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Electro-Optical Imaging Microscopy of Dye-Doped Artificial Lipidic Membrane

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Abstract:

Artificial lipidic bilayers are widely used as a model for the lipid matrix in biological cell membranes. We use the Pockels electro-optic effect to investigate the properties of an artificial lipidic membrane doped with nonlinear molecules into the outer layer. We report here what is believed to be the first electro-optical Pockels signal and image from such a membrane. The electro-optical dephasing distribution within the membrane is imaged and the signal shown to be linear as a function of the applied voltage. A theoretical analysis taking into account the orientation distribution of the inserted dye molecules allows to estimate the doped membrane nonlinearity. Futur extensions of this work to living cell membranes are discussed.

1. Introduction:

As the main component of cellular membranes, lipid bilayers play an essential role as a barrier protecting the cell and its intracellular organelles. The cellular membranes also maintain the solute and ion concentration gradients which induce the equilibrium transmembrane potential. In the case of excitable cells, the plasma membrane ensures the propagation of the action potential.

Various experimental techniques are used in cellular electrophysiology for stimulation and measurement of membrane potentials by use of intracellular electrodes, an approach known as the patch clamp (1). Although these techniques are extremely sensitive and allow to record the electrical activity down to a single ion channel, they lead to cell death and provide the information about the potential in a single location within one neuron. For these reasons new optical approaches using voltage sensitive amphiphilic dyes have been developed and constantly up-graded (2). The electrometric dyes bind to the external cell membranes and contain an electron donor-acceptor group leading to electrochromism and a high hyperpolarizability. The membrane depolarization and the action potential propagation can then be recorded at different points of the cell as a voltage induced fluorescence variation by confocal microscopy (3), or by nonlinear microscopy techniques, namely by second harmonic generation (SHG) (4-6). Voltage-induced SHG is a third order nonlinear process described by an effective rank-four nonlinear susceptibility tensor associated to both quadratic and cubic time-dependant molecular hyperpolarizability tensors, the former is underlying field induced orientational effects and the latter instantaneous four wave mixing. Therefore SHG-based membrane potential measurements monitor the electrically induced changes of hyperpolarizability or/and the alignment of the dye molecules in the bilayer (5, 6). A major interest of nonlinear microscopy lies in its intrinsic three-dimensional sectioning capability related to the two-photon excitation step that occurs mainly in a focal volume together with the low photobleaching of out-of-focus molecules (it should be however noted that the bleaching may be increased in the focal spot due to the use of pulsed femtosecond lasers). It is remarkable that the non-centrosymmetric organization of the dye molecules, inserted exclusively into the external membrane layer, responsible for SHG is also at the origin of the related electro-optical Pockels effect (7). The Pockels effect is a variation of the index of refraction Δn induced by an applied electric field. When probed by a laser beam, Δn induces an optical phase shift which can be detected via an interferometric setup. Since the Pockels

effect is a non-resonant process, it can be measured using a low-power continuous wave laser, which preserves the integrity of the sample, and more generally avoids the use of costly and cumbersome high power short pulse laser focuses.

In this work we present an original electro-optical microscopy study of the variation of membrane potential in model lipid bilayers stained by the potentiometric DI-8-ANEPPS dye. Since lipid bilayers are considered to be an adequate model for the investigation of the electric properties of biologic membranes (8), electro-optical measurements were subsequently performed on a phosphatidilcholine bilayer formed on a parylene biochip. Our results demonstrate the imaging capability of an advanced electro-optical microscope in a microfluidic environment suitable for biology-related studies.

2. Principle and theory:

The electro-optical (EO) effect of a non-centrosymmetric material, or Pockels effect, is the change of the index of refraction of this medium in response to a quasi-static electrical field as applied via adequately shaped electrodes. The index of refraction change can be read-out as an additional small phase shift on a probing light wave. While the Pockels effect stems from a second-order (rank three) nonlinearity, it can also be viewed as a linear optical response in the electric field of the probe light field E_{op} , which depends linearly on the applied quasi-static external electrical field E_S .

