New colour and a self-referenced holographic imaging techniques

Theses of the PhD dissertation

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1. Abstract

Holographic imaging is based principally on the interference of monochrome and coherent waves, thus making colour or fluorescent holographic imaging is a challenge. Here, in my PhD dissertation I introduce new ways to make colour and fluorescent – self-referenced- holographic imaging.

The motivation of my research is to identify the indicator organisms (algae and worms) of the freely flowing water samples. To identify algae, their colour and fluorescent properties are also necessary beside their morphology. These requirements pointed out the ways of my research.

Colour holographic systems are based on the multiplication of different coloured monochrome holographic systems. So, the colour image is created from different coloured monochrome holographic reconstructions. When the target object is moving, the holograms should be captured in the same time, and the hologram reconstructions should carry the image information from the same perspective of the object with the same magnification, position, and field distortion. Although the nowadays setups gives good solutions they are not as robust as to form the basis of an industrial holographic instrument. In my research work I have given new hardware and software solutions to our colour digital holographic microscope (DHM). I have created a new kind of illumination at holography to make a robust device with higher efficiency that was supported by a compact optical system. Also, I have made a new numerical technique to eliminate the diffraction fringes that were appearing on the edges during the numerical wave propagation process.

Self-referenced digital holographic setups give the chance to holographically image fluorescent, self-luminescent, distant or huge objects. These setups are complex or based on a high prized optical element. During my research I realized that a straightforward ring-shaped bifocal lens (RBL) can be applied for self-referenced holography. I have determined the required parameters and their connections (like optical path difference) of the ring-shaped bifocal lens to make self-interference pattern with it. Based on my theoretical model, I designed a useful RBL and make it manufactured. Then I built it into a chosen conventional microscope to make measurements confirming that the theoretical model is correct and using a ring-shaped bifocal lens self-referenced holography is implementable.

2. The colour DHM

My first and second Theses belong to our colour DHM setup that can provide automatic solution for the biological monitoring of drinking and natural waters. The next figure presents its optical setup.

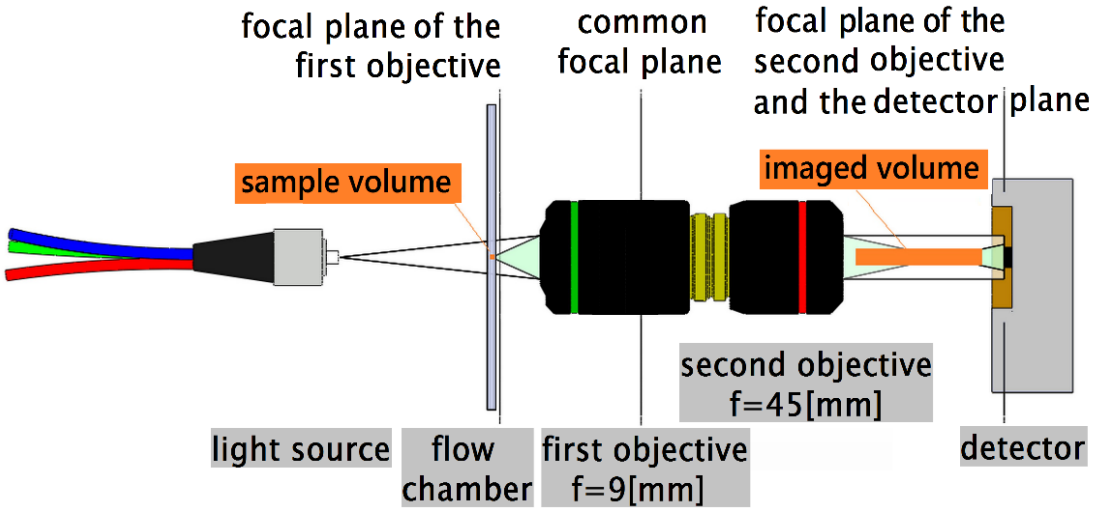

Fig. 1.The optical setup of the colour DHM

The fluid biological sample is flowing through the flow chamber (0.8 mm deep Ibidi®) while the colour DHM captures its holograms. The colour images reconstructed from these holograms. Ordinary microscopes require preparated sample and can record the image of a small depth of view. The DHM can see a volume with a depth 200 times bigger than an ordinary one. Holographic imaging is a two-step method. In the first step, the hologram of the volume/object is captured, and in the second, its 2D image is reconstructed. The hologram is written by a target and a reference beam together. In our in-line holographic setup, the light from the coherence illumination that diffracts on the target is the target beam and the one that goes through the sample without changing its direction is the reference beam. The captured interference pattern of these two beams is the hologram that is positioned to the detector (EPIX SV9C10) by the optics (Olympus LUCPLFLN 20x and Olympus PLN 4x) that makes ensure the Nyquist-Shannon criterion.

With the use of an illumination -that has three different coloured lasers- and a Bayer-pattern camera, three holograms with different wavelengths can be captured from the same object at the same time and from the same point of view to reconstruct the colour 2D image of the object.

