Optical Signal Processing

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Chapter 6
Digital holography for quantitative phase imaging

Digital Holography

- **A digital holography method uses a CCD camera for hologram recording and a numerical method for hologram reconstruction**.
- The reconstruction method simultaneously provides an amplitude and a phase image of the specimen, on the basis of a single hologram.
- Digital holography produces a quantitative phase contrast, meaning that the obtained phase distribution is equal, modulo 2π, to the phase distribution at the surface of the sample.

Digital Holography

- **<u>This means that the obtained phase-contrast image can be directly used for quantitative measurements such as surface profilometry.</u>**
- As shown in Fig. 1, the experimental setup basically consists of a Michelson inteferometer.

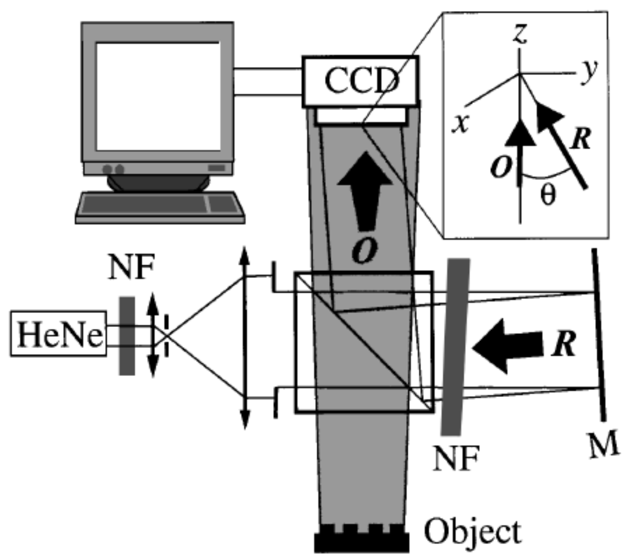

Fig. 1. Experimental setup: NF's, neutral-density filter's; M, mirror; **O**, object wave; **R**, reference wave. Inset, detail showing the off-axis geometry at the incidence of **O** upon the CCD.

Digital Holography

- The beam of a HeNe laser is enlarged to a diameter of ~20 mm by a beam expander including a spatial filter.
- At the exit of the interferometer a hologram is created by the interference between the object wave, **O**(*x*, *y*), and the reference wave, **R**(*x*, *y*).
- **The hologram intensity, is recorded by a standard black-and-white CCD camera**.

$$I_H(x, y) = |\mathbf{R}|^2 + |\mathbf{O}|^2 + \mathbf{R}^*\mathbf{O} + \mathbf{R}\mathbf{O}^*, \qquad (1)$$

- A square image of area $L \times L$ = 4.83 mm × 4.83 mm containing $N \times N$ = 512 × 512 pixels is acquired in the center of the CCD sensor, and a **digital hologram is transmitted to a computer**.

Digital Holography

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

$$I_H(k,l) = I_H(x,y)\mathrm{rect}\left(\frac{x}{L},\frac{y}{L}\right) \times \sum_{k}^{N}\sum_{l}^{N}\delta(x - k\Delta x, y - l\Delta y), \qquad (2)$$

- where k and l are integers ($-N/2 \le k, l \le N/2$) and Δx and Δy are the sampling intervals in the hologram plane; $\Delta x = \Delta y = L/N$.

Digital Holography

- The numerical method that we are using for hologram reconstruction simulates the standard optical reconstruction of a hologram.
- In classical holography reconstruction is achieved by illumination of the hologram with a replica of the reference wave.
- A wave front $\mathbf{\Psi}(x, y)=\mathbf{R}(x, y)I_H(x, y)$ is transmitted by the hologram and propagates toward an observation plane.
- **<u>A three-dimensional image of the object can be observed.</u>**

Digital Holography

- As we reconstruct a digital hologram, a digital transmitted wave front $\mathbf{\Psi}(k\Delta x, l\Delta y)$ is computed by multiplication of digital hologram $I_H(k, l)$ by a digitally computed reference wave, $\mathbf{R}_D(k, l)$, called the digital reference wave.
- **Taking into account the definition of the hologram intensity [Eq. (1)], we have**

$$\mathbf{\Psi}(k\Delta x, l\Delta y) = \mathbf{R}_D(k,l)I_H(k,l) = \mathbf{R}_D|\mathbf{R}|^2 + \mathbf{R}_D|\mathbf{O}|^2 + \mathbf{R}_D\mathbf{R}^*\mathbf{O} + \mathbf{R}_D\mathbf{R}\mathbf{O}^* . \qquad (3)$$

- **The first two terms of Eq. (3) correspond to the zero order of diffraction, the third term to the twin image, and the fourth to the real image.**

Digital Holography

- To avoid an overlap of these three components of **Ψ** during reconstruction, we recorded the hologram in the so-called **off-axis geometry**.
- For this, the mirror in the reference arm, M, is oriented such that the reference wave **R** reaches the CCD at an incidence angle θ (see Fig. 1).
- The value of θ must be sufficiently large to ensure separation between the real and the twin images in the observation plane.
- **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.**

Digital Holography

- For phase-contrast imaging, $\mathbf{R}_D$ must be a digital replica of the reference wave that was used for hologram creation (**R**).
- As Eq. (3) shows, we see that if $\mathbf{R}_D$ is equal to **R**, the product $\mathbf{R}_D\mathbf{R}^*$ becomes $\mathbf{RR}^*=|\mathbf{R}|^2$ and the phase of the twin image (**O**) can be reconstructed.
- $\mathbf{R}_D$ can be a replica of the complex conjugate of **R**.
- In this case the phase of the real image (**O***) can be reconstructed.

Digital Holography

- **<u>If we assume that mirror M reflects a plane wave of wavelength λ, R_D can be calculated as follows:</u>**

$$\mathbf{R}_D(k,l) = A_R \exp\left[i \frac{2\pi}{\lambda} (k_x k \Delta x + k_y l \Delta y) \right], \qquad (4)$$

- where k_x and k_y are the two components of the wave vector and A_R is the amplitude.
- The digital transmitted wave front $\boldsymbol{\Psi}(k\Delta x, l\Delta y)$ is defined in the hologram plane Oxy.
- **<u>The propagation of $\Psi(k\Delta x, l\Delta y)$ is simulated by a numerical calculation of scalar diffraction in the Fresnel approximation.</u>**

Digital Holography

- **The reconstructed wave front Ψ(*m*Δ*ξ*, *n*Δ*η*), at a distance *d* from the hologram plane, in an observation plane *Oξη*, is computed by use of a discrete expression of the Fresnel integral:**

$$\begin{aligned}\Psi(m\Delta\xi, n\Delta\eta) &= A \exp\left[\frac{i\pi}{\lambda d}(m^2\Delta\xi^2 + n^2\Delta\eta^2)\right] \\ &\times \mathrm{FFT}\left\{\mathbf{R}_D(k,l) I_H(k,l) \exp\left[\frac{i\pi}{\lambda d}(k^2\Delta x^2 + l^2\Delta y^2)\right]\right\}_{m,n},\end{aligned} \tag{5}$$

- where *m* and *n* are integers (-*N*/2 ≤ *m*, *n* ≤ *N*/2), FFT is the fast Fourier transform operator, and *A*=exp(*i*2π*d*/*λ*)/(*iλd*).
- Δ*ξ* and Δ*η* are the sampling intervals in the observation plane, and they define the transverse resolution of the reconstructed images.

