In-line color digital holographic microscope for water quality measurements

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ABSTRACT

We introduce a color digital holographic microscope for measuring the biological content of water samples. Our approach uses single shot RGB exposure in an in-line holographic setup to obtain color images. With the application of appropriate numerical algorithms we can fulfill color crosstalk compensation, segmentation, and twin image removal tasks, and we obtain good quality color image reconstructions with 1μm resolution from a 1mm^3 volume. We briefly compare the conventional color CCD/CMOS and the Foveon X3 sensor for color digital holographic applications. The in-line holographic setup and reconstruction algorithms are presented with demonstrative simulations, experimentally captured and numerically reconstructed images.

Keywords: Color Digital Holography, Digital Holographic Microscope, Color crosstalk compensation

1. INTRODUCTION

The possibility to obtain volumetric information from a single image using wave field propagation algorithms makes digital holography a promising method to investigate freely moving biological organisms in water samples. As in ordinary holography, digital holography captures the interference pattern of two beams, namely the object and the reference beam, but instead of using a high resolution holographic recording medium, the digital holographic system uses an image sensor to capture the holograms. The reconstruction of this digitally recorded hologram is done numerically by the simulation of light propagation. Digital holography is frequently used to capture 3D-4D information of objects within a volume.[1,2] In most cases the recorded holograms are monochromatic, however, since the color of the object can carry relevant information several multi-wavelength optical setups emerged. These approaches usually use time multiplexed recordings, where the objects are illuminated by only a single wavelength at a given time, the recording is done by a monochromatic sensor and the captured image is reassembled algorithmically.[3] There are experiments where objects were simultaneously illuminated by several wavelengths[4] and the refractive index of phase samples was determined using multi wavelength illumination.[5] To our knowledge there are methods that use only monochromatic,[6] two-color,[7] or sequentially exposed three color cases,[8] where digital holography is used in microscope. In the case of single shot three-color exposure, color-crosstalk can cause noise by false reconstructions. Our previous in-line approach used a conventional color CCD with a Bayer filter array,[9] whereas here we investigated the behavior of the Foveon sensor with our color compensation method, which is capable to greatly reduce the color crosstalk caused by the overlapping transmission curves. By utilizing the Foveon sensor the sampling issues of the conventional color CCD can be avoided. In the case of in-line holography, for multiple object recognition additional segmentation and twin image removal is required. Our experimental results are presented in the paper.

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2. OPTICAL SETUP

We use an in the in-line holographic setup where the illumination beam also serves as the reference beam. The other frequently used method is the off-axis holographic setup, where an additional reference beam is used which intersects with the object beam at a non zero angle on the hologram plane. Off-axis setups can easily provide better quality images since the twin image and zero order terms are spatially separated from the object during the reconstruction process, and in multi color cases wavelength separation can also be done.[4] However, the off-axis architectures are more complex and costly systems, they can retrieve only approximately 1/3 of the resolution of the image sensor as the object, and thus it inherently sacrifices the majority of the achievable resolution. Although in-line architectures use the full resolution of the image sensor, but due to the overlapping noise terms (twin image, color-crosstalk) additional data processing steps are needed. An other very interesting feature of in-line systems is the capability to work with light sources that have a short coherence length.[10] Our approach uses the in-line setup, and can be seen in Fig. 1. A flow cell of 0.8mm thickness containing fresh water

