# A Digital in-line Holographic Microscope using Fresnel Zone Plate

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Abstract: A digital in-line holographic microscope is presented using Fresnel zone plate. The light impinging on the Fresnel zone plate is divided into a number of diffraction orders. We use the 0rd light, which propagate along the original direction, as the reference beam. And the first order focus is used as a virtual point source after which the sample is placed. The light transmitted through the sample is scattered by the object and the structure information is carried by the light. The interference fringes created by the first and zero order diffraction are recorded by a digital camera. Afterwards, the object information is retrieved using reconstruction algorithm. With the aid of the Fresnel zone plate, an image with higher lateral resolution and lower noise could be obtained. This holographic microscope is tested with several samples and the results show that the lateral resolution is good, and for the phase object, the measured phased difference is accurate compared with the AFM test result.

## 1 INTRODUCTION

Digital in-line holographic microscopy (DIHM) is a type of lensless Fourier transform digital holographic, which the object light and reference light are coaxial, interference pattern are recorded digitally by CCD camera. (Depeursinge, 2011; Kreuzer, 2007; Kreuzer, 2010) The amplitude and phase distributions of the object are obtained by simulating the reconstruction process numerically. Compared with traditional microscopy, DIHM is excellent in simple optical path, speed, real-time, wide field, non-contact, and differential-interference-contrast imaging etc. It is widely applied to measure the three-dimensional shape of the diffraction optical components, interference, deformation, vibration, flow field and particle tracking ,etc.

The most widely used the pinhole DIHM is among the numerous methods. (Garcia-Sucerquia, 2006; Xu. 2002; Granero, 2011; Kuznetsova, 2007) Its main factors affecting the imaging have the following several aspects: the structure size and shape of pinhole; area and pixel pitch of image sensor; zero - order image and twin image. In addition to select a more suitable sensor, composite image is an effective way to expand the area through nine images collected by moving CCD.

Twin image is another factor which affects the performace of the DIHM. Many ways have been proposed to reduce its effect on reconstructed image, for instance, off-axis digital holographic, phase shift, twice positions measurement etc. However, these optical system or process of measurement is complicated. So, we proposed a simple way, using conventional diffraction optical element of Fresnel zone plate (FZP) to solve this problem.

The FZP is laid behind the pinhole to separate the point source into the 0th beam and the 1st beam. The sample is put between the focus of the 1st beam and the CCD along the optical axis. The interference fringe pattern, formed by the two beams, is recorded digitally by CCD. In a different way from point diffraction interferometers, the interference fringe pattern is a Fresnel hologram. A novel reconstruction algorithm is proposed to present the object image. Besides, it also has the following advantages: the third problem proposed in previous paragraph will be solved just through single inversion; expands field of view, improves lateral resolution, increases the space around sample plate to be easy integrated.



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# 2 FZP DIHM CONFIGURATION AND RECONSTRUCTION ALGORITHM

## 2.1 DIHM Configuration

The configuration of the FZP DIHM is shown in fig.1. The laser reaches pinhole through attenuation sheet, reflection in turn. A spherical wave of wavelength λ, emanating from the pinhole which is regarded as the point source in pinhole DIHM, illuminates an object. Without FZP, sample is typically a distance of a few thousand wavelengths from the source, and forms a highly magnified diffraction pattern on a CCD much farther away. The point source is major divided into 0rd scattered spherical wave along the original direction and 1rd gathered spherical wave by FZP. Sample is laid behind the 1rd focus so that object information can be scattered and form interference fringes with 0rd reference beam. In order to satisfy the Nyquist sampling criteria, CCD must be far away from sample to ensure the finest interference fringe can be digitally recorded by CCD.

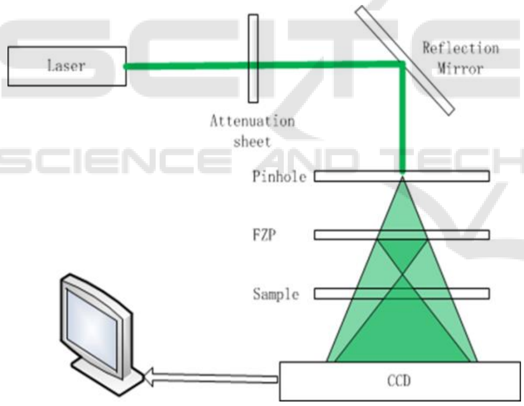


Figure 1: The configuration of the FZP DIHM: laser is focused onto pinhole after attenuation sheet and reflection mirror. The emerging spherical wave is separated major 0rd scattering beam along the original direction as reference light and the scattering object beam after 1rd gathering. The interference pattern or hologram is recorded digitally by CCD.

