Chapter 2
Theory of Holography

Holography has been used as a tool to determine the 3D motion data of swimming microorganisms hence the basic principle of holography is briefly explained in this chapter. A more detailed explanation of holography can be found in the Ph.D. theses of Dr. R. Barth [1] and Dr. M. Schürmann [2] and in the Diploma thesis of T. Gorniak [3] all carried out in our group. Furthermore, this introduction is based on the corresponding chapters in common textbooks [4–10].

To understand the basic holography principles some general wave phenomena are briefly explained in the following.

2.1 Properties of Light Waves

2.1.1 Intensity

When detecting a light wave the crucial parameter is the intensity of the wave. This is true for a human eye as well as for other detectors [11]. A light wave can be described by the wavefunction [5]

$$\psi(\vec{r}) = A(\vec{r})\ \exp\{i\phi(\vec{r})\} \tag{2.1}$$

where $A(\vec{r})$ is the amplitude and $\phi(\vec{r})$ is the phase. The intensity is defined as the square of the absolute value of the wave function

$$I(\vec{r}) = |\psi(\vec{r})|^2 \tag{2.2}$$

In general, the intensity describes how much energy per time is transported to a plane perpendicular to the wave vector. For plane (I_p) and spherical $(I_s(\vec{r}))$ waves it is [10, 11]

$$I_p = |A|^2 \text{ and } I_s(\vec{r}) = \frac{1}{r^2}|A|^2 \tag{2.3}$$

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2.1.2 Interference

Interference is the superposition of two or more waves. Since the wave equation is a linear differential equation the resulting wave function is the linear combination of the individual functions [5]. For two monochromatic waves $(\psi_1, \psi_2(\vec{r}))$ with equal frequency and polarization the total intensity is

$$I(\vec{r}) = |\psi_1(\vec{r}) + \psi_2(\vec{r})|^2 = I_1 + I_2 + 2\sqrt{I_1 + I_2}\cos\{\phi\}. \quad (2.4)$$

The individual intensities are I_1 and I_2 and the phase difference is $\phi = \phi_1 - \phi_2$. Apparently the total intensity is not the simple sum of the individual intensities, but the so called interference term $2\sqrt{I_1 I_2}\cos\{\phi\}$ has to be added. This term can be positive (constructive interference) or negative (destructive interference) and causes the modulation of the intensity visible as dark and bright fringes [5, 10].

2.1.3 Coherence

The basis for interference phenomena is a constant correlation of the phases of the individual waves. This correlation is called coherence and it is distinguished between temporal and spatial coherence. Temporal coherence is understood as the measure of the average correlation for a wave at two points in time (separated by a delay). If the source is not point like but rather extended in one or two dimensions, spatial coherence describes the ability of two points in space to interfere with each other [5].

2.1.4 Diffraction

Another important phenomenon for (light) waves apart from interference is diffraction. If a wave encounters an obstacle with dimensions in the range of its wavelength diffraction occurs which cannot be explained by geometrical optics. The Huygens' Principle gives a qualitative explanation for diffraction. It states that "every point of a wave front can be considered as a point source for a secondary wave. The wave front at any other place is the coherent superposition of these secondary waves" [5]. When a circular aperture (e.g. a 500 nm pinhole) is used with visible light a spherical wave is generated by diffraction which can be used for illumination in a holographic experiment.

2.2 Holography

Standard photography is widely used to conserve moments, but it has the disadvantage that only a two-dimensional projection of the three-dimensional world is stored. Conventional recording media (e.g. CCD-chip, photo plate, …) only respond to the intensity of the light waves. Therefore, according to Eq. (2.12), the phase information ϕ is lost in the image storing process. If the amplitude A as well as the phase ϕ of a wave front in an image can be reproduced, a perfect image of the object is generated which is impossible to distinguish from the original.

