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Compact OAM microscope for edge enhancement of biomedical and object samples

Richard Gozali, Thien-An Nguyen, Ethan Bendau, and Robert R. Alfano[a)]
Department of Physics, Institute for Ultrafast Spectroscopy and Lasers, Complex Light Center, City College of New York, 160 Convent Avenue, New York, New York 10031, USA

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The production of orbital angular momentum (OAM) by using a q-plate, which functions as an electrically tunable spatial frequency filter, provides a simple and efficient method of edge contrast in biological and medical sample imaging for histological evaluation of tissue, smears, and PAP smears. An instrument producing OAM, such as a q-plate, situated at the Fourier plane of a 4f lens system, similar to the use of a high-pass spatial filter, allows the passage of high spatial frequencies and enables the production of an image with highly illuminated edges contrasted against a dark background for both opaque and transparent objects. Compared with ordinary spiral phase plates and spatial light modulators, the q-plate has the added advantage of electric control and tunability. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.5000508]

I. INTRODUCTION

Biological and medical samples are often transparent, showing little contrast under a microscope without the addition of contrasting dyes. However, these dyes can interact with the sample in a way that obscures observation, either by altering or killing the samples. Dark-field microscopy and phase-contrast microscopy are methods of achieving image contrast without the need to stain a sample with a dye. In dark-field microscopy, by rejecting light that transmits directly through a sample and collecting only the light scattered from a material interface, one can achieve a high contrast image of a transparent sample. The background of the image appears dark (hence, "dark field"), and the light-scattering interfaces will appear bright.[1] Phase contrast microscopy provides additional image contrast that includes the internal structure of the sample. In regions with differences in refractive index and path length, transmitted and scattered light will undergo different phase changes. Phase contrast microscopy works by recombining and interfering the directly transmitted and scattered light. In this way, the relative difference in phase between light waves will produce variations in intensity in the image plane that corresponds to different features of the sample.[1] A properly designed filter at the Fourier plane of a 4f system aimed to remove low spatial frequencies can improve imaging through scattering media, for example, by highlighting only the edges of a sample.[2–4] This is termed as "edge enhancement." Spiral plates are also known to produce high-quality edge selective enhancement by acting as spatial filters in standard bright field microscopes. Spiral phase filtering can also achieve orientation-selective edge enhancement. In contrast to isotropic edge enhancement, where all edges are equally illuminated regardless of orientation, orientation-selective edge enhancement is dependent on the orientation of the edges. This allows for further specificity in enhancement of certain features that could be more significant for observation.

A previous experiment of orbital angular momentum (OAM) microscopy has been done by Ritsch-Marte *et al.* producing edge enhancement imaging of different samples where they used a spatial light modulator (SLM) in their setup.[5,6] Problems regarding using the SLM are they can only reflect light, thus leading to a back-folded optical setup and need extra spiral phase filtering for edges to be clearly illuminated.

The q-plate, a new liquid crystal technology developed by Marucci[7] is an optical element with a constant half-wave retardance across its aperture as well as a pattern of electrically addressed birefringent liquid crystals. By running a voltage across the q-plate, the liquid crystals orient such that the alignment of their fast axis rotates around a central topological defect. This central defect causes the central vector components of the beam to cancel and the rotating fast axis imparts a helically varying phase of $\exp[2qi\varphi]$ around the beam, where q is the topological charge, where in this case $q = \frac{1}{2}$, that defines the defect and fast-axis orientations.[8] The helically varying phase $\exp[2qi\varphi]$ is also equal to $\exp[il\varphi]$, where l represents the angular momentum number of a Laguerre-Gaussian beam, with $l = 1$. A q-plate can be tuned by frequency, voltage, and temperature to operate at a range of wavelengths and, if desired, to affect the output polarization of a beam.

The focus of this paper is to demonstrate and design an experimental setup with the use of OAM beams via the convenience of having a q-plate at the Fourier plane of a 4f system in order to produce images with edge contrast enhancement. Using a q-plate at the Fourier plane of a 4f system enables the beam to propagate in the shape of a donut where the illumination of the ring contains only the high spatial frequencies, thus enabling to create an image of an object with edge enhancement. Compared to standard spiral phase plates, a q-plate has the added advantage of tunability by frequency, voltage, and temperature, as well as the ability to be turned on and off unlike static phase plates.[7] In comparison to using an SLM, the implementation of q-plate to a 4f OAM microscopy setup

