

# Confocal Microscopy of Director Structures in Liquid Crystals Connected and Composite Systems

**Ivan I. Smalyukh**

Chemical Physics Interdisciplinary Program and the Liquid Crystal  
Institute, Kent State University, Kent, OH, USA

*We review approaches for simultaneous imaging of three-dimensional director structures and component distributions in composite materials using fluorescence confocal polarizing microscopy. To study dynamic processes in these systems, we use the Nipkow-disk microscope in which the confocal images are obtained within milliseconds. The visualized director fields, free-standing film profiles, and ordered colloidal structures provide insights into the physics phenomena ranging from elasticity-mediated self-organization to anchoring-assisted levitation and dynamics of micron-sized spheres.*

**Keywords:** colloid; defect; fluorescence confocal polarizing microscopy; free-standing film; liquid crystal; surface anchoring

## INTRODUCTION

Orientational order is an important property of liquid crystals (LCs) and other materials [1,2]. Molecular interactions responsible for this ordering are rather weak, so that the spatial structures of the LC director  $\hat{n}(\vec{r})$  can be modified by many factors including surface treatment, temperature changes, flow, colloidal inclusions, magnetic and electric fields, etc [1,2]. Non-destructive imaging of the three-dimensional (3-D) spatial patterns of  $\hat{n}(\vec{r})$  and component distributions in composite LC materials is important for both applied and fundamental

We acknowledge the support of the Institute for Complex Adaptive Matter (ICAM) and International Institute for Complex Adaptive Matter (I2CAM), the National Science

research. Fluorescence confocal microscopy (FCM) is broadly used to study the composite systems in 3-D [3,4] and employs coordinate-dependent dye concentrations (dyes segregate into different components) [4]. Recently, the new class of the detectors allowed researches to develop the Nipkow-disc scanning microscope (with numerous pinholes in the spinning disc) capable of FCM imaging at rates  $\sim 1000$  frames per second [5]. On the other hand, by using (a) fluorescent dye composed of anisometric molecules and (b) polarized excitation and fluorescence detection, one can transform the regular FCM into a technique that visualizes 3-D director fields, called Fluorescence Confocal Polarizing Microscopy (FCPM) [6]. In this approach, the absorption/fluorescence transition dipoles of the used dye molecules homogeneously distribute in the LC sample and follow  $\hat{n}(\vec{r})$ . When confocal imaging is performed with a controlled polarization state, the technique visualizes the 3-D pattern of  $\hat{n}(\vec{r})$ . The director structures are reconstructed based on multiple confocal images obtained for different FCMP polarization states and different sample cross-sections. The technique has been used to study electro-optic effects in nematic LCs [6–8], focal conic domains in smectics [6,8], defects [9,10] and layers undulations [11] in cholesterics, director distortions around beads [12–13], orthogonal director fields in biaxial LCs [14], and the ordered structures in anisotropic colloidal systems [15].

In this article, we review approaches for the simultaneous study of static and dynamic  $\hat{n}(\vec{r})$  in composite and confined materials [6–18]. Fluorescence dye molecules in these composite systems can follow the LC director and also can segregate into different components. The texture analysis is complicated by the non-homogeneity of dye distributions and the finite diffraction-limited FCMP resolution. We therefore use multiple dye labeling and spectral separation of fluorescent signals from specially-selected dyes as well as a comparison with computer simulations. This allows one to decipher  $\hat{n}(\vec{r})$  in confined LCs, free-standing films, phase-separated systems and colloidal suspensions, providing also information on the spatial location of different components. Finally, FCMP and other non-invasive imaging techniques, such as coherent anti-Stokes Raman scattering microscopy, are discussed from the standpoint of applications in the study of composite LC systems.

