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Review

Optical Biosensing of Polarized Light

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Abstract: Interactions between liquid crystal molecules and target analytes open up various biosensing applications for quick screening and point-of-care applications. In this review, we categorized biosensors by type, depending on the liquid crystal mesophase, and considered several applications for the detection of biomolecules, point-of-care diagnostics and environmental monitoring. We also discuss interactions between polarized light and target pathogens dispersed in biological fluids, which result in the change of the polarization state. An array of the Stokes parameters can be compared with the pattern, and a proper pathogen can be manifested. We suggest that a combination of a micropolarizer array and a complementary metal oxide semiconductor sensor is an optimal setup for the detection of pathogens. Herein, we discuss the working principles of liquid crystal biosensors and their fabrication principles. In addition, relevant theoretical and practical issues related to liquid crystal biosensors are outlined. In general, this review gives an in-depth survey of the research on liquid crystal-based sensors, making it easier for researchers to locate their niche and make contributions to this subject from multiple viewpoints.

Keywords: liquid crystal biosensor; liquid crystal droplet; point-of-care testing; liquid crystal mesophases; environmental monitoring

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1. Introduction

1.1. Definition of Liquid Crystal Biosensors

There exist different kinds of biosensors, such as electrochemical, wearable, amperometric, potentiometric, optical, impedimetric and thermometric [1]. Liquid crystal (LC) biosensors belong to the type of optical biosensors, which employ its anisotropy and sensitivity to external stimuli. As it is known, LCs are highly sensitive to environment changes, e.g., temperature, electric fields and surface interactions. The inherent property of LCs to respond to external stimuli gives LC biosensors a high potential to contribute to the new biosensing era. Furthermore, LC-based sensors do not require molecular labels, electric power, intensive labor and complex instrumentation, which makes them extraordinarily attractive in the development of inexpensive and portable devices for point-of-care diagnostics. The main advantage of LC biosensors lies in their label-free and real-time detection capabilities, i.e., that no additional tags or labels are required for signal detection, and the response can be assessed as it occurs. Earlier obtained results demonstrate that LC biosensors are highly sensitive to low concentrations of target molecules. This property makes LC sensors valuable tools for medical diagnostics, environmental monitoring and biotechnology research.

Specific biological recognition elements are immobilized on the surface of LCs. In particular, the target analyte (e.g., antibodies, enzymes and bacteriophage) binds to recognition elements [2]. Consequently, it induces changes in the local surface properties, altering

the alignment of LC molecules. As a result, it is possible to visualize changes in color, light polarization or birefringence.

In order to keep the volume of the review within an acceptable length, we only consider LC-based biosensors, though nanophotonic biosensors based on plasmon resonance have emerged to address the limitations of bioanalytical methods in terms of sensitivity [3]. We also report an increasing number of potential applications of 2D materials in biosensing and healthcare [4]. Liquid crystal biosensors have their own niche applications, mainly due to specific properties that are analyzed below and summarized in the Discussion section.

1.2. Importance of Biosensors in Applications

LC biosensors have gained significant importance in various applications, especially in biomedical applications. Registration of the characteristics of scattered polarized light on biological agents shows a simple example of the relevance of LC biosensors. The currently known methods of direct cultivation, fast cultivation and quantitative determination of potentially pathogenic microorganisms have a number of drawbacks, e.g., not all pathogens have developed nutrient media (not more than 10% of microorganisms), take a long time (up to 1 month for several strains) and require skilled lab personnel. Optical visualization of biological agents does not include the mentioned drawbacks. In general, the importance of LC biosensors can be summarized for the following applications.

1. Medical diagnostics. Liquid crystal biosensors enable the rapid and sensitive detection of specific biomolecules or pathogens in clinical samples, aiding in the diagnosis of diseases [5]. These biosensors have been employed in the detection of various analytes (e.g., proteins, DNA, RNA and viruses), leading to early disease progression and improved patient outcomes.
2. Point-of-care testing (POCT). The label-free and real-time nature of LC biosensors makes them well suited for POCT applications. They allow for on-site and rapid analyses of samples without the need for complex laboratory equipment, enabling timely and convenient medical testing in resource-limited settings [6].
3. Environmental monitoring. LC biosensors can be used to detect pollutants, toxins and contaminants in environmental samples. They offer high sensitivity, making them effective tools for monitoring water quality, air pollution and other environmental hazards [7].
4. Food safety and quality control. Prototype devices based on LC biosensors are known for applications in the food industry to detect pathogens [8], allergens and spoilage indicators. Their ability to detect low concentrations of contaminants is crucial for ensuring food safety and maintaining product quality [9].
5. Biotechnology and life sciences research. Liquid crystal biosensors have found applications in various research areas, including studying protein–protein interactions [10], receptor–ligand binding and cellular signaling processes. Their label-free nature and sensitivity allow researchers to investigate complex biological interactions in real time.
6. Biosensing in wearable devices. The sensitivity and compatibility of LC biosensors with flexible substrates make them promising candidates for integration into wearable health monitoring devices. They can provide monitoring for biomarkers [11], allowing for personalized healthcare applications.
7. Nanotechnology and nanomaterial characterization. LC biosensors are valuable tools for characterizing nanomaterials and nanoparticles [12]. They can detect subtle changes in surface properties and interactions, aiding in the development of novel nanotechnologies.

Thus, LCs have the potential to change the methods of medical diagnostics, environmental monitoring and other fields, contributing to advancements in healthcare, biotechnology and scientific research. In this regard, label-free biosensing techniques become important for the detection of bioanalytes leveraging refractometric imaging [13]. The proceeding subsection discusses the inherent physical properties of LCs, which constitute the working principles of biosensors.