[a)]Author to whom correspondence should be addressed: ralfano@ccny.cuny.edu

does not need to be with backfolded geometry, since q-plates are transmittive and do not need extra use of spiral phase filtering, since a q-plate also acts as a spiral phase filter. Unlike that of phase contrast microscopy, the q-plate does not need an annular diaphragm and diffraction plate to alter the brightness between the in and out of phase light.[1]

II. THEORY

Our 4f imaging system, shown in Fig. 1, works by placing a sample in the object plane of a Fourier transform lens L1 and a q-plate in its Fourier plane. The q-plate, acting as a spatial filter, then transmits light only in a desired spatial frequency range. This light is collected and refocused by a second Fourier transform lens L2, placed such that the object plane of L2 coincides with the Fourier plane of L1. L2 performs an inverse Fourier transform of the transmitted light. The refocused light captured by a digital camera placed at the Fourier plane of L2 then is a reconstructed image of the sample, minus the components of the light filtered at particular frequencies. Analysis of the light in the Fourier plane of a Fourier transform lens imaging an illuminated object shows that general characteristics like shapes and large scale intensity variations are associated with lower spatial frequencies, while finer details like sharp curves and edges are associated with higher frequencies. Therefore, one would expect that the edge enhancement achieved in this experiment is related to an isolation of higher spatial frequencies. The q-plate alters the phase profile of the transmitted beam such that the k-vectors near the central axis of the beam, which pass through the region near the topological defect, rotate so as to point toward the axis. The result is that, due to the "canceling" of the k-vectors near the axis, a dark vortex region appears along the beam axis. This effectively filters out the central and lower spatial frequencies of the incident beam allowing only the higher spatial frequencies near the perimeter of the beam to pass.

The radius of the ring of higher-order Laguerre Gaussian (LG) beams scales positively with the angular momentum number of a Laguerre-Gaussian beam l. We performed a computational analysis of how the distribution of transmitted spatial frequencies might change with increasing value of l to verify that higher-order LG beams will more efficiently filter low frequencies and pass high frequencies. An equation describing the spatial filter $H(\rho,\varphi)$ is being represented by the following components: the beam waist w, the focal length f of the focusing lens, and the value of l. Using the MATLAB program, it will then compute the Fourier transform, $h(r,\theta)$, of a Gaussian beam transmitted through such a filter, display the image produced in the Fourier plane, and plot the distribution of spatial frequencies along a given axial line of the Fourier plane.

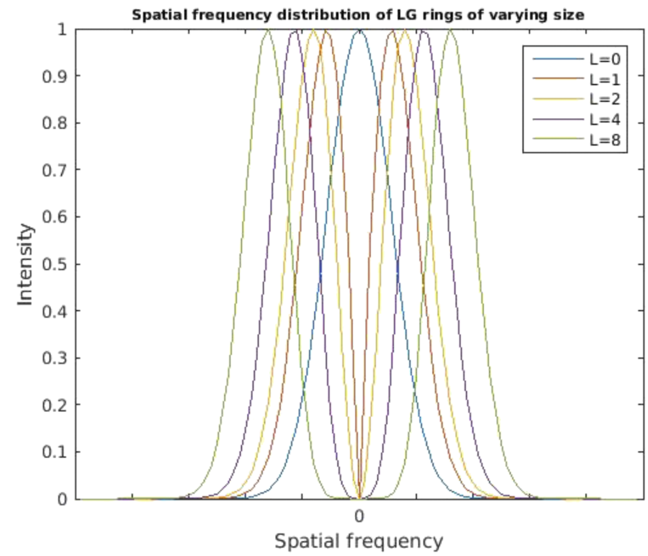

FIG. 2. Vortex beams of higher l number consist of more higher and fewer lower spatial frequencies. Spatial frequency for L = 0 is at the center and spatial frequencies for L = 1, L = 2 L = 4 and L = 8 are both to the right and left of L = 0.

A Laguerre-Gaussian spatial filter for arbitrary values of l can be represented as

$$\mathrm{H}(\rho,\phi) = \left(\frac{\rho}{\mathrm{w}}\right)^{l} \exp\left(-\frac{\rho^2}{w^2}\right) \exp(-\mathrm{i}l\phi), \tag{1}$$

where ρ is the radius, ϕ is the angle of polar coordinates in the Fourier plane, and w is the beam waist.