Digital Holography

- <u>**This transverse resolution is related to the size of the CCD (*L*) and to the distance *d* by (see Chap. 3)**</u>

$$\Delta\xi = \Delta\eta = \lambda d / L\,. \qquad (6)$$

- Note that from Eq. (5) we know that $\lambda d f_x \to \xi,\ \Delta f_x = \dfrac{1}{L}$ so $\Delta\xi = \lambda d / L.$
- 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 $[\mathrm{Re}(\boldsymbol{\Psi})^2] + [\mathrm{Im}(\boldsymbol{\Psi})^2]$ and by the argument $\{\mathrm{atan}[\mathrm{Im}(\boldsymbol{\Psi})/\mathrm{Re}(\boldsymbol{\Psi})]\}$ of $\boldsymbol{\Psi}(m\Delta\xi, n\Delta\eta)$, respectively.

Digital Holography

- The performance of the technique for quantitative phase-contrast imaging was tested with a pure phase object (USAF 1950).
- An example of the results obtained with this object is shown in Fig. 2.

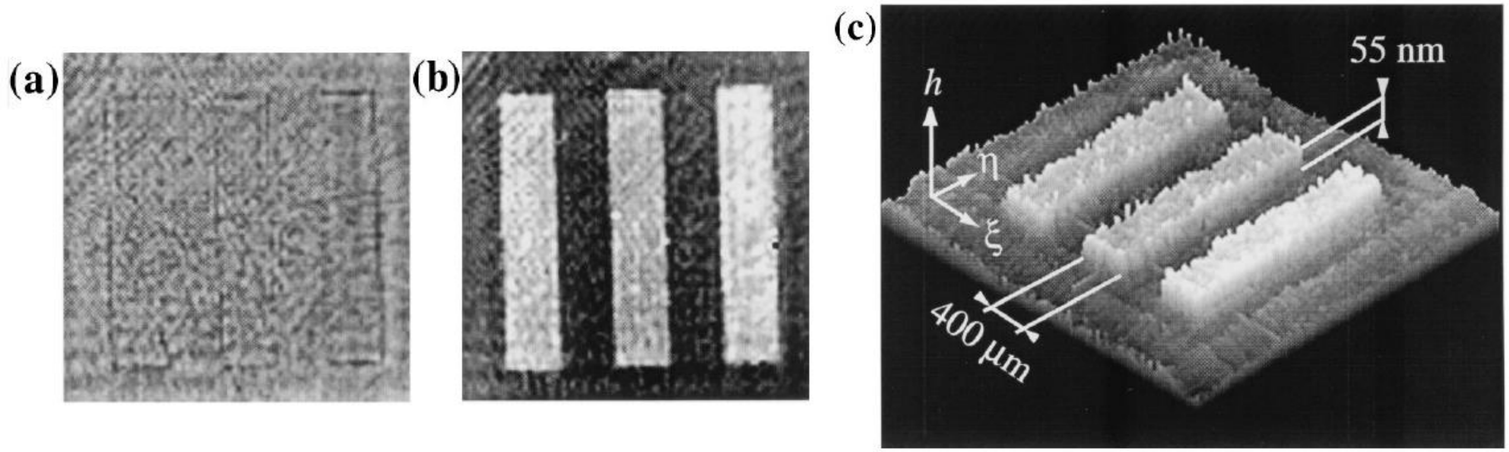

Fig. 2. Reconstructed images obtained with a pure phase object: (a) amplitude contrast, (b) phase contrast, (c) three-dimensional perspective of the reconstructed height distribution (the vertical scale is not equal to the transverse scale).

Digital Holography

- **<u>The reconstructed images present the third element of group 0 of the USAF test target.</u>**
- As expected for such a pure phase object, no contrast in amplitude appears [Fig. 2(a)] because the optical properties are the same over the entire surface of the sample.
- However, the sides of the USAF elements can be distinguished because of edge diffraction.
- **<u>In contrast, one can see in Fig. 2(b) that the elements are clearly revealed in the phase-contrast image.</u>**

Digital Holography

- As shown in three-dimensional perspective of Fig. 2(c), this phase contrast reveals the topography of the sample.
- The height distribution $h(\xi, \eta)$ on the sample surface is simply proportional to the reconstructed phase distribution $\varphi(\xi, \eta)$:

$$h(\xi, \eta) = (\lambda/4\pi)\phi(\xi, \eta). \qquad (7)$$

- **<u>As usual with interferometric techniques, the values of the measured phase are restricted to the [-π, π] interval.</u>**
- **<u>Height differences greater than $\lambda/2$ can be resolved by standard phase-unwrapping methods.</u>**

Digital Holography

- As established by Eqs. (4) and (5), the numerical method for hologram reconstruction involves four parameters, A_R, k_x, k_y, and d.
- **<u>To obtain well-focused reconstructed images we must ensure that the *d* value corresponds precisely to the distance between the object and the CCD during hologram recording.</u>**
- For Fig. 2, d=35.1 cm and the three other parameters are related to $\mathbf{R}_D$ [see Eq. (4)].
- The adjustment of amplitude A_R is not of particular importance, and its value is generally set to unity (A_R=1).

Digital Holography

- The values of k_x and k_y require precise adjustment because these parameters define the phase of the digital reference wave.
- **Their values must be adjusted such that the wave fronts of R_D match as closely as possible the wave fronts of the complex conjugate of the reference wave R.**
- For Fig. 2, the values of these parameters are $k_x = -3.12 \times 10^{-3}$ and $k_y = -5.34 \times 10^{-3}$.

Digital Holography

- The reconstructed phase distribution can also be used for quantitative measurements, as is illustrated in Fig. 3 for surface profilometry.
- A phase profile was extracted from the data corresponding to the image presented in Fig. 2(c).

