5 Digital Holographic Microscopy

5.1 Direct Method

The depth of field of imaging systems decreases with increasing magnification. In microscopy the depth of field is therefore very limited due to the high magnification. The investigation of a three dimensional object with microscopic resolution requires therefore certain refocusing steps. Digital Holography offers the possibility to focus on different object layers by numerical methods. In addition, the images are free of aberrations due to imperfections of optical lenses. Fundamental work in the field of Digital Holographic Microscopy (DHM) has been done by Haddad et al. [46], Kreuzer and Pawlitzek [80], Kebbel, Hartmann and Jüptner [65] and by Coppola et. al. [16, 17].

In order to obtain a high lateral resolution in the reconstructed image the object has to be placed near to the CCD. The necessary distance to obtain a resolution $\Delta\xi'$ with the Fresnel approximation can be estimated with Eq. (3.23):

$$\Delta\xi' = \frac{\lambda d'}{N\Delta x} \qquad ; \qquad \Delta\eta' = \frac{\lambda d'}{N\Delta y} \tag{5.1}$$

The apostrophe is introduced in order to decide between object distance d in the recording process and reconstruction distance d'. We will see that these distances are different for holographic microscopy. With a pixel size of $\Delta x = 10\mu m$, a wavelength of $\lambda = 500nm$, 1000×1000 pixels and a required resolution of $\Delta\xi' = 1\mu m$ a reconstruction distance of $d' = 2cm$ results. At such short distances the Fresnel approximation is no longer valid. The convolution approach has to be applied. On the other hand the resolution of an image calculated by this approach is determined by the pixel size of the CCD, see Eq. (3.33). Typical pixel sizes for high resolution cameras are in the range of $10\mu m \times 10\mu m$, too low for microscopy. Therefore the reconstruction procedure has to be modified.

The lateral magnification of the holographic reconstruction can be derived from the holographic imaging equations, see chapter 2.6.2. According to Eq. (2.70) the lateral magnification of the reconstructed virtual image is:

$$M = \left[1 + \frac{d}{d_r'}\frac{\lambda_1}{\lambda_2} - \frac{d}{d_r}\right]^{-1} \tag{5.2}$$

where d_r and d_r' describe the distances between the source point of a spherical reference wave and the hologram plane in the recording and reconstruction process, respectively. λ_1 and λ_2 are the wavelengths for recording and reconstruction. The reconstruction distance d', i. e. the position of the reconstructed image, can be calculated with Eq. (2.66):

$$d' = \left[\frac{1}{d_r'} + \frac{\lambda_2}{\lambda_1}\frac{1}{d} - \frac{1}{d_r}\frac{\lambda_2}{\lambda_1} \right]^{-1} \tag{5.3}$$

If the same reference wavefront is used for recording and reconstruction it follows $d' = d$. Note that d, d', dr and dr' are always counted positive in this book.

Magnification can be introduced by changing the wavelength or the position of the source point of the reference wave in the reconstruction process. In Digital Holography the magnification can be easily introduced by changing the reference wave source point. If the desired magnification factor is determined, the reconstruction distance can be calculated by combination of Eq. (5.2) and (5.3) with $\lambda_1 = \lambda_2$:

$$d' = d \cdot M \tag{5.4}$$

To enlarge the image the source point of the reference wave needs to be placed at the distance

$$d_r' = \left[\frac{1}{d'} - \frac{1}{d} + \frac{1}{d_r} \right]^{-1} \tag{5.5}$$

The reference wave is now described by

$$E_R(x, y) = \exp\left(-i\frac{2\pi}{\lambda}\sqrt{d_r'^2 + (x - x_r')^2 + (y - y_r')^2} \right) \tag{5.6}$$

where $(x_r', y_r', -d_r')$ is the position of the reference source point in the reconstruction process. The entire process is summarized as follows: After determination of the desired magnification the reconstruction distance d' is calculated with Eq. (5.4). Secondly the source point position d_r' of the spherical reference wave for reconstruction is calculated by Eq. (5.5).

A set-up for digital holographic microscopy is shown in figure 5.1. The object is illuminated in transmission and the spherical reference wave is coupled into the set-up via a semi-transparent mirror. Reference and object wave are guided via optical fibres. For weak scattering objects one can block the external reference wave and work with an in-line configuration.

A digital hologram of a test target is shown in figure 5.2. The corresponding intensity reconstruction is depicted in figure 5.3. The resolution is about 2.2 µm.