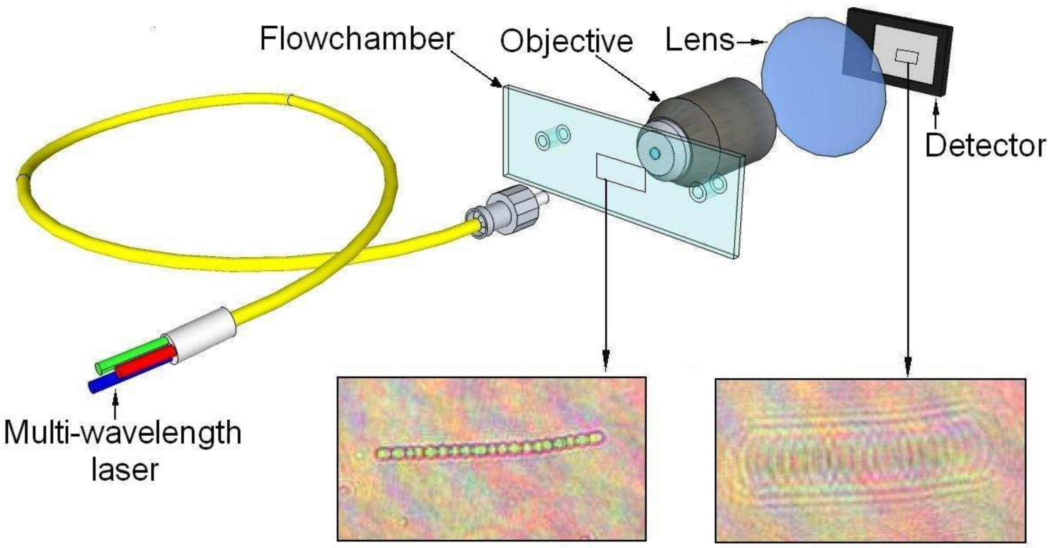

Figure 1. The used in-line holographic optical setup.

algae samples is illuminated by a multi wavelength laser beam. We use an Olympus LUCPLFLN20X microscope objective with an achromatic tube lens of 150mm focal length. Previously we used a conventional Nikon D60 single-lens reflex camera as a detector, but now we investigated the behavior of the SIGMA SD14 which utilizes a FOVEON X3 image sensor.

2.1 Light source

Digital holography assumes that the reference beam is known, therefore usually a distortion free single mode Gaussian beam is applied, where the Rayleigh range of the beam can be either treated as a plane wave or a spherical wave at the detector plane. Traditionally, a nearly perfect spherical wavefront is created by low-pass filtering of a focused laser beam with a pinhole of a few μm in diameter. This pinhole can be treated as a point like light source. Unfortunately, it tends to get blocked by dust or other particles easily. Furthermore, the proper adjustment of such a pinhole to the focused laser beam can be troublesome. Consequently, we implement a point like light source with an alternative method by using the fiber end of a single mode optical cable, which has 4.5μm mode field diameter. We have built an optical system which simultaneously illuminates the sample with red, green, and blue lights, thus it becomes possible to capture holograms of the object in the RGB color scheme at the same time, thus allowing us examine to moving samples. Using a proper detector even video rate color holographic recording can be achieved. We use pigtailed laser diodes as the red (650nm) and violet (406nm) light source, while a frequency doubled Nd:YAG laser is coupled into a fiber to get the green (532nm) one. To achieve simultaneous, parallel illuminating wavefronts fiber couplers are applied to guide all the lasers into a single fiber. We use Thorlabs FC632-50B-FC fiber couplers, which was designed for the 632nm wavelength, thus their performance is not ideal for the other two wavelengths. Nevertheless, cascading them as shown in Fig. 2 with properly set intensities provided us a coherent “white” laser source with nearly perfect spherical wavefronts.

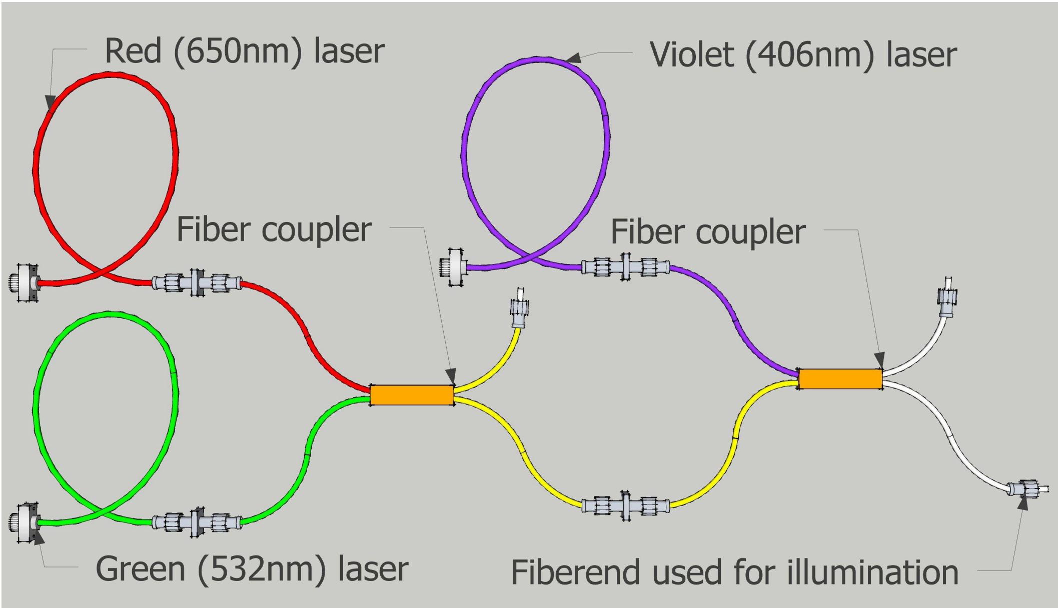

Figure 2. The cascaded fiber couplers used to create a multi-colored light source used for illuminating the object.