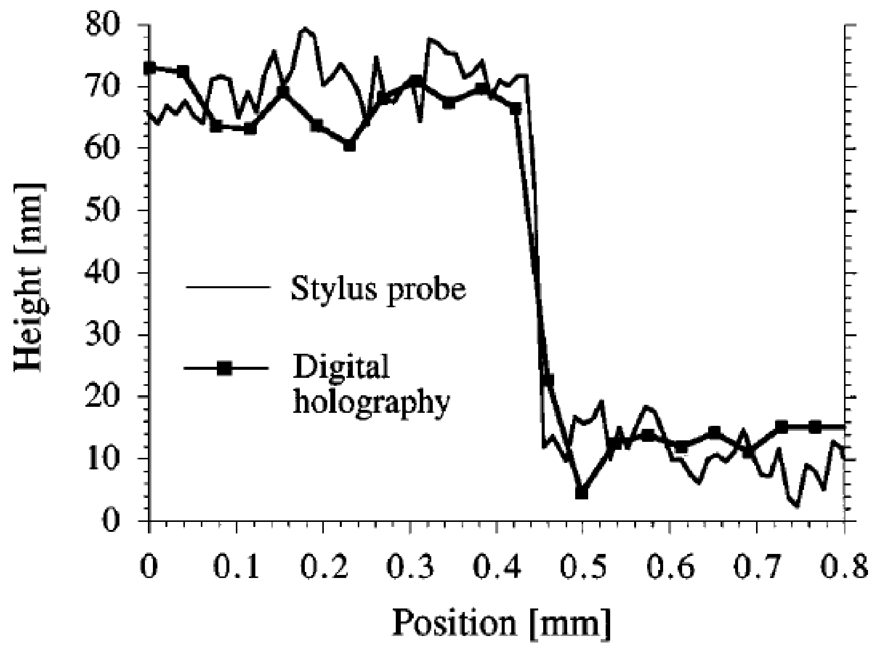

Fig. 3. Step-height measurement.

Digital Holography

- The measured phase values were converted to height measurements by use of Eq. (7) and recorded in the graph shown in Fig. 3.
- **We can see that a step height of ~55 nm is measured with digital holography.**
- For comparison, the same step height was measured by scanning of a contact-stylus probe profilometer (Alpha-step 500) over the corresponding area of the sample: as shown in Fig. 3, the techniques are in excellent agreement.
- **With this surface roughness a resolution better than 10 nm can be estimated for step-height measurement with digital holography.**

Digital Holography

- **<u>Digital holography is a method for simultaneous amplitude- and phase-contrast imaging.</u>**
- In addition, the obtained phase image provides a quantitative measurement of the optical phase distribution at the surface of the object and can be used for many applications.
- The transverse resolution can be improved by addition of magnification optics to the object arm of the interferometer.

Digital Holographic Microscopy

- **The idea of reconstructing a hologram with a computer was proposed for the first time more than 30 years ago by Goodman**.
- At that time the main drawbacks of this numerical approach of holography were the insufficient performance of computers and the lack of adequate devices for digital image acquisition.
- Today these handicaps have been suppressed, and digital holography can be performed efficiently and inexpensively with a charged-coupled device (CCD) camera for hologram recording and a personal computer for the reconstruction.

Digital Holographic Microscopy

- **Digital holography has been applied in various domains such as position measurement, endoscopy, optical coherence tomography, and biomedical microscopy.**
- In a previous lecture, we showed that phase reconstruction is possible by multiplication of the hologram with a computed replica of the reference wave.
- It was also demonstrated that the obtained phase contrast is quantitative and can be used directly for applications in optical metrology.

Digital Holographic Microscopy

- Attractive features of this new imaging technique are the high acquisition rate (video frequency) and the high reconstruction rate, the ability to reconstruct simultaneously an amplitude-contrast and a phase-contrast image.
- It provides precise quantitative information about the three-dimensional structure of the specimen by computational means with a single hologram.
- In the previous optical setup, holograms were recorded with a Michelson interferometer with an object wave directly reflected by the specimen.
- **In this configuration the transverse resolution was limited to approximately 30μm.**

Digital Holographic Microscopy

- **We study a new configuration for microscopic investigations with the same transverse resolution as with classical optical microscopy**.
- This instrument is called a digital holographic microscopy and consists of a microscope objective (MO) that produces a magnified image of the sample that is an object for the hologram creation.
- Since a phase aberration is associated with the use of a MO, a digital method can be developed to correct this aberration.

Digital Holographic Microscopy Designs

- **<u>Two kinds of digital holographic microscopy are presented</u>:** the first one (Fig. 1) is designed for transmission imaging with transparent samples (e.g., biological cells) and the second one (Fig. 2) for reflection imaging.

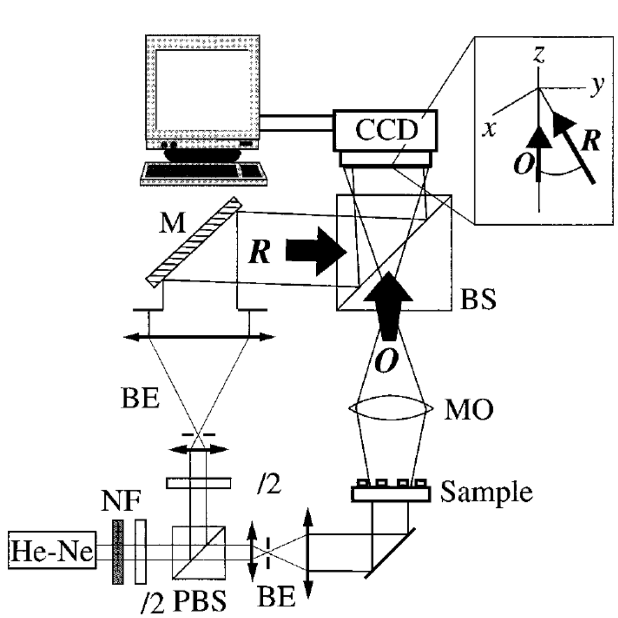

Fig. 1. Schematic of the holographic microscope for transmission imaging. NF, neutral density filter; PBS, polarizing beam splitter; BE, beam expander with spatial filter; $\lambda/2$, half-wave plate; M, mirror; BS, beam splitter; **O**, object wave; **R**, reference wave. Inset: detail showing the off-axis geometry at the incidence on the CCD.

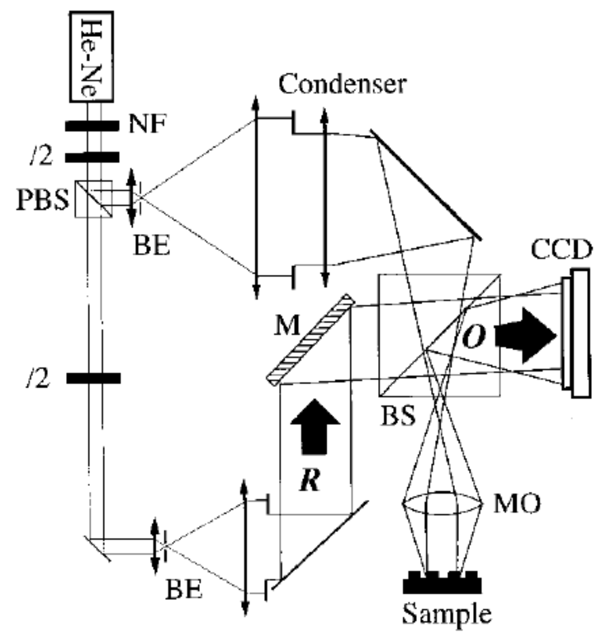

Fig. 2. Schematic of the holographic microscope for reflection imaging. NF, neutral density filter; PBS, polarizing beam splitter; BE, beam expander with spatial filter; $\lambda/2$, half-wave plate; M, mirror; BS, beam splitter; **O**, object wave; **R**, reference wave.

