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Novel alignment technique for LCD-biosensors†

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The directional drying of a low-salt Tris–EDTA (TE)-buffer to give an alignment layer offers a simple, one-step, non-contact procedure for the construction of parallel liquid crystal displays (LCDs), which can be used to amplify the presence of DNA to scales visible to the naked eye, opening up possibilities for easy detection of bio recognition events.

Liquid crystals (LCs) have been shown to behave as excellent molecular magnifying glasses to bring (bio)reactions on the nano-scale into the realm of the naked eye.^{1–10} For example, the binding of proteins to surface-bound receptors in a liquid crystal medium, e.g. bovine serum albumin^{4,5} or streptavidin⁶ to surface-bound biotin or histidine, affects the ordering of LCs in the direct vicinity of the location of the bioreaction. Because of the strong interactions between neighbouring LC-domains, this change in LC director is transmitted into the LC-bulk, thereby amplifying the binding event from nano to millimetre scale.

Unfortunately, the construction of working LCDs as sensors for bioreactions has proven to be labour-intensive in most cases, partly because LC alignment has to be induced by the counter plate of the LCD.⁹ In other examples, chemical modification of the alignment layer is required, entailing either the attachment of a biomolecule,^{1–6} or the synthesis of biomolecules which can chemisorb onto the alignment layer.^{4,9} Here we report the construction of an alignment layer which was constructed by the simple directional drying of a low-salt TE-buffer (Scheme 1). When used in a parallel LCD, this layer was able to induce LC-alignment over about 80% of the surface.

Commonly used spin-coated polyimide (PI) on indium-tin-oxide (ITO) was used as the surface for the application of the buffer. The alignment layer was deposited by blowing a droplet of the low-salt TE buffer back and forth over the surface with a steady nitrogen flow. Parallel LCD cells were made by using a second spin-coated PI–ITO plate, after which the LC (4-*n*-pentyl-4'-cyanobiphenyl, 5CB) was drawn into the LCD in the isotropic phase by capillary action.‡

Atomic force microscopy (AFM) as well as scanning electron microscopy (SEM), confirmed that the drying of the buffer results in a directionally ordered surface (Fig. 1 and supplementary information). Polarising microscopic images showed that

the layer resulting from the drying droplet of TE-buffer was capable of LC alignment in the direction of the nitrogen flow (Fig. 2a). The direction in which the LC was drawn into the LC cell was found not to influence the final direction of the alignment, which was solely determined by the direction of the buffer flow. Although the surface was not as ordered as surfaces used in conventional LCDs,^{11–13} the interaction between the surface and LC was high enough to cause visible alignment of the LC in the parallel cell. However, a twisted-nematic (TN) LC cell showed no significant TN-effect on the TE-covered plate§ (see supplementary information), which indicated that the directional order on the TE-covered plate was not able to overcome the order dictated by the rubbed PI plate. This implied that the anchoring energy, which is an indication of the interaction strength between the TE layer and the liquid crystal, was much smaller than that of the PI-covered plate (which was measured to be $1.5 \times 10^{-5} \text{ J m}^{-2}$).¹¹ Parallel cells made from the directional drying of solutions of sodium chloride or

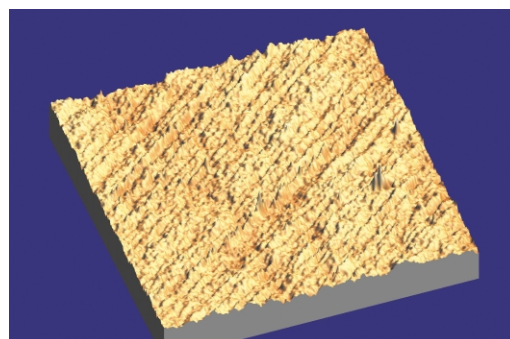


Fig. 1 AFM phase image of the alignment layer created by the directional drying of a TE-buffer. Image is $3.0 \times 3.0 \mu\text{m}$, average layer thickness was 3.3 nm.