The equation for the Fourier transform through a lens of focal length f with a beam of wavelength λ is given by

$$\mathrm{h}(\mathrm{r},\theta) = \frac{1}{\mathrm{i}\lambda \mathrm{f}} \int_0^{2\pi} \int_0^{\infty} \mathrm{H}(\rho,\phi) \exp\left(-i\frac{2\pi}{\lambda f}\rho r \cos(\phi-\theta)\right) \rho \mathrm{d}\rho \mathrm{d}\phi, \tag{2}$$

where the function $h(r,\theta)$ is being represented in polar coordinates with r being the radius and θ being the angle. As seen in Fig. 2, a Gaussian beam with no spatial filtering contains primarily low frequencies centered around the central peak. Here all spatial frequencies are clustered around the beam axis, as expected with a Gaussian distribution. With the spatial filter applied, a cluster of frequencies appear symmetrically on either side of the axis with a region of zero intensity between. For higher values of l, this central zero region increases, and the beam composition takes on primarily higher and higher spatial frequencies. Thus our assumption supports that the high-frequency components of the beam are those that go on to produce the final edge-enhanced image.

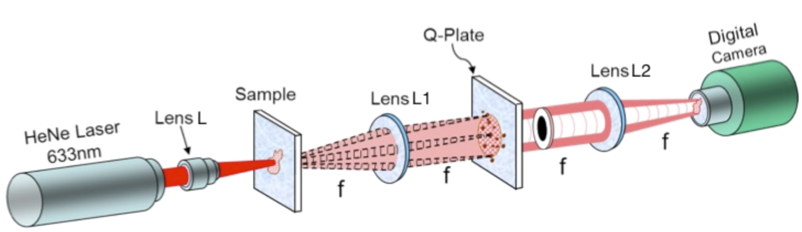

FIG. 1. Diagram of the 4f q-plate dark field OAM microscopy setup.

III. EXPERIMENTAL

The 4f OAM microscope imaging system is shown in Fig. 1. A 633 nm helium-neon laser is focused onto the sample by a microscope objective lens L, and the light is collected by a f = 75 mm focal length lens (L1) situated at a distance f behind the sample. The resulting distribution in the image plane formed by the lens L1 is the Fourier transform of the object. We place the q-plate at this Fourier plane. When there is no voltage across the q-plate, it acts as a simple half-wave retarder. When a voltage is applied with a frequency generator, about 4V at 2 kHz, the liquid crystals reorient and an incident Gaussian beam is transformed into a vortex beam. The incident light is not Gaussian but is the direct and scattered light from the sample. Thus, the q-plate is acting as a filter that overlays the spiral phase qualities of a vortex beam onto the light from the sample. A second 75 mm focal length lens (L2) is placed at the focal distance behind the q-plate, which focuses the light onto a digital camera at the image plane of L2. This image is the reconstructed image of the sample after spatial filtering in which only edges, where the step height of the sample changes, are highlighted and areas of constant height are dark.

IV. RESULTS

The edge-enhancing spatial filtering by the q-plate is demonstrated in Fig. 3 with test samples of thin metal wires. Figure 3(a) shows the image obtained of the metal wires with no voltage applied (i.e., the q-plate acts simply as a constant half-wave retarder and does not produce a vortex) and Fig. 3(b) shows the image obtained with an applied voltage (i.e., the q-plate creates a central vortex, blocking the low frequencies of the beam as described). In Fig. 3(a), the entire length and width of the metal bar are imaged (dark against red background), as expected under standard bright-field imaging circumstances. In Fig. 3(b), with the q-plate on and generating a central vortex, only the edges of the metal bars are illuminated, displaying the edge-enhancement capabilities of the q-plate. Figure 4 shows a similar test with a sample of an opaque number "3" imaged with (a) and without (b) applied voltage. This test displays the isotropic edge-enhancing quality of this microscope, which allows detection of edges that are continuous curves, not simply straight-lines oriented uni-directionally as in Fig. 3. We observe that the edge-enhanced curves of "3" are well-defined (edges of lower intensity are due to lower illumination intensity in that region) and there is no orientation preference in this method.

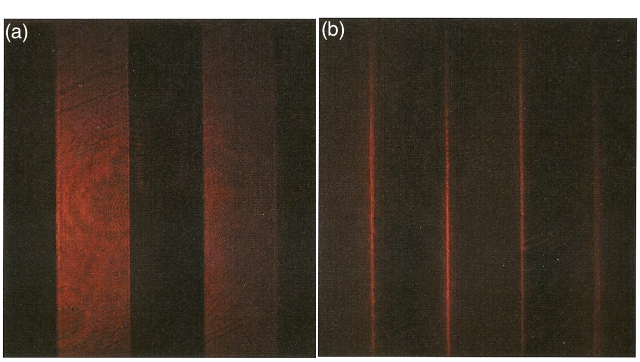

FIG. 3. Metal bars without voltage (a) and with voltage (b) on the q-plate in the 4f system.