The index of refraction change is given by

$$\Delta \left(\frac{1}{n} \right)_i^2 = \sum_j r_{ij} E_j, \quad (1)$$

where n_i is the index of refraction along a combination of x,y,z axes, due to the component of the applied electric field E_S in the j direction E_j . The proportionality factors r_{ij} are the coefficients of the electro-optical tensor (9). Here we assume implicit summation on repetition indices. We choose {x,y,z} as the principal axes of the nonlinear material, i.e. perpendicular to the plan of the doped bilayer (See Fig. 1). z is along the applied field direction and x,y are arbitrary directions in the sample plane.

Depending on the orientation of the molecules inside the medium and the associated geometrical symmetry, some r_{ij} coefficients cancel and some are related. In the case of an artificial bilayer doped with an ensemble of dye molecules, the cylindrical symmetry around the z axis leads to the following tensor (see Appendix):

$$[r_{ij}] = \begin{pmatrix} 0 & 0 & r_{13} \\ 0 & 0 & r_{13} \\ 0 & 0 & r_{33} \\ 0 & r_{13} & 0 \\ r_{13} & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \quad (2)$$

In this notation index ‘3’ is related to the z direction of propagation of the light, perpendicular to the bi-membrane (See Fig. 1).

The induced EO phase shift is

$$\Delta\varphi_i = \frac{2\pi}{\lambda} e \Delta n_i, \quad (3)$$

that can be developed as

$$\Delta\varphi_i = -\frac{\pi}{\lambda} e n_i^3 \sum_j r_{ij} E_j. \quad (4)$$

A signature of the effect can be obtained by rotating the polarization of the linearly polarized incident light. Inspection of the electro-optical tensor matrix shows that the phase shift must not change when the polarization of the incident beam is rotated within the (x,y) plane, as it involves the same r_{13} EO coefficient, in agreement with the cylindrical symmetry about the z axis.

An optical scheme capable of measuring the EO phase shift can measure the electrical field \vec{E} if the EO coefficients are known or conversely the EO coefficients can be extracted when the electric field \vec{E} is known. If both are undefined, it can access to the $E_j r_{ij}$ product.

Assuming a potential of 10mV throughout a membrane of $e=7\text{nm}$ and a realistic r coefficient of the order of 1pm/V , one should be able to measure a corresponding $\Delta\varphi$ dephasing of the order of 10^{-7} radian.

In order to measure this additional EO dephasing contribution, we used an interferometric scheme described below.

3. Sample and optical methods:

3.a EO microscope:

The main parts of the interferometric setup are as follows (See Fig. 2). A continuous wave He-Ne laser is used as the source of light, its beam being subsequently separated in two. One part is focused on the doped lipidic membrane through a $40\times$ microscope objective (Nikon , CFI plan-fluor $40\times$, N.A. = 0.6), then transmitted and collected via an identical microscope objective in forward direction. It is then recombined with the other beam that plays the role of the reference arm in a Mach-Zehnder interferometric scheme (10). The interference optical signal is detected by a photodiode (Hamamatsu). The electric field applied on the sample is

typically modulated at 100 KHz, inducing a modulated phase shift and interference fringes changes at the same frequency. To reach a high sensitivity for detecting a small phase shift variation, a balanced homodyne detection based on two photodiodes is being used (11). A lock-in amplifier (EG&G Princeton applied research) is used to measure this modulated phase shift with high signal-to-noise ratio. The sensitivity of the interferometric signal is maximized if the static optical path difference between the two beams corresponds to $\pi/2$ phase shift. A phase control loop is therefore implemented in the system, with a mirror mounted on a piezo electrical transducer stage (PI ceramic). It is added to the optical path of the reference beam and its translation allows to change the quasi-static optical path length (not shown in Fig. 2). Two half-wave plates (Fichou) allow to control the polarization of the light focused onto the sample.