3. The illumination of the colour DHM

The quality of the illumination determines the quality of the reconstructed image. The wavefront of the illumination is the wavefront of the reference beam. The difference between the wavefront of the reference and the reconstruction beams creates a distortion on the reconstructed image. In a better case, it is only a magnification. The color DHM in its numerical reconstruction uses plane waves for the reconstruction, so the aim is to have a reference beam also with a plane wavefront. Using laser as an illumination, a single mode Gaussian beam can be reached, and there is a small curvature of its wavefront that can be accepted as a plane wave on the surface of the detector. The optical system helps to reach this goal, as it magnifies 25 times the radius of the wavefront.

From a common laser beam a pinhole or a single-mode optical fiber can create single-mode Gaussian beam with spatial filtering.

A colour laser illumination must make the beams with different wavelength collinear, to illuminate the target from the same view. This can be reached either by the use of dichroic mirrors, or with fiber couplers. If there are more light sources, the illumination setup is more expanded and complex, that increase the light loss and can distort the wavefronts.

I realised that it is beneficial to use single-mode optical fibers, because of its many advantages, and it is good to stay clear of the known and mentioned beam unifiers. If all the lasers are coupled in an appropriate single-mode optical fiber, and their output ends are fixed in the same fiber connector tightly and parallel of each other, the colour laser illumination can illuminate the target from the same direction and with the required quality of wavefronts. This illumination can create white light with line spectrum. Using this new concept, it is easy to make colour illumination with different number of different coloured laser sources. The image of the designed and constructed illumination is presented in the next figure.

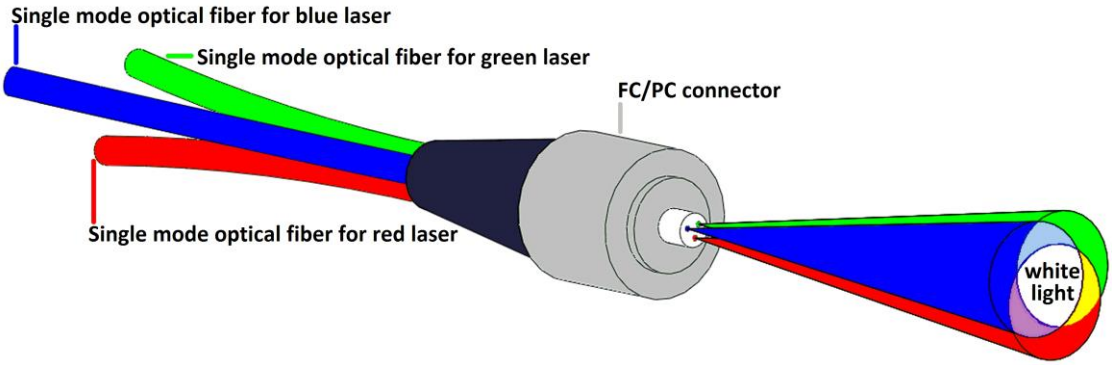

Fig. 2. The new colour illumination for holographic imaging systems. The different single-mode optical fiber ends are tightly packaged in the same connector. This presents three stable and high quality Gaussian beams.

1. Thesis

I designed a new illumination for colour digital holographic imaging that is based on fiber optics. The output ends of the different single-mode optical fibers are tightly bounded in the same connector, so this illumination produces single mode Gaussian beams even in different wavelength for imaging from the same view. It is robust, easy to keep clean, position it and to use. To the free input ends of the fibers the required light source can be connected. It is patented technology, and it is widely used in the commercialized colour digital holographic microscopes.

The related publications: [S1, F1, K1, K2]

4. Colour image reconstruction

In this colour DHM the colour hologram's three colour channels are separately reconstructed with the *Angular Spectrum Method*. When the reconstruction beams with different colours have a parallel wavefront, at the colour image the different channels will not overlap each other precisely, so there will be a chromatic aberration on the image (Fig. 3. a), c)). This aberration is caused by the new illumination, but an illumination based on dichroic mirrors can also produce such a bias. It is easy to make the correction numerically: The difference of the angles of the reference beams (*relative tilt*) should be the same as the reconstruction beams have. But it is not necessary to make parallel the reference and the belonging reconstruction beams. Using this correction, this chromatic aberration disappears as it can be seen in Fig. 3. b), d).

