Hight resolution digital holographic microscope and image reconstruction

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***Abstract*—The current paper presents the development of hight resolution digital holographic microscope and the picture-reconstruction software.**

The application of holography is important because with it the small width depth of traditional microscopes can be avoidable, therefore the three dimension volume can also be reconstructed. Holography makes the examination of liquids more effective, which, for instance, can be used for many blood tests or to supervise the quality of potable water.

I. Introduction

Currently the hologram is stabilised by a digital sensor (CCD camera), and the reconstruction is also calculated by the numerical simulation of the expansion of light-waves. With this, the previously used traditional developing techniques become unnecessary, and it allows the completion of automated measures nearly the actual time

In holography the wave nature of the light is exploited. The light is an electromagnetic vibration. The Maxwell equations describe the interference of powers and charges and the electric and magnetic scope [1].

The interference occurs when two waves encounter; this phenomenon is the basis of holography. The key of this consists that the time the hologram is stabilised, the amplitudes are accumulated. It depends on the phase difference the kind of process that the encounter of the two light waves involves. It can also happen that at the time of encounter the two light waves debilitate or extinguish each other. Due to the great frequency of illuminating light, only within the determined intervals average of intensity can be recorded. If the difference of phases is constant, the resultant of the two waves average intensity of the resultant becomes constant as well. Thus, this typical intensity rate can be seen if the rays are compassed. A stripe system evolves due to the phase difference. The light bars are called interference stripes. However, if the phase difference is inconstant, then the average rates are the same in every single point, thus the emerging picture will be continuous, homogeneous. The condition of creating an interference picture is the constant phase difference. This feature is called coherence- if the two waves are coherent, their phase difference becomes constant as well [2], [3].

During the realization of holography, the object to be imaged should be illuminated with a kind of light which is coherent. Therefore, the laser illumination could be a good alternative because the laser light can be considered a coherent light, thus it gives the body to the creation of interference. Illuminating the object the amplitude and the phase of dispersing waves also change. These waves create the object beam. For the object beam, the reference beam should be added, which is the original illuminating light, and then it can be fixed with a camera. The hologram itself is created by the interference of these two light beams. With this technique only the distribution of intensity can be measured, so the calculation of the phase will be the assignment of the reconstruction. During the process of reconstruction the completed hologram should be illuminated with a so-called restoring, reconstructing wave. In this process the interference picture only serves as a grid; the measure of the light transmission depends on the extent of the fixed intensity in every point [3], [4].

In digital holography, the fixation is made by a digital sensor, for example a CCD camera. The reconstruction of the hologram is implemented by the numerical simulation of the expansion of light. This process also comprises taking samples, which means that the full sign cannot be fixed, just rates in average of specific points. This might severely limit the reconstruction of the picture [2].

Various techniques of hologram realisation are known which allows summaing the object beam and the referent beam. Using the inline arrangement (Fig. 1.) the two beams do not separate, so their axis falls on one straight line. One big drawbacks of this arrangement is that the twin pictures and the zero ordered pictures are situated on each other on the reconstructed picture that is why it is necessary to separate them numerically [5], [6].

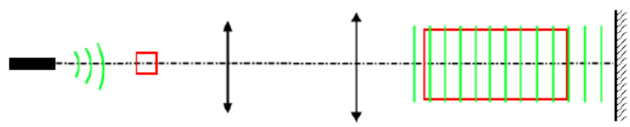

Fig. 1. Inline arrangement for fixing a hologram [7].

One of the big advantages of holographic microscopy is that the focusing can be implemented digitally, therefore the examinable volume is much bigger, and can also be achieved a magnified picture of the examined object without magnifying lens. The process of research and realisation becomes much cheaper and more flexible [8].

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The current paper presents the developement of a digital holographic microscope which is capable producing high-definition holographic pictures of 3D objects and to create a software for the hardware by which the holographic pictures can be reconstructed. The structure of the paper is as follows: In the second paragraph, the Fresnel method in holography will be introduced; in the third paragraph, the holographical registration and reconstruction will be described; the fourth paragraph reviews the development of holographic microscope; the fifth presents the achievements; and the sixth will summarise the study.

