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Microscope system with on axis programmable Fourier transform filtering

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ABSTRACT

We propose an on-axis microscope optical system to implement programmable optical Fourier transform image processing operations, taking advantage of phase and polarization modulation of a liquid crystal on silicon (LCOS) display. We use a Hamamatsu spatial light modulator (SLM), free of flickering, which therefore can be tuned to fully eliminate the zero order component of the encoded diffractive filter. This allows the realization of filtering operation on axis (as opposed to other systems in the literature that require operating off axis), therefore making use of the full space bandwidth provided by the SLM. The system is first demonstrated by implementing different optical processing operations based on phase-only blazed gratings such as phase contrast, band-pass filtering, or additive and substractive imaging. Then, a simple Differential interference contrast (DIC) imaging is obtained changing to a polarization modulation scheme, achieved simply by selecting a different incident state of polarization on the incident beam.

1. Introduction

Spatial light modulators (SLM) are currently used in various applications in optical microscopy, including the generation of an array of optical beams [1], the production of optical traps [2], photo-stimulation [3], the synthesis of arbitrary point spread functions [4], or quantitative phase microscopy [5]. One of the most promising methods is the use of liquid crystal displays (LCD) as programmable phase diffractive optical elements (DOE) placed in the Fourier plane giving access to the spatial frequencies, which can be phased shifted individually, allowing to emulate a wealth of contrast enhancing methods [6,7]. Some of those DOE elements are spiral phase patterns [8,9] giving a strong contrast enhancement of microscopic amplitude and phase samples [10], or phase masks as circular rings or annular structures [11].

In these cases, blazed gratings are displayed on the SLM acting as phase masks that diffract the incoming wave into the first diffraction order image plane [5,6,12,13]. This is mainly due to the SLM flicker effect, that causes a temporal fluctuation of the phase values displayed on the SLM, and is the origin of some important amount of undiffracted intensity [14,15]. So, for such systems it is needed to block the zero-order, and to operate with an off-axis architecture. This operation with a blazed grating reduces substantially the available space bandwidth provided by the SLM. Although a better control of the spatial phase and amplitude modulation of the diffracted beams are a justification to use those systems, what it is true is that off-axis systems have a relevant reduction of light and field of view, as well as some difficulties of stability and optical implementation.

In this work, instead, we use a parallel-aligned Hamamatsu LCOS display, PAL-LCOS-SLM model X10468-01 [16]. Although these LCOS devices suffer from other typical problems of LCOS SLMs like non-uniformities, fringing effects or Fabry–Perot effects [17], they are free of flicker and therefore they can completely eliminate the zero order component of blazed phase diffractive elements, when operated correctly. Therefore, the purpose of this paper is to describe a programmable microscope system where such an LCOS SLM is employed to display Fourier transform filters, with the advantage of operating on axis. We demonstrate this ability by applying some simple pass band filtering operations, as well as some polarization filtering.

Various imaging-based measurement techniques are capable of delivering information in the form of phase, where phase imaging methods and polarized microscopy are used to extract anisotropy or birefringence information. In fact birefringence characterize the differential speed of propagation between two orthogonal polarization states. Several techniques for determining the optical birefringence have been used, including interferometric [18] and polarimetric methods [19,20]. Polarized microscopy is based on optical microcopy techniques involving polarized light. A more

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complex microscopy techniques which take advantage of polarized light include differential interference contrast microscopy (DIC) [21]. DIC works on the principle of interferometry to gain information about the optical path length of the sample, to see otherwise invisible features. Traditionally it is performed using Wollaston prisms to create two slightly offset coherent images that are superimposed in the image plane [21,22]. DIC has been used satisfactorily with SLM [12,23] where Wollaston prism are replaced by a SLM to generate and overlap the two displaced images without taking advantage of the polarization orthogonality of the anisotropy of the LC. In previous methods the shear, orientation and phase difference of the images are all computer controlled and can be varied at video rates. The requirement of computer processing is not always an advantage since the illumination conditions and alignment is critical to perform the correct digital image addition.

In this work we propose the on-axis microscope set-up to implement optical image processing operations by exploiting the phase and polarization properties of the LCOS display. The optical system is equivalent to other typical previous architectures [6,7,12,13,23] where a LC SLM is used as spatial filter in a Fourier plane that is made available by means of a relay optics. There, a phase or polarization filter is displayed, but no zero-order component is obtained. We then use a beam splitter (BS) to recover the information that come from the Fourier plane and recombining it in the final image plane, where a CCD camera is placed. By selecting the proper linear polarization impinging to the LCOS SLM different parallel basic image processing operations are done. In addition we propose a simple architecture to perform DIC microscopy without need to computer processing. We use the polarization properties of the LCD by placing a polarizer oriented 45° angle with respect the two orthogonal polarizations. So the two slightly offset coherent images are superimposed in the final image plane. This is the fundamental work of DIC by separating a polarized light source into two orthogonally polarized mutually coherent parts, which are spatially displaced (sheared) at the sample plane, and recombined before observation. However to produce the two orthogonal polarization quartz prism where used to illuminate the sample, in our proposed optical set-up both images are generated directly with two orthogonal polarization.