3.b Sample composition and fabrication:

Bio chip:

As a sample holder, we use a bio-chip made of parylene. It is encapsulated in a resist that has been photopatterned in three dimensions thanks to a stereolithography process (12). The parylene thin film (20 μ m of thickness) was micromachined using O₂ plasma in reactive-ion-etching through an aluminum mask, which leads to an array of trough holes devoted to the bilayer reconstruction. In each microchamber, 9 holes of diameter 40 μ m allowed the simultaneous formation of 9 bilayers (13-15).

Electrodes:

Two Ag/AgCl electrodes, connected to the solutions from each side of the bilayer, were used to apply a voltage difference. The coating of silver electrodes is deposited by dipping the electrodes for few minutes in a nitric and hydrochloric acid (ROTH) (1/3 vol.vol) concentrated solution. By dipping the electrodes in this bath for a few minutes, we obtained a pure, thin and uniform chloride silver coating.

3.c Mechanism of Bilayer formation:

Prior to this experiment, the bio-chip is first put into an ultra-sound bath in an ethanol solution for 5 minutes, and then in another ultra-sound bath in a Millipore water solution for 2 minutes. It was rinsed with Millipore water, dried with compressed nitrogen, then in an oven (40°C, 10mn).

15 μ l of a buffer solution of K⁺Cl⁻ 0.1M (Ficher Scientific) and MOPS (3-(*N*-morpholino)propanesulfonic acid) 10mM pH7 (Research organics) was inserted in the upper

chamber (c.f. Fig. 3.a). The lipid used was L- α -phosphatidylcholine (Egg, Chicken) (purchased Avanti polar lipids), 20mg/ml dissolved in η -decane (Merck). 8 μ l of the lipid solution was flowed in the lower channels (c.f. Fig. 3.b), followed by the flowing of buffer, which permits the formation of the bilayers (c.f. Fig. 3.c), as described in ref. (12, 13).

Staining of the bilayer:

To generate an EO phase shift the medium has to display a non centrosymmetric statistical arrangement. The lipid molecule by itself satisfied this condition, but in the case of a bilayer the head-tail molecular assembly leads to a centrosymmetric system, and subsequent cancellation of the EO effect. In order to infer electro-optical activity to the membrane we asymmetrically stained the membrane with a non-centrosymmetric probe molecule Di-8-ANEPPS (Invitrogen). This molecule has a long carbon chain, thus adequately lengthening the flip time across the membrane (2, 16). Those properties make from DI-8-ANEPPS a dye that has been widely used for fluorescence or SHG measurement of membranes (17, 18), and as a potential probe (2, 3, 16, 19-23). Its orientation in the membrane was investigated (18, 24), and different models were proposed.

The stock solution was prepared in DMSO (Sigma Aldrich) at 1mg/ml concentration. 3 μ l of the dye was introduced to the upper chamber already filled with the buffer just after the injection of the lipid in the lower channel. Then a glass plate was placed on the bio-chip to cover the upper chamber. It was used to prevent the formation of a curved liquid surface that could act as a micro-lens and thus affect the probe laser beam that passes through it. The glass plate was perforated to insert an electrode inside the buffer solution. Then, as explained above the buffer was introduced in the lower channel and the bilayer formed. A 15-minute waiting time between the introduction of the dye and the start of the measurement allowed the dye to intercalate into the upper layer of the artificial bilayer.

In order to check that the lipidic membrane was organized as a bilayer, a capacitance measurement between both sides of the membrane was made before and after the electro-optical measurement described below. The same capacitance 0,32 μ F/cm² measured at the beginning of the experiment was found also at the end. This is in good agreement with the values of surface capacitance of bilayers (0.4 μ F/cm²) reported in the literature (25).

4. Results:

Once a stable bilayer was formed in the bio-chip as described above, we were able to stain the membrane and then perform the electro-optical measurement. The dye was inserted at one side of the lipidic membrane, and a modulated electric field was applied between both sides of

this membrane. A potential difference up to 10 Volts rms amplitude was applied to the membrane and its electro-optical response was registered, without breaking the membrane.

