High speed water monitoring systems based on Digital Holographic Microscopy

László, Orzó,
Cellular Sensory and Optical Wave Computing Laboratory in MTA SZTAKI
Budapest, Hungary
e-mail: orzo.laszlo@sztaki.mta.hu

Balázs, Wittner
Cellular Sensory and Optical Wave Computing Laboratory in MTA SZTAKI
Budapest, Hungary
e-mail: wittner.balazs@sztaki.mta.hu

Szabolcs, Tőkés
Cellular Sensory and Optical Wave Computing Laboratory in MTA SZTAKI
Budapest, Hungary
e-mail: tokes.szabolcs@sztaki.mta.hu

ABSTRACT

Using Digital Holographic Microscope (DHM) technology - as it is able to alleviate the small depth of focus constraint of the conventional microscopes - the fast screening, detection and evaluation of sparse objects in large fluid sample volumes becomes achievable. We have developed and built automatic DHM based water monitoring devices. The aimed automatic processing and object recognition requires the precise position determination of the detected objects in 3D. This means, proper auto-focusing and object segmentation algorithms have to be applied. We present here the introduced algorithms, which are based on the special, coherent imaging properties of the applied in-line holographic systems. Thanks to the rapid development of the computer technology and the application of parallel computing implementations the digital evaluation of the recorded holograms can be fulfilled almost real time. Using stream processors (graphics processing unit, GPU) it is possible to increase the algorithm speed considerably, without perceptible reconstruction accuracy loss.

Keywords

Automation, Informatics, Digital Holographic Microscopy.

1. INTRODUCTION

Fast screening of fluid samples is a frequently requested task. For example, determining the qualitative and quantitative distribution of algae in water bodies can help to describe and estimate the present state of the ecosystem [1]. Furthermore, the occurrence of some algae or microscopic worms in drinking water wells can indicate the special failure of the filtration, purification process and warns the possible contamination of the water. This, otherwise, can easily result in an emergency situation of the public sanitation.

Automation of physical or chemical fluid sample tests is relatively easy to solve. However, pattern recognition based, automatic morphological examination of microscopic objects can be solved efficiently only for dense samples (e.g. blood, etc.) so far. In the case of sparse samples, we have to look over large volumes to detect the statistically requested number of objects. Automation of sample condensation is hard to solve. It is usu- ally a slow process and frequently destructs the objects in the sample. Therefore, our goal was to construct such a microscope that is able to monitor large volumes automatically and find the sparsely occurring, not marked objects within it at a high speed.

Using conventional microscopes, at the magnification required for the correct pattern recognition ($\sim$1μm) the achievable depth of focus is small (3-5μm). This way only a small layer of the sample can be measured within the observed sample. There are smart, custom flow through measuring chamber designs [2], where a specially planned laminar flow focus the sample into the microscope focal plane. This kind of chambers can be applied in the measurement of dense samples, as in this case the feeding and the forwarding of the samples can be solved more efficiently. Notwithstanding, the measured volume still remains too small.

If we apply the principles of holography we can avoid this constraint of the conventional microscopy. In a hologram we record all the available diffraction information of the coherently illuminated objects and based on these data we can reconstruct their image later at high precision. This way, from a single hologram we can reconstruct all the objects within the observed volume. Especially, we apply Digital Holographic Microscopy, where the hologram of microscopic objects is recorded by a digital sensor (CCD, CMOS) and the objects are reconstructed using numeric simulations of the wave propagation [3, 4, 5]. The applied algorithm makes it possible to digitally set the focus and reconstruct the objects at diverse depths within the entire volume [6] of the measured sample. Using this method, we do not need any physical movement of the target, the sensor or the objective as it would be required in the case of z-scan based methods and do not need the otherwise required large number of image acquisitions.

Using DHM technology we were able to construct a very efficient, high speed fluid monitoring system [7]. Avoiding the small depth of focus constraints, our multi color DHM device [8] is able to monitor $\sim$100 times larger volume than its conventional counterparts. Here, we will present the introduced DHM architectures, show the applied object segmentation and auto-focus algorithms, that applies the inherently coherent imaging features. We show two different DHM constructions aiming at monitoring of different kinds of objects and show their achievable performance. Finally, we outline some other application fields and the directions of further developments.

