A multi-modal stereo microscope based on a spatial light modulator

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Abstract: Spatial Light Modulators (SLMs) can emulate the classic microscopy techniques, including differential interference (DIC) contrast and (spiral) phase contrast. Their programmability entails the benefit of flexibility or the option to multiplex images, for single-shot quantitative imaging or for simultaneous multi-plane imaging (depth-of-field multiplexing). We report the development of a microscope sharing many of the previously demonstrated capabilities, within a holographic implementation of a stereo microscope. Furthermore, we use the SLM to combine stereo microscopy with a refocusing filter and with a darkfield filter. The instrument is built around a custom inverted microscope and equipped with an SLM which gives various imaging modes laterally displaced on the same camera chip. In addition, there is a wide angle camera for visualisation of a larger region of the sample.

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References and links

Optical microscopes are indispensable tools in a vast range of laboratories [1]. Spatial Light Modulators (SLMs) offer remarkable control over a given light field. In optical microscopy, this control has been used to create forms of microscopy more typically implemented with different physical apertures and filters in the optical path. SLMs positioned in the Fourier plane bring a flexibility to optical microscopy which would be difficult to realise with traditional techniques [2–8]. In parallel with these developments, we reported a new approach to stereo microscopy [9]. Normally, a stereo microscope comprises of two objective lenses and a single light source. In our previous approach we used a single objective lens and two light sources combined with a bi-prism in the Fourier plane. This has also been achieved with two different colours of illumination [10]. More recently, the bi-prism was replaced by an SLM, albeit with a
conventional light source [11]. In this work, we use two light sources and a SLM acting as a bispectrum to create a stereo microscope that is also capable of darkfield and defocus in conjunction with stereo microscopy. This new technique improves 3D visualisation, extends the depth of field and could allow stereo microscopy to be used for more weakly scattering objects. Our set up is compatible for using other filters, such as spiral phase contrast [5], double-helix point spread functions [12], Spatial Light Interference Microscopy (SLIM) [13, 14], depth of field multiplexing [4, 15] and Gabor filters [16] amongst others [17–19]. Not only can different filters be applied without changing any hardware, but several filters can be applied at the same time to give multiple modalities simultaneously, offering a unique advantage over any traditional optical element.

2. Design

The inverted microscope design, Figs. 1 and 2, is similar to that of our recent work developing a compact optical tweezer system [20]. Focussing is achieved by moving the objective lens on a motorised stage (LS-50-M, ASI) which is mounted vertically within the aluminium frame of the microscope. Similarly, translation of the microscope sample is achieved using a motorised X-Y stage (MS-2000, ASI). Both these stages are controlled with the ASI MS-2000-WK Multi-
Fig. 2. Photograph of the system. Visible is the optical fibre illumination, motorised stage, SLM and CMOS cameras. The footprint of the system is $45 \text{cm} \times 30 \text{cm}$, and the height is approximately $35 \text{cm}$.

Axis Stage Controller. High power red LEDs (LUXEON Rebel) coupled through the condenser using a short length of acrylic fibre (core diameter = 1.5 mm) provide the illumination. A critical illumination condenser provides a uniform illumination of the sample. The output face of the fibre is imaged on to the sample, with a variable aperture to give brightness and spatial coherence control. An alternative illumination consists of three acrylic fibres positioned a few millimetres from the sample: one on axis, and two at $30^\circ$ for stereoscopic operation [9]. For this illumination, some control of the Numerical Aperture (NA) is given by raising and lowering the fibres, however careful consideration of scattered light from outside the field of view is needed at low NA. Switching between the on axis and stereo illumination is achieved through USB control.