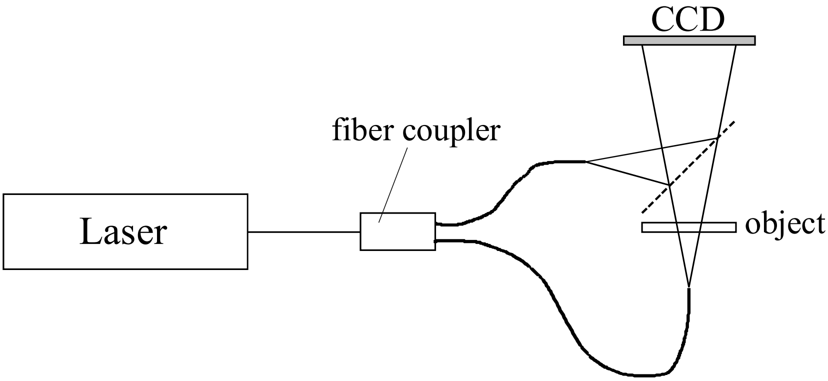

Fig. 5.1. Digital holographic microscope

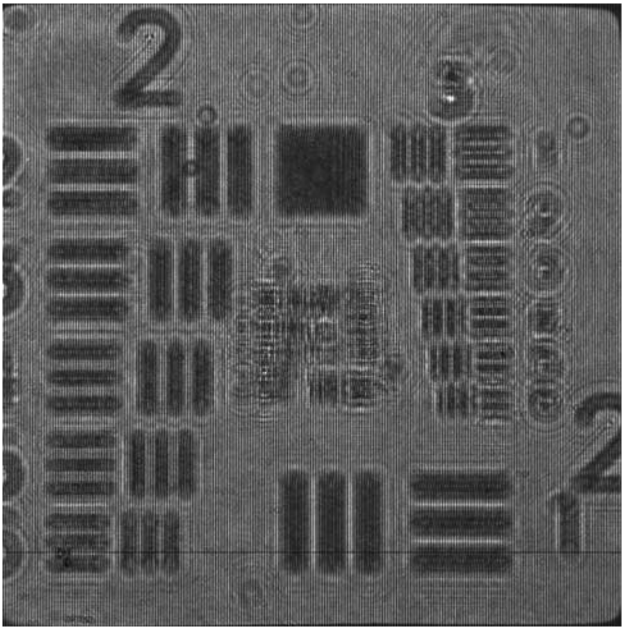

Fig. 5.2. Digital hologram

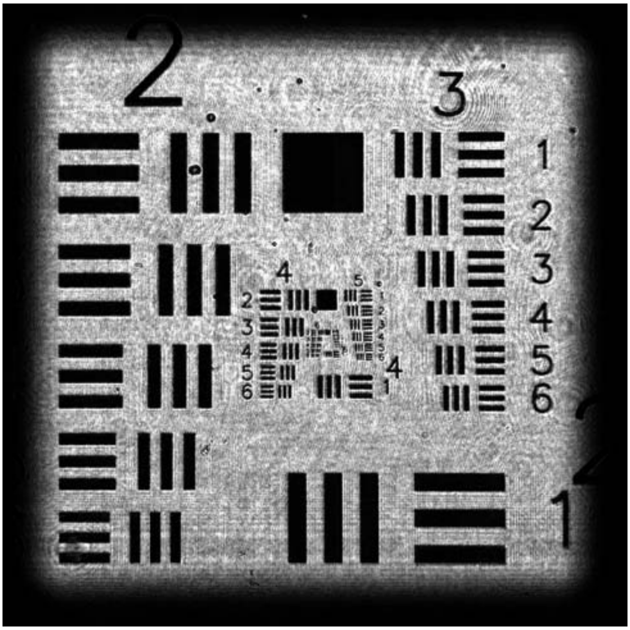

Fig. 5.3. Numerical reconstruction with microscopic resolution

5.2 Phase Shifting Digital Holographic Microscopy

Phase Shifting Digital Holography has been also applied to microscopy [172, 178, 179]. The principle of this method is shown in the set-up of figure 5.4. A light beam is coupled into a Mach-Zehnder interferometer. The sample to be investigated (object) is mounted in one arm of the interferometer. It is imaged onto the CCD target by a microscope objective (MO). A second objective is mounted in the reference arm in order to form a reference wavefront with the same curvature. Both partial waves interfere at the CCD target. An image of the sample superimposed by a coherent background (reference wave) is formed onto the CCD target.

A set of phase shifted images is recorded. The phase shift is realized by a piezo electric transducer (PZT) in the reference arm of the interferometer. From these phase shifted images the complex amplitude of the object wavefront in the image plane can be calculated as described in chapter 3.3.3. Numerical refocusing into any other object plane is now possible with the Fresnel-Kirchhoff integral.

The quality of images recorded with coherent light is in general worse than those recorded with incoherent light due to coherent noise. Dubois, Joannes and Legros developed therefore a phase shifting digital holographic microscope with an ordinary LED as light source [30]. The image quality improves (less speckle noise) due to the reduced spatial coherence of that light source compared to images generated by a laser.

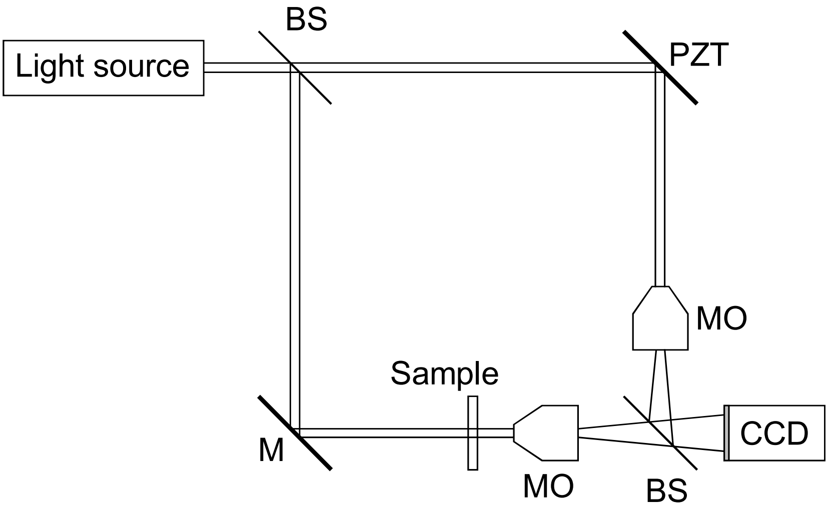

Fig. 5.4. Phase shifting digital holographic microscope