SLM-based Microscopy

Malte Hasler, Tobias Haist, Wolfgang Osten
Institut für technische Optik , Universität Stuttgart
mailto:hasler@ito.uni-stuttgart.de

We present a microscope that has the capability of adapting to various object characteristics by applying different filters in the Fourier plane of the imaging path. To this end we use a spatial light modulator and display different computer generated holograms. This allows us to realize several phase contrast techniques, dynamic aberration correction, pseudostereoscopic microscopy and confocal microscopy.

1 Introduction

Microscopy is a very versatile field in the sense that it offers a wide variety of techniques and methods to analyze all kinds of objects. However, the changeover from one technique to another can be quite time consuming. In order to improe on this we have integrated a spatial light modulator(SLM) which is used as a dynamic Fourier filter. This allows us to implement a number of classical microscopy variants such as Zerike phase contrast and differential interference contrast (DIC). Additionally, we realize stereoscopic imaging that leads to a pseudo threedimensional representation of certain objects. And we can perform confocal measurements by applying the dynamic filtering process in the illumination as well as in the imaging path. The filter manipulation also enables us to correct for aberrations.

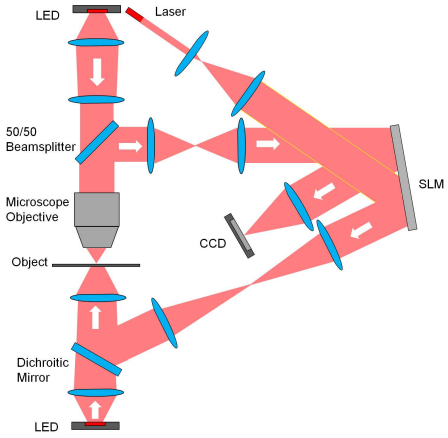

Fig. 1 *Setup for the SLM-based microscope with a SLM controlled imaging pathway*

The core design has been used for various applications [1, 2, 3] (Fig. 1). The object is imaged using an Olympus UMPlanFl ($NA = 0.8$, 50x) microscope objective whose exit pupil is imaged by a Kepler telescope onto a HDTV phase only liquid crystal on silicon SLM (Holoeye Pluto, 1920x1080 pixel, $8\mu\mathrm{m}$ pixel pitch).
For the illumination the system offers three different variants. Two classical Köhler illuminations in transmission as well as reflection based on high power LEDs (OSRAM Diamond Dragon, $\lambda = 632$ nm/$\lambda = 532$ nm). Since the holograms are highly sensitive to dispersion, a 1 nm bandwidth filter is used. The third alternatice is a holographic illumination using a frequency-doubled Nd:YAG laser in combination with the second half of the SLM.

2 Applications

Aberration correction is one important advantage of SLM-based microscopy. In order to correct for various aberrations we employ a correction based on Zernike polynonmials. Through adjustment of the respective coefficients we can directly adjust the amount of any specific aberration in the wavefront. Figure 2 shows the correction on the example of a Siemens star including a comparision with a commercial microscope(Zeiss Ergoplan: Leitz Wetzlar objective lens, $NA = 0.95, l = 540 - 580$ nm).

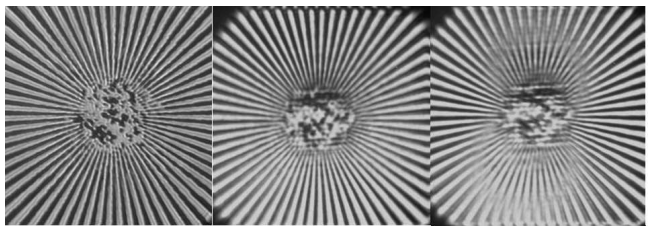

Fig. 2 *Imaging of a Siemens star in transmission with (l) Zeiss Ergoplan (Leitz Wetzlar objective lens, $NA = 0.95, l = 540 - 580$ nm) and (m) the SLM microscope (Olympus UmPlanFl objective lens, $NA = 0.8, l = 633$ nm) with aberration correction, (r) without aberration correction. The half-pitch of the smallest grating structures is 450 nm.*

One important application of SLM-based microscopy is **phase contrast microscopy** [1, 2, 4]. Realizing the Zernike phase contrast is achieved by writing a circle into the phase shifting SLM. If the circle covers the whole image of the illumination source the phaseshift will be analog to the classic phase shift through a phase plate.

Another common phase contrast method is the differential interference contrast(DIC). Through the superposition of two blazed gratings two slightly shifted images interfere in the camera plane. This results in an improved visibility on edges and strong gradients. It is also possible to implement new phase contrast methods, e.g. a vertical version of DIC (V-DIC) which applies the displacement in axial instead of a lateral direction.[5]

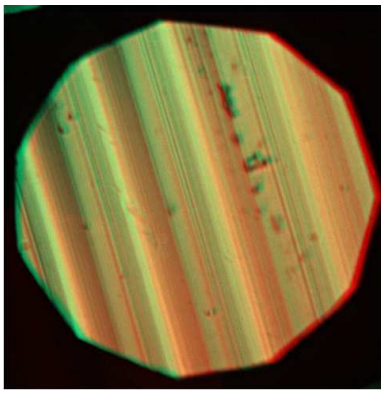

Fig. 3 *Anaglyph of a roughness standard class A grade 1. (3D viewable with red/blue glasses).*

The basic idea of **stereovision** is to image the object from two different directions. This can be realized by dividing the filter in the Fourier plane, since it is an image plane to the objective's entrance pupil. By redirecting the light from both halves of the SLM to differing positions on the camera we achieve the division into two independent images from slightly different angles of vision. The two resulting images can be combined to achieve a threedimensional impression of the object (see fig.3).

The central point of **confocal microscopy** is to find the position in which a measurement point is ideally focused on an object, and from that retrieve the object height at that point. For this, it is necessary to defocus the measurement point side, and to detect the focus position in the image plane. Usually the latter is done by placing a pinhole into the image plane. Since we do ot want to introduce a pinhole into the system, the determination of the correct focal plane has to be done differently. By considering intensity and spread of light on the CCD camera we can, through finding the focal position with the highest intensity maximum and minimal spread, determine the focal plane. The defocus is introduced digitally and synchronization at the imaging and illumination defocus is necessary.

In the experiment a scan was performed on a metallic surface. The defocus per image was in the range of 300 nm. In order to determine the axial position of the object, a range of 40 images was used. A repeated recording of the same object revealed a standard deviation in the peak center position of 24 nm. This results in a 3σ confidence intervall of 144 nm when measuring the exact z position.

3 Conclusion

We presented a microscope that is capable of handling a number of different microscopy methods without relying on any physical modifications. We have shown various kinds of phase contrast methods and examplified the great potential of the SLM in a phase contrast microscope. Additionally we demonstrated its use as a stereomicroscope and the possibility of implementing confocal microscopy.

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References

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