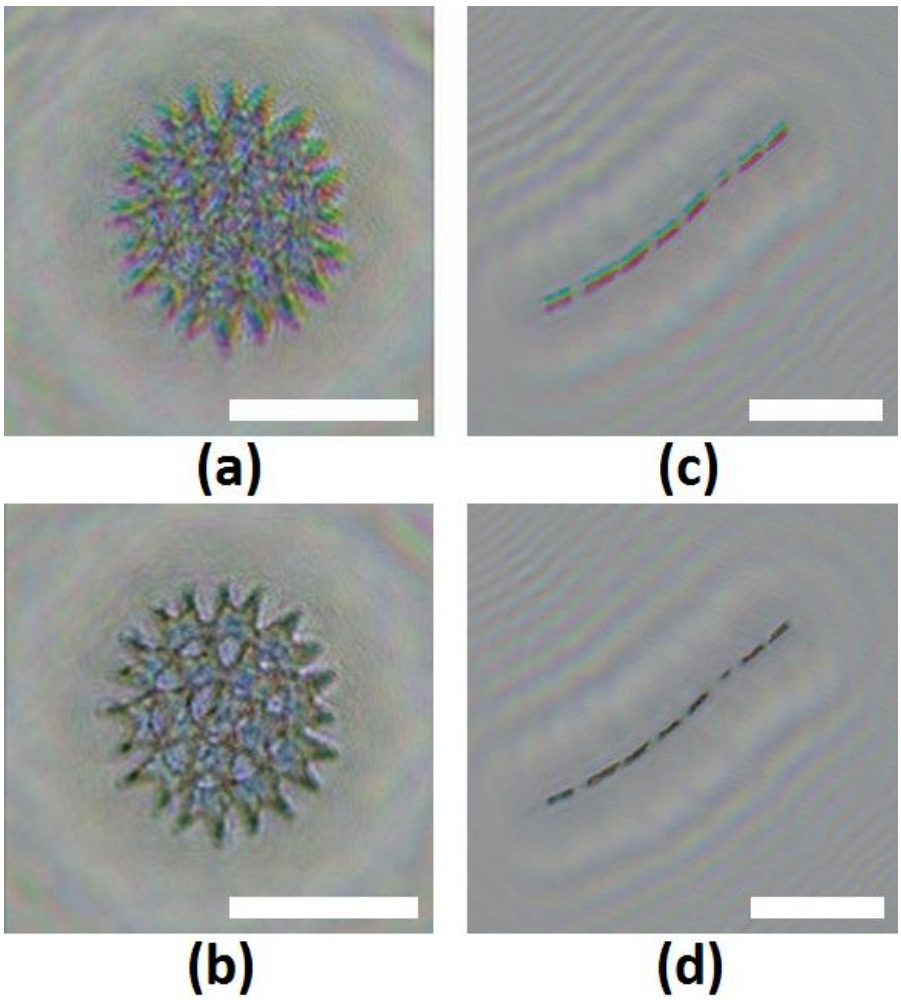

Fig. 3. The hologram reconstructions of Pediastrum and Melosira alga colonies without (a, c), and with (b, d) tilt compensation; (the scale bar is 20 μm).

The fact that the reconstruction beams are tilted causes the strip pattern distribution of the phase. At the numerical reconstruction, the used Discrete (Fast) Fourier Transformation assumes infinite tiling of this strip pattern phase distribution. Observing this distribution, in an ordinary case the tilted phase makes phase jump on the edges (Fig. 4. b)), that cause diffraction noise in the reconstructed image (Fig. 4. d)).

In the literature we can find, that this noise can be eliminated with using field diaphragm, or calculating the diffraction noise and subtract it from the image, or resize the image to ensure continuous phase distribution on the edges (like in Fig. 4. c)). I have given a new and more effective solution: change the tilt to be continuous the phase distribution at the edges.