II. Describing the Fresnel method

In the following sections, the registration of the hologram will be introduced. Let from the ff point-like light source distance R the A registration plane be, which is perpendicular to axis z (Fig. 2.). The instantaneous rate of electric field intensity falling on the optional (y, x) point of the registration plane is [9]:

$$E_t(x,y) = \frac{E_{t0}}{r} exp[i(kr - \omega t)], \tag{1}$$

where

$$r = \sqrt{R^2 + x^2 + y^2}. \tag{2}$$

As the light can be perceived only by the energy falling into the tracking point, therefore the energy falling into (y, x) point should be calculated, as the intensity of light [9]:

$$I_t(x,y) = E_t^*(x,y)E_t(x,y) = \frac{E_{t0}^2}{r^2}. \tag{3}$$

However, if the incident intensity of light is registered, further information belonging to the incident waves phase will be lost. For the proper tracking of the phase, a referent beam should be made. This incident plane wave starting from point ff, should be coherent and identical with the light in frequency, furthermore, parallel with axis z. In this case the referent beams phase is identical everywhere on plane A [9], [10]:

$$E_r = E_{r0} exp[i(kz - \omega t)]|_{z=0} = E_{r0} exp(-i\omega t). \tag{4}$$

Disregarding polarisation, in point (x, y), the previously calculated referent beam and the wave coming from ff light source interfere [9]:

$$\frac{E_{t0}}{r} exp[i(kr - \omega t)] + E_{r0} exp[-i(\omega t - \phi_0)] = \\ = exp(-i\omega t)[\frac{E_{t0}}{r} exp(ikr) + E_{r0} exp(i\phi_0)], \tag{5}$$

where ϕ_0 is the phase difference between the reference and the object beam. Based on these, the intensities are the following [10]:

$$I(x,y) = E^*(x,y)E(x,y) = \\ = \frac{E_{t0}^2}{r^2} + E_{r0}^2 + 2\frac{E_{t0}E_{r0}}{r} cos(kr - \phi_0). \tag{6}$$

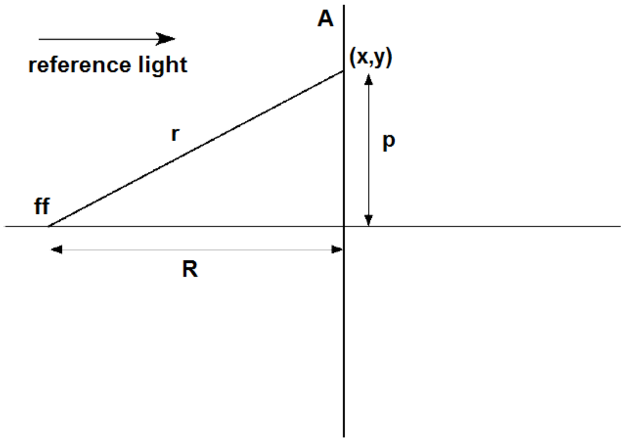

Fig. 2. The registration of the hologram [10].

Consequently, the incident light intensity on plane A will also contain the modulation part of interference apart from the sum of intensity which derives from the two light beam. This modulation preserves quantum kr, which equals to the phase of the spherical wave which is incident on plane A (only if the two light waves are coherent and constant in ϕ_0 time). Based on these it is presumable that $\phi_0 = 0$. Hence, the intensity of the incident light changes periodically between the rates $(E_{t0}/r + E_{r0})^2$ and $(E_{t0}/r - E_{r0})^2$ [10]. This period is determined by function $cos(kr)$,

$$k\delta r = 2\pi n, n = 0, 1, 2..., \tag{7}$$

As a result, we get back the same intensity on the rates [10]. Hence, it could be determined where these rates are situated on plane(x, y) [9]. According to Fig. 2. [9]:

$$r^2 = R^2 + x^2 + y^2 = R^2 + \rho^2 \tag{8}$$

places with identical intensity on plane A, are situated along concentric circles with radius ρ. The $x = y = 0$ point-in intensity, which is determined by the rate of kR, appears at such a distance from the axis as: $r - R = 2n\pi/k = \lambda$ [9].