1.3. Overview of the LC Properties for Biosensors

The anisotropy of LCs represents their key property and includes dependence on the refractive index versus the direction of light propagation and directional property of electrical conductivity. Temperature changes also expose many interesting effects in LCs. All these properties have made LCs attractive for a variety of technological applications. Let us remind the readers of the properties of LCs.

Molecular ordering. Molecules of LCs tend to align along the preferred direction, which is described by a unit vector, called a director **n**. Such molecular ordering gives rise to the unique optical and electrical properties of LCs.

Phase Transition. Liquid crystals exhibit a multitude of phase transitions of the second order, forming different mesophases. Transitions to the mesophases may be brought about in two different ways: one by purely thermal processes and the other by the influence of solvents. The nature of the mesophase depends sensitively on the backbone, the mesogenic unit and the spacers. Consequently, the symmetry of LC phases can be categorized in terms of orientational and translational degrees of freedom.

Response to external fields. LCs are highly sensitive to external stimuli, such as electric fields **E**, temperature changes and mechanical stress. This consideration is based on a classic picture of a nematic LC as a medium with no dielectric dispersion and instant dielectric response; the dielectric torque $\mathbf{M}_d = \varepsilon_0 \Delta\varepsilon (\mathbf{E}\cdot\mathbf{n})\cdot\mathbf{E} \times \mathbf{n}$ is quadratic in the electric field, where ε_0 is electric constant and $\Delta\varepsilon$ is the dielectric anisotropy [14]. The influence of these stimuli induces changes in the molecular ordering and alterations in the optical properties of the materials.

Birefringence. LCs are birefringent materials, i.e., have different refractive indices for light polarized along different axes. This property is critical for their application in displays and sensors.

Flexibility and compatibility. LCs can be incorporated into flexible and deformable materials, making them perfect for applications in flexible displays and wearable electronics.

The combination of these unique properties makes LCs versatile and applicable in a wide range of technologies, particularly in the realm of displays and optics. They allow the precise control of the light transmission, polarization state and phase. A detailed description of how these properties work to detect any changes is discussed in Section 2.

How do all these properties work in the detection of target molecules? The proceeding section summarizes the major points of interaction between LC molecules, target molecules and light.

1.4. Working Principles of LC Biosensors

The working principle of LC biosensors is based on the LC response to the presence of target biomolecules or analytes (e.g., [9]). These biosensors utilize van der Waals and dipole–dipole interactions to detect and quantify interactions between the recognition elements immobilized on the LC surface and the target molecules. A general overview of the working principles is provided below.

1. Surface immobilization is exposed in the immobilization of specific biological recognition elements on the surface of the LC. These recognition elements can be antibodies, enzymes, aptamers or other biomolecules that have a high affinity and selectivity for the target analyte [15]. Therefore, enzyme-based chemical biosensors are applied in biological recognition.
2. Molecular recognition and binding is a selective process that induces changes in the local surface properties around the immobilized recognition elements [16].
3. The molecular rearrangement of LCs in droplets consists in the binding of the target analyte to the recognition elements, which results in changes in the LC alignment near the immobilized biomolecules. Consequently, the anisotropy of LCs enables the observation of the molecular alignment by using polarized optical microscopy.
4. The optical response is exposed in the rearrangement of the LC molecules, which leads to alterations in the optical properties, e.g., birefringence, polarization or light

scattering. These changes can be captured and quantified by using various optical techniques, such as polarized light microscopy, spectroscopy or ellipsometry [17].

5. Signal detection and analysis of the optical response of an LC biosensor can be monitored in real time as biomolecular interactions occur. The captured changes in optical signals are then analyzed to determine the presence and concentration of the target analyte in the sample [18].
6. The calibration and quantification of the LC response to the target analyte consist of measurements of quantifiable internal reference standards, the characteristics of which are measured by experimental observation under differing conditions. The response of the LC biosensor is usually calibrated by using ab initio known concentrations of the analyte. The corresponding calibration curve helps to establish a correlation between the changes in the optical signal and concentration of analyte [19].

Thus, LC biosensors are highly sensitive materials for the detection of low concentrations of target analytes, which makes them valuable tools for biomedical applications and control of the environment. The real-time and label-free nature of these biosensors also make them suitable for point-of-care testing and various monitoring applications. Our further analysis is based on explanations of the orientational switching of LC biosensors by using case studies.

2. Types of LC Biosensors

The birefringent optical response of LC molecules gives rise to bright optical textures under crossed polarizers. Another distinguished feature of LCs is low anchoring energy (10^{-6}–10^{-3} J/m^2). Consequently, the alignment of LCs is sensitive to the chemical structure of the substrates and the presence of various biochemical substances. The long-range orientational order of LCs extends the changes in the interfacial alignment of LC molecules over distances of up to ~100 µm. This results in the amplification of molecular interactions into visible optical signals, which are detectable by polarized optical microscopy (see Section 1.4). The set of biosensor applications and LC properties for biosensors make us believe that the development of LC-based biosensors will result in a new therapeutic window for point-of-care diagnostics (see Figure 1).

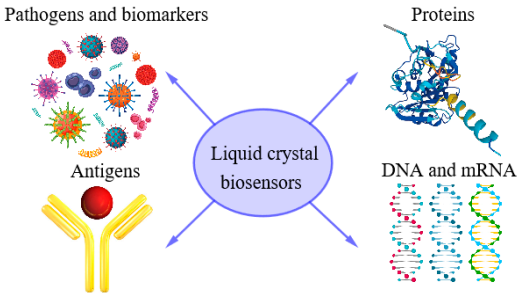

Figure 1. Potential applications of LC biosensors in point-of-care diagnostics.