Digital Holographic Microscopy Designs

- **<u>In both cases the basic architecture is that of a Mach–Zender interferometer.</u>**
- For the present experiments a linearly polarized HeNe laser (10mW) is used as a light source.
- The combination of a neutral density filter, wave plate, and a polarizing beam splitter is used for the adjustment of the intensities in the reference arm and the object arm of the interferometer.
- In the reference arm a half-wave plate is introduced to obtain parallel polarizations at the exit of the interferometer.

Digital Holographic Microscopy Designs

- In each arm, beam expanders, including pinholes for spatial filtering, are introduced to produce plane waves.
- **For transmission imaging (Fig. 1), the specimen is illuminated by a plane wave and the transmitted light is collected by a MO that produces a wave front called object wave O.**
- For reflection imaging (Fig. 2) a lens with a long focal length is inserted between the beam expander and the MO.
- This lens position is adjusted to illuminate the sample with a collimated beam.

Digital Holographic Microscopy Designs

- At the exit of the interferometer the interference between the object wave **O** and the reference wave **R** creates the hologram intensity,

$$I_H(x, y) = |\mathbf{R}|^2 + |\mathbf{O}|^2 + \mathbf{R}^*\mathbf{O} + \mathbf{R}\mathbf{O}^*, \qquad (1)$$

- where **R*** and **O*** are the complex conjugates of the reference wave and the object wave.
- The off-axis geometry is used: the mirror that reflects the reference wave (M) is oriented such that the reference wave reaches the CCD camera with a small angle θ (see Fig.1) with respect to the propagation direction of the object wave.
- A digital hologram is recorded by a CCD camera and transmitted to a computer.

Digital Holographic Microscopy Designs

- The digital hologram $I_H(k,l)$ is an array of $N \times N$ that results from the two-dimensional sampling of $I_H(x,y)$ by the CCD camera:

$$I_H(k, l) = I_H(x, y)\mathrm{rect}\left(\frac{x}{L}, \frac{y}{L}\right) \times \sum_{k=-N/2}^{N/2} \sum_{l=-N/2}^{N/2} \delta(x - k\Delta x, y - l\Delta y), \tag{2}$$

- **Holographic microscopy has been proposed in various configurations: we are working with a geometry including a MO.**

Digital Holographic Microscopy Designs

- **<u>The optical arrangement in the object arm is an ordinary single-lens imaging system (see Fig. 3).</u>**

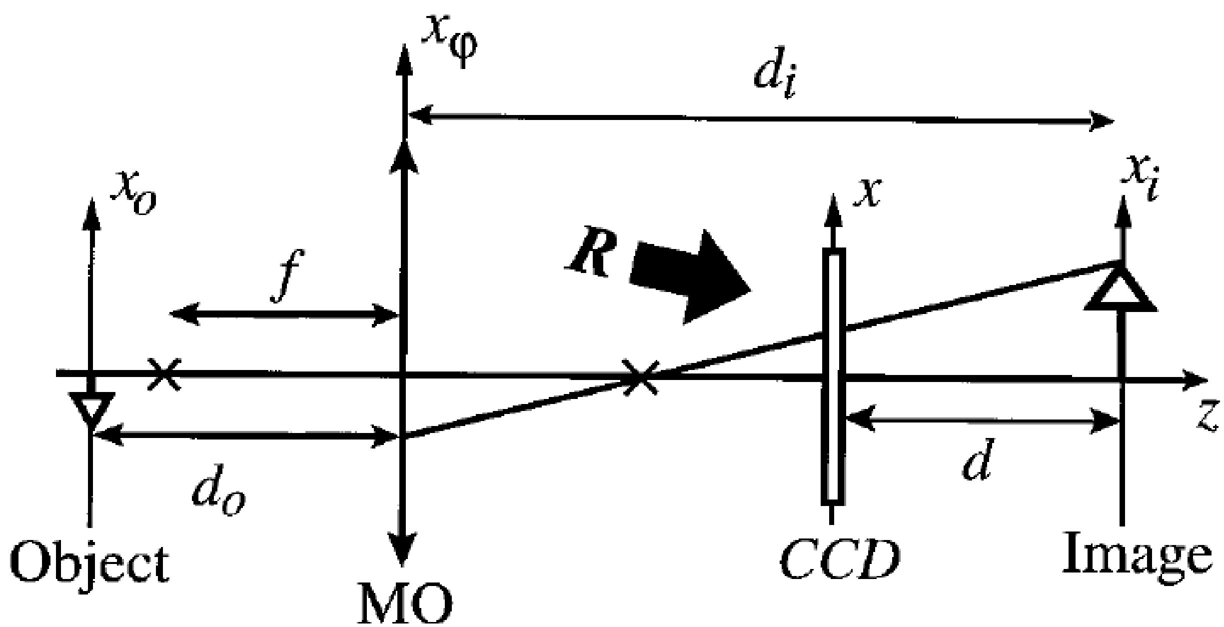

Fig. 3. Configuration for holographic microscopy.

Digital Holographic Microscopy Designs

- The MO produces a magnified image of the object, and the hologram plane $0x$ (the CCD plane) is located between the MO and the image plane ($0x_i$), at a distance d from the image.
- This situation can be considered to be equivalent to a holographic configuration without magnification optics with an object wave emerging directly from the magnified image.
- For this reason the term **image holography** is sometimes used to designate this procedure.
- **Classical microscopy can be achieved with this arrangement by translation of the object or the hologram plane such that the image is focused on the CCD.**

Digital Holographic Microscopy Designs

- A particular case arises when the specimen is located in the object focal plane of the MO.
- In this case the distance between the image and the MO (d_i) is infinite, the hologram is recorded with the Fourier transform of the object field.
- **The reconstruction can be performed by the Fourier transform of the hologram.**
- **The numerical reconstruction method consists basically of calculating the Fresnel diffraction pattern of the hologram.**

