Afocal Digital Holographic Microscopy and its Advantages

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Abstract—Applying afocal optical systems in microscopy, especially in digital holographic microscopy (DHM) have several advantages. We have investigated some possible implementations theoretically and experimentally as well. Space bandwidth product of an afocal system can exceed that of the conventional ones. Afocal systems provide higher resolution and much less distortions. Furthermore, the computational cost of the numerical reconstruction and correction phase is also lower in the case of an afocal optical setup, as it ensures constant lateral magnification within the whole measured volume. We show that the advantage of low distortion is especially enhanced in the case of color DHM. GPU implementation of reconstruction software is demonstrated.

I. INTRODUCTION

In search of finding the most adequate architecture for in-line or on-axis digital holographic microscopy we have tested several setups. In order to get around the limitations due to the restricted resolution of digital image sensors we inserted a magnifying microscope into a Gabor-like in-line setup [1] and [2] as shown in Fig. 1 and Fig. 3. By the end we have chosen an afocal optical system [3], [4] built with two lenses separated by a distance of e:

\[ e = f_1 + f_2 \] (1)

(where \( f_1 \) and \( f_2 \) are the focal lengths of the sequential lenses) forming a confocal telecentric telescopic system as depicted in Fig. 1 and Fig. 3. Three pigtailed lasers have been used with the fiber-ends tightly put together. These endings formed three point-like Gaussian RGB sources. It illuminated the trough-flow cell containing sparse microorganisms. The waves scattered by the objects are imaged through the lens system onto the CCD or CMOS sensor as object waves and the undisturbed part of the illuminating wave-front serves as reference waves. Their interference field is sampled and recorded by the sensor pixels. The Bayer-pattern filter facilitates recording three separate different color holograms. The cross-talk among the colors is eliminated by a numerical method as described in [5], [6] and [7]. For comparison a single lens system is shown in Fig. 2. For the numerical holographic reconstruction we used the angular spectrum wave propagation algorithm implemented on NVIDIA GPUs. The DHM’s main advantage is that we can record a hologram of a volume larger nearly 1000 times as the traditional microscopes and we can reconstruct the images layer-by-layer at real time speed by parallel processing.

We applied our DHMs in the field of monitoring (with recognition/classification) of water born microorganisms based on a morphological color database (mainly that of algae) and sending an alarm signal in case of surpassing the number of microorganisms in a liter of given limits of harmful or indicator species.

It is true that in the case of the in-line and on-axis versions the zero order and the twin image are overlapping the useful image, generating noises. However, there are numerical methods to diminish these noises [8]–[12].

II. MAGNIFICATION OF A SINGLE LENS VERSUS THAT OF AN AFOCAL OPTICAL SYSTEM: ANALYSIS OF DISTORTION AND RESOLUTION

A. Single Lens System

A single lens system according to Fig. 2 is analyzed. Apart from geometrical optical distortions and aberrations of lenses we have to investigate the inherent distortions of optical systems built with perfect: ideal thin lenses. Using a microscope objective of \( f = 5 \) mm focalength, object space distances from the front focal plane between \( z_1 = 1 \) mm; and \( z_2 = 3 \) mm; we get the following Newtonian image distances accordingly

\[ z'_1 = \frac{f^2}{z_1} = \frac{25}{1} = 25.0 \] (2)

\[ z'_2 = \frac{f^2}{z_2} = \frac{25}{3} = 8.33 \] (3)

The related lateral and longitudinal magnification regions:

\[ \beta_{lat} = \frac{f}{z} = \frac{z'}{f} = 5, ..., 1.67 \] (4)

\[ \beta_{long} = \beta_{lat}^2 = 25, ..., 2.78 \] (5)

As the lateral magnification is \( \beta_{lat} = \frac{z'}{f} \), its slope is

\[ \frac{d\beta_{lat}}{dz'} = \frac{1}{f} = const \] (6)

consequently a cubical object (or object space) will be distorted into a pyramidal square frustum image space shown in
Fig. 1. Optical setup with flow-through cell. The insets shows the measured hologram and its reconstruction.

