Digital holography for quantitative phase-contrast imaging

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Short summary:

In the paper, they have presented a new application of digital holography for phase-contrast imaging. The technique uses a CCD camera for recording of a digital Fresnel off-axis hologram and a numerical method for hologram reconstruction. The method simultaneously provides an amplitude-contrast image and a quantitative phase-contrast image.

I. INTRODUCTION

The holographic process is described mathematically as follows:

 $O(x, y) = o(x, y) e^{i\varphi_o(x, y)}$... (1.1)

Is the complex amplitude of the object wave with real amplitude o and phase $\, \varphi_o \,$ and

 $R(x, y) = r(x, y) e^{i\phi_R(x, y)}$... (1.2)

Is the complex amplitude of the reference wave with real amplitude r and phase φ_R

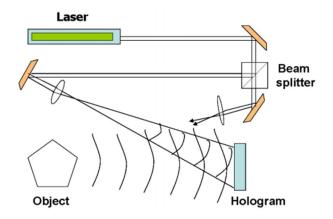


Fig. 1 Construction of a hologram

Both waves interfere at the surface of the recording medium. The intensity is calculated by

$$I_{H}(x, y) = |O(x, y) + R(x, y)|^{2}$$

= (O(x, y) + R(x, y))(O(x, y) + R(x, y))^{*}

$$= R(x, y)R^{*}(x, y) + O(x, y)O^{*}(x, y) + O(x, y)O^{*}(x, y) + O(x, y)R^{*}(x, y) + R(x, y)O^{*}(x, y) \qquad \dots (1.3)$$

$$I_{H}(x, y) = |R|^{2} + |O|^{2} + R^{*}O + RO^{*} \qquad \dots (1.4)$$

Digital Recording

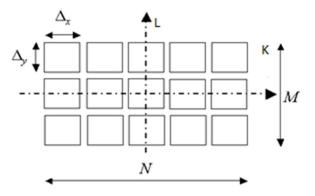


Fig. 2 Concept diagram of a surface-pixel sensor

The digital recording will (depending on the directions x and y on the recording plane) consist of M x N pixels. Each of these pixels is of a dimension $\Delta_x \times \Delta_y$. In CCD sensors, Matrices made up of photosensitive elements called

pixels are generally square-shaped. In our case it is $12 \times 12 \mu m$

In their case, the hologram intensity was recorded by a standard black and white CCD camera (Hitachi Denshi KP-M2).

The two neutral-density filters allow the adjustment of the object and the reference intensities.

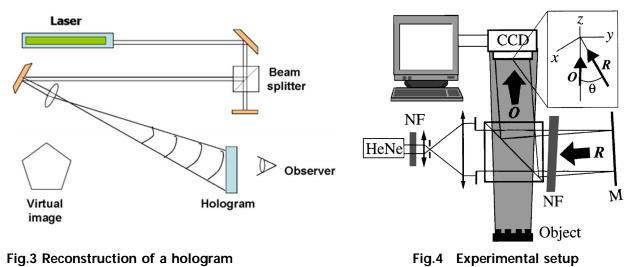
A square image of area LxL (Sensor size) = 4.83mm X 4.83mm containing N x N= 512 x 512 pixels is acquired in the center of the CCD sensor, and a digital hologram is transmitted to a computer via a frame grabber.

The digital hologram $I_{H}(k,l)$ results from two-dimensional spatial sampling of $I_{H}(x, y)$ by the CCD:

$$I_{H}(k,l) = I_{H}(x,y)rect\left(\frac{x}{L},\frac{y}{L}\right) \times \sum_{k}^{N} \sum_{l}^{N} \delta(x-k\Delta x, y-l\Delta y) \qquad \dots (1.5)$$

Where k and I are integers $(-N/2 \le k, l \le N/2)$ and Δx and Δy are the sampling intervals in the hologram plane i.e. pixel size: $\Delta x = \Delta y = L/N$

II. NUMERICAL RESULTS



A wave front $\psi_H(x, y) = R(x, y)I(x, y)$ is transmitted by a hologram and propagates toward an observation plane, where a three-dimensional image of the object can be observed.

For reconstructing a digital hologram, a digital transmitted wave front $\psi_H(k\Delta x, l\Delta y)$ is computed by multiplication of digital hologram $I_H(k, l)$ by a digital computed reference wave, $R_D(k, l)$, called the digital reference wave.