## 2.2 Interference Pattern

Ideally the reference wave emanating from the pinhole is a spherical wave, irrelevant with FZP,

$A_{ref}(\boldsymbol{r}) = \exp(ikr)/r$, where $k = 2\pi/\lambda$ is the wavenumber. $A_{scat}(\boldsymbol{r})$ denotes the object beam, the interference pattern as follows :

$$\begin{aligned} I(\mathbf{r}) &= |A_{ref}(\mathbf{r}) + A_{scat}(\mathbf{r})|^2 \\ &= |A_{ref}(\mathbf{r})|^2 + |A_{scat}(\mathbf{r})|^2 + A^*_{ref}(\mathbf{r}) A_{scat}(\mathbf{r}) + A_{ref}(\mathbf{r}) A^*_{scat}(\mathbf{r}) \end{aligned} \quad (1)$$

As Eq.1, the third term in the second line is the holographic diffraction pattern in pinhole DIHM, because it arises from the superposition of the interference terms between the along the original direction reference wave from point source and the scattered wave from the object. The fourth term is the conjugate function of previous term and also contains the interference between the scattered waves.

The structure of FZP can be expressed as a coordinate transformation structure as follows

$$t_{FZP}(r,\theta) = \sum_{n=-\infty}^{\infty} c_n \exp(-jnar^2) \quad (2)$$

$c_0 = 1/2$, $c_{\pm1} = \mp 1/\pi$, $c_{\pm2} = 0$, $c_{\pm3} = \pm 1/3\pi \dots$.

Where,

The intensity of higher order diffraction is far below 0rd and 1rd, moreover, 0rd is the reference beam and 1rd is the object beam. So Eq.2 can be simplified only including $C_0$ and $C_1$. Then Eq.1 can be rewritten as follows:

$$I(\mathbf{r}) = \left|\exp(j\phi)/2 + A_{scat}(\mathbf{r})/\pi\right|^2 \quad (3)$$

It is obvious that first term in the second line is constant, does not affect the results of the restoration object. Second term is self-modulation image nearly constant. According to third term, object information can be acquired through reverse diffraction transform. In regard to fourth term, the conjugate image will appear if we do the forward diffraction transform.

## 2.3 Reconstruction Algorithm

We use the angular spectrum method to reconstruct the complex amplitude of the object. The O(x, y, 0) stands for the optical field on the object plane and O(x, y, z) stands for the optical field on the CCD plane. The standard angular spectrum theory give us

$$O(x,y,0) = \mathcal{F}^{-1}\left\{\mathcal{F}\{O(x,y,z)\}\exp\left[jkz\sqrt{1-(\lambda f_x)^2-(\lambda f_y)^2}\right]\right\} \quad (4)$$

Where $f_x$ and $f_y$ are spatial frequencies.

For amplitude object, the structure can be recovered by reckoning the amplitude. Phase object must remove the phase envelope, because the reference light is spherical wave, that the object phase contains a phase envelope of a spherical wave in general. Moreover, maybe there are some dirty points on the CCD. Therefore, we use a symmetrical hologram to restore the object. The equation is as follows:

$$\tilde{I}(x,y)=I(x,y)-I_0(x,y) \qquad (5)$$

The former is the hologram with object, the latter not. Regardless of the type of object, light changes only in the object exist. It is efficient to recover the amplitude or phase object。

## 3 EXPERIMENT RESULTS AND DISCUSSION

In order to ensure good coherence on the surface of CCD, 532nm laser is selected for illumination. After all, green light is sensitive for our eyes and CCD. CCD camera comes from Lumenera Company (pixel pitch of 3.5um, 3000×2208 pixels, size of 10.5mm×7.7mm). The distance between CCD and pinhole is 130±10mm (10mm is used to adjust Z axis of CCD). The purpose of such a design is to manufacture prototype consistent with the ordinary microscope in size. The experimental setup is shown in fig.2.

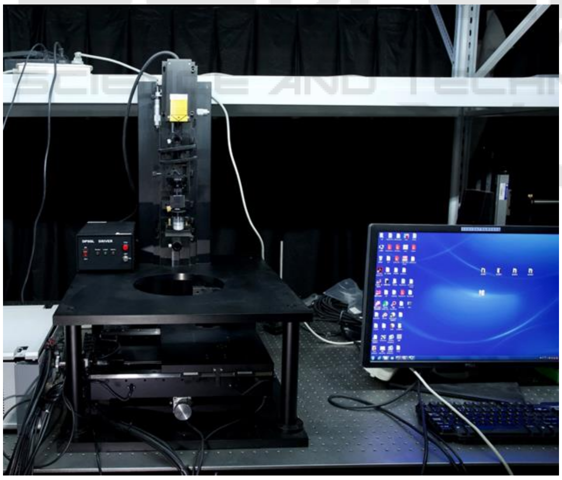

Figure 2: The experimental setup.

One key feature of the DIHM is that it can detect the transparent phase object. Phase object, which is quite common in biological microscopy, does not change the amplitude of the light, but change the phase of the light. Many method, such as Zernike phase contrast or differential interference contrast, has been proposed. However, accurately measure the phase change is still difficult.

To test the capability of our DIHM for phase object detection, we fabricated a silica sample, which is transparent, and with only steps on it, it is a pure phase object. The steps are firstly measure by a atomic force microscope (AFM), the test result is shown in fig.3. According to the test result, the step height is 111nm.