$$r^2 - R^2 = \rho_n^2 = (r + R)(r - R) \approx 2Rn\lambda, \tag{9}$$

where the $r + R \approx 2R$ approximation should be applied. This approximation holds true for every radius that is near to or close to a small angle with the optical axis. This is called paraxial approximation in connection with the optical calculations. The obtained distribution of intensity is like places with identical intensity are situated along concentric circles, and these circles radius is increasing with $\sqrt{n}$. As a result, the condition of holography can be accomplished, which means that the amplitude and phase of the initial light field (the spherical wave E_t) should be recorded simultaneously, if the previously calculated distribution of intensity is recorded. In other words, a kind of transparency should be created which is adequate to the distribution because of its changing capacity according to the location of permeability.

The achieved picture can be considered as a Fresnel type of zone plate. Originally, according to classical optics, a Fresnel plate is a kind of transparency which has the feature that its permeability is 1 at the previously calculated places of positive modulations rates, so it is fully permeable; and at places with negative modulation the permeability is 0, so it is fully capable to absorb. However, if we put the permeability as a continuous function, then we can achieve better result at the application of Fresnel plate. The Fresnel plate should be examined in terms of how it can restore the obtained wave field from recording, meaning that it is capable for producing the original picture of ff point-like light source. In order to restore it, the picture should be illuminated with the original reference light [9], [10] (Fig. 3).

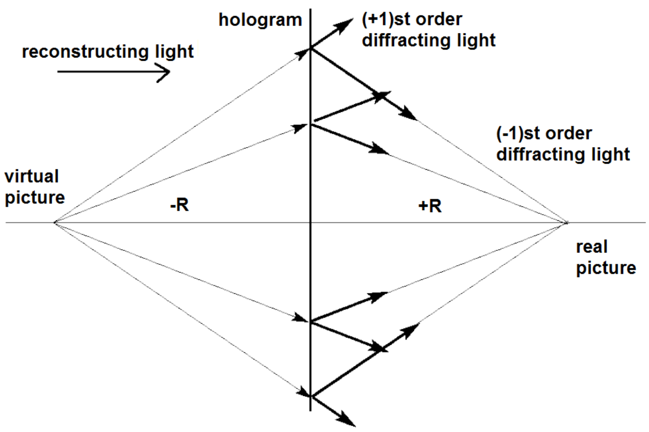

Fig. 3. Diffraction on the Fresnel plate [10].

The restoring light, which is projected on the plate, i.e. on the hologram, should behave according to the rules of diffraction passing on the plate. Regarding Huygens principle, elemental spherical waves spring from every point of the plate where the light is transferred; the resultant light will be the result of the interference of these spherical waves. The illumination of the point from the plate distance R (which is situated on the axis) will be the strongest. In this point the zone plate (9), does not transfer only those waves which can deteriorate the diffracted waves falling from permeable places through the process of interference. As a result, the point-like light source used at the recording can be restored if the plate is illuminated by the reference beam. On the optic axis, every (-1)st of the diffracting light crosses every diffracted light which belongs to the diffractive order. These create a divergent light beam, the diffractive waves of the (+1)st order adequate for these rays. However this created divergent light springs from the place of the original light source, so the virtual picture of the original object can be produced with these light beams [11]. A higher- ordered real and virtual picture can be created from the original object with the help of the higher-ordered waves. During the process of making the hologram the distribution of modulated intensity has to be recorded during the restoring process; hence higher-ordered diffractive waves cannot evolve. Furthermore, the primary ordered diffractive rays will be the biggest in intensity in comparison with the zero ordered light [12].

As a result, during the previously described process, the hologram can be created, reconstructed and restored. It can be seen that the restored holograms picture is not so ideal as it is very difficult to observe the real picture due to the picture has been evolved as the background of the light intensity of zero-ordered beam, which is highly intensive.