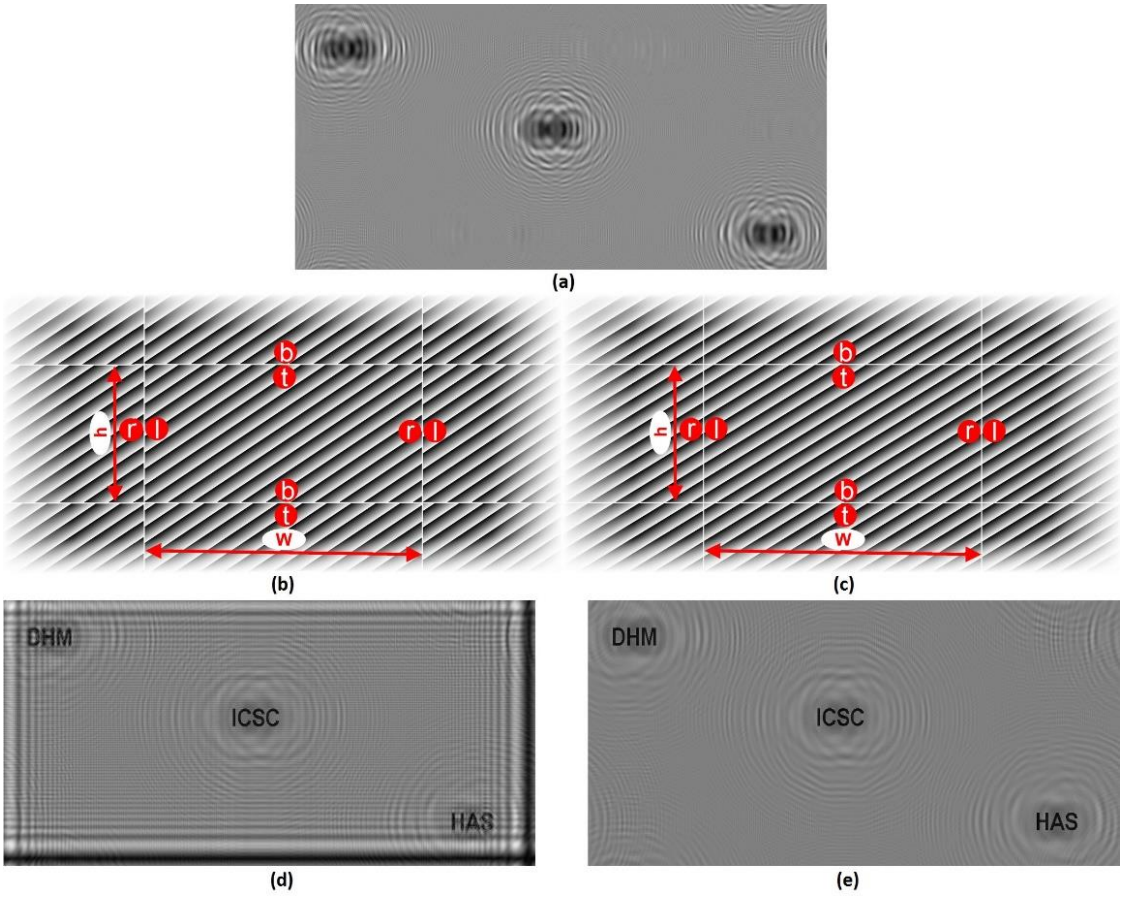

Fig. 4. Reconstruction from a digitally simulated sample in-line hologram (a), using a tilted reconstructing plane wave (b) shows considerable numerical reconstruction artifacts at the hologram borders (d). Discrete Fourier transform implies the infinite periodic tiling of the wave field. In the case of a tilted plane wave this periodicity can introduce severe phase discontinuity (b) where the wave field and its copies meet at the border of the hologram. The top, left, bottom, and right borders are shown in the tiled copies, while 'w' and 'h' denotes the width and height of the detector respectively. The continuities of the phase at the detector boundaries can be ensured by slight modification of the applied plane wave slopes (c). Applying a reconstructing beam with this optimized tilt the artifact is perfectly eliminated (e).

When the tilt is bigger, this modification in the tilt is smaller. Because only the relative tilt is needed between the beams with different colours, their reconstruction beams angle can be increased together to have small enough modification in the tilt -during the elimination of the diffraction noise- to avoid the chromatic aberration.

2. Thesis

I have created a new and a more efficient numerical method to remove the diffractions at the edge of the image that appears due to the use of Discrete (Fast) Fourier Transform during the reconstruction with tilted reconstruction wave. This method modifies the tilt in a way to make equivalent the size of the edge and an integer multiples of the wavelength component that is parallel with that edge. This new method keeps the original size of the hologram and the image; and using optimal parameters, it has no effect on the quality of the imaging; and its computational cost is negligible next to the computational cost of the reconstruction process.

The related publications: [S1,F1]

Closing the topic of the colour DHM, finally I would like to present some reconstructed image made by this device.