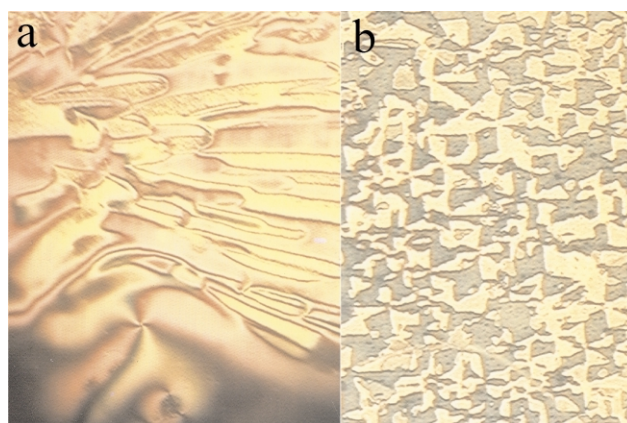
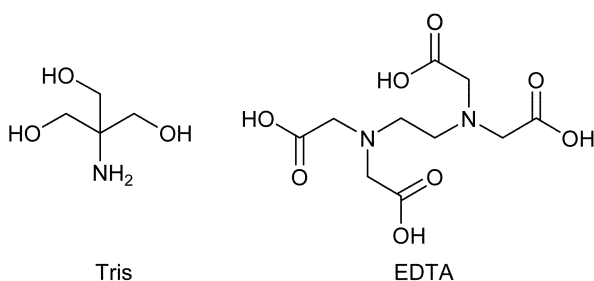


Fig. 2 a) Polarising microscopic image of LC cells with a TE-buffer alignment layer constructed by directional blowing of a droplet across the top of the image (direction of blowing was left to right). Aligned LC domains are visible as elongated structures. The boundary with unaligned LC, resulting in a nematic texture, is clearly visible. b) Adding DNA to the buffer gives a cholesteric “fan-type” LC phase. Images are $1.5 \times 1.0 \text{ mm}$.



Scheme 1 Structure of Tris and EDTA.

† Electronic supplementary information (ESI) available: alignment layer formation and structure, FT-IR spectra and polarising microscopic images. See <http://www.rsc.org/suppdata/cc/b3/b310860k/>

phosphate buffer did not show LC aligning capability, indicating that this phenomenon could not be explained simply by the formation of (micro)crystalline domains on the surface.

It has been shown before that supramolecular interactions can play a pivotal role in the formation of alignment layers for LCD purposes.¹⁴ For a drying TE-buffer, a developing hydrogen bond network was expected to arise. Therefore, the underlying mechanism of the formation of the alignment layer was investigated with FT-IR spectroscopy (see supplementary information).[‡] Upon directional drying of TE-buffer on an AgCl window, the spectra clearly showed that the superimposed OH vibrations sharpened up considerably and shifted 150 cm⁻¹ to lower wavenumbers. The NH vibrations also shifted 30 cm⁻¹ to lower wavenumbers. This suggests the formation of strong, directional hydrogen bonds, extending over the entire surface of the plate, below a certain water content. Also, the C–N and C–O stretch vibrations, which were initially superimposed, resulting in a broad peak at 1059 cm⁻¹, separated and sharpened up to give two peaks at 1057 and 1038 cm⁻¹, respectively, when the water content was decreased. The drying experiment was repeated with D₂O as the solvent. Although assigning peaks in the carbonyl region of the spectrum proved difficult because of the multitude of peaks, several carbonyl peaks decreased in intensity and shifted to lower wavenumbers by about 5 cm⁻¹, indicating the direct involvement of D₂O in the hydrogen bonding.¹⁵ A comparison of the carbonyl regions in the FT-IR spectra of a dried TE-buffer (from water) and a mixture of *solid* Tris–EDTA in the same concentration as the buffer solution, which was ground in a mortar, showed that the carbonyl peaks were much sharper in the dried buffer, indicating that layer formation from solution is required to produce a well-defined hydrogen bond network. Unfortunately, reproducing these results on the PI–ITO plates, which were used in the original experiments, proved difficult because the plates exhibited an IR cut-off at about 2100 cm⁻¹. The OH-vibrations of a non-directionally and directionally dried sample, however, shifted from 3357 to 3227 cm⁻¹, respectively. In addition, both the OH- and NH-vibrations sharpened up considerably upon directional drying, suggesting increased ordering on the surface. Furthermore, three additional NH- and OH-peaks appeared, suggesting several different types of hydrogen bonding were formed upon drying. These combined FT-IR results unambiguously showed that the directional drying of the buffer on a PI–ITO surface indeed increased the surface ordering. Related effects have been reported in the literature.^{16,17}

