Label-Free Liquid Crystal-Based Optical Detection of Norfloxacin using an Aptamer Recognition Probe in Soil and Lake Water

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SUPPORTING INFORMATION

1. Determination of Greyscale Intensity:

For data analysis, the average grayscale intensity of the LC images obtained from Polarizing optical microscopy (POM) studies conducted using a microscope (BANBROS BPL400B) equipped with a digital camera (Euromex Microscopen BV, 5MP) were processed by greyscale intensity quantification of at least 4 TEM grid squares using ImageJ free access software (U. S. National Institutes of Health, Bethesda, MD). The procedure for the same is explained in Figure S1.

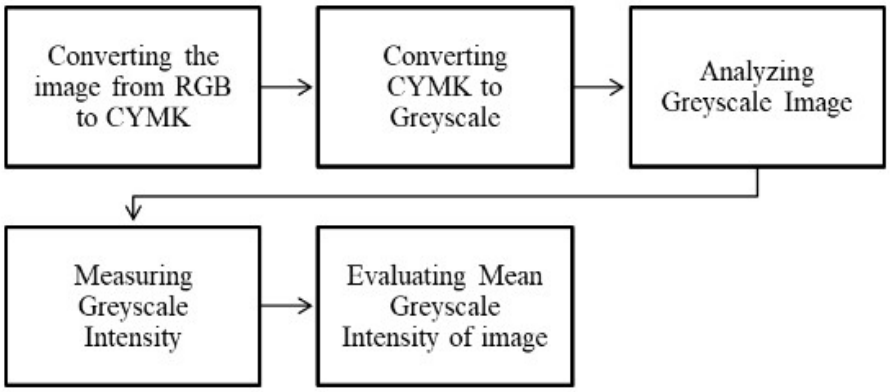

Figure S1: Procedure to obtain average GI from the recorded images.

