

# Fluorescence Confocal Polarizing Microscopy: Imaging Liquid Crystal Director Fields in Three Dimensions

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3D imaging · confocal microscopy · liquid crystals · soft matter

Confocal microscopy<sup>[1–3]</sup> has triggered a new era of instrumentation development in light microscopy. Nowadays, confocal microscopy is an established three-dimensional imaging technique, which has already been used in the biological and medical sciences<sup>[4–6]</sup> for quite some time, while its advantages for application in soft condensed matter systems are only beginning to be explored. This includes colloidal and polymeric systems,<sup>[7–9]</sup> but also liquid crystals, which will be discussed in relation to confocal microscopy in this Highlight.

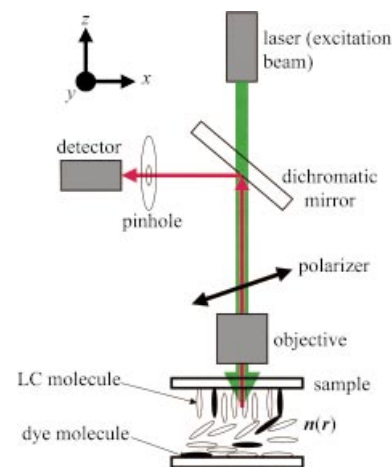
The principle of confocal microscopy relies on a laser beam being focused to a small volume ( $< 1 \mu\text{m}^3$ ) of the sample and rastered across it by a scanning unit which contains two mirrors. A very small pinhole, which is located directly in front of the detector, suppresses all scattered light from outside the focal plane. This technique provides a resolution of approximately  $0.2 \mu\text{m}$  in the  $x$ – $y$  sample plane and of  $0.3 \mu\text{m}$  in the  $z$ -direction through the sample. To achieve contrast in soft matter systems, the objects of interest are generally labelled by a fluorescent tag, for example in the imaging of polymer networks formed by photo-polymerization of reactive mesogens within a liquid crystal host.<sup>[10, 11]</sup> The imaging in fluorescence confocal microscopy (FCM) is based on a concentration gradient of the fluoro-

phore; only the phase separated polymer network is fluorescent, the liquid crystal host is not. The excitation laser beam of short wavelength (usually 488 nm, Ar<sup>+</sup>) passes a dichromatic mirror and is focused by an objective into a small volume of the sample. The fluorescent light of longer wavelength is reflected by the dichromatic mirror, passes the pinhole, and is recorded by the detector. Scanning an area of the sample, a two dimensional  $x$ – $y$  plane of the (fluorescent) polymer network within the liquid crystal is obtained. Thus, by moving the focal plane through the sample to different  $z$ -positions, the various “slices” can be used to reconstruct a three-dimensional image of the network. The same principle is analogously applied to image colloidal particles or aggregates.<sup>[12, 13]</sup>

Liquid crystals (LC)<sup>[14, 15]</sup> are partially ordered, anisotropic fluids. They consist of anisotropically shaped molecules, often rodlike, which form so called calamitic LC phases. The rodlike mesogens can either exhibit solely orientational order of the long molecular axis along a preferred direction, called the director  $\mathbf{n}(\mathbf{r})$ , like the nematic and chiral nematic (cholesteric) phase, or exhibit additional one- or two-dimensional positional order, which leads to the layered structure of the smectic phases. Liquid crystal phases generally exhibit a wealth of defect structures. Due to birefringence, these lead to characteristic textures observed in conventional polarizing microscopy (PM) and are used for identification of the respective phase. A clear drawback of PM is the lack of resolution through the sample (in the  $z$ -direction). Images only contain information on two dimensions, obtained by

optical integration of the director field  $\mathbf{n}(\mathbf{r})$  along the direction of light propagation. Thus, polarizing microscopy is not capable of directly producing a three-dimensional image of the director field of a liquid crystal.

This has now become possible by a combination of polarizing microscopy and fluorescence confocal microscopy, fluorescence confocal polarizing microscopy (FCPM), as introduced by Lavrentovich et al.<sup>[16]</sup> Two simple modifications are made to FCM and sample preparation. A polarizer is added to the FCM setup before the light passes the focussing objective, to result in a setup which is schematically depicted in Figure 1. The



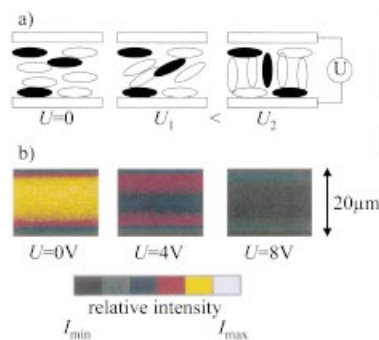
**Figure 1.** Schematic experimental setup of fluorescence confocal polarizing microscopy, FCMP (after ref. [16]).

