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Phase contrast imaging using photothermally induced phase transitions in liquid crystals

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Phase contrast imaging is performed for live biological species using photothermal induced birefringence in dye doped liquid crystals. Using typical 4-f configuration, when liquid crystal cell is at back focal plane of Fourier lens, low spatial frequencies at center of Fourier spectrum are intense enough to induce local liquid crystal molecules into isotropic phase, whereas high spatial frequencies on the edges are not intense enough and remain in anisotropic phase. This results in $\pi/2$ phase difference between high and low spatial frequencies. This simple, inexpensive, all-optical, user-friendly, self-adaptive phase contrast imaging technique using low-power laser offers several distinct advantages. © *2006 American Institute of Physics.* [DOI: 10.1063/1.2397030]

In 1933, Zernike developed a nondestructive mechanism to observe translucent microscopic objects where a phase plate is used to create a $\pi/2$ phase difference between undeviated light and light diffracted by the object, thereby transforming minute variations in phase of the object into corresponding changes in image contrast.[1] This principle is exploited in the commercial phase contrast microscope which is widely used in teaching and research laboratories to view high-contrast images of transparent specimens. One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without being fixed and/or stained. However, there are some disadvantages associated with the commercial phase contrast microscope such as repeated alignment of condenser plate relative to the annular ring. Also it is not ideal for imaging phase objects situated a few millimeters inside scattering media as it uses an incoherent light source. With the growing demand for a variety of imaging modalities, a simple technique for not only imaging phase objects in transparent media but also imaging phase objects in tissuelike scattering media may be useful.

Several concepts are exploited in the past for phase contrast imaging with varying degrees of success.[2–11] We demonstrate here a simple, self-adaptive, all-optical technique which exploits photothermal induced phase transition in dye doped twisted nematic liquid crystals. Nematic liquid crystals (LCs) are composed of rodlike molecules which line up parallel to a preferred direction and hence are anisotropic. Traditionally, an electric field is used to align the liquid crystals, but there is an increasing trend to attain the required phase transition using optical techniques,[12–14] for applications such as image-intensity inversion of amplitude objects and spatial filtering.[15,16] Aligned liquid crystal molecules add

a certain amount of phase to the incident polarized light wave as it passes through. The relative phase retardation is given as $\Gamma = \pi \Delta n d / \lambda$, where d is the cell thickness, λ is the wavelength, and $\Delta n = n_e - n_o$ is the induced birefringence. At low light input intensities, the temperature of the liquid crystal is well below its phase transition temperature (where the liquid crystal molecules undergo phase transition from liquid crystal phase to isotropic phase) T_c. Thus an optimum phase of $\pi/2$ is added to the transmitted beam because of the large birefringence Δn in the liquid crystal phase. However, with increase in the input light intensity, the temperature of the liquid crystal increases due to the absorption by the dye molecules. This results in the increase of ordinary refractive index (n_o) and decrease in the extraordinary refractive index (n_e). For $T \geq T_c$, $n_o = n_e$ and the induced birefringence Δn vanishes. Hence no additional phase is added to the transmitted beam. Therefore, if two light beams of different intensity are incident simultaneously at different spatial locations on the liquid crystal, the local liquid crystal molecules undergo respective intensity dependent liquid crystal phase transitions. This leads to a relative phase difference of $\pi/2$ between these two light beams at the exit plane of liquid crystal cell.

We exploited this intensity dependent liquid crystal phase transitions to generate phase differences among different groups of spatial frequencies in the Fourier spectrum of a phase object as shown in Fig. 1. At position A in Fig. 1, all the waves are in phase before entering the object. As they pass through the specimen, some waves get diffracted because of phase gradients (refractive index differences) in the specimen and accumulate a $\pi/2$ phase as shown at position B. The undeviated waves from those portions of the specimen where there is no phase gradient do not accumulate any phase change. When these waves are Fourier transformed using a simple convex lens, the undeviated light corresponds to low spatial frequencies situated in the center of the Fourier spectrum at the back focal plane of the convex lens. Simi-
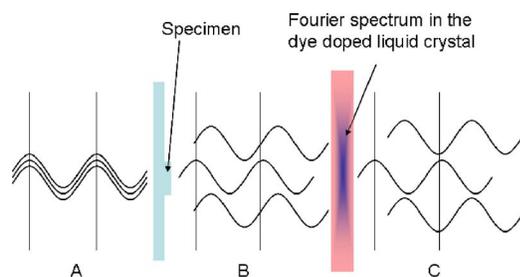