2. CTAB Optimization

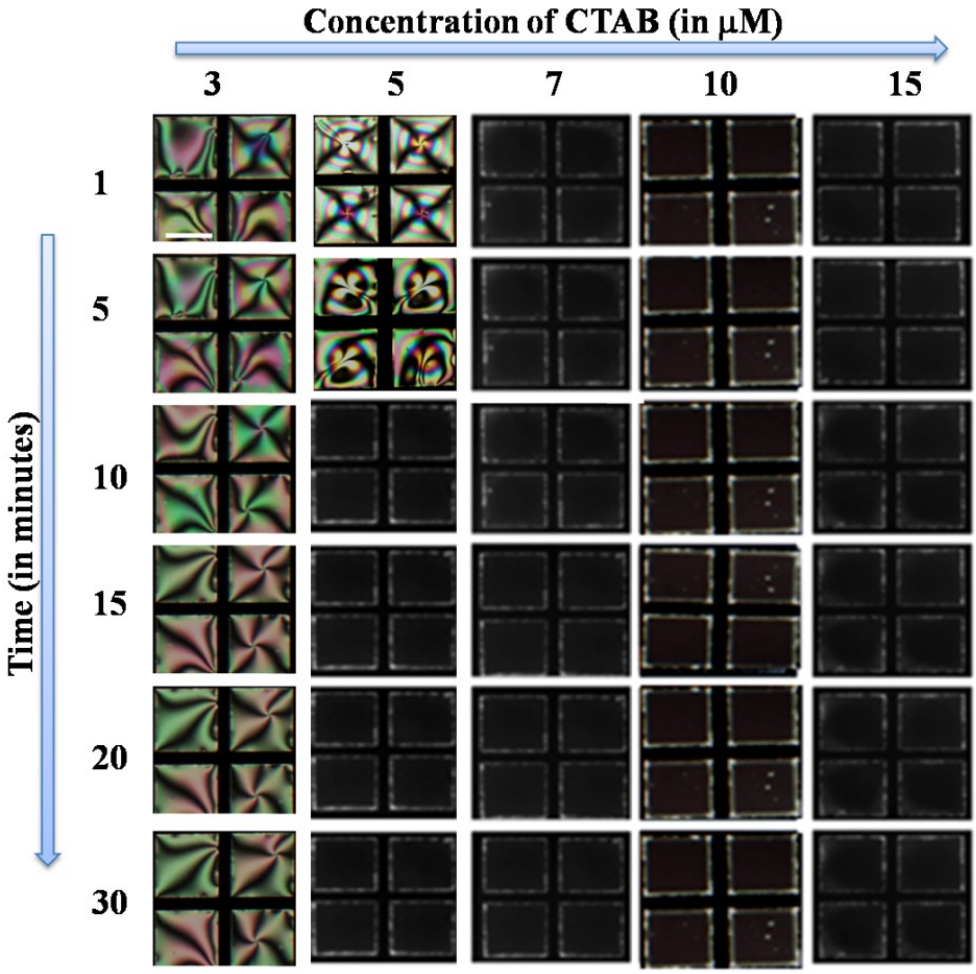

Figure S2: Polarized optical images of 5CB film suspended in gold TEM grids on a DMOAP functionalized glass substrate on addition of an aqueous solution of CTAB at 15 μM, 10 μM, 7 μM, 5 μM and 3 μM concentrations. Images were taken at 1, 5,10, 15, 20 and 30 minutes after addition of CTAB soultion. (Scale bar: 200 μm)

3. NOX Aptamer Optimization

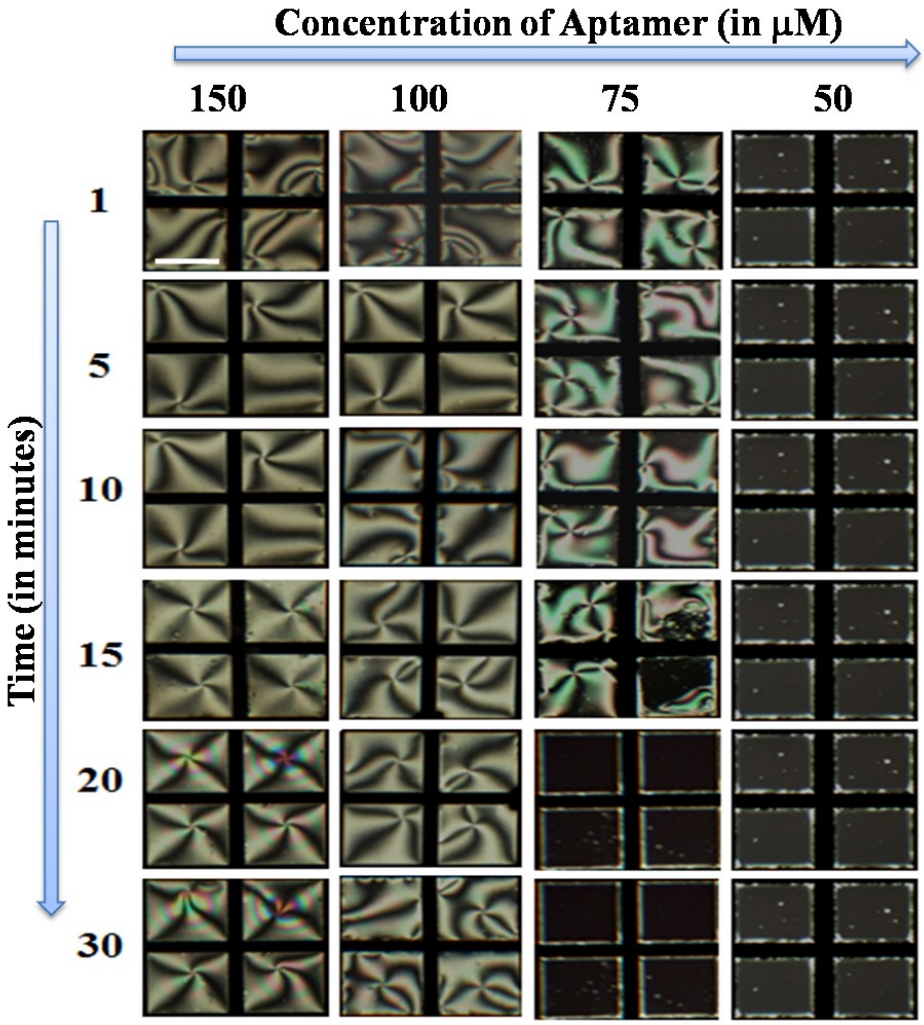

Figure S3: Polarized optical images of 5CB film suspended in gold TEM grids on a DMOAP functionalized glass substrate on addition of an aqueous solution of 7μM CTAB incubated with NOX aptamer concentrations at 150 nM, 100 nM, 75 nM and 50 nM concentrations. Images were taken at 1, 5, 10, 15, 20 and 30 minutes after addition of CTAB-Aptamer soultion. (Scale bar: 200 μm)