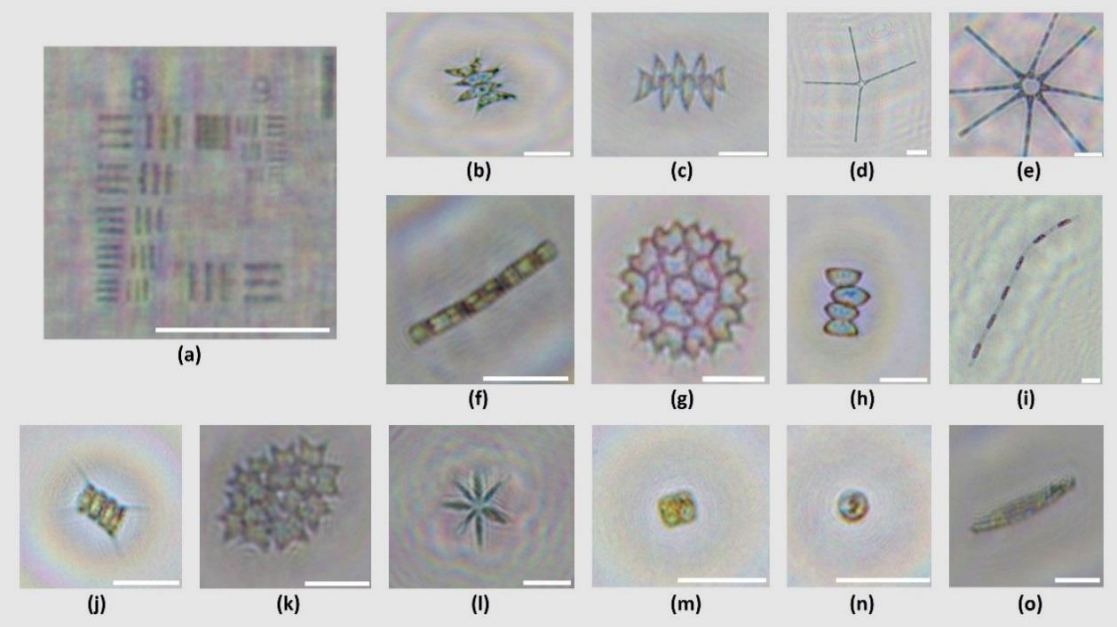

Fig. 5 Reconstruction of an USAF test target hologram (a) and some reconstructed color images of freely floating algae holograms. These algae are classified as Scenedesmus (b, c, h, j), Asterionella (d, e), Melosira (f, i), Pediastrum (g, k), Ankistrodesmus (l), Thalassiosirales (m, n), and Bacillariophyceae (o). (Scale bar is 20 μm.)

5. Self-referenced holography

Fluorescent microscope is widely used in biology. For example, using appropriate fluorescent techniques we can distinguish alive and dead cells in biological samples. Although a color DHM is not able to make fluorescent measurements, but the holography has the advantage of volume measurements, that seems really important in many applications. Therefore, I studied self-referenced holography.

The interference phenomena -which is the basis of the holography- can appear with only one light source light. When the reference and target beams have a common light source, the coherence criteria can be ensured. At self-referenced holography, the light source is not a part of the optical system, it is the sample, the target itself. In this case the illumination of the target (e.g. fluorescent) will be separated to target and reference beams to make the interference phenomena. To make interference the optical path difference between the target and reference beams should be smaller than the coherence length of the used light. However, fluorescent light has a coherence length of cc. 10 μm.

The self-referenced holographic systems that have been described or built can be separated into two main groups: to an interferometer based and a bi- or multifocal based one. For example, the Hariharan-Sen, Mach-Zehnder or Linnik interferometer based setups belong to the first group, and, those that contain Briefringent or diffractive lens or spatial light modulator (SLM) belong to the other. The disadvantages of the first group are the big size that makes it sensitive for vibration, and the used beam splitters, that results in considerable light loss, which can be important as the fluorescence usually provides low light intensities. The other groups has a disadvantages that they are expensive, or uses polarizer, that makes also light loss, but their advantages are the compactness.

Overviewing these, I made a question to myself: Is it *possible to implement self-referenced holography with ring-shaped bifocal lens*?

The Ring-shaped bifocal lens

The ring-shaped bifocal lens (RBL) has an aperture with a round and a ring shape, so the beams that left this lens have round and ring shaped cross sections. This results that the interference

pattern -if we get- that was made by them also has a ring shape. The question is, can we ensure that the zero optical path difference has a zero value inside this ring?

I planned to build this RBL into an ordinary microscope, whose exact optical parameters were not available. *I had to design a that kind of RBL, that ensure an OPD from the target to the ring of the hologram between the target and reference beams, that is smaller than the coherence length of the fluorescent light, but it is better, if it is zero*. To reach this aim, I used the first and the second image planes and the position of the detector (Fig. 6.) as an input to make my calculation that was based on geometrical optics.