The modulated electric field induced a modulated phase shift in the laser beam that probes the EO active sample. While the signal was small, our interferometric setup proved sensitive enough for measuring this electro-optical phase shift through the membrane.

Firstly the electro-optical signal was detected with a good signal-to-noise ratio (SNR). Indeed, for a voltage difference of 5V peak-to-peak amplitude at 100 KHz, the SNR is 10 ± 1 for 20ms integration time. Secondly we observed that the temporal response of the system was fluctuating (Fig. 4), to be probably accounted for by impurities inside the buffer passing through the probe beam during the measurement.

In order to check the origin of the detected signal, we changed the amplitude of the applied potential difference. Temporal signal of the lock-in amplifier was recorded for each modulated voltage amplitude, and the linear dependence of the EO signal on the applied voltage was plotted. Fig. 5 shows a linear dependence, as expected for the linear Pockels effect (See Eq.1). From the slope of this curve we can extract the electro-optical coefficient of the membrane.

The slope $\Delta\phi/E$ is $(43.8 \pm 1.7) \times 10^{-6}$ rad/V, and it is theoretically equal to $\frac{\pi}{\lambda} en^3 r_{13}$ (see Eq. 18 in Appendix). The laser wavelength λ is 632.8nm. It is difficult to give accurate values to the thickness and index of refraction of the membrane for the electro-optical response. Here we make the assumption that a macroscopic description of this ultrathin optical device is valid, which is commonly done for thin films (26). Under this assumption, values $e=7$ nm and $n \approx 1.5$ seem to be safe values, that have also been used in the literature (27). Then one can estimate the membrane EO coefficient $r_{13} = 2.6$ pm/V. This value seems reasonable since it can lead to non-negligible SHG signals as those reported in ref (18).

Fig. 6 shows a typical raster scan of a membrane under a 5V sinusoidal voltage at 20 KHz frequency. The contour of the hole holding the membrane can be distinguished. The signal is higher at the center of the membrane, maybe due to higher density of DI8ANEPPS molecules at the center of the hole due to a possible Gibbs plateau on the circumference of the hole. The inset in Fig. 6 shows another scan during which the membrane broke.

In order to test the symmetry of the dye orientation distribution, the linear polarization of the incoming light was switched perpendicular to the previous one, i.e, from x to y axis in Fig. 1. The results are given in Tab. 1. The signal did not change within 2%, as expected from the cylindrical symmetry about the z cone axis, hence the same electro-optical coefficient is

found in both configurations. We compared such a response to a reference sample made of a LiNbO_3 single crystal plate. This reference is highly anisotropic in its x and y responses, which confirmed the polarization sensitivity of our interferometric detection method. Therefore any change of dye alignment in the membrane can be readily detected by our set-up.

5. Discussion and Conclusion:

In conclusion we demonstrated that the electro-optical effect can be used to quantitatively image a dye-doped artificial membrane. We checked that the EO response is proportional to the applied voltage difference as expected for the Pockels effect. Using a polarization-sensitive measurement, we checked the symmetry of the doped membrane around the direction normal to the bilayer, and proved that this microscopy is able to probe the statistical orientation order of the dye inside the membrane. Estimating the applied electrical field we obtained a reasonable value for the dye-doped membrane nonlinear susceptibility $r_{13}=2.6\text{pm/V}$. If the nonlinear susceptibility is known independently, for instance from SHG measurement, our method provides an optical measurement of the transmembrane potential. This second approach therefore allows to investigate electrical fields in doped lipid membrane.

We note that the above results rely on average values over the membrane. A future analysis taking into account the distribution of the field and exact molecular insertion could be performed by using different dye molecules. At the scale of a single layer of molecules, we may also observe heterogenous physical effects, in terms of thin-film organization and for multicomponent membranes which will require a refined model.

