

## The development of the transmission and reflection type microscope using digital hologram II

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The basic digital hologram principle is described and the transmission and reflection type microscope we are developing is explained in this paper. First, the basic digital hologram principle and the numerical reconstruction process are described. Second, the distinction of this digital hologram microscope layout will be explained.

The basic principle of digital holography involves numerical reconstruction by diffraction process based on Fresnel-Kirchhoff integral in Eq(1).

$$r(\xi, \eta) = \frac{i}{\lambda d} \exp(-i \frac{2\pi}{\lambda} d) \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} R(x, y) h(x, y) \exp\left[-i \frac{\pi}{\lambda} ((\xi - x)^2 + (\eta - y)^2)\right] dx dy. \quad - \text{Eq (1)}$$

In order to reconstruct numerically at distance  $d$  from the surface of the CCD, if the variables are changed and made discrete, the equations becomes <sup>(1),(2),(3)</sup>

$$r(\xi, \eta) = \frac{i}{\lambda d} \exp\left[-i\pi\lambda d \left(\frac{m^2}{N^2 \Delta x^2} + \frac{n^2}{N^2 \Delta y^2}\right)\right] \times \left\langle \sum_{k=0}^{N-1} \sum_{l=0}^{N-1} R(k, l) h(k, l) \exp\left[-i \frac{\pi}{\lambda d} (k^2 \Delta x^2 + l^2 \Delta y^2)\right] \right\rangle \\ \times \exp\left[-i\pi\lambda d \left(\frac{km}{N} + \frac{ln}{N}\right)\right] \quad - \text{Eq (2)}$$

The second and third bracket equations in Eq(2) can be calculated effectively by fast Fourier Transform(FFT). By this numerical reconstruction, not only the intensity but also the phase distribution can be obtained and this is mentioned later more concretely.

After numerical reconstruction, undiffracted reconstruction wave does appear except object information what we want to know. The interference pattern of the object beam and the reference beam from the CCD is obtained. The image obtained by CCD is needed to be reconstructed numerically. The amplitude transmission hologram function is proportional to the recorded intensity by the CDD. In the course of trying to obtain the information about the object, because the object information and the reference information were mixed in the intensity like Eq(3), the image is deformed.

$$I(x, y) = |O(x, y) + R(x, y)|^2 \\ = R(x, y)R^*(x, y) + O(x, y)O^*(x, y) + O(x, y)R^*(x, y) + R(x, y)O^*(x, y). \quad - \text{Eq (3)}$$

These two square terms are called 'DC-term'. To obtain good quality information, the DC-term must be removed: which is called DC- suppression<sup>(4)</sup>.

The phase distribution can be known from the numerical reconstruction. The phase distribution is calculated from the real part and imaginary part of the reconstructed wave like Eq(4).

$$\varphi(\xi, \eta) = \arctan \frac{\text{Im}[r(\xi, \eta)]}{\text{Re}[r(\xi, \eta)]} \quad - \text{Eq (4)}$$

However this phase is wrapped and has ambiguity. Therefore the exact phase distribution can't be known. So, the wrapped phase is needed to be unwrapped. The process is phase unwrapping<sup>(5)</sup>. The phase ambiguity is unwrapped by phase-unwrapping method. There are some algorithms for example, Goldstein's algorithm, Quality-guided path-following algorithm, Mask-cut algorithm. Then, the thin thickness which can't be measured using optical microscope from the unwrapped phase can be gained.

And the distinction of this digital hologram microscope layout in Fig 1 is explained. For microscope, plane wave illumination is essential and difficult. To illuminate object by plane wave using transmission mode is ordinary Machzehnder type. But illuminate object by plane wave using reflection mode is difficult. The idea to solve the problem is adding the focusing lens PIL in Fig.1 to objective lens. This plays a role to illuminate object by perfect plane wave. The RMS wavefront error of lens PIL is 0.001waves in good condition. For example, the beam whose diameter 1.54 $\mu$ m pass through PIL illuminates object field diameter above 300 $\mu$ m about NA 0.8 simulation objective lens<sup>(6),(7)</sup> in Fig 2.

As mentioned above, digital hologram experiment is carried out now. The USAF target was tested, the reconstruction image of that is in Fig 3. After a step, the surface profile of the biological sample will be gained by reflection type and the inter-structure of it at the same position will be gained by transmission type. It is expected that this system will be applied to the field of study like the Biology which 3-D information is needed to.

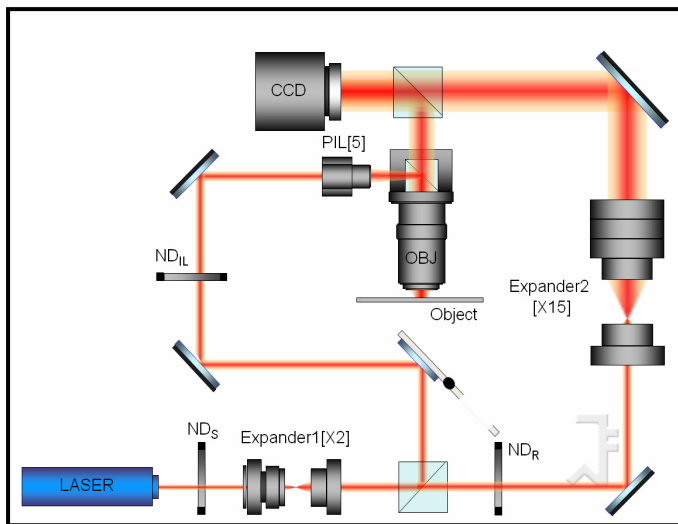


Fig. 1. total system layout

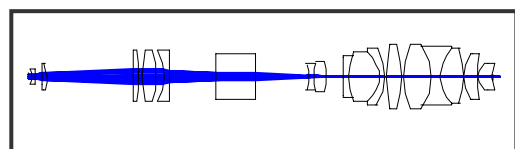


Fig. 2. PIL, BS and objective lens

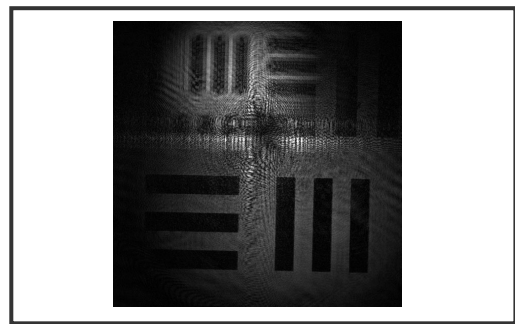


Fig. 3. The reconstruction image

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