A polarising beamsplitter (PBS) splits the light between two arms, one containing a camera for wide field viewing of the sample and the other arm containing the SLM (Boulder Nonlinear Systems, X-Y Series (512 $\times$ 512 pixels)). The reflected light from the PBS is the correct polarisation for the SLM. In order to avoid problems with dispersion when illuminating the SLM using a LED, a bandpass interference filter (636 nm, 10 nm) is placed in the optical path after the SLM. The light diffracted from the SLM is imaged on to a second CMOS camera (Dalsa Genie, HM1020) which has a large sensor area, capable of imaging all diffracted images simultaneously. The overall optical configuration is similar to that of holographic optical tweezers [21,22], where the diffraction from the SLM creates multiple, laterally displaced trapping beams. Here, the SLM now produces displaced images and, just as the trapping beams can be of different beam types, the images can correspond to different Fourier filter functions.
Fig. 3. Illustrative intensity distributions of a 5 μm silica particle in water. The particle is stuck to the coverslip and is moved through the focus of the microscope. A series of images were recorded at 0.5 μm spacing in the axial direction, then four intensity isosurfaces were interpolated from the data (shown in shades of red). In the x – y plane of each plot is an illustrative phase hologram used to Fourier filter the image. A darkfield filter has been used in (a), an annulus filter has been used in (b) and a cubic filter has been used in (c). The annular filter extends the depth of field of the imaging system. The cubic filter also extends the depth of field but in addition introduces a curved intensity profile. All these point spread functions were introduced by the SLM.

3. SLM Fourier filters

Fourier filtering is a common technique applied images to enhance desired information of a sample object [23]. The role of the SLM in our system is to generate different imaging modalities by modulating both the phase and amplitude of the light in the Fourier plane. For example, Fig. 3 shows the image intensity as a function of defocus whilst displaying a darkfield filter, an annular filter or a cubic filter on the SLM. The annular filter is useful as it gives an extended depth of field [24], while the cubic filter produces an Airy point spread function [25]. For each of the following patterns, we use a grating to diffract an image far enough from the zero order (undiffracted) image so that it does not overlap with it. For this to be possible, we first need to partially close the intermediate image iris so that the cone angle of each diffracted image is less than the diffraction angle given by the SLM. As with holographic optical tweezers, where the effective pixel size of the SLM sets an upper limit to the field of view over which traps can be positioned [26], the SLM resolution sets the maximum field of view of the images not to overlap in the camera plane.

As previously reported [27], the ideal kinoform describing the pattern to be displayed on the SLM for a particular imaging mode, \( \phi_m \), is given by,
Fig. 4. Shown here are results from combining the Fourier filters as described in the text while imaging three 5 μm silica spheres suspended in optical adhesive. (a) - (c) Shows individual phase patterns for three imaging modes, namely, double-helix point spread function, defocus and darkfield, respectively. Aberration correction has been incorporated with these filters and each also includes a grating to diffract the image. The greyscale corresponds to $0 - 2\pi$ phase changes. (d) The SLM display, showing the combined filter as described by Equation 2. (e) The resulting image detected by the camera. We extract the subregions of the image indicated, each 220 × 220 pixels. The subregions correspond to each filter; top left: double helix, bottom left: defocus, right: darkfield, centre: undiffracted zero order image.

$$\phi_m = \arg\left(e^{i(\alpha u + \beta v + \phi_{\text{Filter}})}\right),$$  \hspace{1cm} (1)

where $u$ and $v$ are, respectively, the vertical and horizontal distances from the centre of the SLM, $\alpha$ and $\beta$ are constants which define the angle of diffraction and $\phi_{\text{Filter}}$ is the filter function. There are a wide range of different filter types ranging from simple implementations of defocus [4] and darkfield to more elaborate phase contrast techniques [13, 28, 29] and vortex phase masks [5].

One advantage of the SLM implementation is that it is possible to diffract several image modalities at different angles by combining their phase patterns. Several images can be produced simultaneously by the complex addition of individual kinoforms, the argument of which gives the combined kinoform and the simultaneous images,

$$\phi_{\text{combined}} = \arg\left[\sum_{m=1}^{M} R_m e^{i\phi_m}\right],$$  \hspace{1cm} (2)

where $m$ is the imaging mode produced by the SLM, $M$ is the number of diffracted images, and $R_m^2$ is the relative intensity of the image. The potential interference between overlapping images is minimised by suppression of ghost diffraction orders by directing unwanted light into the zero order [30]. This multi-modal approach is made possible by the availability of large sensor arrays capable of working over a large dynamic range inherently arising from the different filter types. The design of the SLM filter to produce these images is exactly the approach used in holographic optical tweezers to produce multiple trapping beams, where each
beam type can be different. Figure 4 shows a set of multi modal images.