FIG. 1. (Color online) Schematic of the phase contrast imaging. (A) All the waves are in phase initially. (B) Diffracted waves (high spatial frequencies) from the specimen accumulates a $\pi/2$ phase difference relative to undeviated waves (low spatial frequencies). (C) High spatial frequencies are of low intensity and do not induce any phase transition in the local liquid crystal molecules. Hence they accumulate another $\pi/2$ phase change relative to low spatial frequencies as they pass through the cell. Thus there is a total of half-wavelength (π) phase change between high spatial frequencies and low spatial frequencies.

larly diffracted light corresponds to high spatial frequencies situated on the edges in the Fourier spectrum. As this Fourier spectrum is incident onto the dye doped twisted nematic liquid crystal cell (DTNLC), due to high intensity of low spatial frequencies, only the liquid crystal molecules situated at the center of the Fourier spectrum transform selectively into the isotropic phase, whereas high spatial frequencies on the edges of the Fourier spectrum are weak enough not to induce any phase transition in the liquid crystal. Thus low spatial frequencies transmit through the self-induced isotropic phase of liquid crystal cell without acquiring any phase difference, whereas high spatial frequencies acquire an additional $\pi/2$ of a wavelength phase change as they transmit through the liquid crystal phase (anisotropic phase) as shown at position C of the Fig. 1. As a result there is net half-wavelength (π) phase difference between high and low spatial frequencies which will interfere destructively and convert phase information of the specimen as image contrast. The addition of dye to the liquid crystal facilitates the required phase transition at low light intensities, an added advantage for noninvasive imaging of live biological specimens or imaging objects in scattering medium. Phase contrast image of live amoeba, which can be visualized only using phase contrast imaging without being fixed and/or stained, clearly identifies nucleus and other internal organelles. This image, Fig. 2, is as the good as the images obtained with a commercial instrument and in
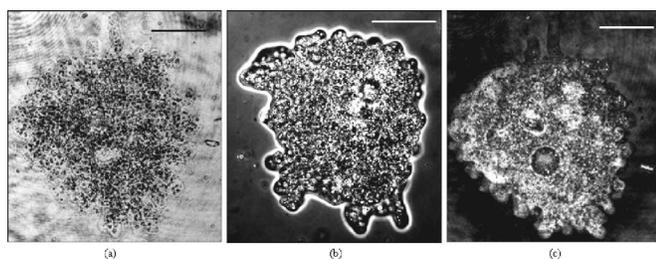

FIG. 2. Phase contrast image of amoeba. The scale bar is $\sim$50 μm. (a) Bright-field obtained at low incident intensity. (b) Phase contrast image of a similar spherical amoeba obtained using commercial phase contrast microscope. (c) DTNLC phase contrast image of the same amoeba shown in (a) at high incident intensity. In both DTNLC and commercial phase contrast images, two larger organelles, nucleus and contractile vacuole, are clearly noticeable in the center of the specimen along with smaller internal organelles and protruding pseudopodia. The DTNLC phase contrast image has more three dimensional representation than commercial phase microscopy image, multiple pseudopodia at varying depth, in better focus.
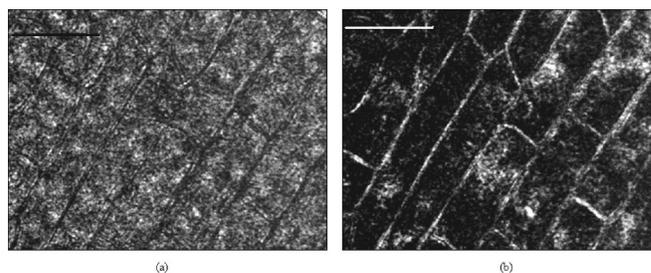

FIG. 3. (a) Bright-field image of onion cells taken with our experimental setup. The onion peel is placed in Intralipid solution (scattering coefficient is 6/cm). (b). Phase contrast image of onion cells; edges of the cell walls and nuclei within a number of these cells are clearly visible with good contrast. The scale bar is $\sim$50 μm.

some cases displays additional features. We also obtained phase contrast image, Fig. 3, for a translucent object (onion peel) placed in a scattering Intralipid solution displaying high-contrast cell walls and the nucleus within.