To avoid this problem, Leith and Upatnieks [13] have developed the procedure that the referent beam should be situated that way that it can close any kind of angle with the optic axis during the recording [7]. It is important that the light used for restoration should be similarly arranged, because the Fresnel zone plate behaves like a lens, so the modelling can also be accomplished with it. The Fresnel plate models the restoring light into a point on the optic axis. Subsequently, this point can be regarded as the focus point of the plate, namely $f = R$. It is true for the picture point deriving from the primary ordered diffraction and evolving k distance from the plate that $\frac{1}{k} = \frac{1}{f} - \frac{1}{t}$, so the lenses behave properly according to the known modelling laws if the plate is illuminated with a point-like light source situated on the axis and placed t distance from the plate.

Applying the Frensel plate as a lens, based on (9) the focal length is $f = R = \rho_1^2/2\lambda$, (the first annuluss radius is expressed with (ρ_1)), so the chromatic mistake of the process can be very big when the Fresnel plate is used for this purpose.

Regarding these conditions, the Fresnel method is traditionally applied for those modelling projects, where in the given wave length those lens to be used cannot work due to strong absorption [7], [10].

III. The Holographic Registration and Reconstruction

In the previous section it was mentioned that it is preferable that the reference beams direction which is used for creating a hologram is not identical to the optic axis. Disregarding the electromagnetic waves time course, in this case

$$R_r = E_{r0}exp[ik(R + xsin\Theta)], \tag{10}$$

where Θ is the closed range of the referent beam and axis z [1]. Axis x falls in the intersection of the plane of the hologram, rather the optic axis and the referent beams plane. This feature can be seen on Fig. 4 [11].

The wave coming from the object [4]:

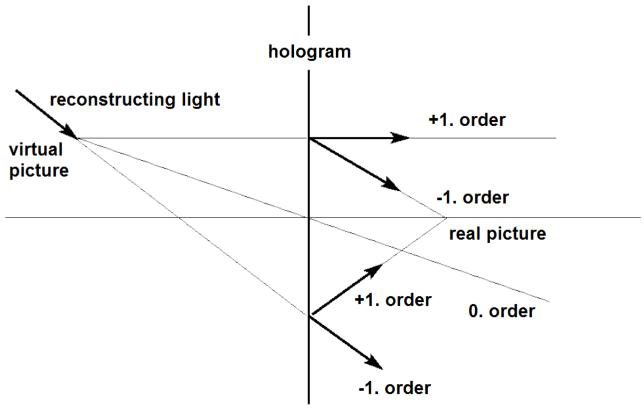

Fig. 4. Restoring hologram with a referent beam out of axis [10].

$$E_t = \frac{E_{t0}}{r} exp(ikr) \approx \frac{E_{t0}}{R} exp[ik(R + \frac{\rho^2}{2R})]. \quad (11)$$

Applying the paraxial approximation, the incident intensity of plane (x, y) is [4]:

$$I(x, y) = \frac{E_{t0}^2}{R^2} + E_{r0}^2 + 2\frac{E_{t0}E_{r0}}{R} cos[k(\frac{\rho^2}{2R} - xsin\Theta)]. \quad (12)$$

The permeability of the registered plate is [4]:

$$T(x, y) = K_1 + K_2 cos[k(\frac{\rho^2}{2R} - xsin\Theta)], \quad (13)$$

where $K1$ and $K2$ are constants. If it is illuminated by a plane wave from under angle Θ, the rate of field strength will be from l distance behind the plate [10].

$$E(\xi, \eta, \zeta) = B \int\int T(x, y) exp(-ikxsin\Theta) E_l(\xi - x, k) dxdy, \quad (14)$$

where

$$E(\xi - x, k) = -\frac{i}{\lambda l} exp[ik(\zeta + \frac{(\xi - x)^2}{2l} + \frac{(\eta - y)^2}{2l})], \quad (15)$$

which is called the impulse response of open field [10]. Conducting the previous equations, two rectangular coordinate systems are needed. The first is the system (x, y, z) which is fixed to the object, and the second is sytem (ξ, η, ζ) which is defined in the picture field and its nucleus in the object field can be find on place $z = l$ [10].