2. OUR DIGITAL HOLOGRAPHIC MICROSCOPE DESIGN

In DHM implementations, when relatively high resolution and simple optical setup is required, usually an in-line holographic setup is applied, because this architecture provides an efficient utilization of sensor resolution [4, 9]. Especially, the application of the conventional Gabor holographic architecture is preferred [10], where coherent illumination is used in a more or less conventional digital microscope setup. This way, the illumination supplies the reference wave field, too. This architecture provides a really simple measuring setup (Fig 2) and it is applicable whenever the wave field of the objects can be regarded as only a small perturbation of the reference. That is, sparse objects, with small absorption are to be recorded [4, 11]. Using such architectures, even lensless DHM systems are realizable [12, 13]. We also apply a lensless setup, when the required resolution is not too high (see later in Section 5). Proper coherent illumination is provided by fiber coupled lasers. Fiber end provides even spherical wave- front and we do not need any spatial filtering as it was customary done in some earlier approaches [4]. Due to the aimed microscopic imaging application, lasers of small coherence length are satisfactory. Microscope objectives were applied to achieve the required magnification. Nyquist sampling criteria can be accomplished much easier if we implement some magnification. Otherwise, the pixel size and aperture of the applied high resolution image sensor limit the achievable resolution of the reconstructed images.

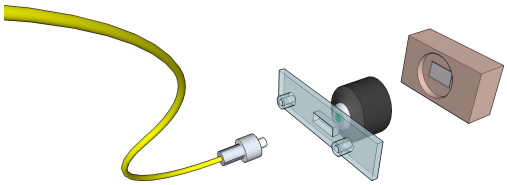

Figure 1: Optical setup of our Digital Holographic Microscope system.

Quality of the recorded holograms can be considerably deteriorated by the diffractions on debris, deposits residing in the flow-through cell or in the optical system. To avoid this contamination of the reconstructions we measure differential holograms, that is we subtract the steady background from each measured hologram.

Digital reconstruction process, however, requires immense computation power. By the application of parallel computing hardware, especially stream processors (GPU), close to real time reconstruction speed can be achieved [14, 15]. Using our DHM setup 3D tracking of the objects within the volume is also achievable [16]. Conversely to other approaches, every object can be reconstructed with its actual 3D positions from a single hologram, without the need of time consuming, iterative processes [17].

3. HOLOGRAM AUTO-FOCUSING AND OBJECT SEGMENTATION

The goal of the DHM device is to reconstruct the objects occurring within the flow-through measuring chamber. We fulfill these reconstructions of the captured differential holograms object-wise. That is, in every step a single object is segmented, reconstructed and its contribution to the hologram is also recovered. This way, step by step, all the objects and their corresponding in-line holograms will be revealed.

To fulfill this task we apply a holographic object detection algorithm. This algorithm finds the reconstruction distance and support of the different objects constructing the hologram. As the objects are usually in different depths within the sample, therefore they have diverse reconstruction distances. These, locally distinct depth keys can be exploited to find and segment the objects efficiently. This way, object segmentation of a volumetric in-line hologram is much easier, than that of conventional microscope images, where the overlap of the objects and debris are obstructing the proper segmentation. To define support and reconstruction distance of the object we digitally simulate the hologram reconstructions at different distances by simulating the wave field propagation of a sub-sampled hologram. For digital wave field propagation we applied the angular spectrum method Eq. (1) [6, 15]. According to the angular spectrum method [18], $h_d(.)$ denotes the d distance wave field propagation operation:

$$h_d(E(x,y)) = \mathcal{F}^{-1}\{\mathcal{F}\{E(x,y)\}e^{ik_z(u,v)d}\}, \qquad (1)$$

where $\mathcal{F}$ and $\mathcal{F}^{-1}$ denote the direct and inverse Fourier transform operations, while

$$k_z(u,v) = \begin{cases} 2\pi\sqrt{\frac{1}{\lambda^2} - u^2 - v^2}, & \text{if } \frac{1}{\lambda^2} - u^2 - v^2 > 0 \\ 0, & \text{otherwise.} \end{cases}$$