Hologram Reconstruction

- **<u>Figure 4 defines the considered geometry.</u>**

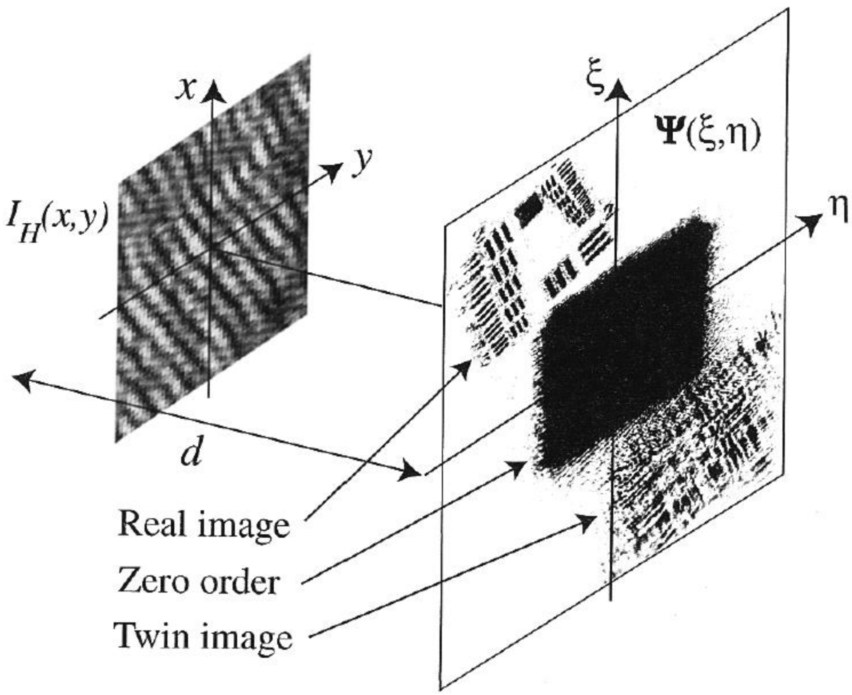

Fig. 4. Geometry for hologram reconstruction. $0xy$, hologram plane; $0\xi\eta$, observation plane; d, reconstruction distance; $\Psi(\xi, \eta)$, reconstructed wave front.

Hologram Reconstruction

- The result of the calculation is an array of complex numbers called reconstructed wave front **Ψ**, which represents the complex amplitude of the optical field in the observation plane $O\xi\eta$.
- The distance between the hologram plane Oxy and the observation plane is defined by the reconstruction distance d.
- <u>**If the hologram is recorded without a MO, *d* must be equal to the distance between the object and the CCD to obtain in-focus reconstructed images.**</u>

Hologram Reconstruction

- **<u>In holographic microscopy, image focusing occurs when the reconstruction distance is equal to the distance between the CCD and the image during the hologram recording (*d* in Fig. 3).</u>**
- In classical holography the reconstruction is carried out by illumination of the hologram intensity with the reference, and the reconstructed wave front is defined as follows:

$$\Psi = \mathbf{R}I_H = \mathbf{R}|\mathbf{R}|^2 + \mathbf{R}|\mathbf{O}|^2 + |\mathbf{R}|^2\mathbf{O} + \mathbf{R}^2\mathbf{O}^*. \quad (3)$$

- The two first terms of Eq. (3) produce a zero order of diffraction, the third term produces a twin image, and the fourth produces a real image.

Hologram Reconstruction

- As a consequence of the off-axis geometry these different terms are reconstructed at different locations in the observation plane (see Fig. 4).
- In classical holography we usually observe the twin image that appears to be emitted by a virtual replica of the object located at its initial position, i.e., behind the hologram.
- **Here, if the reconstruction distance *d* is positive, the reconstruction takes place in front of the hologram.**

Hologram Reconstruction

Numerical Calculation of Fresnel Diffraction

- In the Fresnel approximation the reconstructed wave front can be written as

$$\begin{aligned}\boldsymbol{\Psi}(\xi, \eta) = A \exp\left[\frac{i\pi}{\lambda d}(\xi^2 + \eta^2)\right] \\ \times \iint I_H(x, y) \exp\left[\frac{i\pi}{\lambda d}(x^2 + y^2)\right] \\ \times \exp\left[\frac{i2\pi}{\lambda d}(x\xi + y\eta)\right] \mathrm{d}x\mathrm{d}y,\end{aligned} \qquad (4)$$

- where λ is the wavelength, and $A = \exp(i2\pi d/\lambda)/(i\lambda d)$ is a complex constant.
- As presented by Eq. (4), the Fresnel integral can be viewed as a Fourier transform, in the spatial frequencies $\xi/\lambda d$ and $\eta/\lambda d$, of the function

$$I_H(x, y) \exp\left[\frac{i\pi}{\lambda d}(x^2 + y^2)\right]. \qquad (5)$$

Hologram Reconstruction

- For rapid numerical calculations a discrete formulation of Eq. (4) involving a two-dimensional fast Fourier transform can be derived directly:

$$\Psi(m,n) = A\exp\left[\frac{i\pi}{\lambda d}\left(m^2\Delta\xi^2 + n^2\Delta\eta^2\right)\right] \times \mathrm{FFT}\left\{I_H(k,l)\exp\left[\frac{i\pi}{\lambda d}\left(k^2\Delta x^2 + l^2\Delta y^2\right)\right]\right\}_{m,n}, \quad (6)$$

- where k, l, m, n are integers ($-N/2 \le k, l, m, n \le N/2$), and $I_H(k,l)$ is the digital hologram (Eq. (2)).

Hologram Reconstruction

- **<u>The values of the sampling intervals in the observation plane ($\Delta\xi$ and $\Delta\eta$) is related to the size of the CCD (L) and to the distance d by</u>**

$$\Delta\xi = \Delta\eta = \frac{\lambda d}{N\Delta x} = \frac{\lambda d}{L}, \qquad (7)$$

- where the relationship between the sampling intervals in the space (0*x*) and Fourier (0*v*) domains in discrete Fourier-transform calculations ($\Delta v=1/(N\Delta x)$) is considered.

Hologram Reconstruction

- **<u>In a setup without a MO, $\Delta\xi$ defines the transverse resolution in the observation plane.</u>**
- For a typical reconstruction distance d=30cm with a wavelength λ=633nm and a CCD size L=5 mm, the transverse resolution of the imaging system is limited to $\Delta\xi$ = 38 µm.
- In holographic microscopy $\Delta\xi$ defines the resolution with which the magnified image of the object is reconstructed, **<u>a transverse resolution equal to the diffraction limit of the MO can be achieved</u>**.

Hologram Reconstruction

Phase-Contrast Imaging

- For phase-contrast imaging, the digital hologram has to be multiplied by a digital reference wave $\mathbf{R}_D$, which must be replica of the experimental reference wave $\mathbf{R}$.
- **In classical holography, the same operation is performed optically when the hologram is illuminated with the reference wave.**

Hologram Reconstruction

- **<u>If we assume that a perfect plane wave is used as reference for hologram recording, R_D is calculated as follows</u>**:

$$\mathbf{R}_D(k, l) = A_R \exp[i(2\pi/\lambda)(k_x k\Delta x + k_y l\Delta y)], \quad (8)$$

- where A_R is the amplitude, Δx and Δy are the sampling intervals in the hologram plane, and k_x, k_y are the two components of the wave vector that must be adjusted such that the propagation direction of $\mathbf{R}_D$ matches as closely as possible that of the experimental reference wave.