In the following equations the semi-bold $\boldsymbol{\xi} = (\xi, \eta, \zeta)$ and $\boldsymbol{x} = (x, y, z)$ mark the adequate coordinate systems vectors. Substituting these on $l = R$ we got the following expression [10]:

$$E(\xi, \eta, \zeta) = C_1 exp(-ik\xi sin\Theta) + \\ +C_2 exp[\frac{ik}{4R}((\xi - 2Rsin\Theta)^2 + \eta^2)] + C_3\delta(\xi, \eta). \quad (16)$$

In (16), the first part means the zero ordered diffraction (a plane wave in parallel with the incident wave), the second part gives a concentric shaped wave front with its nucleus on the holograms illuminated side in a plane from R distance, and it falls in a point where has coordinates $(\xi = 2Rsin\Theta, \eta = 0)$. This result in a virtual picture. The third part of the equation gives the real picture of the object point, which can be find on the revenge side of the hologram and emerges R distance from it in a point with coordinates $(\xi = 0, \eta = 0)$, on the optic axis. It can be seen from previous calculations and equations that with this method the virtual picture, the real picture and the zero ordered diffraction beam can be separated. The case have been proved by Leith and Upatnieks [13], that if we add a reference plane beam $E_r(x)$ to the wave front $E_t(x, y)$ arriving on the surface of the shooting at an object and we detect the resultant intensity with the belief that we will get a hologram as a result, then illuminated with ray $E_r^*(x)$, the reverse side of the hologram illumination, the adequate distance from the original and the real picture of the object will be created. Instead of angle Θ, using angle $-\Theta$ the complex conjugation of referent beam can be created. The virtual picture of the hologram will be draw up at the original place of the object that the hologram should be illuminated with the original wave $E_r(x)$ [9], [10].

IV. Developing a Holographic Microscope

In the following sections, the completed hight resolution digital holographic microscope will be introduced.

The reason why this device should have built is because there were no available holographic pictures which had the appropriate definition for the reconstruction by software; in addition, the parameters of the found pictures were not known. For instance, one of these parameters is what can be found in the original picture, which camera-object distance has been used, or which wave-lenght was the illuminating light. Without these parameters and skills, it is impossible to develop an accurate reconstructional software.

A. Applied components

Fig. 5. shows the components of the digital holographic microscope.

The three main components of the developed device are the CCD camera, pinhole and source of light.

1) Source of light: As a source of light an RGB LED was applied, where the different colours light on different wave length. Hence, the illuminating lights wave length can be known accurately during the software developement.

2) RGB LED controller: In order to drive the controller, a separate control module was needed to switch the different channels of the LED separately and together as well.

3) Source of light and the pinhole: On the source of light, a holder was put inconsistently, functioning as the pinholes socket as well. This was necessary not letting the light escaping elsewhere but the pinhole. The pinhole was manufactured by Edmund Optics.

Table I contains the RGB LED's wavelength.

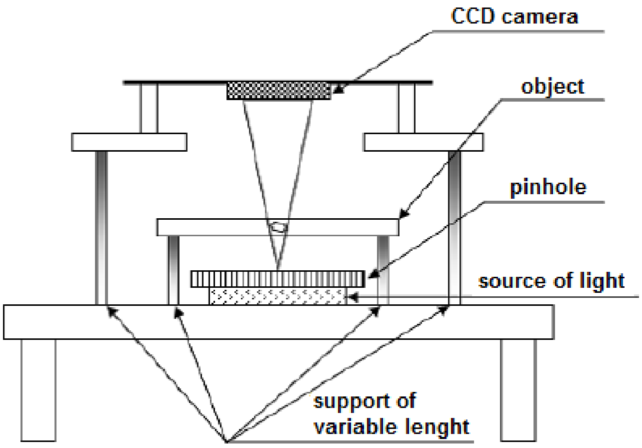

Fig. 5. The construction of the holographic microscope [2].