The u and v symbols denote the Fourier frequencies in the x and y directions, respectively (the illuminating wavelength is λ). We use some local image quality measure (focus measure; e.g. Tenengrad or Local energy of the gradient image [3]) to detect if the object is in focus [15]. Although several alternative focus measures have been investigated earlier [3, 19], but these were applied only for off-axis holograms or conventional microscopic images [20]. The in-line holograms inherent twin image noise can deteriorate the success of these, earlier proposed auto-focusing algorithms, as diffractions can produce false extremes of the focus measure. We can avoid this problem by the application of a so-called in-line hologram segmentation method (see later). To define the contribution of a segmented object to the measured hologram we need only the support and reconstruction distance parameters of the corresponding object. Our experiment shows that even a coarse approximation of these parameters is sufficient for the correct operation of the algorithm. We define these parameters object-wise. Using the focus measure we generate two maps: the 'Focus Maximum Map' that contains the pixel-wise maximum of the focus measure within the simulated wave field propagation distances, and the corresponding 'Distance Map' that shows the distance, where this focus maxima are achieved [15]. As a single object is usually reconstructs only in a special distance from the hologram, support of the object is determined from the 'Distance Map', while the selection of the highest contrast object can be done by using the 'Focus Maximum Map'. If we remove the object contributions from the hologram, and recalculate these maps we can segment and reconstruct, step by step, all the objects within the volume.

To determine the individual objects contribution to the measured hologram we developed a simple non-iterative hologram segmentation algorithm which is able to estimate the individual objects contribution to the measured hologram. Conversely to the earlier approaches

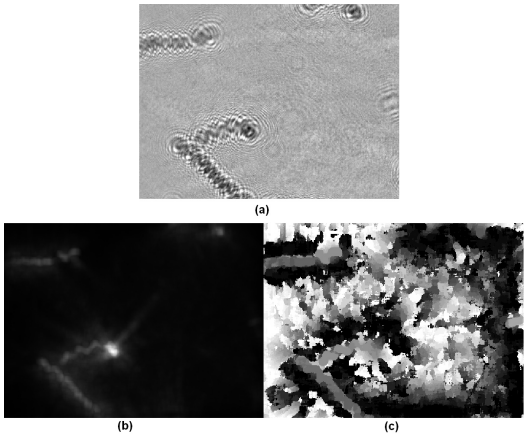

Figure 2: From a measured hologram (a) we can define the 'Focus Maximum Map' (b) showing the highest focus measures during the different distance reconstructions and the corresponding 'Distance Map' (c).

[21], it does not aim at parallel, complete phase retrieval of all the objects, which seems a really challenging task if high resolution microscopic reconstructions are needed, but only a simple straightforward estimation of the in-line hologram of the object is considered.
This algorithm is based on the inner structure of the in-line holograms. It approximates the in-line hologram of the object at high precision from the original hologram using only the support and reconstruction distance information. It takes only four field propagation steps to fulfill the hologram segmentation.

Summing up the proposed segmentation algorithm:
• Reconstruct the object by simulated propagation of the hologram.
• Fill the unsupported part of the reconstructed object with the background amplitude.
• Propagate backward this wave field to the hologram plane.
• Subtract twice the real part of the result from the original hologram.
• Propagate this modified hologram wave field to the object plane.
• Fill the unsupported part of the reconstruction with the background amplitude.
• Propagate backward this wave field to the hologram plane and subtract it from the modified hologram.

Using the above delineated object segmentation method including the introduced hologram segmentation technique we are able to find and reconstruct all the objects recorded in our DHM device. To demonstrate the device performance we show a couple of measured holograms with the corresponding reconstructions in Fig. 3.

4. DETECTION OF ALGAE BY DHM

We have developed a high resolution DHM water monitoring device that aims at the detection and classification of different kinds of algae. This device applies an afocal optical setup which provides even magnification (x5) within the whole observed volume. This way, submicron resolution can be achieved within a cubic millimeter observed volume. As color information seems essential for correct classification we apply three different fiber coupled lasers (red, green, blue) and record the corresponding holograms by a color CMOS sensor. Color crosstalk is diminished by the application of spe-