2.2 Detector

The image sensor is the most crucial part in a digital holographic optical system. The pixel pitch, the effective area, and the image quality of the sensor is one of the main limiting factors for the observable volume size and for the resolution of the whole microscope. Most single chip color cameras use a Bayer filter to capture color information. This filter is a 2D array of color filters with RGB colors shown in Fig. 3a. The sensors photosensitive elements have similar absorption spectra, but the transmission characteristics of the superimposed Bayer filter makes them color sensitive. However, the transmission spectrum (see Fig. 3b) usually overlap and thus the obtained image contains considerable color crosstalk. In our previous setup we used a Nikon D60 camera which uses a Bayer filter array. Since the transmission spectrum of the Nikon D60’s Bayer filter is not available we measured the transmission of each of the wavelengths we used. Table 1 shows the results for reference. Using a

Table 1. Normalized transmission of the Bayern pattern’s color filters at the Nikon D60.

Illumination wavelength	Red Filter	Green Filter	Blue Filter
650nm	1	0.042838	0.005157
532nm	0.066383	1	0.032673
406nm	0.178884	0.115504	1

detector with a Bayer filter also causes sampling problems of the wavefront, and thus various color artifacts. The color crosstalk of a Bayer filter array is relatively small, but the sampling method of these sensors makes color crosstalk compensation problematic. These difficulties can also be overcome by using a direct image sensor such as the Foveon X3. Unlike the Bayer filter array, the Foveon image sensor takes advantage of the fact that red, green, and blue light penetrate silicon to different depths, thus it is capable to capture the full range of colors at each pixel location. (See Fig. 3c) The main weakness of this sensor is the considerable higher color crosstalk