One can make several comments on Figure 1. Proteins are involved in all vital processes within cells and organisms, such as cell signaling and the immune response. LC-based biosensors have also been developed for protein and peptide detection, e.g., amyloid fiber formation in Alzheimer's disease is a lipid membrane-associated process, since lipids lead to the acceleration of aggregation by several orders of magnitude [20]. Based on a coated lipid, it was demonstrated that LCs can serve as markers for distinguishing molecular secondary structures in proteins of protein aggregates [21]. Functional interfaces for the detection of environmental pollutants, toxic metal ions and disease-causing pathogens were earlier fabricated by using LCs (e.g., [22,23]). Cancer biomarkers (proteins, DNA, RNA, cell-surface receptors, antibodies, etc.) that are present in serum, blood or tumor tissues can be visualized for assessment and the forecasting of cancer [24]. Another recent application of LC biosensors was demonstrated in the specific sensing of goat Immunoglob-

ulin G by a nematic liquid crystal material [25]. The listed potential applications reinforce our observation that differences in LC features develop early in response, and they seem to persist.

2.1. Nematic LC-Based Biosensors

Biological agents, biomarkers and lightweight molecules are essential indicators of biological processes. In particular, biomarkers are attracting close attention in diagnosing diseases and the optimization of treatments based on accurate measurements of biomarkers in body fluids. The investigation of methods for the identification of cancer cells is necessary for early detection and proper selection of the treatment method.

In order to address the inherent limitations of bioanalytical methods in terms of early sensitivity, we suggest taking advantage of the sensitivity of LCs, which is affected by the bounding surfaces and optical anisotropy. This enables us to transfer chemical and biological changes in tissues into the changes of scattered polarized light. It is clear that multiple light scattering induces changes in the degree of beam polarization and Stokes parameters. Accordingly, uncertainty arises in the expected properties of polarized light. A combination of changes in polarized light, induced by biological objects, opens up an opportunity to extract information about the structural state of a sample (tissues, cells and saliva) and the presence of morphological changes. These changes can be detected by, e.g., a cosine similarity approach.

Earlier published results showed that it is technically possible to assemble a matrix of micropolarizers to detect the Stokes parameters and obtain multiple microscopic images of tissues [26]. Like a vast majority of commercially available camera systems, image sensors were originally designed to capture intensity and color. However, these sensors are "blind" to the polarization state. Capturing the polarization state of light reflected or emitted by objects in an input image scene provides valuable information about the geometrical, physical, chemical, etc. properties and a series of numerical data for a number of machine vision problems, e.g., automatic target detection. Let us consider the method of the optical detection of pathogens in detail.

Multiple light scattering on biological objects (see the input image scene in Figure 2) develops a pattern with the set of Stokes parameters. If the object has pathogens, then the Stokes parameters will differ from the sample. The subsequent registration of the Stokes parameters $\left(S_0'', S_1'', S_2''\right.$ and $\left.S_3''\right)$ by using a complementary metal oxide semiconductor (CMOS) sensor and a collection of polarized light microscopy images obtained by light scattering in the sampled biological medium and a biological medium with morphological changes make it possible to form a bank of sampled data (see Figure 2). A schematic representation of the biosensor enables measuring the Stokes parameters of circularly polarized components. These components are expressed as follows:

$$S'' = M_{\mathrm{LP}} \cdot M_{\mathrm{ret}} \cdot S \tag{1}$$

where M_{LP} is the Mueller matrix of an ideal linear polarizer, and M_{ret} is the Mueller matrix of the retarder. Since only the input Stokes parameter are measurable, the intensity of the emerging light from the retarder–polarizer combination is $S_0'' = I(\theta, \varphi)$.

In fact, each set of (θ, φ) corresponds to one optical polarizing device. Mathematically, at least four intensity measurements with four different optical polarizing devices having four different values of (θ, φ) are needed to extract all the Stokes parameters in expression $I(\theta, \varphi)$. The four Stokes parameters can then be expressed by the linear combinations of the emerging light intensities after transmitting through the four different optical polarizing devices. This analysis suggests that there should be an assumption that the polarization state of scattered light is spatially uniform. We hypothesize that changes in the polarized light traveling through biological objects make it possible to extract information about the structural state of the sample (tissues, cells, saliva and biomarkers) and the presence of morphological changes.

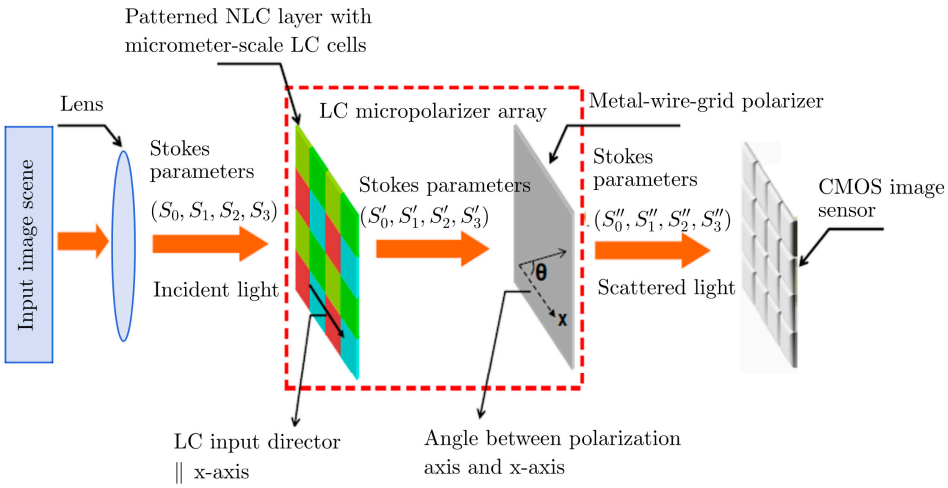

Figure 2. Schematic representation of an LC biosensor with a micropolarizer array.