TABLE I
RGB LED COLOURS WAVELENGTH

Color	Wavelength
Red	625nm
Green	525nm
Blue	470nm

4) CCD camera: The produced holographic picture should be fixed, for this purpose the Logitech HD Pro Webcam C910 was used. Its advantage is that it has a relatively big CCD sensor.

5) The object plate: For easier testability, the pictures were made from a USAF 1951 resolution testing plate. Fig. 6. presents the USAF 1951 plate.

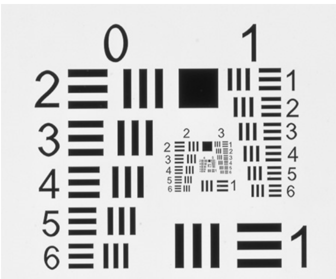

Fig. 6. USAF 1951 resolution testing plate.

On the resolution testing plate, the lines are ordered in groups, and these groups are classes. The resolution is determined based on the thickness of the lines.

B. Composition

At the composition it was emphasized that the distance between each plate could be relatively easily configured, because at the initial state for assessing these distances the plates should be often modified. Furthermore, to get a cheap device which is easy to be build and compiled constraints were used on the simple materials.

TABLE II
THE IDEAL DISTANCE OF THE PLATES

The distance of light source and object beam:	3mm
The distance of object beam and CDD sensor:	13-41mm

Each plate on which the camera, the object plate and the mainboard is placed are uniquely formed. These plates are fixed to each other on the corners; hence, twisting these allows the adjustment of the distance of the plates. On the corners of the plates a kind of gripper is constructed which allows to converge and abduct these by twisting the screws. In every four corners the distance of the plates are separately adjustable, so the accidental gripping inaccuracy can also be corrected (Fig. 7.).

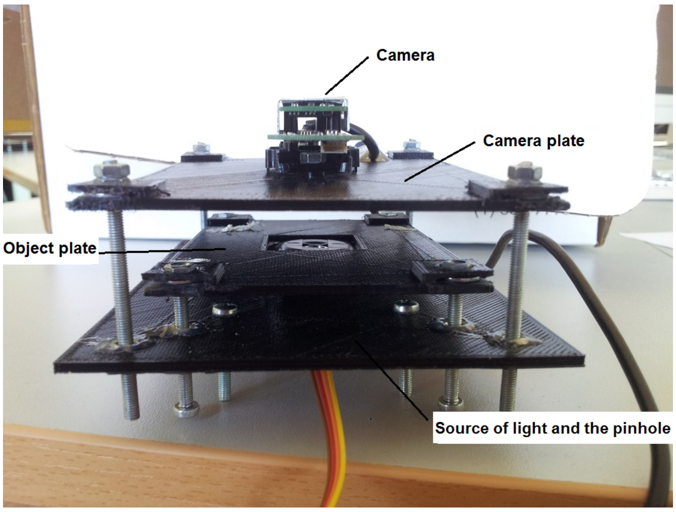

Fig. 7. The finished holographic microscope, side view.

C. Operation

The operation of the device can be easily introduced. The light beams starting from the light source placed underneath come through the 15μm diameter pinhole, afterwards the object plate, and finally fall into the CCD sensor where the picture is produced. On the LED controller the channels can be switched individually, so the object can be illuminated with different wave length lights, and later on it will be possible to make a more detailed reconstruction.

D. Parameters

In the production of the pictures the plates with different distance play a main role. These distances should be set in a way that the maximum resolution can be achieved (39μm). The resolution is determined by the distance of the light source and the object plate, and the distance of the light source and the camera. Table II summarizes the used distances.

V. ACHIEVEMENTS

The pictures made by the accomplished hardware have been elaborated and reconstructed with a self-developed software.