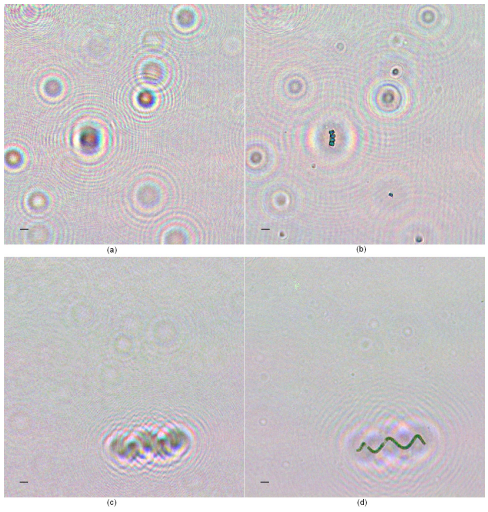

Figure 3: Using our color DHM setup and the proper reconstruction algorithm we can reconstruct the image of microscopic objects ((b) and (d)) from the corresponding measured holograms ((a) and (c)).

cial preprocessing of the raw data [8]. In Fig. 4 a prototype of the alga monitoring device is shown and a set of measured microscopic objects. Although, this device

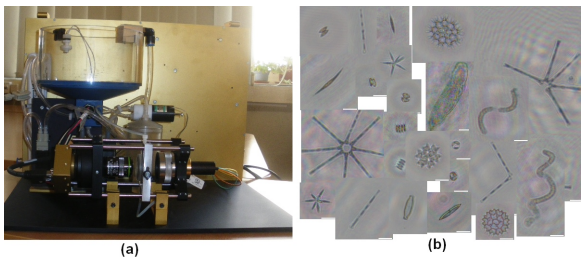

Figure 4: Color Digital Holographic water monitoring Microscope (a). A set of different microorganism reconstructions (b) demonstrates the performance of the device.

is built to detect and classify algae, it is able to de- tect larger microscopic objects (e.g. worms), too. The developed prototype system can be efficiently used for monitoring the ecological state of water bodies or the filtration efficiency of some kind of water wells.

5. DETECTION OF WORMS BY DHM

There are fluid monitoring tasks, where larger objects are to be detected, that have no special coloration. This can be solved by a much more simple device. It uses only a single laser and does not apply any kind of optics, but the magnification is achieved by the different (spherical) illuminating and (plane) reconstructing wave fields. We have developed and built such a lensless DHM setup that aims at worm monitoring. Fig. 5 shows the prototype of the worm monitoring DHM (AquaScope (Knot LLC)) and a set of reconstructed microscopic worms.

This device can be efficiently used in waterworks, where it can continuously monitor the state of the water filters and the filtration process. Although, the device has lower resolution ($\sim 3\mu$m), than that of the algae monitoring one, but it can measure much larger volume ($\sim 500 mm^3$/hologram).

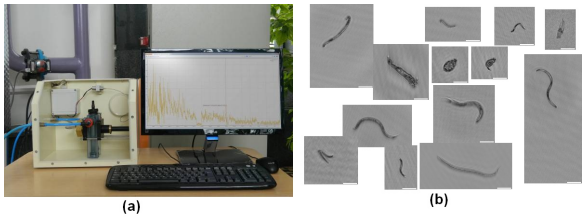

Figure 5: AquaScope (Knot LLC), a water monitoring Digital Holographic Microscope aiming worm detection (a). A set of different worm reconstructions (b) demonstrates the performance of the device.

6. CONCLUSION

Different DHM based water monitoring devices have been constructed. These are able to inspect sparse fluid samples ∼50-100 times higher speed than that of conventional microscope counterparts. Using volumetric DHM systems object segmentation is alleviated as depth keys can be efficiently utilized. We are looking for new application fields for our DHM devices. Especially, evaluation of automated pollen measurement is aimed, when the pollen grain is collected and trapped in fluid samples. Applying the proper (auto) fluorescent tags of the target objects we will be able to construct a DHM based, special, high speed, flow-cytometer, that provides morphological information of the detected objects, too (e.g. aiming it blue-green algae recognition).

7. ACKNOWLEDGEMENT

This work was funded by the Hungarian National Office for Research and Technology (NKTH 1981822A) project entitled Water Biology Digital Holographic Microscope (DHM) as an early warning environmental system. The preparation of this presentation has also been supported by the INARMERA-ICT project (Grant Agreement Nr. 294943, web: www.inarmera-ict.eu).

REFERENCES

[1] R. Stevenson and J. Smol, "Use of algae in environmental assessments," pp. 775–804, 2003.

[2] C. Simonnet and A. Groisman, "Two-dimensional hydrodynamic focusing in a simple microfluidic device," *Applied Physics Letters*, vol. 87, pp. 114104, 2005.