detection is not anymore based on a spatial concentration gradient of the fluorophore, but instead a homogeneously distributed fluorescent dye within the liquid crystal is used. The polarizer selects the polarization of the excitation as well as the fluorescent beam, thus the recorded

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fluorescent light reflects the spatial distribution of the director field  $\mathbf{n}(\mathbf{r})$  assuming that the dye orients parallel to the liquid crystal director. This requires the selection of an adequate fluorescent probe for different liquid crystals. The dye concentration as well as the light intensity have to be small in order to avoid director field distortions due to the Janossy effect<sup>[17]</sup> (light-induced reorientation of dyes). Special care has also to be taken in the interpretation of images obtained from samples with large-pitch helical superstructures (twisted nematics) because, for light propagating along the twist axis within the Mauguin regime ( $d\Delta n \gg \lambda/2$ , for a cell gap  $d$ , birefringence  $\Delta n$ , and wavelength of light  $\lambda$ ), the polarization of light follows the liquid crystal director field  $\mathbf{n}(\mathbf{r})$ .

Lavrentovich and co-workers<sup>[16]</sup> have nicely demonstrated the applicability of fluorescence confocal polarizing microscopy for three-dimensional imaging of liquid crystal director configurations on two selected examples: focal conic domains of the smectic A phase, as well as the Fredericksz transition of a nematic LC with positive dielectric anisotropy. The latter demonstrates especially clearly the advantages of FCPM over PM. The Fredericksz transition is the general electro-optic effect exploited in practically all commercial display applications of liquid crystals today. Depending on the applied field amplitude, the director reorients from a planar (horizontal) to a homeotropic (vertical) orientation with respect to the substrate plates (Figure 2a). This reorientation can be followed in PM as a change in transmitted light intensity, but no direct information about the director field  $\mathbf{n}(\mathbf{r})$  across the cell is available. In contrast, FCPM provides full information about the director configuration along the z-direction of the liquid crystal cell, demonstrating a) an increasingly deflected director from the planar orientation with increasing field amplitude, b) the reorientation starting in the middle plane of the cell ( $z = d/2$ ), and c) boundary layers with planar alignment even for large field amplitudes (Figure 2b). This is a direct experimental confirmation of earlier computer simulations.



**Figure 2.** a) Schematic illustration of the Fredericksz effect in a nematic liquid crystal with positive dielectric anisotropy. b) Cross sections of a nematic liquid crystal cell under electric field application, as determined in ref. [16] by FCPM (reprinted from ref. [16], with permission from Elsevier Science).

Fluorescence confocal polarizing microscopy (FCPM) does in fact literally add a new dimension to polarizing microscopy of liquid crystals. The molecular orientation in defect structures like Schlieren brushes in nematics and smectic C (SmC) phases or oily streaks in cholesteric phases can now be investigated directly by a true 3D imaging technique. But also for investigations of zigzag defects, domain walls in ferroelectric liquid crystals, or horizontal SmC\* chevron configurations, FCPM is an experimental technique of great potential. Perhaps even structures of frustrated LC phases, like twist-grain boundary phases, especially the undulated UTGBC\* texture, can now be eluded in more detail. Following the LC director profile in polymer-network stabilized and polymer-dispersed liquid crystals touches questions of a more applicational background, referring to reflective displays. How do micron-sized inclusions, air bubbles, (surface modified) colloidal particles (of different shape) and so forth alter the director field of a liquid crystal? What is the structure of defects introduced by particle inclusion? Obviously, the potential of fluorescence confocal polarizing microscopy is not limited to the study of 3D liquid crystal director fields, but can be applied to all anisotropic systems provided that a suitable (anisotropic) fluorescent dye can uniformly be dispersed within the sample. An example would be morphological changes of anisotropic polymers during tempering. For the future, time-

resolved FCPM would add yet another dimension to imaging in soft matter systems, allowing in situ 3D investigations, for example, of evolving structures during polymerization in anisotropic media, monitoring the changes of liquid crystal director fields during the (slow) motion of included particles or during aggregation processes, or following the dynamics of defect creation and annihilation. It is evident that fluorescence confocal polarizing microscopy provides a very valuable experimental tool in soft condensed matter research and opens a large potential for investigations on numerous anisotropic systems with a variety of open questions to be answered.

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