A key component of the setup depicted in Figure 2 is the two-dimensional micropolarizer array, which is fabricated by using photoalignment technology [26]. Here, the photoalignment technique provides an opportunity to produce a number of matrix patterns with the resolution of 100 nm for the subsequent identification of biological objects [27].

The collected data of the Stokes parameters and images obtained by polarization optical microscopy are the basis for their subsequent investigations by means of data mining methods [28].

2.2. Cholesteric LC-Based Biosensors

In comparison to NLCs (see Section 2.1), cholesteric liquid crystals (CLCs) have a helical arrangement along the orientation of the director. This mesophase is characterized by the helical pitch p, which is the distance corresponding to the 2π rotation of mesogens along the helix director (see Figure 3a). If the pitch length is of the same order of magnitude as the wavelength of visible light, then the cholesteric phase selectively reflects visible light.

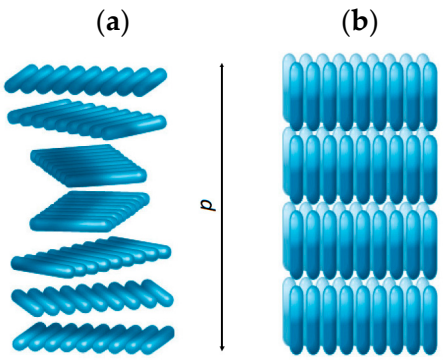

Figure 3. (**a**) Schematic representation of a cholesteric mesophase structure; p is the helix pitch. (**b**) A smectic mesophase structure.

In other words, the intrinsic periodicity and cholesteric mesophase structure possess a Bragg selective light reflection band (photonic band gap) with the central wavelength λ_c defined by the identity

$$\lambda_c = np \tag{2}$$

for normal light incidence and reflection, where n is the mean refractive index of the LC compound. The reflectance is limited by 50% of the ambient, unpolarized light, because only circularly polarized light of the same handedness as the helix is reflected. This

selective reflection of light makes them unique materials with a significant advantage over other mesophases.

The sensors described in Sections 2.1 and 3 employ either a confined homeotropic or planar alignment of LCs with a typical film thickness of several micrometers. Droplets (i.e., LC-in-water emulsions) represent another type of LC arrangement and are rapidly emerging as promising candidates for LC-based sensing. Droplets have attracted attention because of the micrometer-sized geometric confinement of LCs in various configurations. This confinement depends on how the LC orientation at the interface influences the LC structure throughout the droplet. In comparison with planar LC films, the preparation of droplets does not require a substrate and grid structure to support and confine LCs. Moreover, LC droplets with a large surface area-to-volume ratio and topological defects allow detecting a variety of targets, which makes them an excellent candidate for sensing applications.

The fabrication of an LC droplet sensor is mainly based on the LC long-range order and birefringence. The goal of an LC droplet sensor is to design a modified sensitive surface with specific recognition of the target molecules. Under the action of the modified molecule, the LC molecules arrange themselves [29]. However, this alignment is hindered in the presence of the target analyte. LC droplets are produced by using a microfluidic device and have either bipolar or radial configuration due to the orientational ordering of LC mesogens within a droplet (see Figure 4). Notably, the droplets also adopt other configurations, which are not discussed here [10].

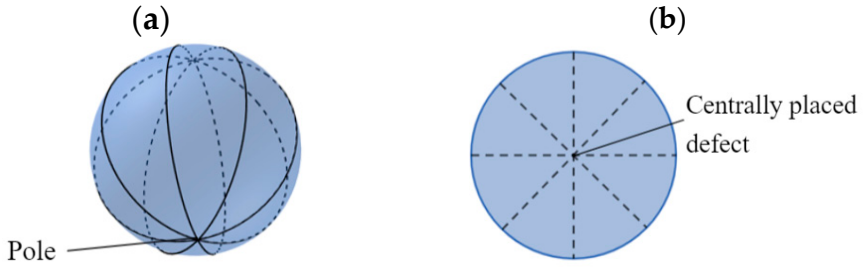

Figure 4. (**a**) Bipolar and (**b**) radial configurations of an LC droplet.

The parallel alignment of LC mesogens to the surface of a droplet with two diametrically opposite surface point defects at the poles results in bipolar configuration (Figure 4a), whereas the radial configuration of LCs forms an orthogonal alignment to a droplet surface with centrally placed defects (Figure 4b).