4. **Optimization of Observation Time:**

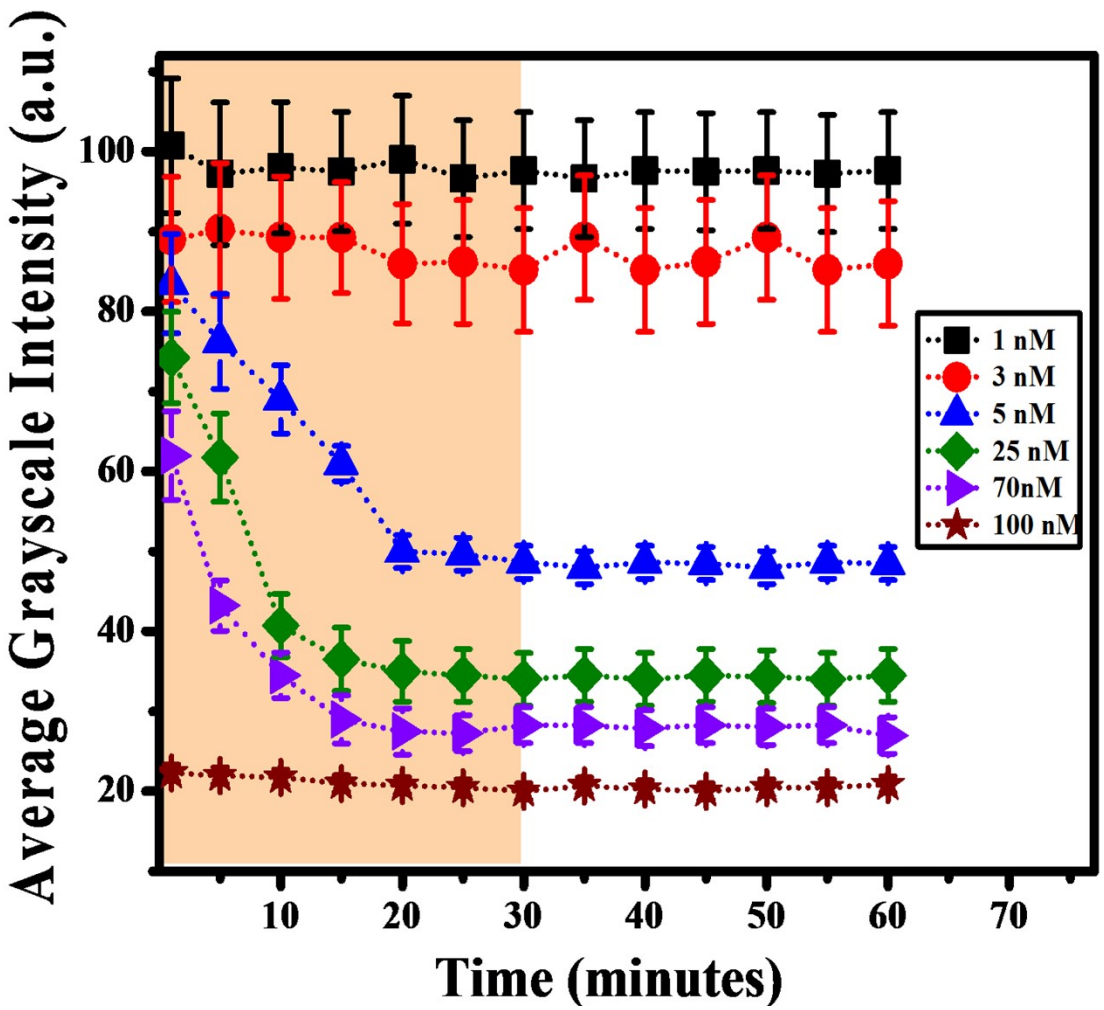

Figure S4: Plot comparing the average greyscale intensity of POM images at different concentrations of NOX recorded for 1 hour at regular intervals. No perceptible changes could be observed beyond 30 minutes for any of the concentrations.