Finally we note that we observed that a modulated sinusoidal potential difference of 1 Volt rms amplitude and at a relatively high frequency, varied from 15 to 100 kHz, was not invasive to our membrane, until voltages reaches 4Vpp (frequency = 20KHz) (supplementary information available). In this case the bilayer was not stained by Di-8-ANEPPS, and the electrical capacitance of the 9 simultaneous bilayers was 33pF. In another experiment with stained lipidic membranes, some of them could withstand voltage application up to 20Vpp, at 20KHz signal, without damage. This is quite surprising since it is well known that when a DC potential difference higher than 1V is applied to the bilayer, it is depolarized by the corresponding static electric field and the membrane is broken (28-30). The enhanced robustness of the membrane to higher voltage is probably linked to the sinusoidal character of the potential difference. This unexpected result has to be investigated in future work.

Many developments of the techniques are being now envisioned. For instance, in order to fully characterize the orientation order of the dye in the artificial bilayer, the average tilt angle $\langle\theta_0\rangle$ of the molecule being given by the coefficient ratio $r_{13}/r_{33} = \langle\tan(\theta_0)\rangle/2$ (see Appendix Eq.15), a light polarization in the z axis direction could be applied on the membrane by focusing an inhomogeneously polarized beam (31). New bio-chips where the

buffer solution in the upper chamber is controlled can also be designed to allow us to change the dye concentration during a single experimental measurement and insert membrane proteins that have the potential to control membrane channel opening and the related ionic concentration that are responsible for membrane potential variations.

It can also be envisioned to probe the depolarization of plasma membrane after stimulation and in long term the propagation of an action potential in excitable cell. Some bottlenecks have however to be considered. Indeed such internally generated the electrical field is a self triggered train of pulses rather than a sine wave at a definite frequency. Therefore, a new asynchronous detection technique has to be developed. While such a goal, under investigation in our lab, is challenging, the results presented here are promising to further explore our optical method onto an all-optical patch-clamp technique.

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APPENDIX

Relation between the nonlinear susceptibility and molecular orientation:

The electro-optical (EO) effect is the change of the refractive index of a medium in response to a quasi-static electrical field. This effect is related to second-order nonlinearity and the related EO susceptibility is given by :

$$\chi_{ijk}^{(2)}(\omega + \Omega; \omega, \Omega) = N f^2(\omega) f(\Omega) \iint \beta_{ijk}(\omega + \Omega; \omega, \Omega) P(\check{\rho}) d\check{\rho}, \quad (5)$$

where $\chi_{ijk}^{(2)}(\omega + \Omega; \omega, \Omega)$ is the nonlinear second-order susceptibility, ω is the optical frequency, Ω is the applied electrical field frequency, which can be assumed to be quasi-static compared to that of the optical frequency. N is the density of non-linear chromophore and β is their molecular hyperpolarisability, $\check{\rho}$ is the solid angle related to the orientation of the molecules, as defined for each molecule by its set of Euler angles. $f(\omega)$ and $f(\Omega)$ are the local field factors at optical frequency and quasi-static frequency. Referring to x,y,z laboratory frame, i,j,k are the macroscopic coordinates. The β tensor of the molecule is known and simple in the axes X,Y,Z associated to the geometry of the molecule. It can be assumed to be one dimensional (e.g. $\beta = \beta_{zzz} z \otimes z \otimes z$), in agreement with the quasi 1-D structure of the dye and related intra-molecular charge transfer that underlies β tensor. The associated coordinates are I,J,K. Taking into account the transformation from X,Y,Z to x,y,z for laboratory axes, Eq. 5 becomes:

$$\chi_{ijk}^{(2)}(\omega + \Omega; \omega, \Omega) = N f^2(\omega) f(\Omega) \sum_{IJK} \iint \beta_{IJK}(\omega + \Omega; \omega, \Omega) P(\check{\rho}) (\hat{I} \cdot \hat{i}) (\hat{J} \cdot \hat{j}) (\hat{K} \cdot \hat{k}) d\check{\rho}. \quad (6)$$

In the case of a bilayer having a thickness e , doped by a nonlinear dye with a surface density N_s we have $N = N_s/e$.