A microfluidic technique can be used to overcome the size–polydispersity bottleneck in the fabrication of LC droplets; therefore, wider applications of advanced photonic devices are becoming possible [30]. To apply CLC droplets in biosensing, their surfaces must be coated with a sensitive material to the external stimuli of interest, and this response should change the CLC droplet configuration. Consequently, a functionalized compound known as PAA-b-LCP (PAA and LCP denote poly (acrylic acid) and poly (4-cyanobiphenyl-4-oxy-undecylacrylate), respectively) was developed. These droplets have become pH-sensitive. Being a weak polyelectrolyte at a low pH, protonated PAA exposes a neutral charge, and it shrinks, being a bulk material (Figure 5a). High values of pH result in deprotonated PAA with a negative charge state, as shown in Figure 5b,c. The charged state of PAA favors a homeotropic alignment. Therefore, bright field images of CLC droplets look different for various concentrations of a chiral dopant and different pH. Thus, the helical structure of CLC droplets is pH-sensitive. This structure provides the basis for sensing pH-perturbing target analytes [31].

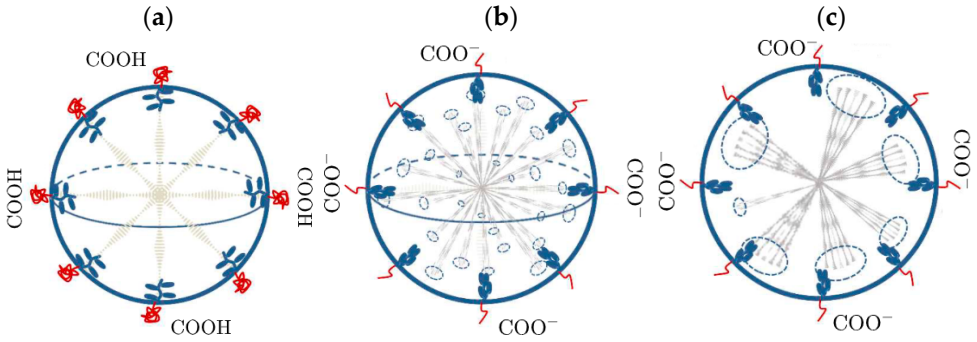

Figure 5. Working design principles of CLC configurations for (**a**) low and (**b**,**c**) high pH.

pH-responsive and color-inducing CLC droplets at high concentrations are leveraged as a platform for developing new biosensors by immobilizing enzymes on the surface. The droplets have high sensitivity, good selectivity and fast responses to the presence of glucose and cholesterol [29].

Thus, microfluidic techniques provide a promising tool for the fabrication of uniform and reproducible LC-based sensing platforms, but the performance of LC droplet-based biosensors strongly depends on the droplet density.

2.3. Smectic LC-Based Biosensors

Smectic LCs represent another well-known mesophase that has been extensively explored. Like CLCs, smectic LCs also possess a layered structure with a certain periodicity. A layered structure of smectic LCs results in the highest order parameter value among all LCs:

$$\sigma = \langle \frac{3\cos^2\alpha - 1}{2} \cos\left(\frac{2\pi}{d} z\right) \rangle, \quad (3)$$

where α is the angle between the director and long molecular axis, d is the layer spacing and z is the position of the molecular center (see Figure 3b). A variety of smectic LCs, such as SmA, SmB and SmC, have been deeply investigated, but their applications in biosensing are difficult due to their strong intermolecular bonding. Meanwhile, a recent study reported the crystallization of cholesterol (concentrations in ethanol of 100, 200, 300 and 400 mg/dL) in combination with ferroelectric liquid crystal (FLC) in the form of cholesterol needles (Figure 6) [32]. Optical microphotographs showed gradual changes in the texture with the increase of the concentration of cholesterol, i.e., the change in the induction of the needle-like lines perpendicular to the alignment or parallel to the sematic layers. The angle between the rubbing direction and polarizer (analyzer) axis was 45°. Cholesterol crystals are optically anisotropic and have the tendency to rotate the plane of polarized light; hence, a slight change in transmission through these lines was also observed. It means that the region of lines is optically active, i.e., the lines are a combination of FLC and cholesterol. If these lines are dominated by the FLC molecules, then the transmission must change with the application of the electric field.

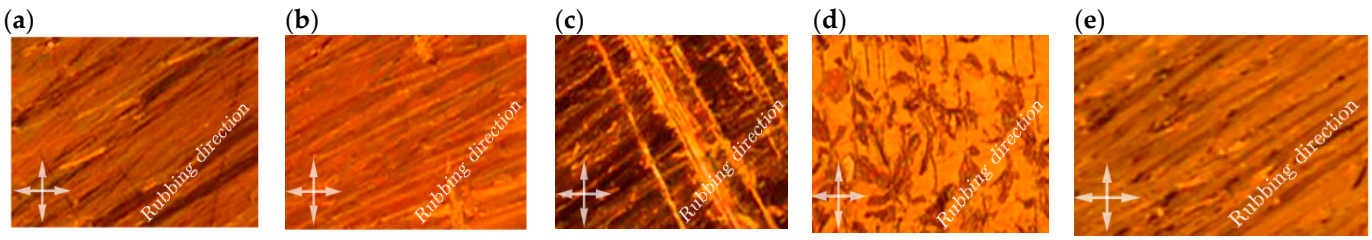

Figure 6. Optical microphotographs of cholesterol with various concentrations in ethanol: (**a**) 100 mg/dL, (**b**) 200 mg/dL, (**c**) 300 mg/dL, (**d**) 400 mg/dL and (**e**) a pure FLC mixture under crossed polarizers (double-headed arrows).

This behavior of lines was analyzed in the presence of an electric field at room temperature. The application of an electric field confirms that the lines are not due to only FLC molecules but also due to the crystals of cholesterol.

The study of the appearance of cholesterol parallel to the smectic layers is the first observation to the best of the authors' knowledge and is significant to understanding the interaction of cholesterol-like molecules and the FLC matrix. Our understanding of the behavior of cholesterol additions on the properties of FLC is helpful in the investigation of the possibilities of FLCs as a sensing element in the biosensing of cholesterol and other molecules.