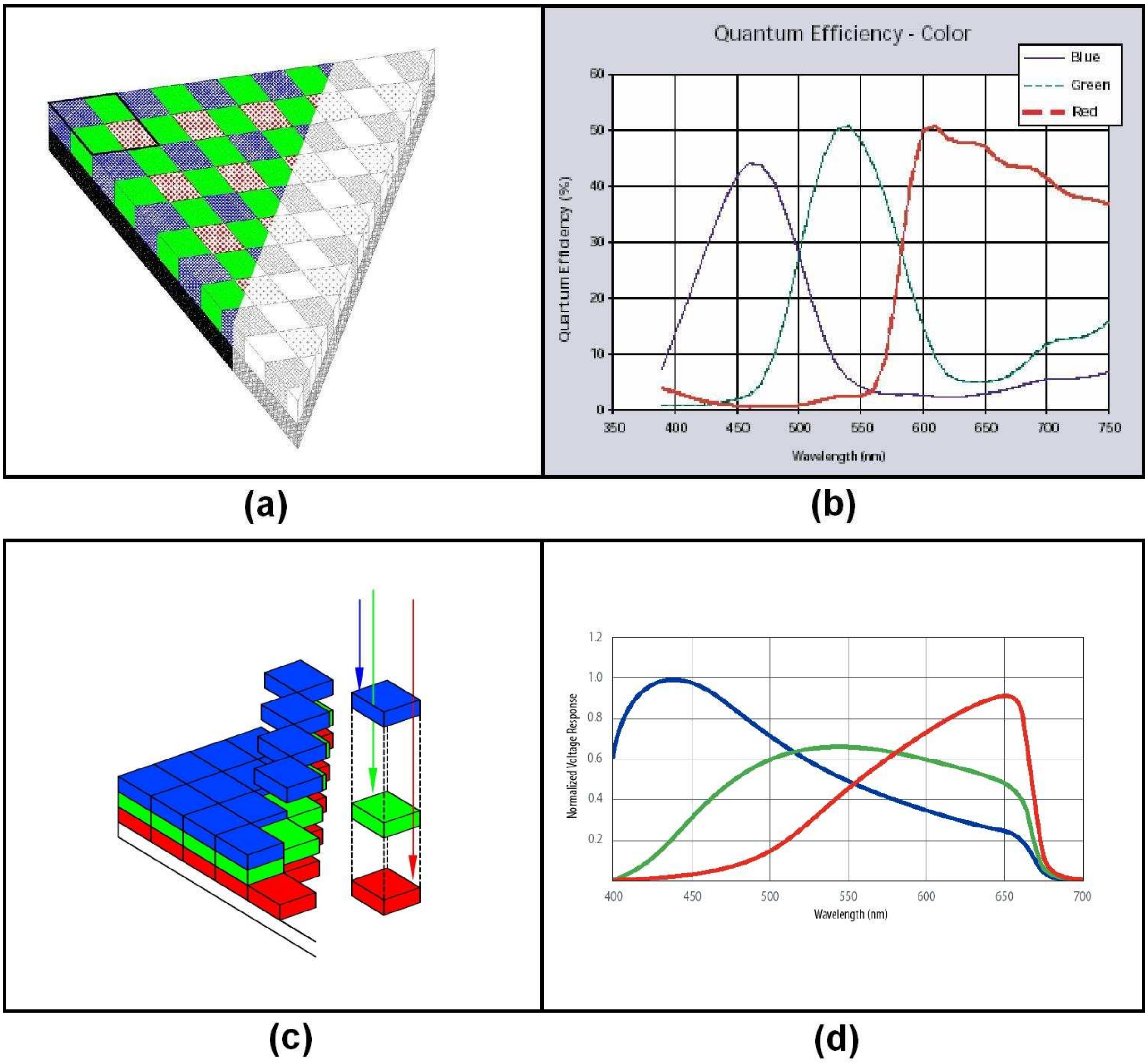

Figure 3. a) Bayer filter array on the detector surface. b) Usual color sensitivity spectrum of a color CMOS sensor with Bayer filter array. c) The color sensitive pixel structure of the Foveon X3 sensor d.) Color sensitivity spectrum of the Foveon X3 image sensor.

which can be seen in Fig. 3d. We also measured the color crosstalk of the Foveon image sensor (see Table 2) at the used wavelengths.

3. LIGHT PROPAGATION ALGORITHM

There are three main methods for digital emulation of propagating wave fields between parallel planes.[11] These methods are the single Fourier transform based Fresnel method, the convolution based Fresnel method, and the angular spectrum method. They commonly use fast Fourier transform to calculate the propagated electric field distribution. During our measurements we used the angular spectrum method because it works even for small propagation distances, as does not use paraxial approximations. An extension of this method was recently found by Matsushima,[12] which improves the accuracy of the angular spectrum method for larger distances by proper sampling.

Table 2. Normalized transmission of the Foveon X3 sensors color channels at the Sigma SD14.

Illumination wavelength	Red channel	Green channel	Blue channel
650nm	1	0.585268	0.301762
532nm	0.503208	1	0.852527
406nm	0.062338	0.076821	1

3.1 The angular spectrum method

In the current algorithm however we use the conventional angular spectrum method, which is the solution of the Rayliegh-Sommerfeld scalar diffraction integral.[13] Using $E(x, y, 0)$ as the monochromatic electric field distribution at the hologram plane ($z = 0$) the electric field distribution at a $z \neq 0$ plane will be:

$$E(x,y,z) = \iint E(x',y',0) \frac{e^{i\frac{2\pi}{\lambda}r'}}{r'} \frac{z}{r'} \left(\frac{1}{2\pi r'} + \frac{1}{i\lambda} \right) dx'\, dy', \tag{1}$$

where $r' = \sqrt{(x-x')^2 + (y-y')^2 + z^2}$ and λ is the wavelength. Using

$$h(x,y,z) = \frac{e^{i\frac{2\pi}{\lambda}r'}}{r} \frac{z}{r} \left(\frac{1}{2\pi r} + \frac{1}{i\lambda} \right), \tag{2}$$

where $r = \sqrt{x^2 + y^2 + z^2}$, Eq. (1) can be rewritten in a convolution form

$$E(x,y,z) = E(x,y,0) * h(x,y,z). \tag{3}$$

Fourier transforming Eq. (3) we get

$$\mathcal{F}\{E(x,y,z)\} = \mathcal{F}\{E(x,y,0) * h(x,y,z)\} = \mathcal{F}\{E(x,y,0)\} \cdot \mathcal{F}\{h(x,y,z)\}. \tag{4}$$