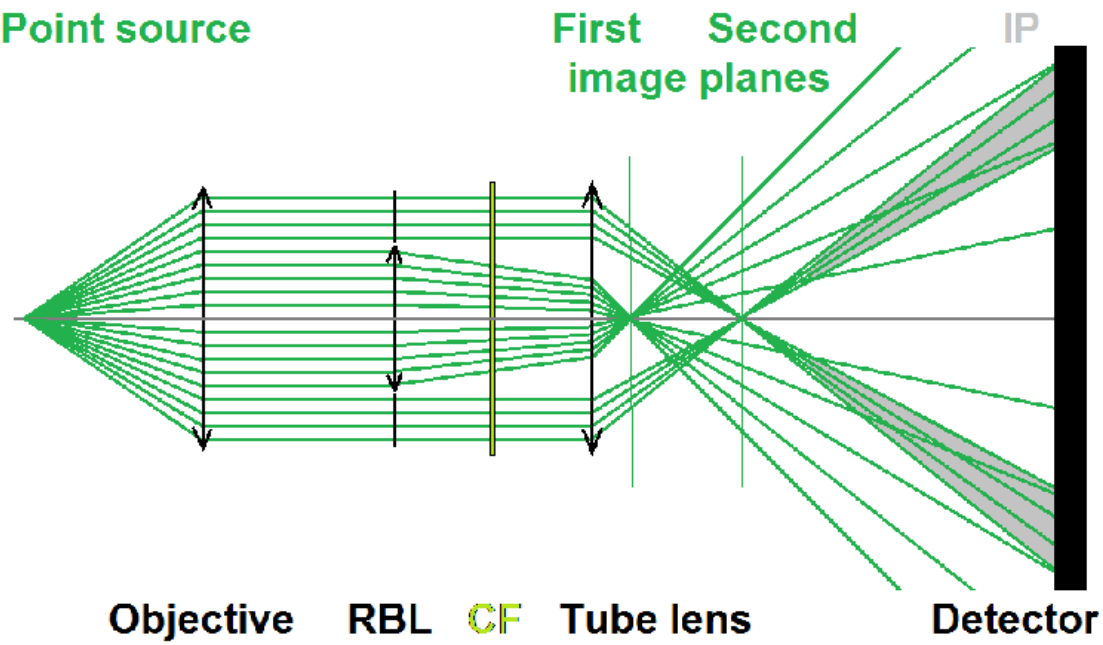

Fig. 6. The scheme of the RBL based self-referenced holographic microscope setup. The objective collimates the light of a point source. This light beam is spatially divided by the RBL. The colour filter (CF) is primarily used to cut off the excitation light. The tube lens adjusts the divergence of the two beams. Finally, the detector captures the digital hologram. IP denotes the grey area, where the two beams can interfere with each another.

The manufactured RBL that was designed by me I inserted into an Olympus IX71 microscope. With this setup, from the ® of the USAF test target I captured images that can be seen below.

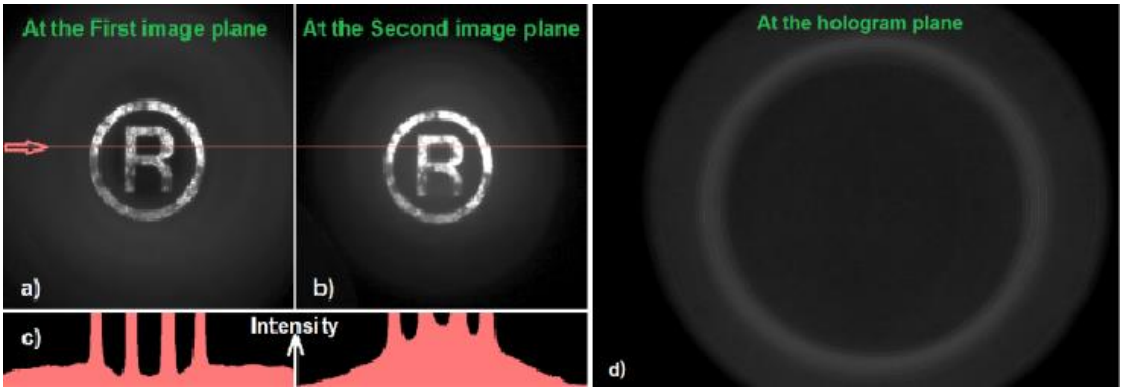

Fig. 7. Images made by the RBL. a) The image made by the central beam. The background pattern is the intensity distribution of the ring-shaped beam. b) The opposite of a). c) is the intensity distribution along the red line, and d) is the hologram of the same target.

Results

In my research I was interested in that the RBL is able or not able to produce a hologram, and from this hologram is it able to reconstruct any image (Fig. 8.) of some quality? In my measurements, I used most the USAF test target, which has many different sized details (Fig. 9.). I also placed the target into different depth (Fig. 10.).