In the experiment we used a liquid crystal cell containing 90° twisted nematics. The cell walls are unidirectionally rubbed poly(vinyl alcohol)-coated glass substrates with the two directions arranged in a crossed configuration. The cell ($\sim$10 μm) is filled with a uniform mixture of liquid crystal 4-cyno-4′-pentyl1-1,1′-biphenyl (K15, EM Industries, $T_C \approx 35$ °C) and absorbing dye N-ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo)aniline (Disperse Red 1, from Aldrich) which has an absorption peak around 502 nm. We adopted simple 4-f configuration for the phase contrast imaging. Phase object is uniformly illuminated by a spatially filtered and well collimated 480 nm laser beam. The object information is Fourier transformed onto the DTNLC cell using a 10 cm focal length biconvex lens, and the inverse Fourier transform is imaged onto the charge-coupled device (CCD) camera using another 10 cm focal length biconvex lens. Neutral density filter is used to control the incident laser light intensity that illuminates the phase object. At low incident intensities, the CCD captures the bright-field image. As the incident intensity is increased to an optimum value generating the required $\pi/2$ phase difference between high and low spatial frequencies, the CCD captures the phase contrast image. It is observed that typical incident power required to induce the $\pi/2$ phase difference is $\sim$3 mW. The system is self-adaptive in the sense that no optical alignment is required when the object is changed or the microscope objective is changed for a different magnification. As this system does not use annulus-phase plate combination, we do not have the disadvantage of frequent alignment as in the case of commercial version.

Figure 2(a) shows the bright-field image of amoeba taken using our experimental setup at low intensity. This bright-field image of the amoeba is a two dimensional structure with no clear edges and its two larger organelles are displayed as clouded areas in the center of the specimen. Figure 2(b) is the phase contrast image of a similar spherical amoeba taken with the commercial phase microscope Leitz model SM-Lux. This image projects a slightly better three dimensional view, and the nucleus and contractile vacuole are more obvious than with bright-field microscopy though they are not sharply focused. In addition we also see many smaller sharp internal organelles with good resolution. These features inside the cytoplasm are more clearly seen than in the case of the bright-field microscopy image. Figure 2(c)

shows the DTNLC phase contrast image which clearly improves on the commercial phase contrast image. Organelles are more noticeable than with bright-field microscopy; the nucleus, contractile vacuole, and smaller organelles that move within the cytoplasm shine or have a real edge and visible volume. It has more three dimensional representation than the commercial phase microscope image, with multiple pseudopodia at varying depth in better focus. The spherical nature of the amoeba is also much more noticeable than with either bright-field or the commercial phase microscope.

To illustrate the versatility of our technique, we also performed phase contrast imaging for a phase object placed in a scattering medium and the results are illustrated in Fig. 3. Because the thin layer of onion peel is so translucent, phase contrast or staining is needed to have a good image of the cell wall, vacuole, and nucleus. The onion peel is placed in a 2 mm glass cuvette filled with uniform mixture of 100 ml of water and 3 ml of Intralipid (Liposyn II, Hospira, Inc.). It is common practice to use such a solution as scattering medium. The reduced scattering coefficient of the solution is about 6/cm. Figure 3(a) is a bright-field image. The cell walls are visible and a nucleus is noticeable in the picture. The DTNLC phase contrast image in Fig. 3(b) shows edges of the cells with much better contrast and the nuclei within are also clearly visible. A striking feature worth mentioning is that the edge effect is very noticeable in this image.

We observed that it is possible to obtain similar phase contrast imaging results using simple aligned nematic liquid crystals and doped with any absorbing dyes, for example zinc 2,9,16,23-tetra-*tert*-butyl-29H,31H-phthalocyanine as the absorbing medium with peak at around 677 nm and two rubbed substrates of the cell aligned at 10° to each other. Thus by selectively choosing the absorbing medium, the proposed technique can be used for any wavelength region. It may be worth mentioning that 700 nm could be used as it is ideal for *in vivo* imaging of tissue.

This simple phase contrast imaging technique offers several advantages. Positive as well as negative phase contrast images can be achieved by simply varying the incident intensity. The required amount of the phase difference can be achieved by the right combination of birefringence and cell thickness as the phase shift accumulates with length of the birefringent material. A unique feature of the proposed system is that by simply placing a polarizer (analyzer) after the liquid crystal cell, the configuration can be transformed into a self-adaptive spatial filtering system.

In summary, we demonstrated a simple, inexpensive, all-optical, self-adaptive phase contrast imaging technique. It is based on the spatial frequency intensity induced liquid crystal to isotropic phase transition at the Fourier plane where the liquid crystal cell is kept. The addition of absorbing dye to liquid crystals facilitates phase transition temperature at very low incident intensities—ideal for phase contrast imaging of live biological species and phase objects buried in a scattering medium. Bright-field and phase contrast images are obtained with the touch of a button that increases incident intensity, without any alignment or adjustments in the system. A laboratory version with inexpensive optical components gives images comparable to commercial instruments with high quality optics. Unlike commercial phase contrast microscopy, the technique is self-adaptive to changes in shape, size, and magnitude of phase variations of the objects. Also the technique is not limited to a single wavelength, and by addition of a suitable dye (or broadband absorbing dyes) the system can be used for any wavelength of interest.

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