5. pH Response of Sensor :

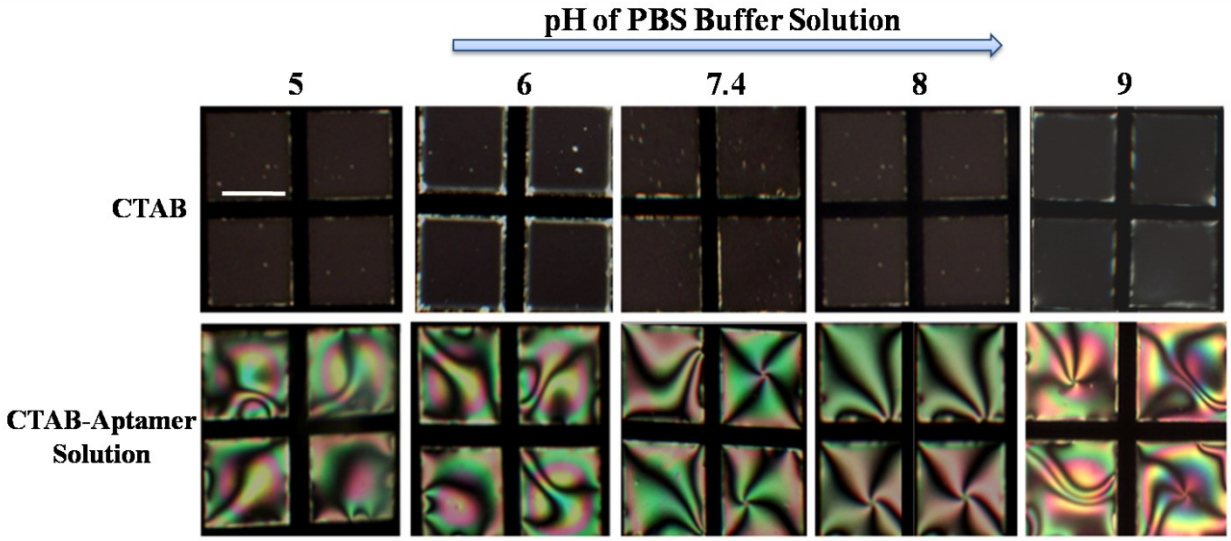

Figure S5: Polarized optical images of 5CB film suspended in gold TEM grids on a DMOAP functionalized glass substrate on addition of an aqueous solution of 7μM CTAB and 7μM CTAB +100 nM Aptamer in 10mM PBS solution having pH levels at 5, 6, 7.4, 8 and 9. (Scale bar: 200 μm)