2.4. Polymer-Stabilized Blue-Phase Liquid Crystal Biosensor

A number of optical properties of polymer-stabilized blue-phase liquid crystals (PS-BPLCs) make these materials excellent for optoelectronics. Earlier studies revealed that PS-BPLCs are sensitive to xylene, heptane, cyclohexane, dichloromethane and ethyl alcohol vapors [33]. The effect of vapors on PS-BPLC sensors consists of a change of color from blue to green. The reason for this effect is the change in the refractive index and the anchoring energy between LC molecules and the polymer network induced by the diffusion and adsorption of volatile vapors. Then, subsequently holding the PS-BPLC sensor in the air leads to recovering of the original color. This PS-BPLC sensor also had a good selectivity, sensitivity, high repeatability, insensitivity to humidity, fast response and long-term stability. To be more precise, we consider in detail the working principle of PS-BPLC sensors.

The fabrication of an LC cell includes the following steps: an LC compound must be heated up to an isotropic state and stirred for 30 min; then, the compound must be injected into the LC cell without an initial alignment (Figure 7a). The thickness of the LC cell should be about 20 μm. At 44.6 °C, the sample must be irradiated with ultraviolet light (5 mW/cm^2) for 30 min (Figure 7b). After removing the upper glass substrate of the LC cells, the PS-BPLC film is obtained, and the hue of the PS-BPLC textures turns from green to blue due to the deformation collapse of the lattices.

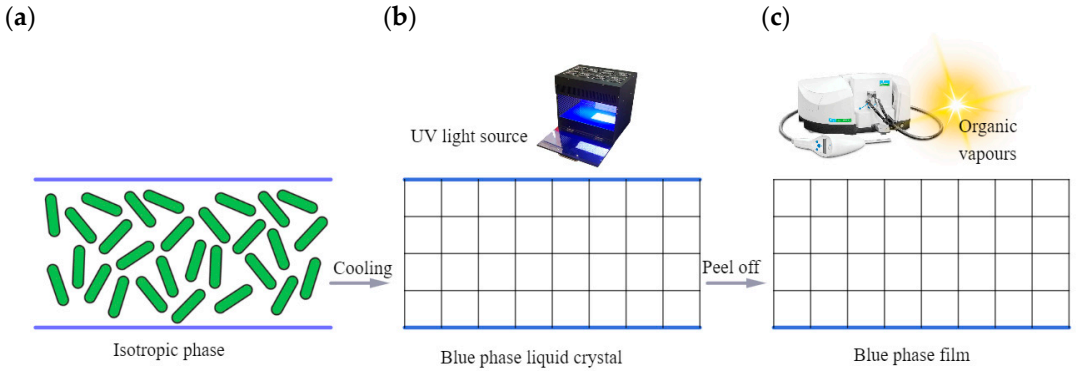

Figure 7. Sketch of an experimental sensor for the detection of organic vapors by using a PS-BPLC. The LC mixture was filled in a cell and heated to the isotropic phase (**a**); then, the LC mixture was carefully cooled to the blue phase and exposed by UV light with an intensity of 5 mW/cm^2 for 30 min to ensure complete polymerization (**b**). Then, a PS-BPLC film was obtained after peeling off the top glass substrate. The platelet texture change of the PS-BPLC is observed using a spectrometer (**c**).

In order to evaluate the sensing properties of the sensor, the PS-BPLC film is placed in a closed chamber at room temperature. Organic vapors are injected into the closed chamber by a pipette. The sunlight is reflected by the PS-BPLC film, and a fiber spectrometer connected to a computer detects the reflection spectrum (see Figure 7c). The detected red shift of the PS-BPLC sensor increases from 10 to 40 nm when the copolymer concentration decreases from 16 wt% to 10 wt%.

In addition to PS-BPLCs, CLCs were also applied in microfluidic devices for gas sensing applications. The photonic band gap (Equation (2)) of CLCs is also very sensitive

to temperature and a volatile organic compound [34]. This can be done by applying a thin layer of CLCs directly to microfluidic devices and then protecting it with a layer of a gas-permeable membrane. In this case, gas molecules permeate through the membrane and induce the color change of CLCs. An important finding of this application is that perceptible changes in color can be detected with a spectrometer within seconds of exposure of the LC film to the chemical environments.

3. Explanation of LC Behavior

Nearly all LC biosensors are based on the orientational switching mechanism of long molecular axes. As a result, a variety of schemes of LC-based biosensing platforms have been developed. Below, we aim to address the following questions: (i) molecular interactions that lead to changes in LC alignment and (ii) the role of surface functionalization and recognition elements in biosensing.

The easiest way for direct observations of the changes in LC alignment is polarized optical microscopy. For example, bovine serum albumin (BSA) is used as a probe protein for the detection of heavy metal ions (Pb^{2+} and Hg^{2+}) via the formation of chelating complexes. A biosensor with BSA without heavy metal ions shows dark optical images due to the homeotropic alignment of the NLC, suggesting that BSA does not change the topology of the surface (see Figure 8a). However, heavy metal ions in BSA induce optical changes in POM images due to the distortion in the alignment of LC molecules (Figure 8b) [35]. The subsequent amplification of molecular interactions due to the long-range orientational order makes the presence of heavy metal ions detectable by polarized optical microscopy. As a result of these working principles, one can observe bright images.