Hologram Reconstruction

Digital Correction of the Phase Aberrations

- **As shown in Fig. 5 the MO produces a curvature of the wave front in the object arm.**

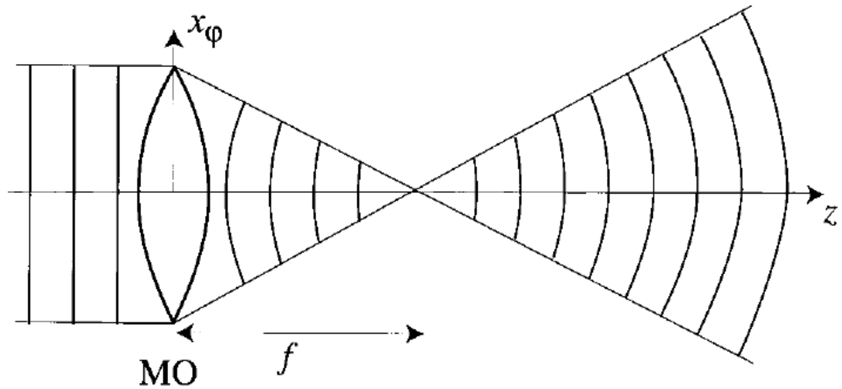

Fig. 5. Schematic of the wave-front deformation by the MO.

- This deformation affects only the phase of the object wave and does not disturb amplitude-contrast imaging.
- **However, for phase-contrast imaging or, more generally, in any interferometric system, this phase aberration must be corrected.**

Hologram Reconstruction

- In interference microscopy this problem is solved experimentally by insertion of the same MO in the reference arm, at equal distance from the exit of the interferometer.
- This arrangement requires that, if any change has to be made in the object arm, then the same change must be precisely reproduced in the reference arm such that the interference occurs between similarly deformed wave fronts.
- **We design a digital method that allows us to perform the correction by multiplication of the reconstructed wave front with the computed complex conjugate of the phase aberration.**

Hologram Reconstruction

- If we assume a monochromatic illumination, the relation between the optical Fields $\mathbf{U}_i(x_i, y_i)$ in the image plane and $\mathbf{U}_o(x_o, y_o)$ in the object plane can be described as follows:

$$\mathbf{U}_i(x_i, y_i) = \int\int \mathbf{h}(x_i, y_i; x_o, y_o)\mathbf{U}_o(x_o, y_o)dx_o dy_o, \quad (9)$$

- where $\mathbf{h}(x_i, y_i; x_o, y_o)$ is the point-spread function.
- If the image plane and the object plane form an object–image relation,

$$\frac{1}{d_i} + \frac{1}{d_o} = \frac{1}{f}, \qquad (10)$$

Hologram Reconstruction

- <u>h(x_i, y_i; x_o, y_o) can be written as follows (in two dimensions, *x* and *z*, for simplicity).</u>

$$\mathbf{h}(x_i, x_o) = C\exp\left(\frac{i\pi}{\lambda d_i}x_i^{\,2}\right)\exp\left(\frac{i\pi}{\lambda d_o}x_o^{\,2}\right) \times \int P(x_\phi)\exp\left[\frac{-i2\pi}{\lambda}\left(\frac{x_o}{d_o}+\frac{x_i}{d_i}\right)x_\phi\right]\mathrm{d}x_\phi, \tag{11}$$

Refer to Eq. (5-28) in Fourier Optics

- where $0x_\varphi$ is the coordinate of the MO plane, $P(x_\varphi)$ is the pupil function of the MO, and *C* is a constant.
- Note that it's just a Fourier transform of the pupil function $P(x_\varphi)$.

Hologram Reconstruction

- **Under the assumption of a perfect imaging system of magnification $M=d_i/d_o$**, for which a point of coordinates (x_o, y_o) in the object plane becomes a point of coordinates ($x_i = -Mx_o$, $y_i = -My_o$) in the image plane, the integral in Eq. (11) can be approximate by a Dirac δ function.
- If we replace x_o with $-x_i d_o/d_i$ in the quadratic phase term preceding the integral, we can write

$$\mathbf{h}(x_i, y_i; x_o, y_o) \cong C \exp\left[\frac{i\pi}{\lambda d_i}\left(1 + \frac{d_o}{d_i}\right)(x_i^2 + y_i^2)\right] \times \delta(x_i + Mx_o, y_i + My_o). \qquad (12)$$

Hologram Reconstruction

- In other words, relation (12) means that the image field is a magnified replica of the object field multiplied by the quadratic phase term.
- **It also means that the phase aberration can be corrected by multiplication of the reconstructed wave front with the complex conjugate of the phase term that precedes the δ function in relation (12).**

Hologram Reconstruction

- **Therefore, an array of complex numbers called digital phase mask Φ(*m, n*) is can be calculated:**

$$\Phi(m, n) = \exp\left[\frac{-i\pi}{\lambda D}(m^2\Delta\xi^2 + n^2\Delta\eta^2)\right], \qquad (13)$$

- where *D* is a parameter that must be adjusted to compensate the wave-front curvature.
- In accordance with relation (12) we have

$$\frac{1}{D} = \frac{1}{d_i}\left(1 + \frac{d_o}{d_i}\right). \qquad (14)$$

- We show that high-quality phase-contrast images can be obtained with this model.

Hologram Reconstruction

- <u>**Finally, taking into account the developments made in Eqs. (6) and (7), the complete expression of the reconstruction algorithm becomes**</u>

$$\begin{aligned}\boldsymbol{\Psi}(m, n) = A\boldsymbol{\Phi}(m, n)\exp\left[\frac{i\pi}{\lambda d}(m^2\Delta\xi^2 + n^2\Delta\eta^2)\right] \\ \times \mathrm{FFT}\left\{\mathbf{R}_D(k, l)I_H(k, l)\right. \\ \left.\times \exp\left[\frac{i\pi}{\lambda d}(k^2\Delta x^2 + l^2\Delta y^2)\right]\right\}_{m,n}. \qquad (15)\end{aligned}$$

Hologram Reconstruction

- Since **Ψ**(*m, n*) is an array of complex numbers, we can obtain an amplitude-contrast image by calculating the intensity,

$$I(m, n) = \mathrm{Re}[\boldsymbol{\Psi}(m, n)]^2 + \mathrm{Im}[\boldsymbol{\Psi}(m, n)]^2, \quad (16)$$

- and a phase-contrast image by calculating the argument,

$$\phi(m, n) = \arctan\left\{\frac{\mathrm{Im}[\boldsymbol{\Psi}(m, n)]}{\mathrm{Re}[\boldsymbol{\Psi}(m, n)]}\right\}. \quad (17)$$

Hologram Reconstruction

- **The reconstruction algorithm [Eq.(15)] involves four constant parameters:** *d*, the reconstruction distance, k_x and k_y for the calculation of the digital reference wave [Eq. (8)], and D for the calculation of the digital phase mask [Eq. (13)].
- These reconstruction parameters represent physical quantities (distances and angles) and could be measured experimentally.
- **However, their values must be defined with such a high precision that it is more efficient to adjust them digitally.**

Results and Discussion

- Figure 6 presents a digital hologram recorded with the experimental setup presented in Fig. 2, with a MO of magnification M = 40 and of numerical aperture N.A.= 0.65.

