Compact diffraction phase microscopy for quantitative visualization of cells in biomedical applications

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Abstract. We consider a simplified and compact scheme of interference phase microscopy using a diffraction grating and spatial filtering of the diffracted field, i.e., diffraction phase microscopy. The scheme and the parameters of the device with the possibility of using the optical system of a smartphone and its software are analysed. The results of experimental determination of the spatial structure parameters of erythrocytes are presented.

1. Introduction
In the methods of optical phase microscopy the interference image of the studied object is analysed, in which the phase shifts of the light wave, reflected by the object or passed through it, manifest themselves. Such methods include the phase contrast method [1-3], the interference microscopy [4-6] and holographic microscopy [7-10]. In these methods, in one way or another, a coherent reference wave is formed and superposed onto the object image to form an interference image of the object. The computer processing of the interference image allows the reconstruction of the phase profile of the object field and the determination of structure optical characteristics of the object, e.g., morphological parameters of biological objects. The accuracy of such reconstruction and the spatial resolution are increased with the growth of the spatial frequency of the interference fringes of the image. The interference fringes with the highest frequency are obtained in the holographic microscopy with off-axis (oblique) reference beam [8,9]. However, the formation of such reference beam is associated with the complication of the optical scheme of the microscope and decrease of its noise immunity. This drawback is largely overcome in the so-called diffraction phase microscopy, in which a classical light microscope is used, equipped with the additional diffraction module, including a diffraction grating and an optical system for spatial filtering of the wave fields diffracted by the grating [11-15]. The diffraction module in this system is actually an interferometer, in which the diffraction grating plays the role of the amplitude splitter for the initial optical field. At the same time the optical system of the diffraction module by its construction is an imaging system that can operate in the mode of essential magnification of the image, and, therefore, in the microscope mode. In this relation, the diffraction module can be used autonomously without the microscope to form the magnified interference image of the studied object. In this case, the system of diffraction phase microscopy becomes compact, more simple and mobile. Moreover, the use of smartphone optics, computing and data processing software...
The aim of the present work was to develop a compact diffraction phase microscope and to study the possibility of using it for phase microscopy without the classical light microscope. In the paper, we consider the scheme of the compact diffraction phase microscope, its possible parameters, the procedures of interference image processing, and the experimental results, obtained with the laboratory prototype of the compact diffraction phase microscope.

2. Scheme design

The diffraction phase microscope is a usual (classical) microscope equipped with additional diffraction module aimed at the formation of interference image and quantitative phase visualisation of the object structure [12,13]. The diffraction module incorporates the diffraction grating placed in the front focal plane of the objective of the telescopic optical system that consists of two objectives (lenses) with coincident focal planes, a spatial filter, placed in the coincident focal planes of the objectives, and a matrix (CMOS or CCD) detector of the interference image. Depending on the used detector, the recording rate can be as large as a few hundreds of frames per second [13,14]. Every frame of the interference image contains all information necessary for subsequent determination of the optical parameters of the object. In the case of using an optical microscope, the module is installed instead of the ocular lens so that the diffraction grating is located in the plane of the object image or in the immediate vicinity of it. The field of the object image incident on the diffraction grating is separated into the diffraction orders. The spatial filter entirely transmits only the first diffraction order, which is used as an object wave, and the wave not scattered by the object in zero diffraction order that plays the role of a reference wave in the back focal plane of the second objective of the telescopic system, where the image of the object it repeatedly formed in the field of the diffraction order, transmitted by the filter. The interference of the reference wave and the field of the object image yields the interference image, which as a result of recording actually yields the hologram of the focused object image. The period of the carrier hologram structure, the interference fringes, equals the period of the diffraction grating image, formed by the telescopic system with the specified magnification.

No essential difference in the operation of the diffraction module will arise, if instead of the object image the object itself will be placed near the grating, and the optical field scattered by the object will be forwarded into the diffraction module. In this case, the diffraction module operates as the primary imaging system, which at sufficiently large magnification can be considered as a microscope. The spatial filtering of the object wave field and the formation of the interference image makes this instrument to be an interference (phase) microscope.

Figure 1 presents a schematic diagram of such use of the diffraction module. The transparent object is illuminated with a plane wave from a laser source. When passing through the object, the wave acquires spatial phase shifts, i.e., the spatial phase modulation, the magnitude of which is dependent on the local thickness of the object and the refractive index of the object and the environment. As a result of this modulation the object field arises, including the scattered and non-scattered components. The diffraction grating decomposes the object field into diffraction orders. To get the interference image, the spatial filter is used that entirely transmits the first diffraction order and the non-scattered component of the object field in the zeroth order of diffraction. In the back focal plane of the second objective the superposition and the interference of non-scattered plane wave and the obliquely incident wave field of the object image occurs.