## EXPERIMENT

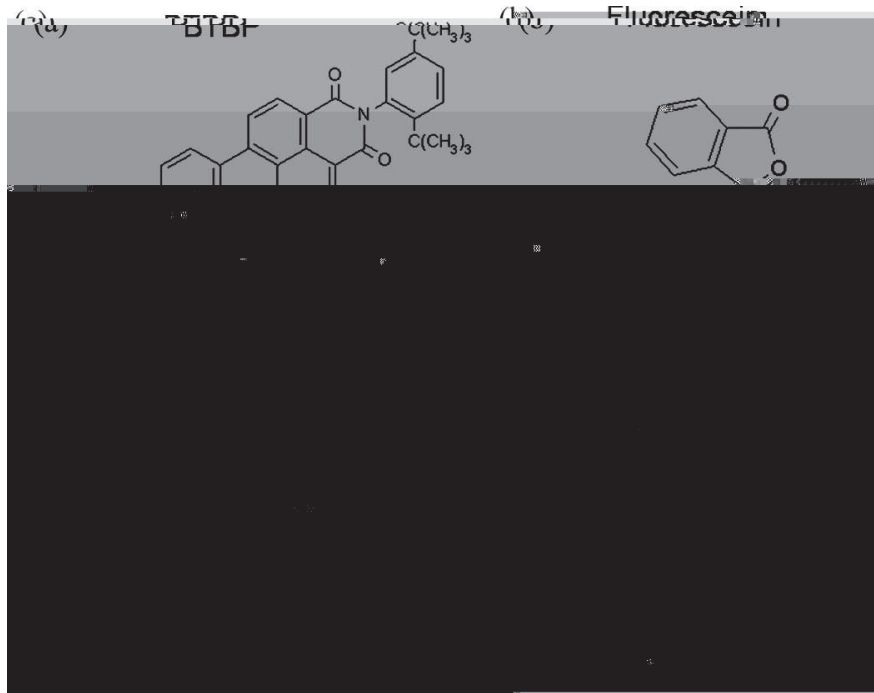
### Experimental setup

Figure 1a shows the FCMP set up based on an Olympus Fluoview BX-50 confocal microscope. An achromatic linear polarization rotator



**FIGURE 1** (a) Two-channel FCPM and (b) fast FCPM based on a Nipkow-disk confocal microscope.





**FIGURE 2** Chemical structures of the used fluorescent dyes

different components, as discussed below for specific examples. Dyes used for director imaging, such as the *n,n'*-bis(2,5-di-*tert*-butylphenyl)-3,4,9,10-perylenedicarbonylimide (BTBP, Fig. 2a), have relatively short fluorescence lifetime ( $\tau_f = (3.7 - 3.9)\text{ns}$  for BTBP [19]) which is smaller than the characteristic time of rotational diffusion  $\tau_D \approx 10\text{ ns}$  in LCs. Therefore, molecule orientations during absorption and emission are assumed to be the same [6,7]. The translational diffusion coefficient for most dye molecules in LCs is  $D \sim 10^{-10}\text{ m}^2/\text{s}$ . Therefore, to diffuse a distance  $L = 1\ \mu\text{m}$ , the dye molecule would need time  $t \approx L^2/D \approx 10\text{ ms}$ , which is much larger than the time during which the fluorescent light is emitted. Therefore, the dye molecule emits within the same diffraction-limited volume in which it was excited.

#### Orientation of Rotator or FC M

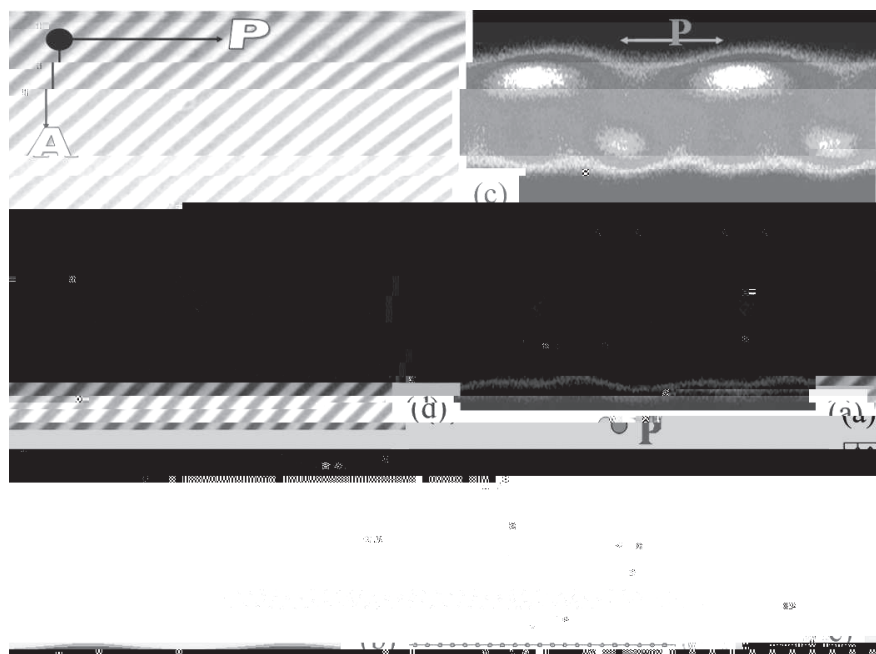
In principle, the FCPM polarization can be changed by rotating a polarizer in the common path of excitation and emission light (Figure 1). However, this approach has disadvantages: (a) the excitation intensity