A possible drawback of a microscope incorporating an SLM in the optical path is that the SLM itself introduces aberrations as it is not optically flat. However, this can be corrected for with a corresponding phase pattern displayed on the SLM. Not only can we correct for aberrations of the SLM itself, but also other aberrations in the optical train or even within a sample. We do this by manually tuning coefficients of Zerinke polynomials whilst imaging a known object, typically a 2 \( \mu \)m silica bead. With adjusting the focus of the microscope and iterating the coefficients, we were able to dramatically improve the image and remove the worst of the system’s aberration [31]. Figure 5 gives details of the aberration correction. Whilst successful in correcting the system aberrations, imaging an unknown object through a dynamic aberrating layer would require some kind of reference, such as a focused laser spot in the object plane. The system could then be used as a SLM-based Shack-Hartman wavefront sensor [32].

3.1. Holographic stereo microscopy

As discussed, Bowman et al. [9] created a novel microscope, optimised for 3D visualisation and particle tracking. The approach uses a single microscope objective lens and a custom illumination consisting of two optical fibres at an angle around 30° to the optical axis, yielding two views of the sample. This method enables the three dimensional tracking of particles without the need for complex beam steering optics or the requirement for pre-recorded template images. It is also easily scaled to track multiple particles simultaneously, and has been employed to track the motion of non-spherical particles in a novel form of scanning probe microscopy [33].

The two images are from different angles of illumination, and, just as parallax vision gives us depth perception, depth information can be obtained from the images. A particle moving in \( \z \) direction would move from left to right in one image and from right to left in the other. It is simple then to attain the \( \delta \z \) displacement, as,
Fig. 6. 3D position coordinates can be obtained using the software’s particle tracking capabilities. (a) An image of the SLM display where the greyscale represents $0 - 2\pi$ phase change. Two apertures can be seen corresponding to the two views of the sample, each with a different grating so that the images from these angles don’t overlap in the image plane. (b) and (c) The different images extracted from an image recorded on the CMOS camera. The images are of the same object, 2 $\mu$m polystyrene spheres undergoing Brownian motion in water. (d) Stereo visualisation of particle positions. The images (b) and (c) are colour coded and overlapped. With 3D stereo glasses, the user can visualise the scene with depth perception. Images (e) and (f) are of the same cheek epithelial cell nucleus as viewed with the stereo microscope. Viewing the sample from two directions affords additional geometric information about the cell structure.
\[ \delta z = \frac{\delta x}{2 \tan \theta}, \]  
\hspace{1cm} (3)

where \( \delta x \) is the difference in the \( x \) position of the particle in the two images and \( \theta \) is the angle of the optical fibres to the vertical axis (i.e. the fibres are separated by \( 2\theta \)).

Illuminating at two angles corresponds to two positions in the Fourier plane and around these positions is the scattered light from the particles in the sample. For each of these light fields, a wedge prism deflects the light so that they do not overlap in the image plane. Also, a twin circular aperture needs to be placed just in front of the prisms in order to restrict high angled scattering from one view contributing to the image for the other view. In [11], Hasler et al. an SLM was used to create stereo images with just a single, on axis, illumination. As discussed by the authors, this approach leads to an inversion of the image for some samples owing to the division of the scattered field in the Fourier plane. By using two light sources we effectively have two separate light fields which, in the Fourier plane, only overlap at high spatial frequencies. By blocking these areas with the twin circular aperture we can obtain images suitable for particle tracking and free from inversion, albeit with a high frequency cutoff.