Calculating the Fourier transform of the kernel leads to

$$\mathcal{F}\{h(x,y,z)\} = \iint h(x,y,z) e^{-i2\pi(ux+vy)} dx\, dy = e^{i2\pi \cdot w(u,v) \cdot z}, \tag{5}$$

where the symbols u, v, w are the Fourier frequencies in the x,y, and z directions, respectively. However these frequencies are not independent thus:

$$w(u,v) = \begin{cases} \sqrt{\frac{1}{\lambda^2} - u^2 - v^2} & \text{if } u^2 + v^2 \leq \frac{1}{\lambda^2} \\ 0 & \text{otherwise} \end{cases} \tag{6}$$

Consequently the angular spectrum method calculates the scalar electric field in a z distance as follows:

$$E(x,y,z) = \mathcal{F}^{-1} \left\{ \mathcal{F}\{E(x,y,0)\} \cdot e^{i2\pi \cdot w(u,v) \cdot z} \right\}. \tag{7}$$

This is the core of our light propagation algorithm.

3.2 Multi-wavelength algorithm

A quick overview of the algorithm can be seen in Fig.(4). We start with the raw sensor data of the captured hologram. Due to the overlapping transmission curves, a color crosstalk compensation has to be made on the red, green, and blue components, to acquire the three holograms of the object at the different wavelengths. By using the angular spectrum method on one of the holograms the whole volume is reconstructed layer by layer to find the position of the objects. If there are two objects close to each other laterally, but at different depths the diffraction pattern of the first object is usually so spread out that it overlaps with the second object at the plane where the second object is in focus. To eliminate this problem we use a segmentation process where the objects in the examined volume are removed one after an other, so only the examined object remains. The next step is a twin image removal process to achieve the best possible image quality. The three holograms are then recombined to the color image which is then processed by an object classification algorithm to recognize the different types of algae in the water sample.

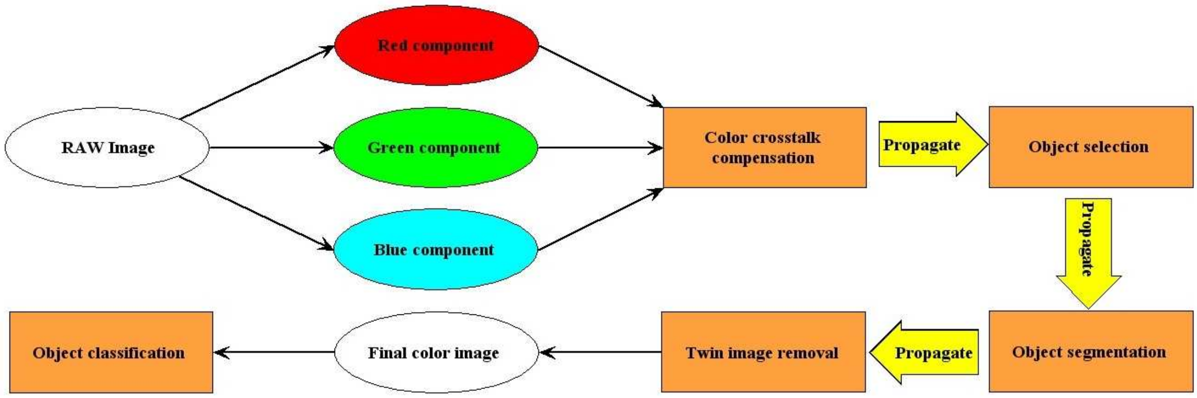

Figure 4. The schematics of the algorithm.

4. ERROR COMPENSATIONS

4.1 Color crosstalk compensation

As mentioned before, to record the color digital hologram the sample is illuminated with the combination of three laser beams with the wavelength of 406nm, 532nm, and 635nm. Each of these waves create their own separate hologram, but the Sigma SD14's Foveon X3 image sensor captures it at the same time. Due to the considerable color crosstalk shown in Table 2 the three holograms are mixed at the color channels of the sensory data. Because of the wavelength difference each hologram has to be processed individually, thus the first task is to separate them. We found a very simple method to overcome this problem. Using the values in Table 2 we can calculate the effect of the overlapping spectrum. Using