Parallel cells made with a directionally dried urea solution, also showed limited LC alignment capability (see supplementary information), further suggesting that hydrogen bonding may play a role in the formation of the anisotropic surface. In contrast to alignment layers of a TE-buffer, these cells lost their aligning capabilities within one day.

The method presented here has the advantage of eliminating an extra step in the construction of most LCD-biosensors, *i.e.* the separate formation of an alignment layer. Biomolecules or biosensors of interest can now be deposited together with the alignment layer in one step: when parallel cells, made by directional drying of a TE-buffer containing λ -phage DNA, were imaged through the polarising microscope, a cholesteric “fan-type” LC phase was found along the path of the drying droplet (Fig. 2b), embedded in the normal nematic phase expected for 5CB. The twist angle (defined here as the angle at which transmittance of light through the cell is optimal) also differed from the expected 0° for a normal parallel cell, implying a chiral conformation of the LC. Furthermore, SEM confirmed the presence of the DNA on the buffer surface (see supplementary information). Unfortunately, the stability of the DNA in the LC environment was rather limited, as most cells made with DNA lost their chiral phase within several days, giving rise to a normal, nematic texture. The cells also suffered from thermal degradation: a triple heating–cooling run between

room temperature and 50 °C resulted in a normal nematic LC phase. Also, the stability of the alignment layer itself was found to be limited, as most cells lost their alignment after seven days (see supplementary information). These drawbacks, however, do not diminish the usefulness of this method in the field of biosensors.

The method we present here provides a very simple and non-labour-intensive way of making an LCD which is capable of detecting DNA on a surface, without the need for chemical amplification of the generated signal (*e.g.* Polymerase Chain Reaction to amplify the amount of DNA, or chemical modification with fluorescent probes). This opens up a myriad of possibilities for detecting bio reactions on DNA.

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Notes and references

[‡] ITO plates were cleaned with ozone (100 l h⁻¹) for three hours, before being spin coated with Polyimide Pyralin PI2555 (HD Microsystems) at 5000 rpm for 20 s and baked at 120 °C for 90 min. TE buffer consisted of 10 mM Tris-HCl and 1 mM of EDTA at pH 7.6. TE-buffer in D₂O was brought to pH 7.6 by the addition of NaOD in D₂O. λ -Phage DNA from *E. coli* (48502 base pairs, 31.5 MDa) was purchased from Sigma. Alignment layers were made by blowing the drop of buffer (with DNA) up and down the PI plate with a nitrogen flow at room temperature. Mylar (6 μ m) was used as a spacer between the two plates. LCDs were filled with 5CB in the isotropic phase at 40 °C (T_{NI} = 35.3 °C), and were submitted to a heating–cooling cycle between 20 and 40 °C three times.

Silver chloride windows were covered with 5 drops of buffer solution and directionally dried as described before. At t = 5, 10 and 15 min, the drying was stopped and any remaining fluid blown off the window onto a tissue.

Studies regarding further reactions and recognition events amplified by these bio-LCDs are currently underway.

§ A TN-LC cell was made by using a velvet-rubbed PI–ITO plate as counter plate, which was rotated 90° with respect to the buffer-covered plate. The cell was analysed by examining the difference in transmission of incident light between parallel and crossed polarisers.

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