The light intensity distribution in the interference pattern in the plane of the matrix photodetector can be presented in the following simplified form [16]:

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\[ I(x, y) = I_0(x, y) + I_R(x, y) + 2\sqrt{I_0 I_R} \cos \left( \frac{2\pi}{\Lambda} \left[ x \sin(\alpha) + y \cos(\alpha) \right] + \varphi(x, y) \right), \]  

(1)

where the coordinate axes \( x, y \) are directed along the rows of photodetector pixels, \( I_0(x, y) \), \( I_R(x, y) \) are the intensity distributions of the object and reference fields, respectively; \( \Lambda = Md \) is the period of interference fringes, \( M \) is the image magnification by the optical system, \( d \) is the period of the diffraction grating; \( \alpha \) is the angle of the grating orientation with respect to the rows of the photodetector pixels; \( \varphi(x, y) \) is the phase incursion acquired by the wave passing through the object.

For the plane illuminating wave and sufficiently smooth optical inhomogeneities of the object the function \( \varphi(x, y) \) can be expressed as

\[ \varphi(x, y) = \frac{2\pi}{\lambda} h(x, y) (n(x, y) - n_0), \]  

(2)

where \( \lambda \) is the wavelength of light, \( h(x, y) \) is the spatial relief of the object surface, \( n(x, y) \) is the spatial distribution of the refractive index in the object, \( n_0 \) is the refractive index of the environment.

In fact, the resulting system of interference fringes in the object image is a system of carrier fringes of a hologram structure. The digital record of such interference image is a digital hologram of the focused image. The numerical processing of such digital hologram, e.g., using direct and inverse Fourier transforms and spatial filtering, allows the reconstruction of the phase shift spatial distribution of the object field and, therefore, the spatial variations of the optical thickness of the object.

**Figure 1.** Optical scheme of compact diffraction phase microscope setup. LS, light source; S, sample; G, grating; L_1, L_2, lenses; SF, spatial filter; Cam, CMOS camera, \( f_1, f_2 \), focal distances of \( L_1 \) and \( L_2 \) lenses, respectively.

### 3. Technical features

In the present work, we considered the possibility of designing a compact diffraction phase microscope resistant to external vibrations, with formation of high-frequency interference fringes in the object image located in the photodetector plane. For implementing these intentions and providing the possibility of further improvement of the compact interference module parameters and/or upgrading the system for 3D visualisation of phase objects and using it in combination with the digital camera of a smartphone, we found the parameters of the interference module that are to satisfy a number of technical requirements.

1. For spatial filtering of wave fields diffracted by the diffraction grating it is necessary that the diffraction orders \( \pm 1 \) hit the aperture of the first objective of the system (\( L_1 \) in Fig.1). According to our estimates, this is fulfilled under the condition that the period of the grating \( d \geq (3/2)\lambda/NA_1 \), where \( NA_1 \) is the numerical aperture of the first objective of the interference module. In this case the transverse size \( \Delta x_0 \) of the object resolved by the system, e.g., red blood...
cell in the blood smear, should exceed the grating period at least by two times, $\Delta x_0 \geq 2d$. Then the diffraction orders of the object field will not overlap in the back focal plane of the objective, and the efficient spatial filtering of the object light field is possible. In the interference image of the object, formed in the back focal plane of the second lens $L_2$ of the module (Fig.1), about three interference fringes will appear.

2. For recording a digital hologram it is necessary that the matrix photodetector can resolve the carrier interference fringes of the image with the mean period $H$ expressed as $M_d H = \Delta$, where $M$ is the transverse magnification of the system, determined by the ratio of focal lengths of the objectives, $M = f_2/f_1$. This requirement is equivalent to the fulfillment of the Nyquist condition that for the record of a digital hologram can be presented in the form $\Delta x_D \leq \Lambda_H/2$, where $\Delta x_D$ is the pixels period in the matrix photodetector [17-19]. Taking into account the object image field phase variation that give rise to the variation of the fringes period, this inequality should be written as $\Delta x_D \leq \Lambda_H/3 = M_d / 3$ [19].

3. The integration of the requirements 1 and 2 leads to the generalized condition of optical spatial filtering of diffracted fields and recording the digital hologram formulated as $\Delta x_0 \geq 2d \geq 6\Delta x_D f_1/f_2$ with $d \geq (3/2)\lambda/NA_1$.