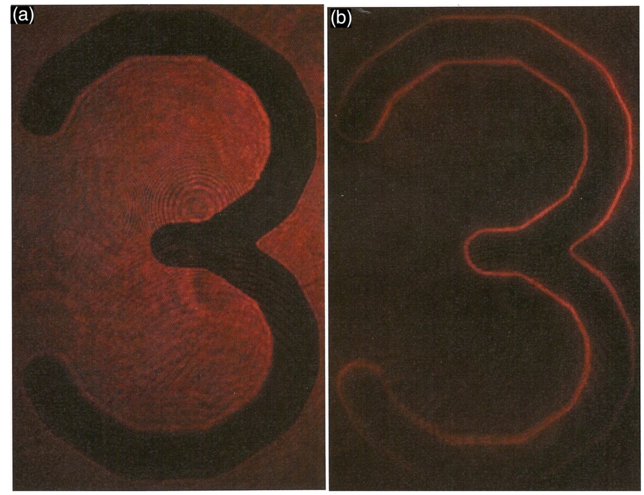

FIG. 4. Opaque number 3 without voltage (a) and with voltage (b) on the q-plate in the 4f system.

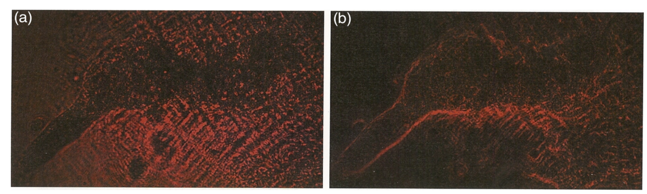

FIG. 5. Amoeba without voltage (a) and with voltage (b) on the q-plate in the 4f system.

Figure 5 demonstrates the edge-enhanced imaging capability for translucent media, such as biological samples. An amoeba imaged using the q-plate spatial filter shows both enhanced edges and reduced scatter noise from the glass microscope slide, whereas using standard bright-field imaging, the scattering from the slide would obscure the edges. We expect that the imaging of biological media will be the primary use of this method.

V. CONCLUSION

The addition of an active q-plate with charge q or any other OAM producing instrument, such as spiral phase plates or spatial light modulators, at the Fourier plane of a 4f imaging system acts as a frequency-selective spatial filter that provides enhanced contrast of sample edges. In our 4f system, this filtering is done in the Fourier plane of the first Fourier transform lens. Thus, those rays which comprise the reconstructed image of the object formed by the inverse Fourier transform lens do not include the central and lower spatial frequencies from the object. This method produces a degree of edge enhancement similar to established phase contrast imaging using static spiral phase plates, but with the added benefit of tunability for laser wavelength and the ability to turn on and off the filter. Therefore, this work has demonstrated a new compact handheld OAM microscope capable of edge enhancement imaging, shown in Figs. 3–5. Thus, we have demonstrated the viability and ease-of-use of a q-plate spatial filter microscope that can be used to obtain images of microscopic biological samples that highlight significant features, such as relative size and detailed features, that are easily obscured under standard bright-field imaging circumstances.

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[1]S. Kumar, *Textbook of Microbiology* (Jaypee Brothers Medical Publishers Ltd., New Delhi, 2012), pp. 14–15.
[2]G. E. Anderson, F. Liu, and R. R. Alfano, Opt. Lett. **19**, 981 (1994).
[3]G. Z. Wang, X. Liang, L. Wang, P. P. Ho, and R. R. Alfano, Opt. Lett. **20**, 1498 (1995).
[4]R. R. Alfano, W. B. Wang, L. Wang, and S. K. Gayen, in *Photonics, Volume IV: Biomedical Photonics, Spectroscopy, and Microscopy*, edited by D. L. Andrews (John Wiley & Sons, Inc., Hoboken, 2015), Chap. 9, pp. 367–412.
[5]S. Furhapter, A. Jesacher, S. Bernet, and M. Ritsch-Marte, Opt. Express **13**, 689–694 (2005).
[6]M. Ritsch-Marte, Philos. Trans. R. Soc., A **375**, 20150437 (2017).
[7]L. Marrucci, J. Nanophotonics **7**, 078598 (2013).
[8]L. Marrucci, C. Manzo, and D. Paparo, Phys. Rev. Lett. **96**, 163905 (2006).