Fig. 2. That can be easily compensated numerically. However, for the sensor matrix (a CCD or CMOS camera chip) that records the hologram this image is the holographic object. The effective size of the sensor chip — determined by the Nyquist sampling criterium: the interference fringes produced by the waves of the object and the reference beams must be at least twice larger then the sensor pitch — plays as an aperture. The resolution (the size of smallest resolved dot):

$$d_{lat} \simeq \frac{\lambda}{2NA} \tag{7}$$

The smallest numerical aperture NA of the system will limit the resolution. This optical system has two apertures: the first is the objective lens aperture size, the second is the CCD’s size. In this architecture the objective lens has the smallest NA for the farthest object point of the cube (here asquare cross-section is shown):

$$NA_{lens} \simeq \frac{D/2}{z_2 + f} \tag{8}$$

The CCD sensor has the smallest numerical aperture for the farthest image point (the right corner points of the pyramid in Fig. 2): it is the sine of the half viewing angle of the sensor size seen from the image (holographic object) points.

$$NA_{lens} \geq \sin \left( \frac{\theta}{2} \right) \tag{9}$$

We will see bellow that the afocal system results in higher NAs for the worst case (for the farthest points), because there is no enlarged lateral magnification for the farthest image points. In both the single lens case and the afocal case the longitudinal magnification is the square of the lateral magnification.

Having large lateral magnification the farthest image points will be too far-away from the sensor resulting in the decrease of resolution. So, an optimal magnification should be chosen. However, we have to take into consideration that for a magnified image point the necessary $NA_{sensor}$ is decreased by $\frac{1}{\beta_{lat}}$.

B. Afocal System

The two lens afocal system and its advantageous features: It has constant lateral magnification

$$\beta_{lat} = \frac{f_2}{f_1} = \text{const.} \tag{10}$$

but quadratic longitudinal magnification:

$$\beta_{long} = \left( \frac{f_2}{f_1} \right)^2 = \beta_{lat}^2 \tag{11}$$

transforming a cube into a square based prism [4]. This results in lower distortion of the object-fields image, and needs less
correction computations. This is crucially important because only the correct superimposition of the reconstructed RGB images can result in a good quality color image. This registering process can be done in software way by changing the simulated illuminating rays direction until exact overlapping of the RGB images is reached. The numerical aperture of the total system is determined by the smallest $NA_{min}$ within the system times the magnification of the region of $NA_{min}$. Definition of $NA$ for an arbitrary point in the holographic object space: $NA$ is the n times the sine of the half viewing angle of the aperture seen from the object point considered. Where n is the refractive index. From the figures you can see that the CMOS sensor chip’s viewing angle from a distant image point (produced by the lens(es)), that is an object point for the sensor, is much smaller in the single lens case than in the afocal case. The latter one is a telecentric system, this advantageous feature is stemming from the nature of afocality. That is why the afocal optical system can reach a higher resolution for the distant object points.

### III. Measurements

We have built an afocal DHM system with the following parameters:

- $f_1 = 9mm$; $f_2 = 45mm$; $e \simeq 54mm$;
- $\beta_{lat} = 5$; $\beta_{long} = 25$
- size of the sensor surface: 6.10mm(H) x 4.58mm(V)

As the distance between the two lenses was only approximately the sum of their focal lengths, they formed a not precise afocal system.

Because of the slight deviation from exact afocality a minor decrease of field of view and that of the resolved object details is measured. A volume of about 2.5 mm by $1.27 \ mm^2 = 3.2 \ mm^3$ has been imaged and reconstructed.
The lateral resolution of the optical system is about:

\[ d_{lat} \approx \frac{\lambda}{2NA} = \frac{0.532}{2 \times 0.45} \mu m \geq 0.6 \mu m \]

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### IV. Conclusion

From our theoretical and experimental studies it turned out that the in-line DHM setup with a built-in afocal magnifying microscope has higher resolution and even the total space-bandwidth-product (SBWP) is higher than that of the single lens or lenless holography. The SBWP (multiplied by the number of quantization levels) is the measure of the captured and transmitted information. The on-axis afocal setup is even better because in this case the reference beam(s) is(are) undisturbed. The off-axis setup’s great advantage is the inherent spatial separation of the zero order and the twin image from the reconstructed image, however, its great disadvantage lies in the fact that in this case about only 1/8th part of the sensor area is utilized, 7/8th part of the information bearing SBWP is lost. It is true that in the case of the in-line and on-axis versions the zero order and the twin image are overlapping the useful image, generating noises. However, there are numerical methods to diminish these noises. As we have already mentioned, our DHM system is applied mainly for continuous and automatic checking of potable waters, however it is also used for studying the ecological status of natural water bodies [13].

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### REFERENCES