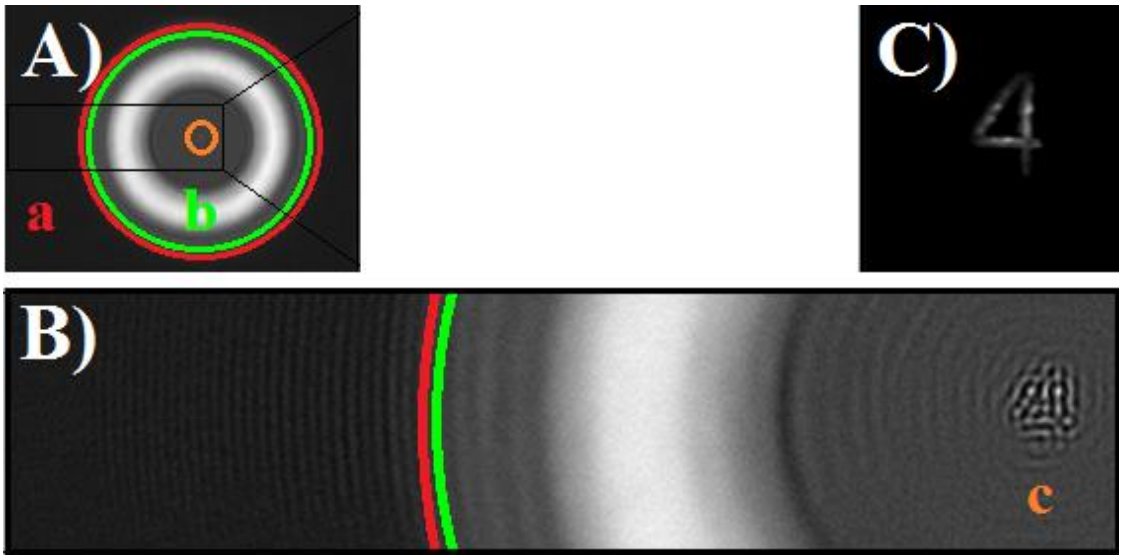

Fig. 8. A reconstruction of a hologram made by the RBL based setup. A) The whole reconstruction. B) A part of the reconstruction, where the twin image (fringes out of the red circle), the zero order (inside the green circle) and the reconstructed image (in the middle) can be seen. C) The photo of the test target. We can observe the similarities and the differences on the images (e.g. details of the structure of the object, the background, diffraction fringes).

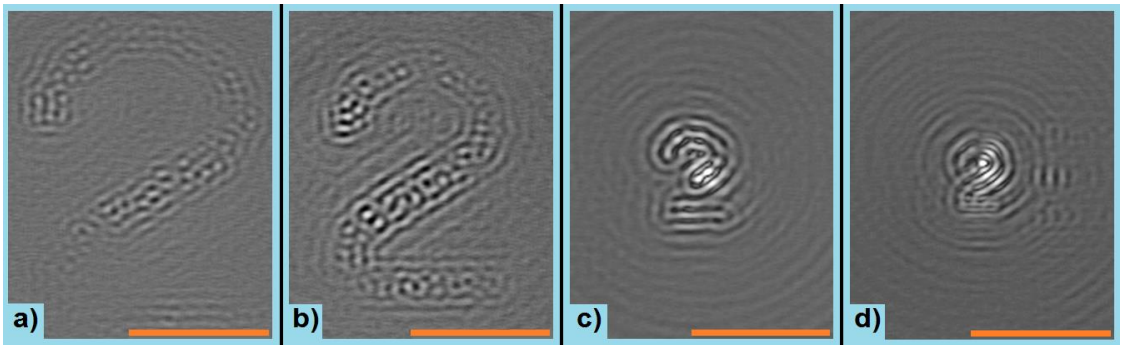

Fig. 9. a), b), c) and d) Targets that have different size, but have the same shape like "2", and are in the same depth. The orange scale bars are 200 µm long.

