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#### ABSTRACT

Unique lateral shearing properties between ordinary and extraordinary rays appearing in twisted nematic liquid crystal cells are investigated. The small lateral shear is useful for differential interference contrast imaging systems, and a potential of electrically tuning of shear distance and/or bias retardation is demonstrated to optimize the imaging.

#### **1 INTRODUCTION**

Double refraction is a typical optical property appearing in birefringent material, which is caused by the laterally spatial separation between ordinary and extraordinary rays. Since the shear can also occur in normal liquid crystal (LC) displays and it may deteriorate image quality, the lateral shear is usually suppressed within small enough. The small lateral shear, created by using optically uniaxial crystal prism, is generally utilized for differential interference contrast (DIC) imaging, which is one of the powerful microscopic observation tools for transparent samples such as bio cells. Some DIC observation systems have also been investigated by using LC cells to introduce some electronic tunability. [1-4] Half of the ideas are based on the combination with normal DIC prisms [1,2], and the others are based on the utilization of spatial light modulator [3] and LC prism [4]. On the other hand, we are proposing another method based on the basic double refraction phenomena of uniaxial crystal. The simple lateral shear created by LC cells is useful for DIC observation system, since small LC cells are easy to install into the microscope optics, and it is easy to drive directly by PC interface, which may provide another functionality to the conventional DIC imaging system. [5-7]

In this work, we focus on the unique lateral shearing properties appearing in twisted nematic (TN) cells, and investigate a potential application to DIC imaging system, which can introduce some tunability by using the LC cells.

#### 2 FUNDAMENTALS

Figure 1 shows a typical optics of DIC microscope system, which consists of a pair of DIC prisms. In this case, lateral shear is created by the combination of angular

separation between ordinary and extraordinary rays through DIC prism with a help of condenser or objective lenses, where the DIC prisms are set on the focal plane of each lenses. Phase compensation between them is achieved by the precise DIC prism configuration, which consists of two identical but orthogonal optical axes triangular prisms.

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## Fig. 1 Optical configuration of typical DIC microscope system by using solid DIC prisms.

Figure 2 shows a schematic draw of the creating phenomena of DIC by lateral shearing interference. Interference generally occurs by combining two wave-fronts, which are classified to test wave and reference wave. Here, since a little laterally sheared own wave-front is used as the reference, phase difference between two wave-fronts, which creates the interference contrast, is proportional to the derivative of the test wave-front. This is the reason why this kind of interference is called DIC. If we imagine the simple mesa shape wave-front as shown in Fig.2, edge of the mesa is enhanced or diminished according to the derivative of the wave-front structure along the shear direction. This phenomena

generally creates unique image contrast characterized by shining and shadowing edges, which makes us to feel 3D like image. DIC contrast is sensitive to the steep refractive index change, even though the difference is small because the derivative is essential. Then, DIC is very popular as a powerful tool for the observation of such as transparent test sample embedded in transparent background, which is usually very difficult to see by the normal microscope observation.



Fig. 2 Creation principle of differential interference contrast (DIC) by lateral shear interference.



Fig. 3 (a) Molecular orientation state of TN cell under voltage application. (b) Lateral separation phenomena between ordinary and extraordinary rays in the TN cell.

Liquid crystal layer confined in planar cells can be described as a stack of thin uniaxial crystals, and each layer creates a very small lateral shear according to the inclination angle of LC molecules. Since the total lateral shear of LC cell is determined by the sum of that created in each layer, it is easily understand that the shear becomes maximal under low voltage application. Especially, twisted nematic (TN) cells show unique shearing phenomena due to the twisting structure of optical axis as shown in Fig.3. The small shear direction rotates 90° inside the cell according to the twisting structure, and the extraordinary

ray finally separates 45° oblique direction. Polarization direction rotates 90° at the same time under low voltage level by the TN effect.