We assume that the molecules are oriented inside the membrane in a cone with a symmetry around the normal z to the membrane surface as shown in Fig. 1 and Fig. 7, θ_0 being the angle of this cone with respect to the normal.

In the case of a dye oriented inside a membrane one, can express the probability of orientation

as: $P(\check{\rho}) = \frac{\delta(\theta - \theta_0)}{2\pi \sin \theta_0}$, therefore:

$$\chi_{ijk}^{(2)}(\omega + \Omega; \omega, \Omega) = \frac{N_s}{e} f^2(\omega) f(\Omega) \beta_{zzz} \frac{\iint \delta(\theta - \theta_0) (\hat{I} \cdot \hat{i}) (\hat{J} \cdot \hat{j}) (\hat{K} \cdot \hat{k}) d\theta d\varphi}{2\pi}. \quad (7)$$

This cylindrical symmetry leaves only two independent non-zero coefficient component of the $\chi^{(2)}$ tensor, namely:

$$\chi_{zzz}^{(2)} \text{ and } \chi_{xxz}^{(2)} = \chi_{yyz}^{(2)} = \chi_{yxy}^{(2)} = \chi_{zzx}^{(2)} \quad (8)$$

with :

$$\chi_{zzz}^{(2)} = \frac{N_s}{e} f^2(\omega) f(\Omega) \beta_{zzz} \cos^3 \theta_0, \quad (9)$$

and

$$\chi_{xxz}^{(2)} = \frac{N_s}{e} f^2(\omega) f(\Omega) \beta_{ZZZ} \frac{\sin^2 \theta_0 \cos \theta_0}{2} . \quad (10)$$

Electro-optic coefficients:

The electro-optical coefficients are related to the second-order susceptibility (32), by the relation

$$r_{ijk}(\omega) = - \frac{2\chi_{ijk}^{(2)}(\omega+\Omega; \omega, \Omega)}{n_{ii}^2 n_{jj}^2} . \quad (11)$$

We can write those coefficients in the contracted indices mode where ijk will become lk and l stands for a combination of ij (33). It follows the Tab. 2.

From the above geometry and dye characteristics, the electro-optical coefficient tensor matrix of our doped artificial bilayer takes the form:

$$\begin{pmatrix} 0 & 0 & r_{13} \\ 0 & 0 & r_{13} \\ 0 & 0 & r_{33} \\ 0 & r_{13} & 0 \\ r_{13} & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \quad (12)$$

where

$$r_{13} = - \frac{N_s}{en^4} f^2(\omega) f(\Omega) \beta_{ZZZ} \sin^2 \theta_0 \cos \theta_0 , \quad (13)$$

$$\text{and } r_{33} = - \frac{N_s}{en^4} f^2(\omega) f(\Omega) \beta_{ZZZ} 2 \cos^3 \theta_0 . \quad (14)$$

These expressions show the dependence of the electro-optical coefficient on the orientation angle θ_0 of the dye. The ration between the two coefficients is directly linked to this angle:

$$\frac{r_{13}}{r_{33}} = \frac{\tan(\theta_0)}{2} . \quad (15)$$

EO induced phase shift:

The additional EO phase shift across a membrane of thickness e , when the beam is polarized along the \hat{i} direction, can be expected as:

$$\Delta\varphi_i = \frac{2\pi}{\lambda} e \Delta n_i , \quad (16)$$

so that :

$$\Delta\varphi_i = - \frac{\pi}{\lambda} en_i^3 \sum_j r_{ij} E_j , \quad (17)$$

where E_j is the applied quasi-static electric field.