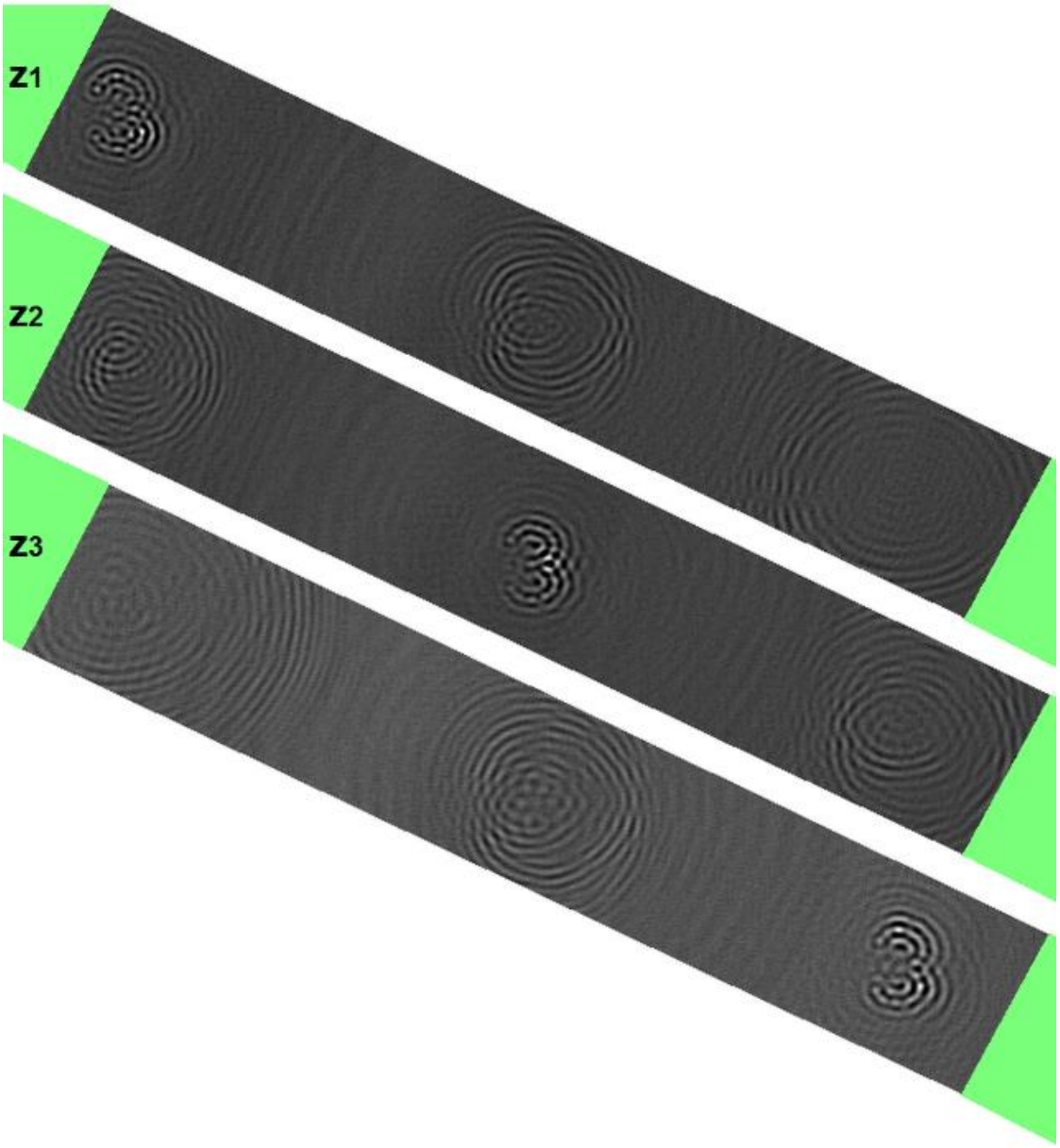

Fig. 10. Targets in different depth. The same target with a shape "3" was placed in different x, y, and z coordinates. "z2" is the focal plane of the objective (but not the setup, because hologram is captured), "z1" is closer to the objective with 0.5 mm, and "z3" is out with 1 mm. The captured three holograms are added together to make one hologram. After smoothening its background, I reconstructed the different depths that can be seen here.

Conclusion

I imagined, designed and asked for the manufacturing of the ring-shaped bifocal lens (RBL), that I inserted into a common microscope. I was the first, who created self-referenced holographic image with an RBL based self-referenced holographic microscope. With these measurements I proved the possibility to make self-referenced holographic imaging with a ring-shaped bifocal lens.

3. Thesis

I was the first, who proposed Ring-Shaped Bifocal Lens (RBL) to make self-referenced holography. I have designed then built it in a conventional microscope to make self-referenced holographic microscope. The RBL created hologram has a ring shape. I have shown by measurements that RBL is a tool to make self-referenced holography having light, with short coherence length such as fluorescent light; and with the RBL an order of magnitude larger depth can be captured with one exposure than using a conventional microscope with similar conditions. After reconstructing a ring-shaped hologram, in the image plane the image and the twin-image are clearly separated. With the use of the RBL it is now possible to create simple, robust and cheap self-referenced holographic systems.

The related publications: [F3, K3, K4]

The Author's Publications

patent

[S1] **Kiss Márton**, Szatmári István, Orzó László, Göröcs Zoltán, Tőkés Szabolcs; "*Háromdimenziós színes képet alkotó berendezés*" HU000229591B1, 2011.

article

[F1] **M. Z. Kiss**, B. J. Nagy, P. Lakatos, Z. Göröcs, S. Tőkés, B. Wittner, and L. Orzó, "Special multicolor illumination and numerical tilt correction in volumetric digital holographic microscopy," *Opt. Express*, vol. 22, pp. 7559–7573, Apr 2014.

[F2] **M. Z. Kiss**, "Ring-shaped bifocal lens used for fluorescent self-referenced holographic imaging" EOS-JRP, 2016.

[F3] P. Bawuah, **M. Z. Kiss**, P. Silfsten, C.-M. Tåg, P. A. C. Gane, and K.-E. Peiponen, "Far infrared (Thz) absorption spectra for the quantitative differentiation of calcium carbonate crystal structures: Exemplified in mixtures and in paper coatings," *Optical Review*, vol. 21, no. 3, pp. 373–377, 2014.

conference

[K1] Z. Göröcs, L. Orzó, **M. Kiss**, V. Tóth, and S. Tökés, "In-line color digital holographic microscope for water quality measurements," 2010.

[K2] Z. Göröcs, **M. Kiss**, V. Tóth, L. Orzó, and S. Tőkés, "Multicolor digital holographic microscope (DHM) for biological purposes," in *BiOS*, p. 75681P, International Society for Optics and Photonics, 2010.

[K3] **M. Z. Kiss**, "A new compact self-referenced holographic setup tested on a fluorescent target," in *Digital Holography & 3-D Imaging Meeting*, p. DTh1A.7, Optical Society of America, 2015.

[K4] **M. Kiss**, Z. Gorocs, and S. Tokes, "Self-referenced digital holographic microscopy," in *Cellular Nanoscale Networks and Their Applications (CNNA), 2012 13th International Workshop on*, pp. 1–4, Aug 2012.

[K5] **M. Zs. Kiss**, "Proper autofocus for better particle measurements," *International Society for Optics and Photonics, Practical Holography XXXIII: Displays, Materials, and Applications, (SPIE), pp 107-115, 2019*