Our DIC system is attained by just sandwiching the test sample between a pair of identical TN cells in Fig.3. When the ordinary and extraordinary rays are separated laterally by the 1<sup>st</sup> TN cell, a large phase retardation is induced between them. However, the retardation can be compensated by the 2<sup>nd</sup> identical TN cell, because the extraordinary and ordinary rays are exchanged after passing through the 1<sup>st</sup> TN cell. The laterally separated rays are combined at the same time, although both components shift laterally from the initial position, and the direction is 45° oblique.

#### 3 EXPERIMENTAL

Laterally shearing phenomena are observed directly by using thick TN cells as shown in Fig.4, where the cell thickness is 50  $\mu$ m. Test sample is glass rod spacer with a diameter of 10  $\mu$ m, which are spread on a cell surface. We can focus on each thin bright line appearing on glass spacers without voltage. (Fig.4.(a)) When a voltage is applied a little above threshold value, a line is separated horizontally. (Fig.4(b)) As increasing the voltage, the separation tends to increase as shown in Figs.4 (c) and (d). The separation distance becomes maximal around 1.7 ~ 1.8V in this case, however, the image contrast tends to decrease probably due to the change of polarization state.



# Fig. 4 Observation of lateral shearing phenomena by using glass rod spacers. Here, 50 $\mu$ m thick TN cell is used to visualize the lateral separation.

Voltage change of lateral shear distance measured by the microscope observation is shown in Fig.5, where 40  $\mu m$  thick TN cell is used. Square plots and broken line

show the measurement results, and solid line shows the calculation results based on the LC molecular orientation state. Both are well coincident, and we can estimate actual shear phenomena starting from the calculation of LC molecular orientation.

Actual DIC observation examples of human cancer cells are shown in Fig.6. Here, 10 µm TN cells are inserted into the optics of normal polarization microscope system. Bio cells are the suitable test sample to show the advantage of DIC observation. Because bio cells are normally transparent, and they are embedded in water if in-situ observation is mandatory. Transparent sample on the transparent background is one of the most difficult samples to observe by using normal microscope as shown in Fig.6 (a). Observation image without voltage is almost the same with that of normal microscope observation. When a voltage above the threshold value is applied to the TN cells, typical DIC image of cancer cells appears as shown in Fig.6 (b). DIC observation can detect a small refractive index difference if the boundary is critical, because the contrast is created proportionally to the differential coefficient of the refractive index. Each cell boundary along the direction perpendicular to the shear direction is enhanced by shining or shadowing, which makes us to imagine 3D structure. The DIC contrast and/or background brightness are changed by changing the applied voltage as shown in Figs.6 (c) and (d). We believe that the voltage tuning function becomes an advantage of LC DIC observation system to optimize the individual sample observation.



Fig. 5 Measurement and calculation results of the shear distance as a function of applying voltage. Here, 40 μm thick TN cell is used to observe the shear distance directly, broken line and solid line show the results of measurement and calculation, respectively.

In this case, the shear distance is probably up to 1 $\mu$ m, and then there is almost no fatal degradation of image quality by the lateral shearing. Another concern is that 2<sup>nd</sup> TN cell is set at between the test sample and objective,









Fig. 6 DIC observation of human cancer cells by using TN LC DIC microscope system, where cell thickness is 10  $\mu$ m, and  $\times$ 60 objective is used.

and some degradation of imaging must occur in comparison with the normal DIC system, because the objective is designed for direct observation of test sample. However, the degradation may not so significant in this stage because we can recognize here the precise internal structure of each cancer cell by using  $\times$  60 objective.

### 4 CONCLUSIONS

A simple DIC observation system is demonstrated by using a pair of TN cells. Typical shining and shadowing contrast is observed under small voltage application. Although tuning range is limited around  $1.0V \sim 1.7V$ , observation image can be optimized by adjusting applying voltage according to each test sample. The voltage tunability is the most advantageous point when we introduce LC cells to DIC system, which is usually difficult in the conventional system.

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