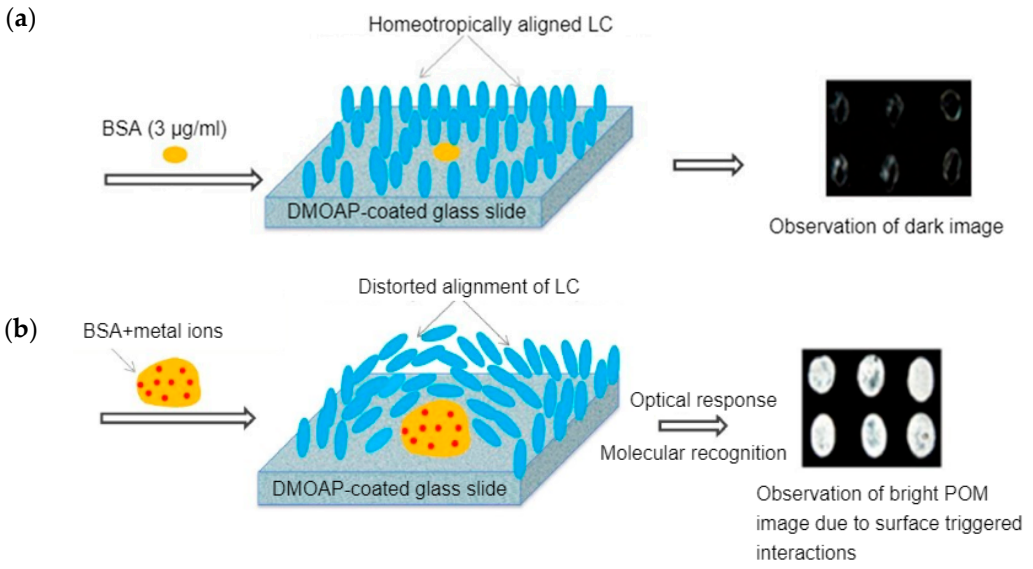

Figure 8. Schematic representation of a BSA-based biosensor for the detection of heavy metal ions: (**a**) without heavy metal ions, where DMOAP denotes dimethyloctadecyl-[3-(trimethoxysilyl)propylammonium chloride, and (**b**) the distorted alignment of a LC induced by BSA with heavy metal ions.

Another case study refers to the spontaneous assembly of phospholipids at planar interfaces between thermotropic LCs and aqueous phases. It gives rise to the patterned alignment of LCs that reflects the spatial and temporal organization of phospholipids. Strong and weak specific binding events involving proteins at these interfaces change the structures of phospholipids and trigger orientational transitions in LCs. The fluid nature of interfaces makes it easy to observe the lateral alignment of proteins by using the orientational response of LCs [36]. Molecular interactions between biotargets and LCs are triggered by treating the substrates with a biological analyte. Aqueous interfaces have

advantages over solid surfaces, since they preserve the functionality of biomolecules and provide high mobility of biotargets. Purified water induces the planar alignment of LCs. The optical image depicted in Figure 9a shows transmitted polarized light through NLC (5CB) films, which are deposited into pores (width ≈ 283 μm; depth ≈ 20 μm) of a golden grid, supported by octadecyltrichlorosilane (OTS)—coated glass substrates. Surfactants or self-assembled biomolecules (e.g., lipids) induce a homeotropic alignment of the LCs at the interface [36], which is observed by polarized optical microscopy (see Figure 9b,c).

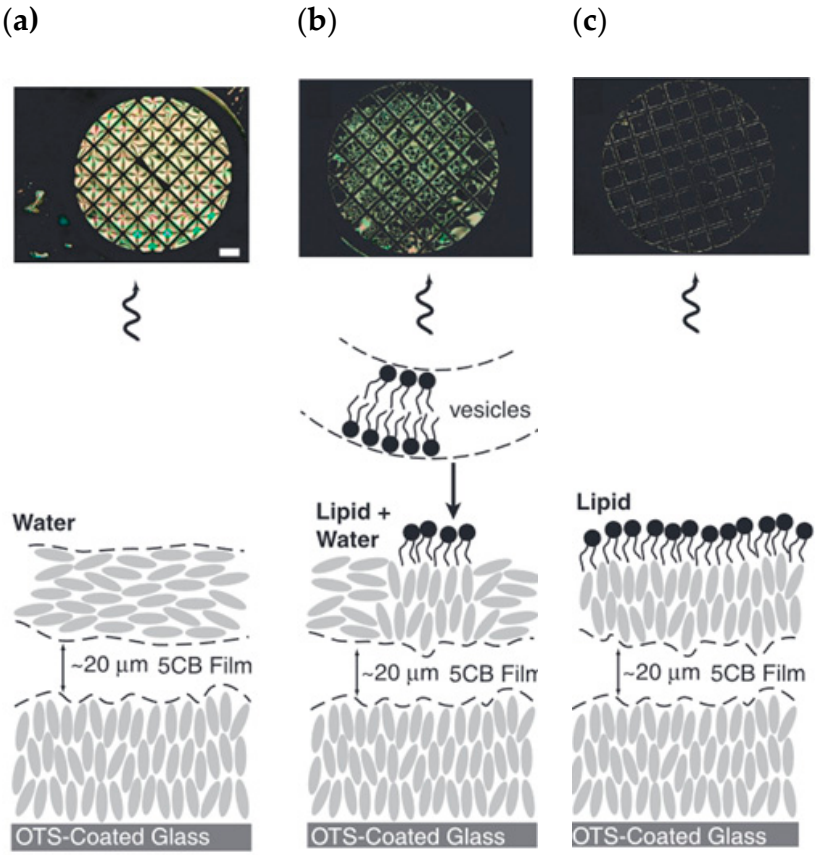

Figure 9. (**a**) Polarized optical microscopy images, and the schematic representation of 5CB anchoring and the state of the aqueous 5CB interface right after the injection of the dispersion of vesicles formed from 0.1 mM L-DLPC in tris-buffered saline. Scale bar: 300 μm; (**b**) optical image and schematic representation of 5CB anchoring after ≈10 to 20 min of contact with the vesicle dispersion of L-DLPC; (**c**) optical image and schematic representation of 5CB anchoring after 2 h of contact with the vesicle dispersion of L-DLPC.