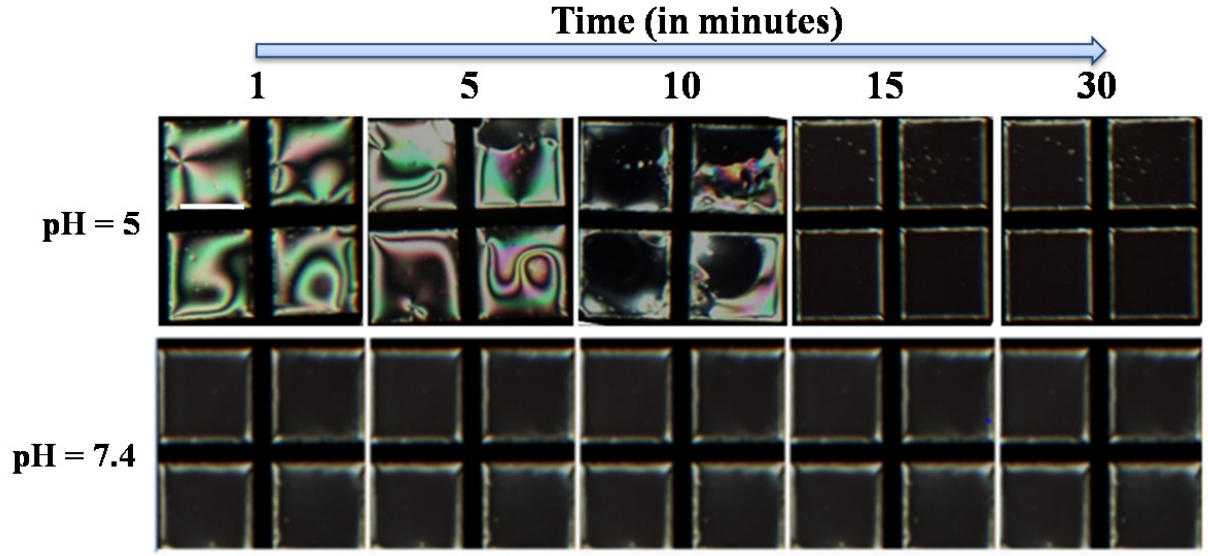

Figure S6: Polarized optical images of 5CB film suspended in gold TEM grids on a DMOAP functionalized glass substrate immobilized with 7 μM CTAB and 100 nM aptamer in the presence of 100nM NOX dissolved in 10mM PBS solution having pH=5 and pH=7.4. (Scale bar: 200 μm)

6. Sensor Response to varying Ionic Strength of Buffer:

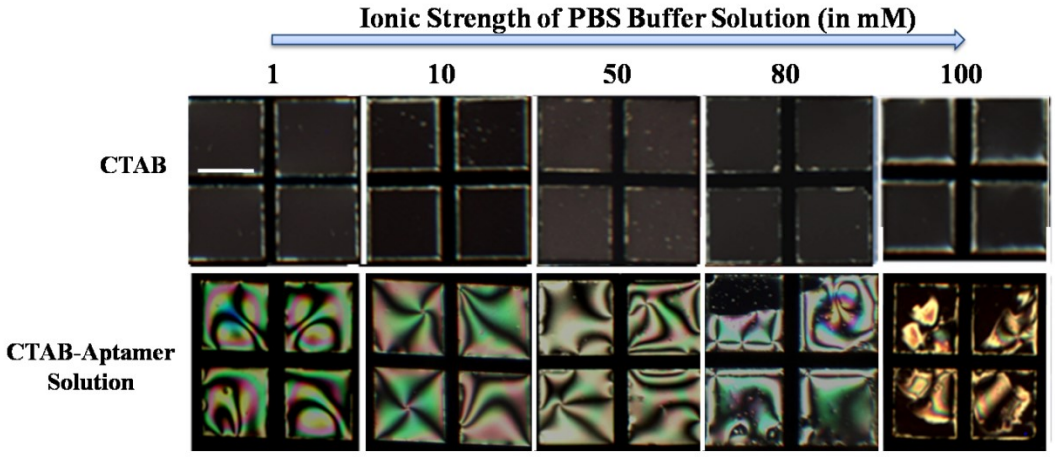

Figure S7: Polarized optical images of 5CB film suspended in gold TEM grids on a DMOAP functionalized glass substrate immobilized with 7μM CTAB and 7μM CTAB +100 nM aptamerdissolved in PBS solution having ionic strength between 1mM to 100mM . (Scale bar: 200 μm)