**resolution that can be achieved using an immersion oil 60X objective with  $NA = 1.4$  is  $\sim 0.2 \mu\text{m}$  in the lateral plane perpendicular to the microscope's axis and  $\sim 0.6 \mu\text{m}$  along the axis [3,4]. However, resolution is usually worse in birefringent media such as LCs, in which**







**FIGURE 4** (a) Polarizing microscopy texture of a field-induced periodic structure in a thin cholesteric cell. (b) Computer-simulated director field in the cell's vertical cross-section. (c,d) Experimental vertical cross-sections of the structure for two orthogonal FCPM polarizations and (e) Computer-simulated FCPM texture corresponding to (d). For details on computer simulations of (b,e) see Refs. [7,8]. The fluorescence intensity color scale is the same as in Figure 2. The black line in (a) indicates the location of FCPM cross-sections (c,d).

experiment. Since the cell thickness and cholesteric pitch are of the same order as the spatial resolution, the features of FCPM textures are determined not only by  $\hat{n}(\vec{r})$  and FCPM polarization, but also by effects of finite resolution [6,7]. For example, in the regions at bounding plates with  $\hat{n}(\vec{r})$  parallel to  $\hat{P}$ , the fluorescence signal does not drop to zero at the LC-substrate interface but is blurred over the distance determined by the resolution. The fluorescence signal in each pixel of the FCPM image is an integral of fluorescence intensity over a diffraction-limited volume determined by resolution:  $I_{FCPM}(x, y, z) \propto \int \int \int I(x', y', z') T(x - x', y - y', z - z') dx' dy' dz'$ , where  $T$  is the weight function that in simulations is assumed to be of the Gaussian type [7,8]. Therefore, the comparison of experimental FCPM textures with computer-simulated  $\hat{n}(\vec{r})$  and textures accounting for the finite resolution (Fig. 4) is important to identify the director structures.

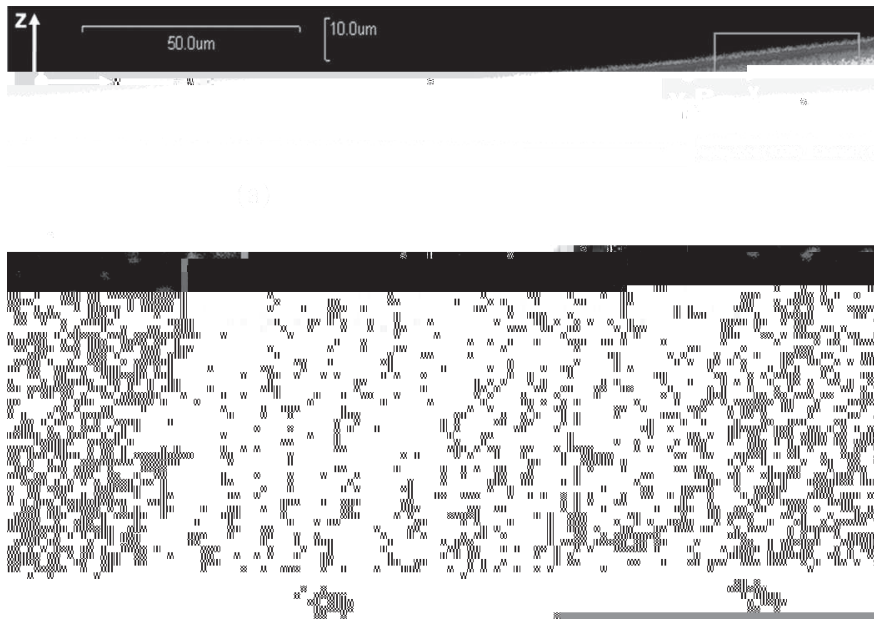
Once the basic features of a director structure are deciphered, it can be further studied, say, as a function of applied electric field. Figure 5 shows the FCPM vertical cross-sections of a cholesteric cell in the  $\pi$ -Grandjean zone for different applied voltages. Starting from the