The paper is organized as follows: after this introduction, Section 2 introduces and described the optical setup and the main elements. Then, in Section 3 we present some experimental results on the application as different Fourier transform filters based on the phase-only modulation operation of the display. These results show how the microscope filter operates on axis. Then, Section 4 introduces some other filtering operations based on the control of the state of polarization, which can be considered as a DIC equivalent. Finally, Section 5 present the conclusions of the work.

2. Optical system

The optical setup is shown in Fig. 1. A He–Ne laser ($\lambda=633$ nm) is spatially filtered and collimated, and illuminates the sample. Then an infinity corrected microscope objective is used, a 10X objective from Nikon, with a focal length of $f_{obj}=20$ mm. The Fourier transform (FT) of the sample transmittance is obtained in the back focal plane of the objective. However, it is difficult or even impossible to access this FT plane (FT plane 1 in Fig. 1). This, following previous systems [5,6], we use a relay optics composed of two converging lenses L1 and L2, with focal lengths $f_1=175$ mm and $f_2=400$ mm respectively, to image FT plane 1 onto FT plane 2, where the LCOS SLM can be placed. The relay optics can be viewed as a 4f system from FT plane 1 to FT plane 2. An intermediate image plane is produced in between (image plane 1). This magnification allows to have a FT size that can be manipulated with the LCOS SLM.

The LCOS SLM is used to display a diffractive mask that performs the Fourier transform filtering operation. Here we use a Hamamatsu LCOS display, parallel aligned PAL-LCOS-SLM model X10468-01, with 792×600 pixels, 20×20 μm^2 pixel size and video-rate operation (60 Hz). The LC director is oriented horizontal with respect to the laboratory frame. This device is a programmable linear retarder with the extraordinary axis oriented horizontally, and the ordinary axis oriented vertically. Therefore, it produces a phase-only modulation for linearly polarized light oriented horizontally. A phase retardation variation of 2π radians is obtained for the operating wavelength of 633 nm for a gray level variation of 196 levels. The electronics of the SLM is corrected to provide a linear relation of the retardance variation with the addressed gray level.

Light reflected back from the SLM is isolated from the input beam by means of a non-polarizing beam splitter (NPBS). A final converging lens (L3), with focal length f_3, performs a final FT operation. The final magnification of the microscope can be adjusted by adjusting the location of lens L3 and the CCD detector. We use a CCD camera from Basler, model scA1390-17fc, with 1392×1040 pixels.

Two rotatable polarizers are included in the system (P1 and P2), before and after the NPBS. The input polarizer (P1) is used to select the polarization of the beam impinging the LCOS SLM. Polarizer P2 is used to select the output transmitted polarization. The polarization of the beam illuminating the sample is selected

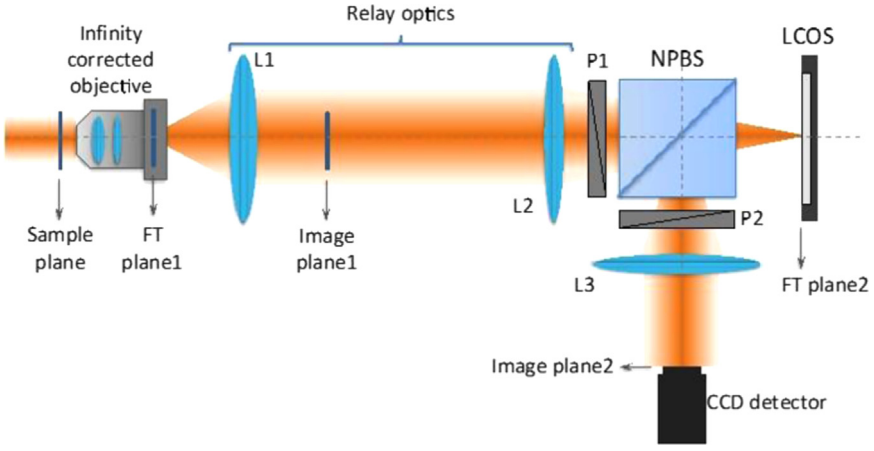

Fig. 1. Scheme of the optical setup. L denotes a converging lens, and P denotes a linear polarizer.