2.2.1 Principle

Denis Gabor observed a possibility to record the phase additionally to the amplitude and subsequently to reconstruct the object wave [12]. Since any light sensitive media—as described above—is only able to store the amplitude of a wave, Gabor encoded the phase information of the object wave ψ_{obj} by recording an interference pattern of the object wave ψ_{obj} and a reference wave ψ_{ref}. According to Eq. (2.4) the observed intensity of the interference pattern is

$$\begin{aligned} I(x,y) &= \left|\psi_{\text{ref}} + \psi_{\text{obj}}\right|^2 \\ &= \psi^*_{\text{ref}}\psi_{\text{ref}} + \psi^*_{\text{ref}}\psi_{\text{obj}} + \psi_{\text{ref}}\psi^*_{\text{obj}} + \psi^*_{\text{obj}}\psi_{\text{obj}} \\ &= A^2_{\text{ref}} + A^2_{\text{obj}} + 2A_{\text{ref}}A_{\text{obj}}\cos\left\{\phi_{\text{ref}} - \phi_{\text{obj}}\right\} \\ &= I_{\text{ref}} + I_{\text{obj}} + 2\sqrt{I_{\text{ref}}I_{\text{obj}}}\cos\left\{\phi_{\text{ref}} - \phi_{\text{obj}}\right\} \end{aligned} \tag{2.5}$$

and is called a hologram [5, 10]. The recorded object can be reconstructed either by illumination of the reference wave [4, 5, 10] ψ_{ref} or by a numerical reconstruction [13]. The latter is widely used today and makes holography feasible for many applications [14–22].

Holography can be performed by using plane or spherical reference waves and it is also possible to work in different setup geometries. Gabor himself used the so called in-line geometry. This setup is also used in the course of this thesis and is therefore introduced in the following. The other geometries like off-axis and Fourier geometries are not discussed but a detailed description can be found in literature [1, 4, 5].

2.2.2 In-line Holography

The characteristics of the in-line geometry are that the source of the reference, the sample and the recording screen are placed on one axis. Typically, the recording screen is orientated perpendicular to the optical axis. In Fig. 2.1, a schematic drawing of Gabors In-line setup is shown.

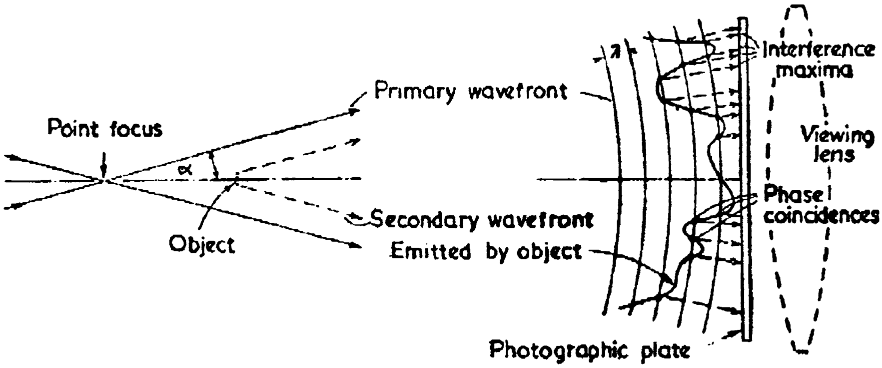


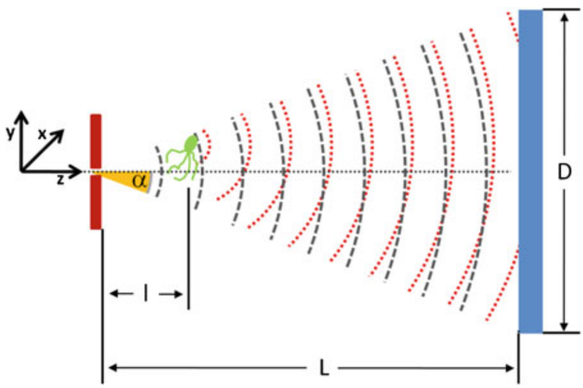
Fig. 2.1 In-line holography setup as published by Gabor in his article "A New Microscope Principle" [12]

The object of interest is placed in a certain distance to the recording screen and is illuminated with a spherical reference wave ψ_{ref} propagating from the source towards the screen. The wave which is diffracted at the sample forms the object wave ψ_{obj} which interferes with the undiffracted part of the reference wave ψ_{ref}. The resulting interference pattern is observed on the recording screen and is called a hologram.

The easiest way to achieve a magnification in the in-line geometry is to use a spherical reference wave for recording and a plane wave with the same wavelength during the reconstruction. If the distance of the recording screen to the point source is L (see Fig. 2.2), and the object is placed in a distance l to the point source, the magnification is [5, 7]

$$M = \frac{L}{l}. \tag{2.6}$$

Fig. 2.2 Schematic drawing of an in-line geometry setup. The pinhole detector distance is L. The sample (*gray*) is positioned in a distance l from the pinhole and the detector size is D. The reference wave $\boldsymbol{\varphi}_{\text{ref}}$ is illustrated in *black*, dashed and the object wave $\boldsymbol{\varphi}_{\text{obj}}$ in *gray*, dotted. The angle\alpha denotes the half-angle of beam spread



Using this approach and especially in combination with a numerical reconstruction, in-line holography can be used as a powerful microscopy technique [15, 17, 18, 20].