$$\overline{\overline{T}} = \begin{bmatrix} 1 & 0.503208 & 0.062338 \\ 0.585268 & 1 & 0.076821 \\ 0.301762 & 0.852527 & 1 \end{bmatrix}, \tag{8}$$

effect of the overlapping sensitivity curves can be calculated as:

$$\begin{array}{ccccc} \overline{M} & = & \overline{\overline{T}} & \cdot & \overline{R}, \\ \begin{pmatrix} M_{red} \\ M_{green} \\ M_{blue} \end{pmatrix} & = & \begin{bmatrix} 1 & 0.503208 & 0.062338 \\ 0.585268 & 1 & 0.076821 \\ 0.301762 & 0.852527 & 1 \end{bmatrix} & \cdot & \begin{pmatrix} R_{red} \\ R_{green} \\ R_{blue} \end{pmatrix}, \end{array} \tag{9}$$

where $\overline{M}$ is the measured intensity vector and $\overline{R}$ is the real intensity vector. Here by multiplying Eq.(9) with $\left(\overline{\overline{T}}\right)^{-1}$ from the left we get:

$$\begin{array}{ccccc} \overline{R} & = & \left(\overline{\overline{T}}\right)^{-1} & \cdot & \overline{M}, \\ \begin{pmatrix} R_{red} \\ R_{green} \\ R_{blue} \end{pmatrix} & = & \begin{bmatrix} 1.407488 & -0.677852 & -0.035667 \\ -0.846575 & 1.477796 & -0.060752 \\ 0.297002 & -1.055312 & 1.062555 \end{bmatrix} & \cdot & \begin{pmatrix} M_{red} \\ M_{green} \\ M_{blue} \end{pmatrix}, \end{array} \tag{10}$$

Using this simple matrix multiplication we can compensate for the color crosstalk of the sensor. Figure 5 shows the process on a hologram which was recorded with a single wavelength (650nm).

4.2 Segmentation

Reconstruction of the in-line holograms is a hard problem not only due to the twin image (zero order terms) caused noises,[14] but the neighboring objects diffraction can also distort the reconstructed image quality. Furthermore, the common phase retrieval algorithms do not tolerate this type of biases.[15] Therefore, first we segment the

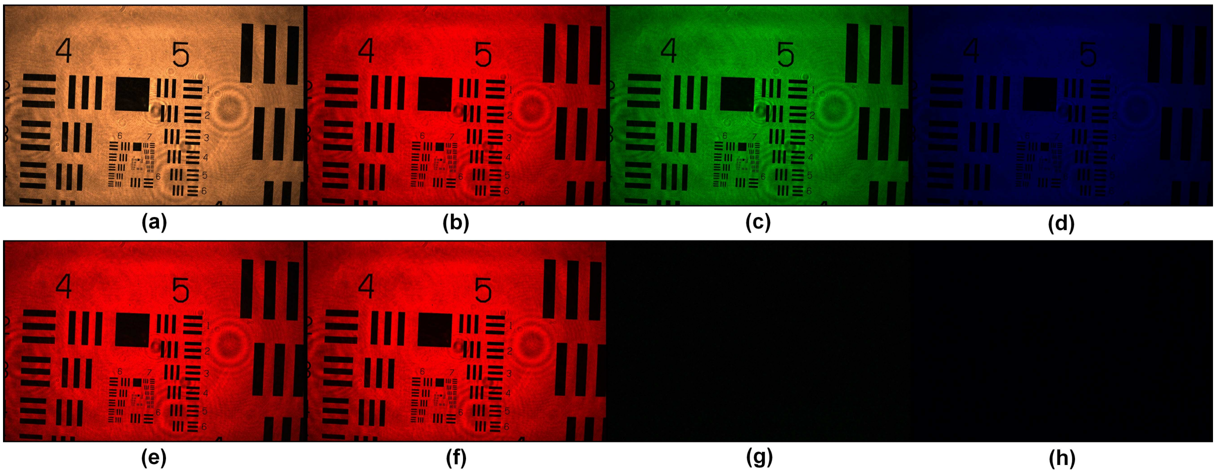

Figure 5. Experimental images of the color crosstalk correction method. a) Original hologram created by a single wavelength (650nm) laser illumination. The intensity values of the b) red, c) green, and d) blue pixels of the original hologram, respectively. e) Color crosstalk compensated hologram. The intensity values of the f) red, g) green, and h) blue pixels of the color crosstalk compensated hologram, respectively.

recorded hologram according to the occurring objects sub-holograms. This segmentation is based on the objects reconstruction distance and their corresponding spatial supports, which can be determined by an algorithm that use an appropriate focus measure.[16] The algorithm is based on the special inner structure of the in-line holograms. The support of the segmented object decrease the aperture of the other objects and thus decrease the achievable resolution, but since we can achieve almost perfect segmentation, all the diffraction caused by the other objects becomes negligible. Figure 6 shows that the segmentation of the nearby objects can remove their diffraction on the resulting reconstructions. Diffraction pattern of the other objects are abolished almost perfectly, and this way enhancing the quality of the reconstruction.