4. **Experimental results**

In the present work we used the laboratory prototype of a compact diffraction phase microscope with the overall dimensions $250 \times 100 \times 100$ mm and the optical magnification $M = f_1/f_2 = 4$. As the object of study the blood smears on glass slide were used. The visualisation of a blood smear was performed using the first objective with the parameters $NA = 0.5, f_1 = 25$ mm. The object was illuminated by a quasiplane wave from a semiconductor laser module ($\lambda = 650 \mu$m). A cover glass ($d_{cg} \approx 120 \mu$m) was placed between the grating and the object to protect the grating from damage because of the interaction with the object.

Holographic diffraction grating with a period of $10 \mu$m (100 lines per mm) was used. The grating was placed perpendicular to the optical axis so that its grooves form an angle of $45^\circ$ relative to pixel rows of the matrix.

In the experiments the high-speed digital camera (CMOS, aca2500-14gm, Basler, Germany) with the pixel size $a = 2.2 \mu$m was used to record interference images. The digital holograms were processes using the software based on double Fourier transform [20,21], written in our laboratory in the LabVIEW programme environment. For recording digital hologram of a focused image the reconstruction procedure for the complex field amplitude includes three sequential numerical procedures, namely, the Fourier transform of the digital hologram that yields the spatial spectrum, the spatial filtering of the hologram spectrum with extraction of the spectrum of the object field complex amplitude, and the inverse Fourier transform for retrieving the complex amplitude of the object image field. As a result of numerical processing the ultimate phase image of the blood smear was presented in the frame, determined by the field of view and having the dimensions $8.25 \times 8.25$ mm$^2$. The visualisation and postprocessing of the recoded interference patterns takes nearly 40 seconds for each frame.

Figure 2d presents the reconstructed image of the human blood smear for the interference pattern, shown in Fig. 2a. Using the above algorithms, the phase shift map $\phi(x, y)$ is first calculated over the entire plane of the image field of view. Then the latter is used to calculate the distribution of the
sample optical thickness $l(x, y) = \hat{h}(x, y)h(x, y)$, which is presented as the reconstructed image (Fig. 2d). The mean value of the red blood cell optical thickness in this experiment amounted to $l_m \approx 0.8 \mu m$, the spread of values was $\Delta l \approx \pm 0.2 \mu m$. Only the separately located erythrocytes were taken into account in the calculation of the optical thickness. The recognition of the appropriate images was executed automatically using the vision algorithms [22].

![Interference pattern](image1)

![Amplitude image](image2)

![Image of 2D spatial spectrum](image3)

![Reconstructed 3D image](image4)

**Figure 2.** a) Interference pattern recorded using the compact diffraction phase microscope; b) Amplitude image reconstructed from the interference pattern; c) Image of 2D spatial spectrum obtained using the direct Fourier transform of the hologram; d) Reconstructed 3D image of the blood smear in the entire field of view of the microscope.

### 5. Prospective studies

The positive results of the present study demonstrate the possibility of implementing a compact diffraction phase module as the phase microscope using the optics of a smartphone and its digital camera for subsequent quantitative reconstruction of the object image. Figure 3 presents the possible scheme of such instrument.

For imaging an object in the back focal plane of the smartphone objective in compact interference module an additional lens ($L_3$ in Fig. 3) must be installed in such a manner that its front focal plane coincides with the plane of the object image in the back focal plane of the lens $L_2$.

The present instrument can be used as a portable autonomous analyser of blood structure parameters with subsequent transmission of data to clinics and health-care institutions via the mobile network. It can also find application in the technological control of production quality, where the use of large-sized microscopes is hard or impossible. One can expect that the technological progress in the field of processors and photodetectors for the next generations of smartphones will allow the use of already known numerical algorithms for real-time hologram processing in mobile phones.
Figure 3. Optical scheme of compact diffraction phase microscope to be used in combination with the optical system and digital camera of smartphone: LS, light source; S, sample; G, grating; L₁, L₂, L₃, lenses; SF, spatial filter; IP, image plane; Obj, smartphone objective; Cam, smartphone camera, f₁, f₂, focal distances of L₁ and L₂ lenses, respectively.

6. Conclusion
In the present study, a compact diffraction phase microscope with the illumination system in transmission of the object was considered. The preliminary results demonstrate the possibility of a new modification of diffraction phase microscope, the compact diffraction phase microscope. The reconstruction of the 3D structure using the compact diffraction phase microscope and the obtained quantitative data on the cell thickness agree with those reported in the literature [23]. Using additional algorithms in the postprocessing of interference images, the microscope can be used to determine the number of leucocytes in blood or to detect the cells with atypical shape, the presence of which may be a symptom of anaemia, cancer, or diseases of the bone marrow. In future we plan to combine the compact diffraction phase microscope with the camera of a cellular phone, which will allow the examination under the conditions, making it impossible to use more perfect methods and instrumentation, e.g., in the areas remote from the laboratory.

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