Examination of the mobility of phospholipids plays a key role in transducing biomolecular interactions at biological membranes. This model permits studying the reorganization of proteins and lipids after binding. Orientational transitions of LCs are applied to characterize biological target information by using polarized optical microscopy. However, the qualitative detection of target molecules represents a difficulty. The study of the spontaneous assembly of phospholipids provides principles for the label-free monitoring of aqueous streams for molecular and biomolecular species without the need for complex instrumentation.

4. Issues and Challenges

In summary, LC biosensors offer unique advantages in terms of sensitivity, label-free detection, real-time monitoring and portability. However, like any technology, LC biosensors also have specific limitations, such as potential challenges with multiplexing and the need for specialized readout equipment. The choice of biosensing technology ultimately depends on the specific application and the desired performance characteristics.

Regarding LC biosensors based on golden nanoparticles for amplification of a signal, one can note that the experimental cost is relatively high. Some metal materials can be dispersed in solvents as inorganic nanocores to form nanoparticles as an alternative to Au nanoparticles [37]. The structure of microchannels and the impact of the phase viscosity and flow rate of the liquid on the formation of the LC alignment based on microfluidic technology need to be further investigated.

Future research can also involve integration of the biomedical applications of polarimetry and image analysis. One possibility is to use high-resolution information of the characteristics of scattered polarized light on biological agents (see Figure 2) and the subsequent application of neural network techniques for comparison between the result and a pattern.

5. Discussion

Our review of biosensing technology shows that the basic principles of LCs open up interesting opportunities for the design of commercial devices. For widespread clinical application, the development of biosensors must be continued in the direction of point-of-care testing; that is, sensors must be portable, cost-efficient and user-friendly. The penetration of biosensing innovations will not be achieved unless (i) long-term stability and selectivity are significantly improved, (ii) environmental susceptibility to the deterioration of biorecognition elements is insignificant and (iii) highly standardized bio-nanointerfaces are readily available.

We saw that the application of responsive compounds like BSA with NLCs for analytes detection (Section 3) usually changes the optical properties of a material due to the inherent molecular ordering of LCs. The fact that we can take advantage of this property for a wide range of analytes speaks strongly in its favor, as does the fact that the optical response of such systems is visualized by using polarized optical microscopy. The technological trend of marrying microscopic label-free imaging techniques with machine learning opens up an opportunity for the interpretation of biomedical tests by using only mobile devices. Our idea of capturing the polarization state by using a patterned NLC layer and CMOS sensor is consonant with earlier mentioned trends on refractometric imaging [13].

We have also seen that the detection limits of biosensors are limited by multiplexing and the need for specialized readout equipment and functional materials. However, the typical detection times are much shorter than in culture-based detection (Section 2.1). The fact that both detection methods are accessible is another point to show the flexibility of this emerging technology. Thus, LC sensors are just a category of optical sensors, and their performance is often compared to other optical approaches. Below, we provide a general comparison with some other sensor technologies, which follow from the physics of their effects (see Table 1).

The choice between various sensing technologies depends on the specific application requirements. LC sensors are particularly attractive for their versatility and ability to respond to a wide range of stimuli, making them suitable for diverse sensing applications. Researchers often select technology based on sensitivity, selectivity, response time and ease of integration into the target system. The price of LC mixtures is usually cost-effective in comparison with specific sensor technologies.

Table 1. Comparison of sensor technologies.

Sensor Technology	Advantages	Disadvantages
Local surface plasmon resonance sensors	High sensitivity to changes in refractive index at the sensor surface, real-time monitoring of biomolecular interactions	Limited to surface interactions, require precise control of experimental conditions.
Fiber optic sensors	High sensitivity, immunity to electromagnetic interference, remote sensing capabilities.	Complex instrumentation, potential for signal loss in long fibers.
Photonic crystal biosensors	High sensitivity, label-free detection, tunable optical properties.	Fabrication challenges, sensitivity to environmental conditions.
Liquid crystal sensors	Versatility in detecting various physical and chemical parameters; dynamic response to external stimuli (e.g., temperature, pressure and analyte presence); real-time and reversible sensing; flexibility for integration into different devices, including wearable systems.	Sensitive to the chemical structure of LC mixtures, specific LC alignment conditions are needed

6. Conclusions

Here, we demonstrate how to use polarized light, the anisotropy of LCs and molecular ordering in biosensors to extract features from complex microscopic pathogens. Although the molecular mechanism of how LC molecules interact with immobilized molecules on their surface is clear, LC biosensors require further exploration. Our results in photoalignment hint at a fresh approach for the design of LC-based sensors based on the characterization of the Stokes parameters. We believe that predesigned LC self-assembled structures across multiple length scales will open up opportunities for designing dynamic functional bionanomaterials for biosensing devices.

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Conflicts of Interest: Vladimir Chigrinov was employed by the company Nanjing Jingcui Optical Technology Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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