4.3 Twin image removal

The hologram of the segmented object is still an in-line hologram and so it is contaminated by twin image and zero order noises. As the estimated object support and reconstruction distance is known we can apply the well known Gerchberg-Saxton-Fienup phase retrieval algorithm .[17,18] Our solution extends this method to the recorded multi-wavelength holograms. This way we can remove the the twin image diffraction pattern from the reconstructed object as shown in Fig. 7.

4.4 Aberration compensation

Although the main advantage of digital holography is the ability to reconstruct sharp images at different layers of a volume algorithmically, thus virtually increasing the depth of field of a microscope system, there are other benefits of knowing the whole wavefront. For example, after obtaining the recorded holograms, we can correct the aberrations of the optical system numerically. Using color holography the most common aberrations are the lateral and the transversal chromatic aberration.[3] Since we used a high end microscope objective, these chromatic aberrations were not observable, but we would like to note that using a less sophisticated microscope objective they cause considerable distortions. In these cases measuring the transfer function of the system for the whole volume, one can design an inverse transformation method to compensate for chromatic, and some other type of aberrations as well.[19]

5. CONCLUSION

Our main goal is to develop a Color Holographic Video Microscope to measure the biological content of water samples. The introduced color crosstalk compensation makes it possible to use simultaneous three-color illumination, and this way to record a hologram of the examined volume with three colors at video frame rate, since