2.2.3 Numerical Reconstruction

In digital holography the hologram is recorded digitally and can be reconstructed numerically. To perform this calculation the propagation and thus the complex amplitude of the wave is computed numerically. The derivation of the wave front in a specific plane is called numerical reconstruction [13]. These reconstruction planes correspond to a focus plane in standard light microscopy. Thus, a stack of various amounts of two-dimensional reconstruction planes derived from a single hologram provides information of the complete observation volume.

The reconstruction algorithm is based on the calculation of the Fresnel-Kirchhoff Integral [13, 23]

$$K(\vec{r}) = \iint_S I\left(\vec{\xi}\right) \exp\left\{-\frac{i2\pi}{\lambda}\frac{\vec{\xi}}{\xi}\right\} d\vec{\xi} \tag{2.7}$$

where $\vec{r} = (x, y, z)$ is the position vector indicating a point in the observation plane, $\vec{\xi} = (\xi, \eta, L)$ denotes the coordinates on the screen at a distance L to the point source, λ is the wavelength, and $I\left(\vec{\xi}\right)$ is the intensity pattern of the hologram. The integral extends over the surface S of the recording screen. The Eq. (2.7) is only valid for a spherical source wave and under the assumption of the validity of the Fraunhofer condition [24]. The absolute value of $|K(\vec{r})|$ corresponds to the intensity distribution $I(x, y, z)$ in the reconstructed xy-layers along the z-axis.

In contrast to a photographic plate a CCD chip is discontinuous and consists of discrete pixels. Therefore the coordinates in the Eq. (2.7) have to be expressed as a discrete grid. Due to the non-linearity in the phase vector, Eq. (2.7) is extremely time-consuming to calculate. Kreuzer succeeded to develop an algorithm which is capable of removing the non-linearity in Eq. (2.7) and allows an exact and fast calculation [13]. More details on the algorithm can be found in the patent of Kreuzer [13] or in the Ph.D. thesis of Barth [1].

2.2.4 Resolution

In analogy to standard light microscopy, the resolution in digital in-line holography (DIH) is determined by the numerical aperture (NA) and the used wavelength. In general the NA is defined as

$$\mathrm{NA} := n \cdot \sin\alpha \tag{2.8}$$

where n is the refractive index of the medium (1.0 for vacuum, 1.33 for pure water) and α is the half-angle of beam spread (see Fig. 2.2).

If the used wavelength and the pinhole diameter are of the same order in dimension the achieved resolution is not dependent on the pinhole diameter. This case is achievable if visible light is used as a source wave. In DIH the numerical aperture is determined by the pinhole-detector distance (L, see Fig. 2.2) and the size of the detector (D) if the latter is completely illuminated. Otherwise only the illuminated fraction of the screen has to be taken into account. By using visible light as a source wave it is possible to illuminate the complete detector. The numerical aperture in DIH is given by

$$\mathrm{NA} = \frac{\frac{D}{2}}{\sqrt{L^2 + \left(\frac{D}{2}\right)^2}}. \tag{2.9}$$

Since $L \gg D$ the approximation $\sqrt{L^2 + \left(\frac{D}{2}\right)^2} \cong L$ is valid. Therefore the NA in DIHM is given as

$$\mathrm{NA} = \frac{\frac{D}{2}}{L}. \tag{2.10}$$

The achievable resolution in DIH is subject to many studies and a detailed explanation can be found in Jericho et al. [16], Garcia-Sucerquia et al. [25] and Barth [1]. For the theoretical lateral and depth resolution follows

$$\delta_{\mathrm{lateral}} = \frac{\lambda}{\mathrm{NA}} \tag{2.11}$$

$$\delta_{\mathrm{depth}} = \frac{\lambda}{2(\mathrm{NA})^2}. \tag{2.12}$$

According to those equations the achievable resolution for the setup used in the course of this thesis is

$$\delta_{\mathrm{lateral}} = 2.3\ \mu\mathrm{m} \text{ and } \delta_{\mathrm{depth}} = 5.6\ \mu\mathrm{m}. \tag{2.13}$$

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