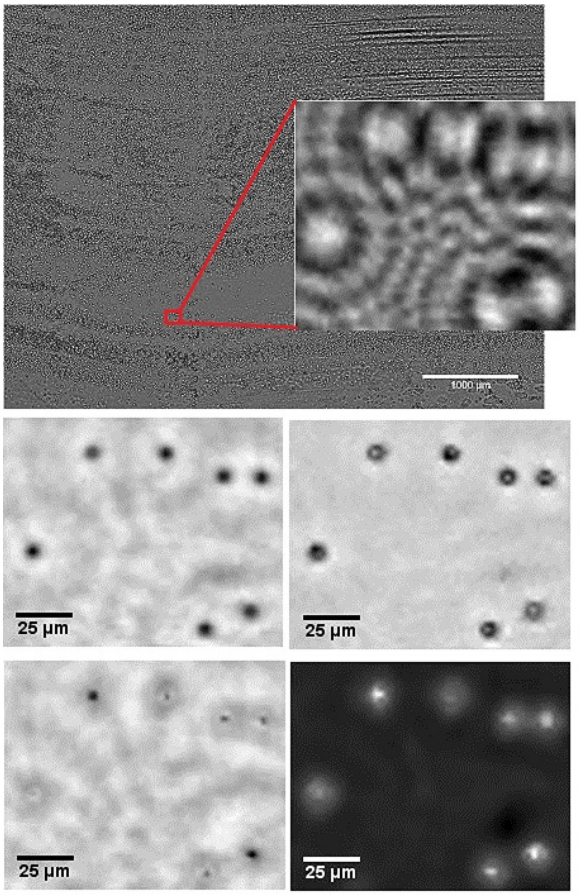

Figure 3: Hologram of blood smear (top), amplitude image after reconstruction of the red marked area without phase retrieval algorithm (middle row left), with phase retrieval algorithm (middle row right), phase image without phase retrieval algorithm (bottom left) and with phase retrieval algorithm (bottom right).

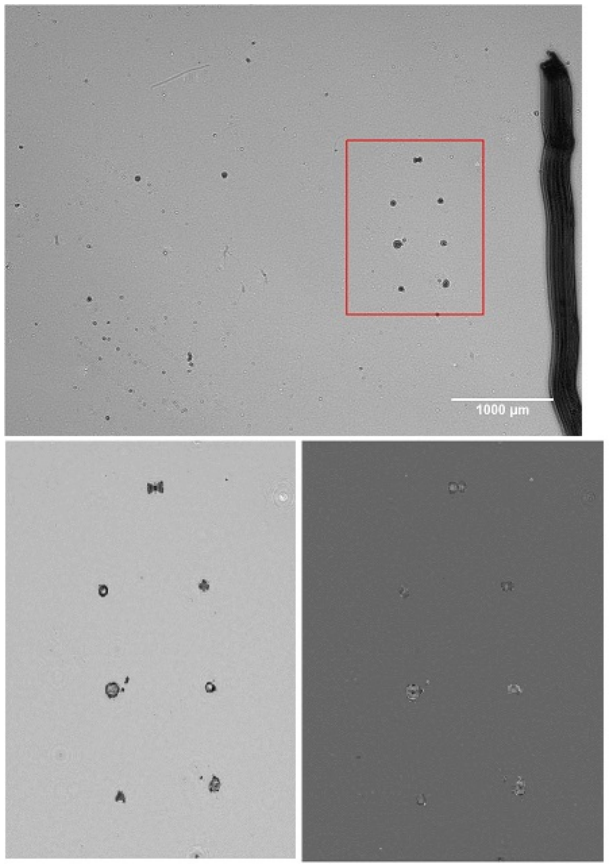

Figure 4: Hologram of diatoms (top), amplitude and phase image (bottom).

ation. All the diatoms can be easily identified and counted in the amplitude and phase images after reconstruction and phase retrieval algorithm. Figure 5 highlight again the elementary diatoms identifier on a light microscope image of the same sample.

4 Conclusion

Our system is a very simple, compact and low-cost possibility of realizing a microscope capable of imaging biological samples with a wide field of view. The availability of the phase provides topographic information of the sample extending the application of this system to be not only for biological samples but also for general transparent microstructure. It is suitable for fault detection, shape and roughness measurements of these structures.

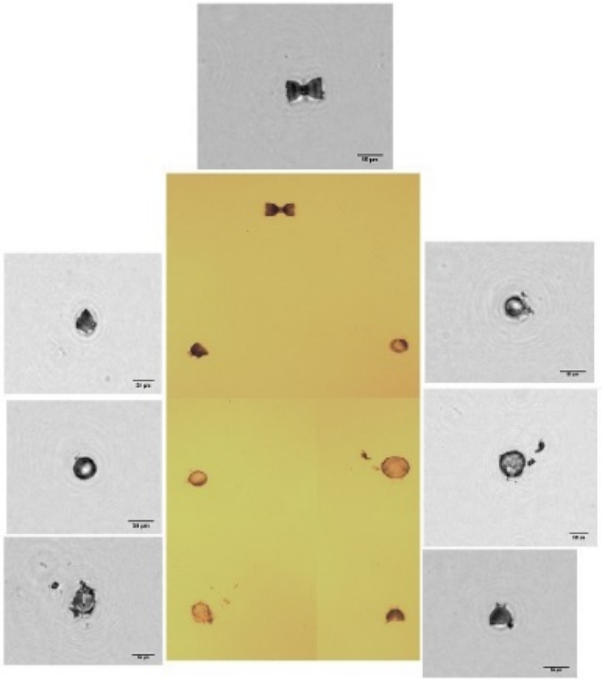

Figure 5: Light microscope image of diatoms (middle), individual identified diatoms with our system all round.

Author's Statement

Conflict of interest: Authors state no conflict of interest. Material and Methods: Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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