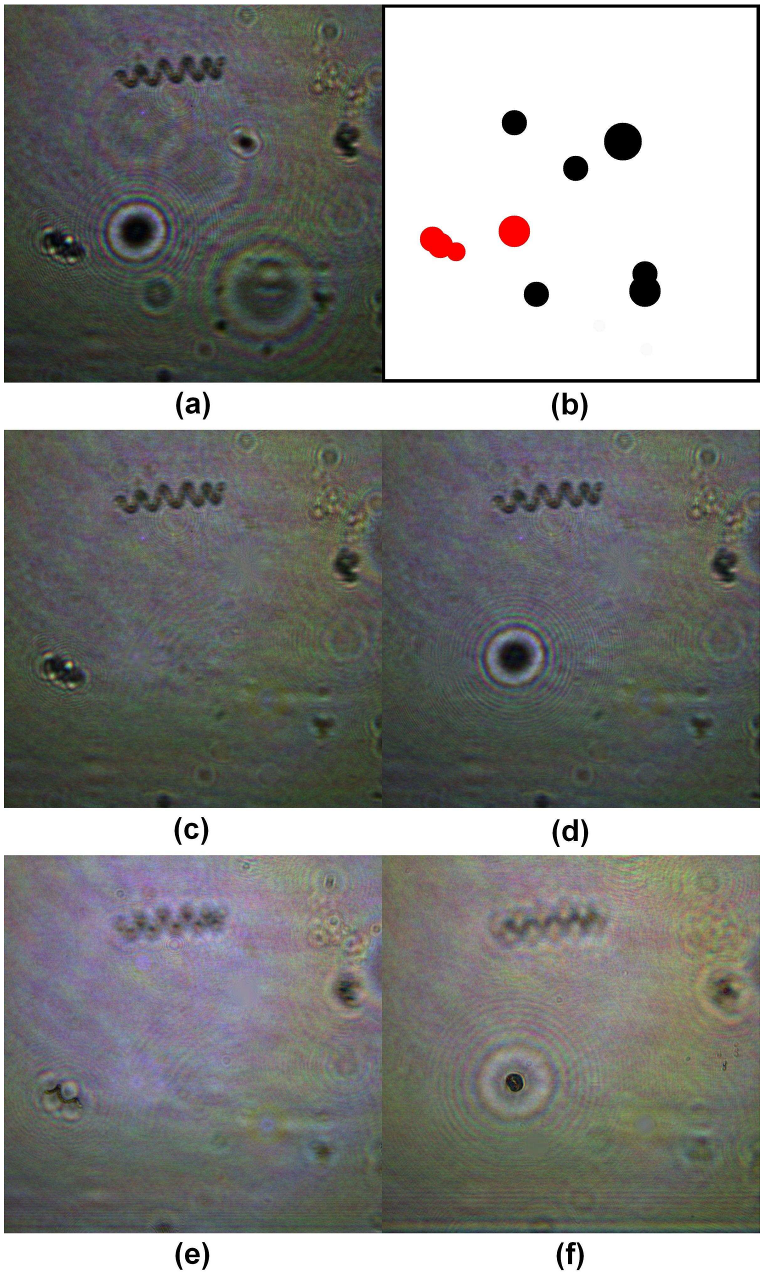

Figure 6. Experimental images of the segmentation method. a) Original hologram. b) Object selection map. The objects we are interested in are marked red, the objects that cause noise and needs to be removed are marked black. c) The segmented hologram of the first and d) of the second object, respectively. e) The propagated sharp image of the first object, f) and of the second.

the illuminating colors are not time multiplexed. The color crosstalk usually causes smaller noise then the twin image. However, as twin image elimination is a deconvolution task, its success and quality considerably depends on the noise of the input in-line hologram. This way, without color crosstalk compensation, there is little hope of good quality twin image removal. Our earlier sampling method[9] can cause aliasing, which can be avoided by using the Foveon X3 sensor. The introduced color compensation method can also be used in the case of off axis architectures, where the effect of color crosstalk seems more crucial as the twin image noise does not exist. An in-line three color (RGB) digital holographic microscope setup using a Sigma SD14 camera was presented. We

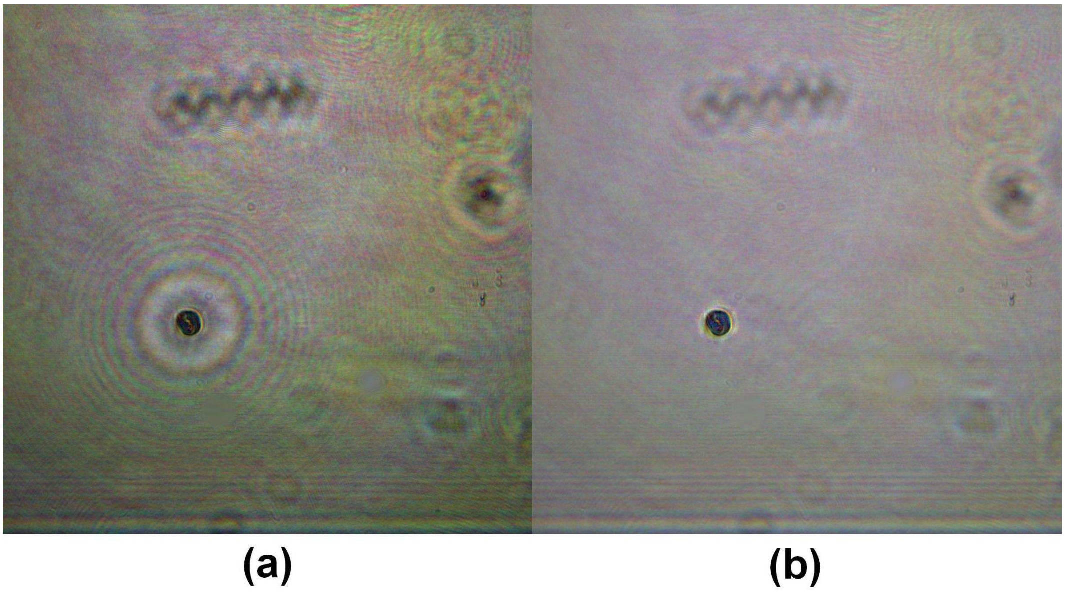

Figure 7. Experimental images of the twin image removal method. a) Original reconstructed image. b) Reconstructed image after twin image elimination.

detailed the color compensation method, which is capable to greatly reduce, the color crosstalk caused by the overlapping color sensitivity spectrum of the image sensor. We gave experimental results of our segmentation and twin image removal algorithm. The performance of our color DHM is demonstrated by reconstructed images from a volume of flowing water containing algae.

ACKNOWLEDGMENTS

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”.

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