If we assume that mirror M reflects a plane wave of wavelength λ then R_D can be calculated as follows:

$$R_D(k,l) = \underbrace{A_R}_{1} \exp\left[i\frac{2\pi}{\lambda}(k_xk\Delta x + k_yl\Delta y)\right] \qquad \dots (1.6)$$

Where k_x and k_y are the two components of the wave vector and A_r is the amplitude. $k_x = -3.12 \times 10^{-3}$ and $k_y = -5.34 \times 10^{-3}$

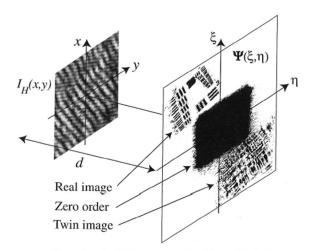
Taking into account the definition of hologram intensity [Eq. 1.4], we have

$$\psi \ (k\Delta x, \Delta y) = R_D(k, l) I_H(k, l) \qquad \dots (1.7)$$
$$= R \ |R|^2 + R \ |Q|^2 + R \ R^* Q + R \ R Q^*$$

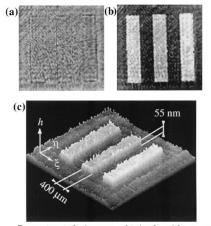
$$= \underbrace{R_D \left| R \right|^2 + R_D \left| O \right|^2}_{Zero \ order \ of \ diffraction} + \underbrace{R_D R^* O}_{Twin \ image} + \underbrace{R_D RO^*}_{real \ image}$$

To avoid an overlap of these three components of ψ during reconstruction, they recorded the hologram in the so-called off-axis geometry. For this purpose the mirror in the reference arm, M is oriented such that the reference wave **R** reaches the CCD at an incidence angle θ . The value θ must be sufficiently large to ensure separation between the real and the twin images in the observation planes. However, θ must not exceed a given value so that the spatial frequency of the interferogram does not exceed the resolving power of the CCD.

$$\theta \le \theta_{\max} = arc \sin\left(\frac{\lambda}{2\Delta x}\right)$$
 ... (1.8)



Geometry for hologram reconstruction. ∂xy , hologram plane; $\partial \xi \eta$, observation plane; d, reconstruction distance; $\Psi(\xi, \eta)$, reconstructed wave front.



Reconstructed images obtained with a pure phase object: (a) amplitude contrast, (b) phase contrast, (c) three-dimensional perspective of the reconstructed height distribution (the vertical scale is not equal to the transverse scale).

The reconstructed wave front $\psi(m\Delta\xi, n\Delta\eta)$, at a distance d from the hologram plane, is computed by use of a discrete expression of the Fresnel integral:

$$\psi(m\Delta\xi, n\Delta\eta) = A \exp\left[\frac{i\pi}{\lambda d} (m^2 \Delta\xi^2 + n^2 \Delta\eta^2)\right] \times FFT \left\{ R_D(k,l) I_H(k,l) \exp\left[\frac{i\pi}{\lambda d} (k^2 \Delta x^2 + l^2 \Delta y^2)\right] \right\}_{m,n} \qquad \dots (1.9)$$

Where m and n are integers $(-N/2 \le m, n \le N/2)$, FFT is the fast Fourier transform operator, and $A = \exp(i2\pi d/\lambda)/(i\lambda d)$

 $\Delta \xi$ and $\Delta \eta$ are the sampling intervals in the observation plane and define the transverse resolution of the reconstructed image.

This transverse resolution is related to the size of the CCD (L) and to the distance d by,

$$\Delta \xi = \Delta \eta = \lambda d / L \qquad \dots (1.10)$$

The reconstructed wave front is an array of complex numbers. The amplitude and the phase contrast images can be obtained by calculation of the square modulus.

References

- [1] M.Pluta, Non-standard techniques for phase contrast microscopy, Vol. 6 of Advances in Optical and Electron Microscopy
- [2] U.Schnars and W. Juptner, Appl. Opt. 33, 179 (1994)
- [3] E. Cuche, P. Poscio, and C. Depeursinge, Proc. SPIE 2927, 61 (1996)