The twin aperture needs to be designed for a particular magnification or illumination angle, \( \theta \), as these set the position of the light fields in the Fourier plane. With the use of an SLM here, we can make the stereo microscope more flexible, see Fig. 6. With no mechanical change to the system described in section 2, the SLM can be used in place of the twin apertures and prisms. The size and position of the apertures on the SLM are now reconfigurable, meaning we can switch between microscope objectives and have corresponding SLM phase patterns ready to compensate for the new position and size of light fields for each view. We can also experiment with different angles of illumination and adjust the SLM display accordingly, which may be more suited to particular applications. The approach works well for particle tracking spheres, but it’s also possible to image extended objects, Figs. 6(e) and 6(f). Viewing from two different directions can give additional information of the shape of the structure, however, the large separation angle between the two views means 3D visualisation of such objects is difficult (if the images were to be colour coded and overlapped).

4. Combination of holographic stereo microscopy with holographic lenses

As a demonstration of the flexibility of the system, we combine our holographic stereo microscopy with holographic lenses and darkfield imaging. We image a 5\( \mu \)m silica bead with stereo illumination and an objective 100\( \times \), NA = 1.3 (Zeiss), Figs. 7(a) and 7(b). With stereo microscopy, particle tracking accuracy and precision is limited by the defocusing of the tracked object: the more the particle moves away from the focal plane, the less reliably can we measure its position. Adjusting the focus of the objective lens to keep the particle in focus during a measurement is not always desirable. Adjusting the focus holographically has the advantage of having no mechanical movements, as well as maintaining the parallax displacement if separate lenses are aligned to the axis of each view. This means we can focus the image without causing a lateral shift, i.e. the measured \( x \) position in each view is unchanged. The range of this technique is now limited by the field of view, rather than the depth of focus of the microscope. In Fig. 8, we show the improvement to the accuracy of the \( z \) position measurement as a function of distance from the focal plane. Without the lens correction, the tracking error increases quickly outside of the depth of focus of the microscope [9]. However, with the application of holographic lens correction, the error falls to a mean of 5.5nm across a 50\( \mu \)m range.

We note that this stereo visualisation approach has parallels with human vision, where each eye has its own lens. This addition of a lens could be of benefit to 3D tracking with a closed loop adjustment of the focus to optimise the trackability of a particle without changing its position
Fig. 7. Recorded images of a 5 μm silica bead fixed in position on a microscope slide. We illuminated the bead with stereo illumination, so that the bead translates as we adjust the focus of the microscope objective over a 35 μm range. We diffract the left and right views to different positions on the camera then extract, colour and overlap the images. (a) The SLM display along with stereo images as the bead goes through the focus of the microscope. (b) the SLM display used to refocus the bead as we change the focus of the objective lens. The refocusing does not change the position of the bead. A different holographic lens is needed for each focal position, shown is an example of the lens used at the 10 μm position. (c) Darkfield stereo images. (d) Darkfield stereo with focus correction.
Fig. 8. Measurement of the accuracy of $z$ position measurement as a function of distance from the focal plane. The error is calculated from measuring the standard deviation of a series of position measurements of a 5 $\mu$m silica particle stuck on the coverslip. The improvement is due to the increased contrast brought about by keeping the particle in focus.

in the image.

Again, the flexibility of the SLM implementation means that we can introduce a darkfield filter to the stereo imaging with no additional system complexity, Figs. 7(c) and 7(d). A use of the dark-field stereo could be improving the contrast for the 3D trajectory tracking of individual water-borne single-celled organisms. In principle, all the benefits of using an SLM in the Fourier plane can be transferred to the stereo microscope, however care may be needed regarding the coherence of the illumination.

5. Conclusions

We have developed a multi-modal microscope capable of stereo imaging, aberration correction and a range of other imaging modalities. The microscope uses a spatial light modulator to Fourier filter the images and generate different imaging modalities laterally displaced on the same camera. Depending on the application, existing filters can be optimised or new filters created and combined with stereo imaging. Novel Fourier filters can be developed with software and tried out without additional hardware components. As an example, we demonstrated holographic stereo microscopy, and used the flexibility of the SLM to improve the range of the technique with the addition of a holographic lens, and holographic stereo darkfield microscopy, which could allow stereo microscopy to be used with weakly scattering objects.

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