**threshold,  $\hat{\mathbf{n}}(\vec{r})$  continuously changes with voltage increase up to  $\sim 8$  V at which a transition to a homeotropic texture takes place. At voltages  $> 8$  V,  $\hat{\mathbf{n}}(\vec{r})$  is unwound and vertical everywhere but in the thin regions next to the substrates. This is visualized by the FCPM optical slice of**

## **Shape of Meniscus and Director Structures in Free-standing Films**

**One of the fascinating properties of lamellar LCs is that they can form thin free-standing films [1,2]. Recently, the attention has been drawn to the meniscus region of free-standing SmA films [20,21], in which the film thickness usually changes in a broad range from nanometers to**





**FIGURE 7** Chains of focal conic domains in the meniscus region of free-standing film as visualized by FCPM: (a) vertical  $xz$ -section; (b,c)  $xy$ -sections with the polarizer (b) along the thickness gradient ( $x$ -direction) and (c) perpendicular to the thickness gradient (along the  $y$ -direction).

Figure 7 [22]. Up to the thickness  $(20-40)\mu\text{m}$ , the elliptical FCD bases are located approximately in the middle plane of the film. This observation can be explained by the homeotropic anchoring at the LC-air interface which favors the locations of the elliptical bases in the film's middle plane (because the surface anchoring energy at air-LC interfaces is minimized). In the thicker parts of the films, the FCD elliptical bases are often displaced from the middle plane but always remain in the film bulk and rather far from the surfaces. The size of FCDs increases with film thickness within  $(10-40)\mu\text{m}$ . When the film thickness exceeds  $(50-70)\mu\text{m}$ , the FCDs can be located at different levels of the vertical cross-section, Figures 6,7. Thus, FCPM allows one to simultaneously study both LC film profiles and the respective director structures.

### Co-orientation of Director Structures and Director Structures

Now we discuss the FCPM applications to LC emulsions and suspensions that show wealth of fascinating phenomena such as elasticity-mediated colloidal interactions [23]. We use colloidal system of glycerol droplets at the LC surface, Figure 8, obtained as in Ref. [15].



polar; their solubility in glycerol is much better than in 5CB; after the glycerol-LC phase separation, the Fluorescein molecules stay in glycerol whose polar molecules contain the same hydroxyl groups as the dye. Nile Red molecules have hydrophobic tails and anisometric shapes, similar to the LC; therefore, this dye stays predominantly in the LC after the phase separation. Nile red molecules are well aligned by the LC matrix, as needed for the LC director imaging. The maximum absorption wavelength of Fluorescein matches the wavelength of Ar-Laser excitation, 488 nm, whereas the Nile Red dye is efficiently excited by Kr-Laser at wavelength 568 nm. The emission crosstalk between the two fluorophores is negligible, which allows one to separate the fluorescent signals from the dyes using interference filters. Fluorescence is detected in the spectral ranges 510–550 nm from Fluorescein and 585–650 nm from Nile Red.

FCPM textures of the sample's vertical cross-section, Figure 8, demonstrate that glycerol droplets are trapped at the LC-air interface; top droplet parts are protruding from the nematic film, Figure 8. The polarized fluorescence signal from Nile Red visualizes  $\hat{n}(\vec{r})$  around

bounding plates, the particles produce elastic distortions, Figure 9. The director structure is axially symmetric with respect to the axis orthogonal to the substrates and crossing the particle's center. By applying DC electric fields  $\sim 5 \text{ V}/\mu\text{m}$ , the particle can be shifted across the cell's vertical cross-section; the shift direction is determined by the voltage polarity. Using polarized FCPM signal from BTBP, we determine both  $\hat{n}(\vec{r})$  and the particle positions in the cell, Figure 9. Figure 9a,b shows spatial displacements of particles in the vertical cross-section; the respective changes of  $\hat{n}(\vec{r})$  are also visualized. A par-



**forces acting on the micrometer-sized particle are negligible and the equilibrium particle's position is usually symmetric with respect to the bounding plates. Thus, FCPM allows one to explore dynamics in composite LC systems.**

## **C NCL I N AND L K**

**We have demonstrated that Fluorescence Confocal Polarizing Microscopy allows one to image 3-D director fields not only in the spatially-homogeneous (in terms of composition) LCs, but also in the confined and composite LC materials. The technique also visualizes**

**neglected. Another advantage is that FCPM imaging of the composite**

- [19] Ford, W. E. & Kamat, P. V. (1987). *J. Phys. Chem.*, **91**, 6373.
- [20] Pieranski, P. et al. (1993).