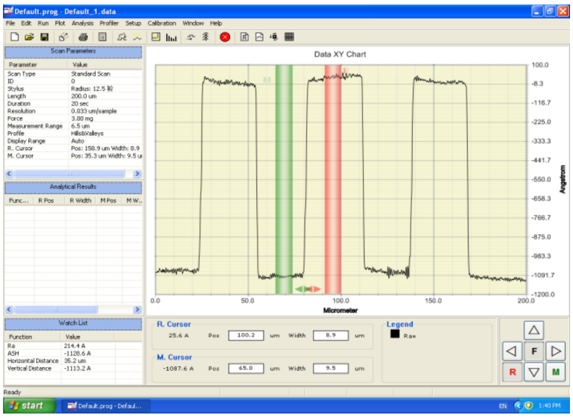

Figure 3: The profile of the silica sample with etched bars.

Then we use the DIHM to measure the height of the bars. The hologram captured by CCD camera is shown in fig.4. Auxiliary spokes is shown in the hologram, for the bars in the center are relatively small and difficult to find .

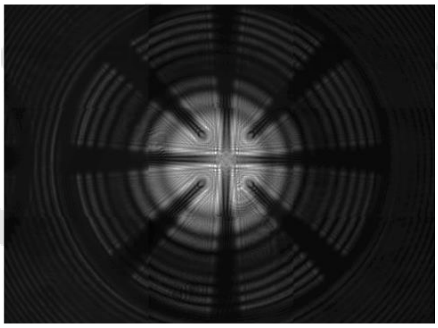

Figure 4: The hologram of the silica phase object.

After the hologram is recorded, we use the numerical method discuss in section II to reconstruct the profile of the bars. The reconstructed image is shown in fig.5 , and the height of the step is also shown, the result is 100nm, which is very close to the result measured by AFM.

To verify the capability of our DIHM, many other samples were observed using our experimental setup. In fig.6, the wings of a fly and a bee is observed in detail, the reconstructed image of the wings shows that our DIHM can be applied to observe varies type of samples.

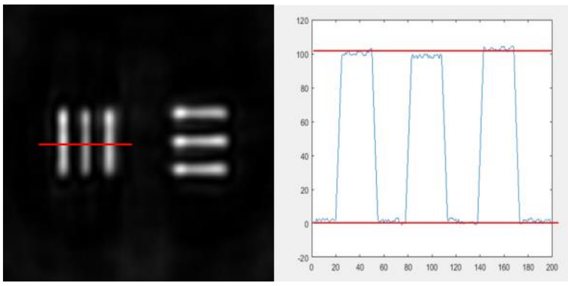


Figure 5: The reconstructed image of the bars and the measured height.

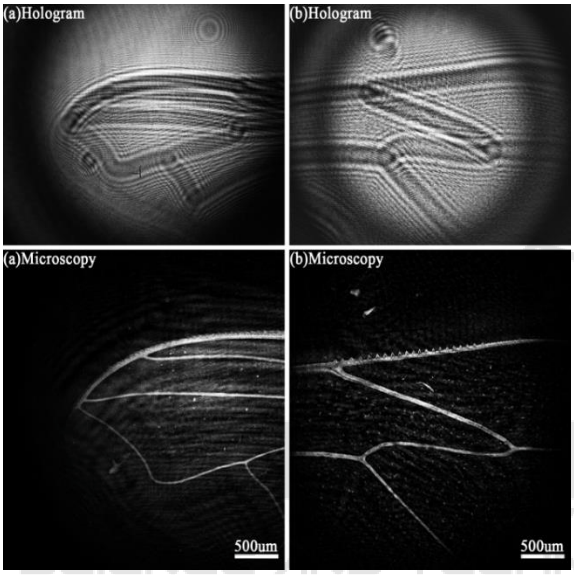


Figure 6: The hologram and the reconstructed image of a fly wing (a) and bee wing (b).

## 4 CONCLUSIONS

Digital in-line holographic microscopy using Fresnel zone plate is a quick, large visual field and no damage measurement method based on pinhole DIHM. 0rd scattered spherical wave and 1rd object spherical wave, emerging from FZP, form the interference fringes on the surface of CCD. 1rd spherical wave is gathered to a virtual point source, and sample is putted behind the focus. So the measurement object is easy to replace, because the distance between FZP and sample is farther than pinhole to sample in pinhole DIHM. Furthermore, the field of view is expanded and the resolution is improved.

Continuing to improve resolution of the optical system, identification of interference fringes must be promoted. Using a smaller pixel pitch CCD, a shorter focal length FZP and composite picture through moving the CCD to collect nine images are efficient ways to make the resolution into Nano-scale at present. Moreover, structured light may be a way to increase contrast ratio between interfere pattern and background and narrowband filter is possible to remove unwanted lights.

The microscopy measurement system can be used in detection of Micro / Nano element, measurement and reconstruction of three-dimensional shape of optical element, biological recognition, path tracking of plankton etc.

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