[3] I. Bergoënd, T. Colomb, N. Pavillon, Y. Emery, and C. Depeursinge, "Depth-of-field extension and 3d reconstruction in digital holographic microscopy," *Proc. SPIE*, vol. 7390, pp. 73 901C–1, 2009.

[4] J. Garcia-Sucerquia, W. Xu, S. Jericho, P. Klages, M. Jericho, and H. Kreuzer, "Digital in-line holographic microscopy," *Applied optics*, vol. 45, no. 5, pp. 836–850, 2006.

[5] T. Colomb, N. Pavillon, J. Kühn, E. Cuche, C. Depeursinge, and Y. Emery, "Extended depth-of-focus by digital holographic microscopy," *Optics letters*, vol. 35, no. 11, pp. 1840–1842, 2010.

[6] K. Matsushima and T. Shimobaba, "Band-limited angular spectrum method for numerical simulation of free-space propagation in far and near fields," *Optics Express*, vol. 17, no. 22, pp. 19 662–19 673, 2009.

[7] Z. Göröcs, M. Kiss, V. Tóth, L. Orzó, and S. Tőkés, "Multicolor digital holographic microscope (dhm) for biological purposes," *Proc. SPIE*, vol. 7568, pp. 75681P, 2010.

[8] Z. Göröcs, L. Orzó, M. Kiss, V. Tóth, and S. Tőkés, "In-line color digital holographic microscope for water quality measurements," *Proc. SPIE*, vol. 7376, pp. 737614, 2010.

[9] J. Sheng, E. Malkiel, and J. Katz, "Digital holographic microscope for measuring three-dimensional particle distributions and motions," *Applied optics*, vol. 45, no. 16, pp. 3893–3901, 2006.

[10] D. Gabor, "A new microscopic principle," *Nature*, vol. 161, no. 4098, pp. 777–778, 1948.

[11] F. Pellistri, C. Pontiggia, L. Repetto, and E. Piano, "Gabor's hologram in a modern perspective," *American journal of physics*, vol. 72, no. 7, pp. 964–967, 2004.

[12] S. Jericho, J. Garcia-Sucerquia, W. Xu, M. Jericho, and H. Kreuzer, "Submersible digital in-line holographic microscope," *Review of scientific instruments*, vol. 77, pp. 043706, 2006.

[13] C. Oh, S. Isikman, B. Khademhosseinieh, and A. Ozcan, "On-chip differential interference contrast microscopy using lensless digital holography," *Opt. Express*, vol. 18, pp. 4717–4726, 2010.

[14] T. Shimobaba, Y. Sato, J. Miura, M. Takenouchi, and T. Ito, "Real-time digital holographic microscopy using the graphic processing unit," *Optics Express*, vol. 16, no. 16, pp. 11 776–11 781, 2008.

[15] L. Orzó, Z. Göröcs, I. Szatmári, and S. Tőkés, "Gpu implementation of volume reconstruction and object detection in digital holographic microscopy," in *Proceedings of IEEE Conference on Cellular Nanoscale Networks and Their Applications (CNNA)*. IEEE, 2010, pp. 1–4.

[16] M. DaneshPanah and B. Javidi, "Tracking biological microorganisms in sequence of 3d holographic microscopy images," *Optics Express*, vol. 15, no. 17, pp. 10 761–10 766, 2007.

[17] J. Hahn, S. Lim, K. Choi, R. Horisaki, and D. Brady, "Video-rate compressive holographic microscopic tomography," *Optics Express*, vol. 19, no. 8, pp. 7289–7298, 2011.

[18] J. Goodman, *Introduction to Fourier optics*. Roberts & Company Publishers, 2005.

[19] P. Langehanenberg, G. von Bally, and B. Kemper, "Autofocusing in digital holographic microscopy," *3D Research*, vol. 2, no. 1, pp. 1–11, 2010.

[20] F. Groen, I. Young, G. Ligthart, and U. Wiley, "A comparison of different focus functions for use in autofocus algorithms," *Cytometry Part B: Clinical Cytometry*, vol. 6, no. 2, pp. 81–91, 1985.

[21] L. Denis, C. Fournier, T. Fournel, and C. Ducottet, "Numerical suppression of the twin image in in-line holography of a volume of micro-objects," *Measurement Science and Technology*, vol. 19, pp. 074004, 2008.