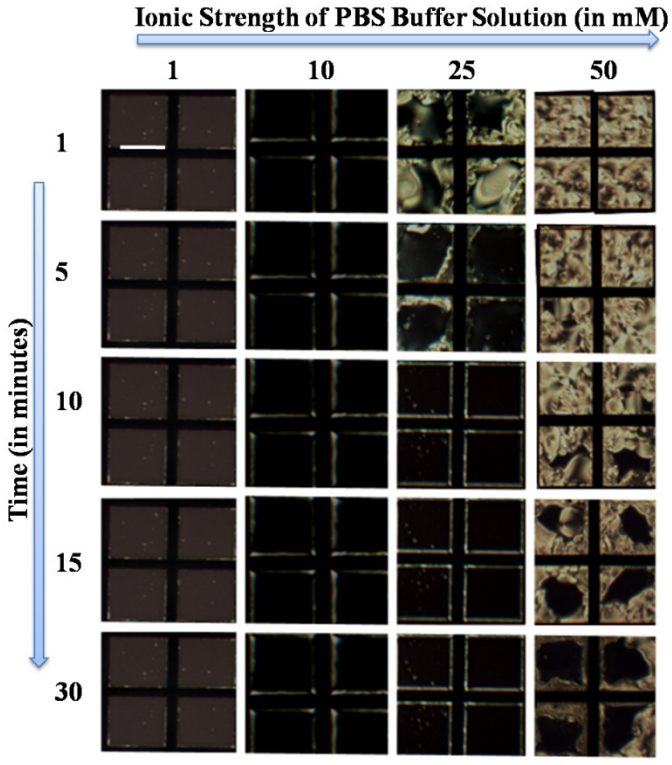

Figure S8: Polarized optical images of 5CB film suspended in gold TEM grids on a DMOAP functionalized glass substrate immobilized with 7 μM CTAB and 100 nM aptamer in the presence of 100 nM NOX dissolved in PBS solution having ionic strength between 1 mM to 50 mM. (Scale bar: 200 μm)

7. Interference Study:

To further enhance our understanding of the selective nature of the biosensor, a qualitative interference study was performed at two concentrations of NOX (50 nM and 20 nM). At each concentration, three solutions of the test samples were prepared containing 90% NOX with 10% interferents, 50% NOX with 50% interferents and 20% NOX with 80% interferents. The interferent solution contained equal volumes of five different interferents: Amoxicillin (AMX), Tetracycline (TCY), Paracetamol (PCM), Ciprofloxacin (CPR) and Enrofloxacin (ENR), at 50 nM or 20 nM concentrations corresponding to the NOX concentration of the solution. Each sample was incubated with 7 μM CTAB and 100 nM NOX Aptamer for 1.5 hours before observing them under the POM for at least 30 minutes. After 30 minutes under the POM, it was observed that for 50 nM NOX concentration, there was no change in the homeotropic alignment after 30 minutes, implying that the detection of NOX was seamless and undisturbed by the interferents. At 20 nM NOX concentration, there was a consistent dark state at 90% NOX volume (10% interferents). However, there was a significant delay in return to the dark stage at equal volumes of NOX and interferents and regions of planar/tilted orientations remained simultaneously with homeotropic regions even after 30 minutes. At 20% volume of 20 nM NOX (80% interferents), no return to the dark state could be observed.

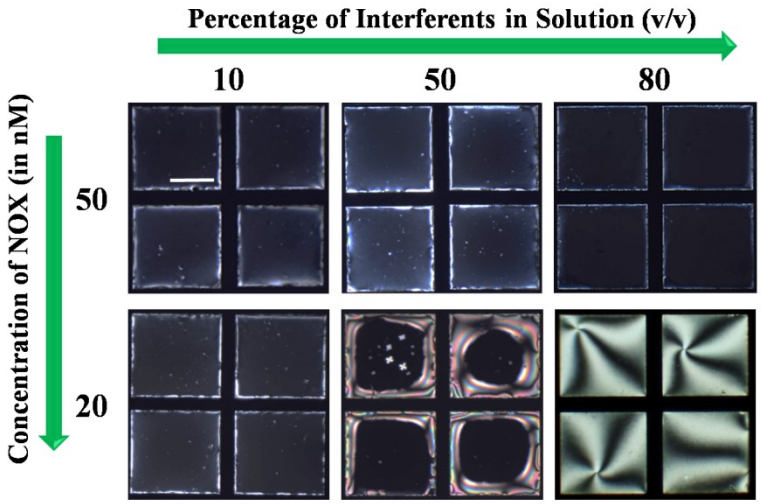

Figure S9: Polarized optical images of 5CB film suspended in gold TEM grids on a DMOAP functionalized glass substrate immobilized with 7 μM CTAB and 100 nM aptamer in the presence of five interferents (AMX,TCY,PCM,CPR and ENR) at two concentrations of NOX (50 nM and 20 nM) observed for 30 minutes. At each concentration, three solutions of the test samples were prepared containing 10%, 50% and 80% interferents by volume. The interferent solution contained equal volumes of five interferents at concentrations equal to NOX. (Scale bar: 200 μm)