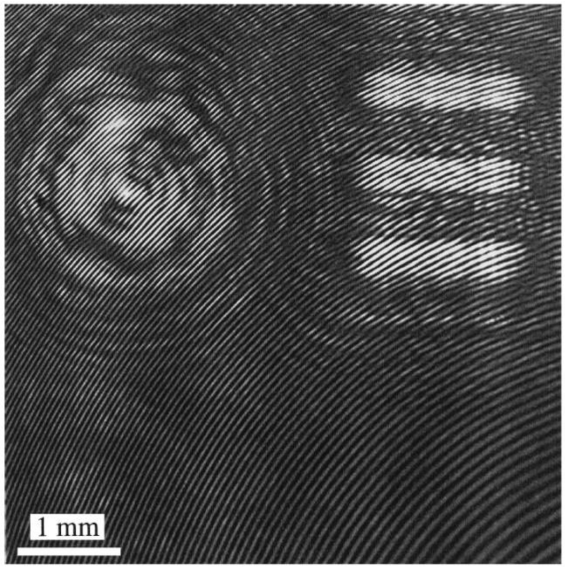

Fig. 6. Digital hologram recorded by the CCD camera.

Results and Discussion

- To study the transverse resolution limit of the system, a test target [USAF (U.S. Air Force) 1950] was used as a sample.
- This standard test object contains horizontal and vertical three-bar patterns in the form of a reflecting chromium coating set on a glass substrate.
- **<u>We can see in Fig. 6 that the hologram appears as a pattern of interference fringes.</u>**

Results and Discussion

- The corresponding reconstructed amplitude- and phase-contrast images are presented in Figs. 7(a) and 7(b), respectively.
- These images show a selected region of interest (200 × 120 pixels) containing the real image.

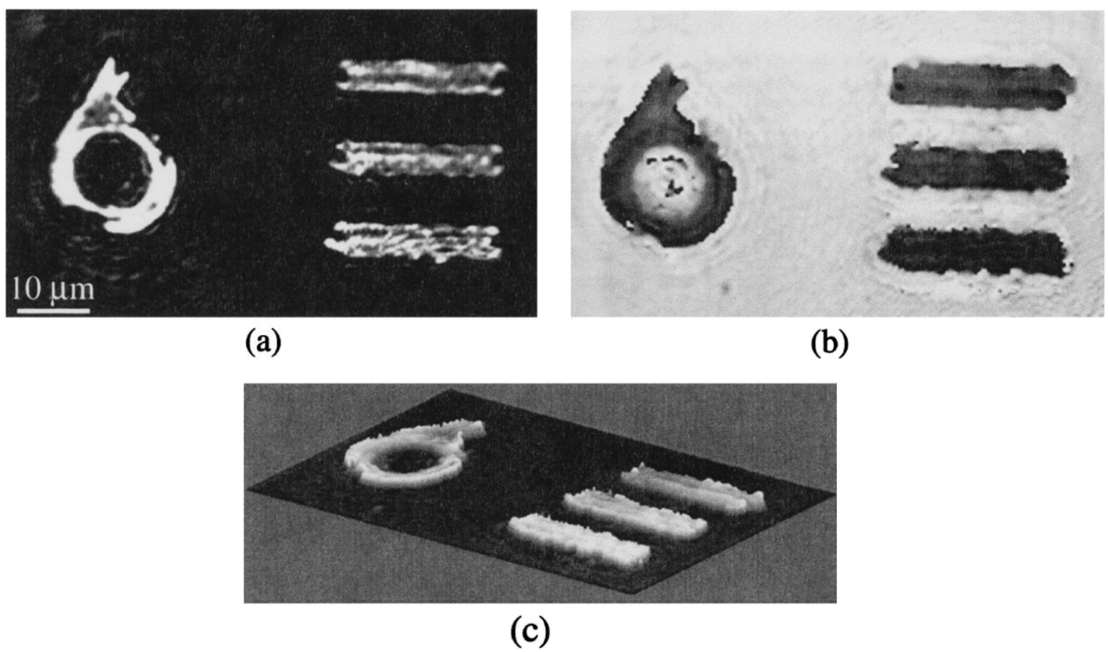

Fig. 7. Reconstructed images corresponding to the hologram presented in Fig. 6. (a) Amplitude-contrast image, (b) phase-contrast image, (c) the reconstructed phase distribution presented in a three-dimensional perspective.

Results and Discussion

- From Fig. 7, a transverse resolution smaller than 1 μm has been achieved.
- **<u>This result agrees with the predicted resolution limit of the MO as calculated according to the Abbe criterion (0.61λ/N.A.= 0.59 μm).</u>**
- Figure 7(c), which presents the reconstructed phase distribution: the phase contrast image provides information about the three-dimensional structures of the sample.
- It is also important to note that the obtained phase values are restricted to the [-π, π] interval.
- **<u>If the sample has phase variations higher than the wavelength, phase-unwrapping methods must be applied to reconstruct the topography.</u>**

Results and Discussion

Adjustment of the Reconstruction Parameters

- The reconstruction algorithm [Eq. (15)] involves four constants called reconstruction parameters.
- These values are fixed for a given experimental configuration but **must be modified if the reference-wave orientation is changed or if the sample is translated along the optical axis.**
- We consider how the reconstruction parameters can be adjusted by showing examples of images that have been reconstructed for different values of these parameters.

Results and Discussion

- **<u>The first parameter that has to be adjusted is the reconstruction distance *d*.</u>**
- Fig. 8 presents three amplitude-contrast images of the USAF test target obtained for different values of *d*, we can see that the reconstruction distance is related to image focusing.