Examining the pictures it was turned out that the best quality reconstruction can be achieved by pictures made with green illumination. In green illumination the lines and the item numbers can also be seen much sharper. The most optimal camera distance has been proven 13mm. In this setting the archieved resolution became the device's and software's best resolution, which is 39 micron. For the reconstruction of a picture the estimated time needed is cc. 2-3 seconds with an average, recent computer (CPU: Intel i5, RAM: 4-8GB). Nowadays, the usage of holographic picture reconstruction is accessible, quick and simple.

Results of the USAF 1951 resolution testing plate can be seen in Fig. 8.

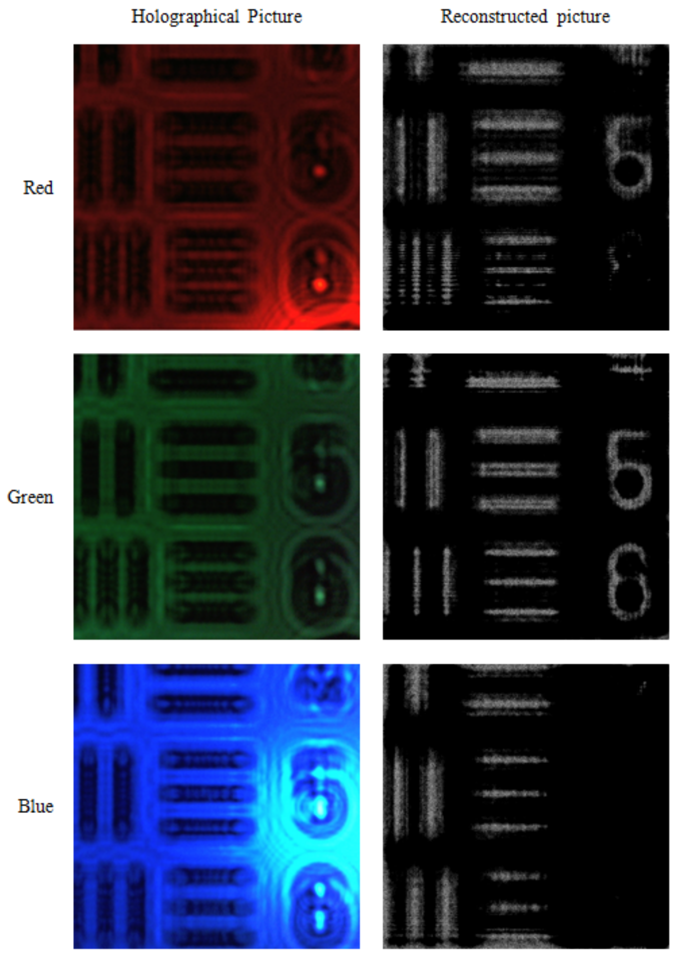

Fig. 8. Pictures made with 13mm camera-object distance (class 3 items 5-6).

VI. Conclusion

In short, the reason why the hight resolution digital holographic microscope has been made is that there would be a possibility to take high-definition pictures with making parameters which are known the wave-lenght of the illuminating light, the camera-object distance, or what the picture depicts. It was necessary for making a more accurate holographic picture reconstruction software. In favour of the system's bigger resolving power, the pictures have been taken after a USAF 1951 resolution testing sample. On the basis of the sample's topology and calibration, the device's resolving power can be determined, which is 39 micron in the presented installation. This resolution has been archieved by the green illuminating light, and the 13 mm camera-object distance. Both the hardware and software of the device has been developed, by which high-definition digital holographic pictures can be taken about objects, and the original object's virtual picture can also be reconstructed.

VII. Further work

Henceforth, it is planned to build a professional holographic microscope, which is capable for increasing the resolving power and each setting can be reconstructed accurately, by the set-up of the microscope. The development of a module is also planned which will help taking pictures on liquids as well. Meanwhile, further development of the software is also planned, as in order to get a more accurate and bigger device in resolution numerous changes in the software are needed. Modifications are needed to examine liquids, and there it should be taken into consideration that the reconstruction should be taken in a moving system, so the compensation of the motion should be expected.

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