Flexible Adaptive Phase Contrast Methods Using a Spatial Light Modulator

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In this paper we demonstrate the high flexibility and potential of a microscopic setup using a spatial light modulator in the pupil plane to realize established and new phase contrast imaging methods.

1 Introduction

Imaging biological objects often is a challenge as most of biological specimens are mainly phase objects. This leads to a reduced contrast. To overcome this problem without using dyes, different phase contrast imaging methods were developed. The most established methods in conventional microscopy are the so-called Zernike phase contrast and Nomarski differential interference contrast (DIC) [1]. To realize those methods in conventional microscopy, different microscopic equipment like special microscope objectives or filters are needed. Each of these methods with their static elements represents a trade-off, as ideal phase contrast imaging depends strongly on the object. A variation of different methods is difficult and expensive. Therefore, we use a phase–only spatial light modulator (SLM) in the pupil plane to realize different phase contrast methods. The filter, which is displayed by the SLM, can be changed and varied pixelwise and, therefore, offers a high flexibility. Additionally, it is possible to adapt the filter to the object and even to vary different methods and their parameters in real time. As there are no mechanical vibrations, it is possible to easily combine those images digitally to benefit from the advantages of different methods.

2 Setup

Figure 1 shows the schematic setup of our adaptive phase contrast imaging system.
To avoid speckles or other interference effects, illumination is done using an incoherent Köhler illumination with an LED (OSRAM Diamond Dragon) and a bandpassfilter of a bandwidth of 1 nm at 633 nm. Compared to a standard laser illumination, which is normally used in SLM-applications, this leads to an increased resolution due to a high numerical aperture (NA) illumination. The object is imaged via a Zeiss Achroplan 20$\times$/0.45 microscope objective onto a CCD-camera (Allied Vision Pike F-145B). The phase–only spatial light modulator (Holoeye HEO 1080 P) is positioned in the pupil plane of the object plane, where freely programmable phase filters can be displayed.

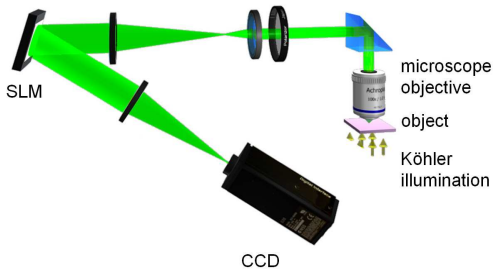

Fig. 1 *Schematic setup of the phase contrast imaging system using a spatial light modulator as phase filter.*

3 Implementation of basic phase contrast methods

It is possible to implement established phase contrast techniques with this setup [2] and at the same time even improve those methods due to new degrees of freedom.
A Zernike phase contrast filter consists of a constant phase with an additional phase offset in the central area to shift the zeroth diffraction order by $\pi/2$ (positive phase contrast) respectively $-\pi/2$ (negative phase contrast). Figure 2 shows cells of human oral mucosa in bright field (left) without any filter applied, in positive (middle) and negative (right) phase contrast, realized by the SLM.

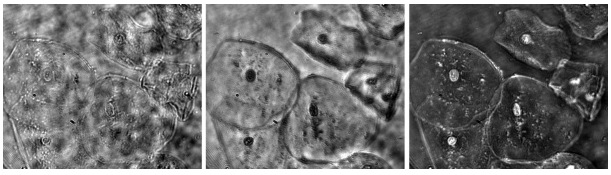

Fig. 2 *Human oral mucosa cells imaged in bright field (left) without any filter applied, in positive (middle) and negative (right) phase contrast, realized by displaying a Zernike filter by the SLM.*

By adjustig the diameter and the phase shift of the phase shifting filter [3] it is possible to reduce artifacts like halo effects, which exists in conventional Zernike phase contrast. Additionally quantitative phase measurements can be easily implemented adapting algortihms of phase-shifting interferometry [4].

To realize DIC in conventional microscopes, the illumination path is splitted and recombined, after passing the object, by a wollaston prism respectively. Therefore, there are two images with a slight offset interfering. Mathematically this represents a derivation of the phase distribution.
In our setup we realize this offset by two superimposed gratings with slightly different grating periods, as described by Lohmann [5]. By adjusting the period of the gratings it is possible to adjust the filter to the phase gradients represented by the objects. Figure 3 shows DIC for two filters with different grating periods.

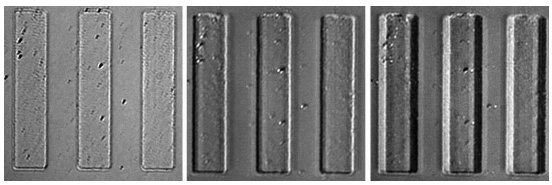

Fig. 3 *Bright field (left) and DIC with different grating periods (middle and right) of a technical phase object.*

4 Implementation of new phase contrast methods

Due to the freely programmable phase filter it is possible to implement new filters. We present here two heuristically developed filters. The W-DIC filter (Warber-DIC, see fig. 5) is related to DIC, as the two gratings represent different periods, but leads to different results. By adding an additional offset to the zeroth order it is possible to enhance fine structures (W-DIC Z, see fig. 4). More details are presented by Warber et al. [6].

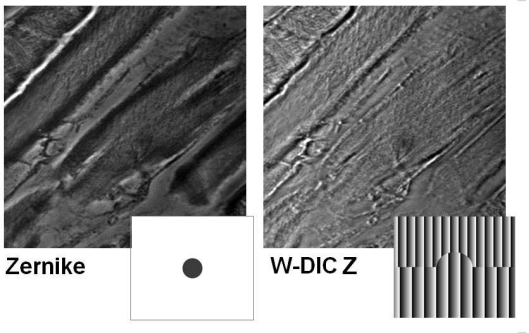

Fig. 4 *New method W-DIC Z implemented in comparison to Zernike phase contrast (left).*

As the filters can be changed in video real-time, it is possible to record various images of the same specimen without any mechanical movement between different recording. Using this advantage, we combine various images using image fusion. Figure 5 shows one simple example of possible recombination. Here, two images taken with a W-DIC filter with a different filter direction were combined by adding the difference of both images to the left image. As these filters are direction sensitive, the resulting image contains information of both directions.

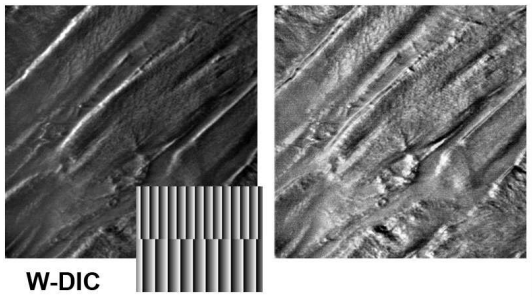

Fig. 5 *Image fusion (right) by recombination of images taken with different filter directions. One of the images, which was used for the recombination is shown on the left.*

5 Conclusion

We have presented a flexible microscopic setup including a spatial light modulator which enables to realize well-established phase contrast imaging methods without any additional equipment. Changing of filters is possible in real time without any mechanical vibrations. Therefore, this methods opens the possibility to realize completely new methods as well as combining images by image fusion to increase information in one image. A choice of options was presented to demonstrate the new flexibility and high potential in biological imaging.

Acknowledgement

We would like to thank the BMBF for financial support (BMBF 13N8809)

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