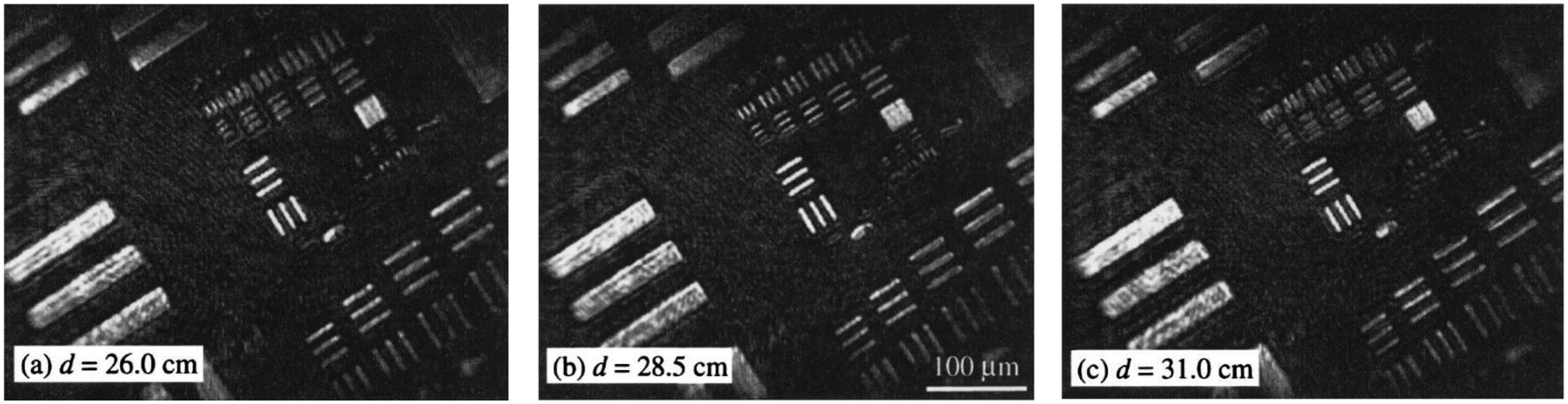

Fig. 8. Amplitude-contrast images obtained for different values of the reconstruction distance d. (a) Out of focus image (d too short), (b) in-focus image, (c) out of focus image (d too long).

Results and Discussion

- The procedure for the adjustment of *d* can be considered to be the digital counterpart of image focusing in classical microscopy in which the same operation is performed by translation of the sample along the optical axis.
- **Autofocus methods can be used to adjust *d* automatically.**

Results and Discussion

- For the reconstruction of phase-contrast images three other reconstruction parameters have to be adjusted: k_x and k_y for the calculation of the digital reference wave and D for the calculation of the digital phase mask.
- Figure 9 shows five phase images of an epithelial cell obtained for different values of k_x, k_y, and D.

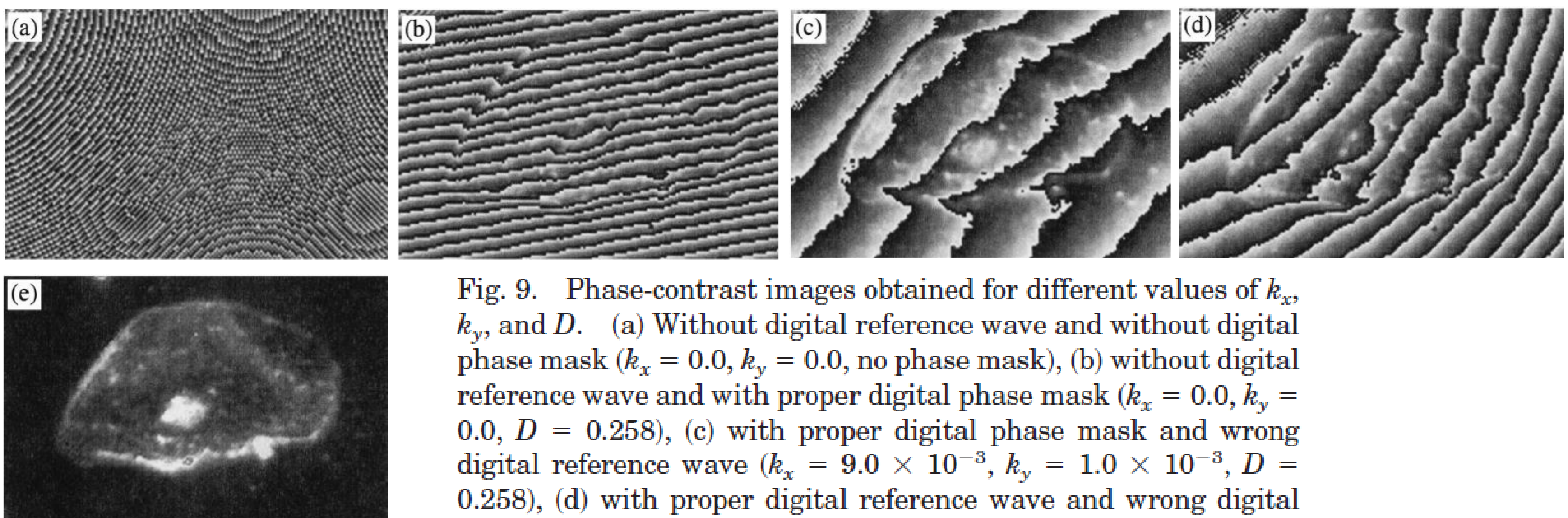

Fig. 9. Phase-contrast images obtained for different values of k_x, k_y, and D. (a) Without digital reference wave and without digital phase mask ($k_x = 0.0$, $k_y = 0.0$, no phase mask), (b) without digital reference wave and with proper digital phase mask ($k_x = 0.0$, $k_y = 0.0$, $D = 0.258$), (c) with proper digital phase mask and wrong digital reference wave ($k_x = 9.0 \times 10^{-3}$, $k_y = 1.0 \times 10^{-3}$, $D = 0.258$), (d) with proper digital reference wave and wrong digital phase mask ($k_x = 11.08 \times 10^{-3}$, $k_y = 2.05\ 10^{-3}$, $D = 0.240$), (e) with proper digital reference wave and proper digital phase mask ($k_x = 11.08 \times 10^{-3}$, $k_y = 2.05\ 10^{-3}$, $D = 0.258$).

Results and Discussion

- Fig. 9(a) presents the extreme case of an image obtained without a phase mask and without a digital reference wave ($k_x = k_y = 0$).
- On the other hand, Fig. 9(e) presents the case of a phase-contrast image obtained with properly adjusted reconstruction parameters.
- Figure 9(b) presents an image obtained without a digital reference wave but with the appropriate digital phase mask (D correctly adjusted).
- We can see that straight fringes with a fixed orientation appear in Fig. 9(b).

Results and Discussion

- <u>**These fringes indicate jumps of the phase values between -π and π.**</u>
- <u>**These phase jumps appear when a phase difference between the wave fronts of the digital reference wave R_D and the wave fronts of the experimental reference wave R is equal to a multiple of 2π.**</u>
- As shown in Fig. 9(c), the procedure for the adjustment of k_x and k_y is to check out the size of the space between these fringes until they completely disappear.

Results and Discussion

- As shown in Fig. 9(d), if the digital reference wave is correctly defined but the *D* value is slightly modified, the digital phase mask can not properly correct the phase aberrations of the MO and curved fringes appear on the reconstructed image.
- **The procedure for the adjustment of *D* consists of removing the fringe curvature.**