In the configuration where the applied electric field is in the direction normal to the membrane i.e. along z , the only nonzero component of the electrical field is E_3 , then :

$$\Delta\varphi_1 = - \frac{\pi}{\lambda} en^3 r_{13} E_3 , \quad (18)$$

$$\Delta\varphi_2 = - \frac{\pi}{\lambda} en^3 r_{13} E_3 , \quad (19)$$

$$\Delta\varphi_3 = - \frac{\pi}{\lambda} en^3 r_{33} E_3 . \quad (20)$$

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	EO phase shift ($\times 10^{-4}$ rad)	
Polarization of the light	Artificial membrane	LiNbO ₃ Crystal
x	2.09 ± 0.14	1.82 ± 0.15
y	2.12 ± 0.23	20.12 ± 2.32

Tab. 1: Table showing the EO phase shift in radiant for two perpendicular light polarizations passing through the sample: (left) in the case of an artificial membrane the EO phase shift is the same for both polarizations (5 volts at 20 KHz), and (right) in the case of a reference sample made of LiNbO₃ crystal plate the ratio between the two signals is around 10 (modulation of 10 volts at 20KHz).

<i>ij</i>	11	22	33	23	13	12
<i>l</i>	1	2	3	4	5	6

Tab. 2: Table of contracted indices.

Fig. 1 : Principle of probing the electro-optical effect of a one-side dye-doped membrane. Inset: orientation distribution of the dye molecules in the upper layer.

Fig. 2: Simplified scheme of the interferometric microscope used to detect the electro-optical phase shift associated to the dye-doped membrane. S: cw HeNe laser source – O: microscope objective (40×, N.A. 0.6) – P: photodiode – G: high frequency generator – L: lock-in amplifier.

Fig. 3: Steps of bilayer formation: (a) insertion of the buffer and the dye in the upper channel, (b) injection of the lipid in the lower channel, (c) injection of the buffer solution in the lower channel and formation of the bilayer.

Fig. 4: Electro-optical temporal response of the bilayer without any modulating field, and then while applying 5V as a difference of potential modulated at 20 KHz frequency. The signal to noise ratio is around 10.

Fig. 5: EO phase shift vs applied modulated voltage amplitude

Fig. 6 : A scan image of the EO response of dye doped bilayer. Inset: destruction of the bilayer during a scan.

Fig. 7 : Molecule orientation with respect to the coordinate axis.

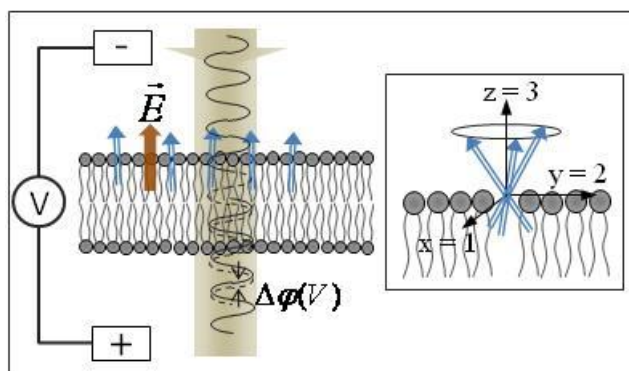


Fig. 1

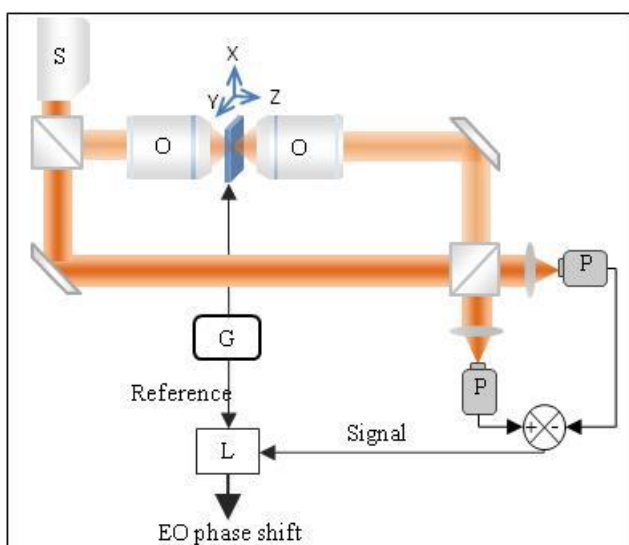


Fig. 2

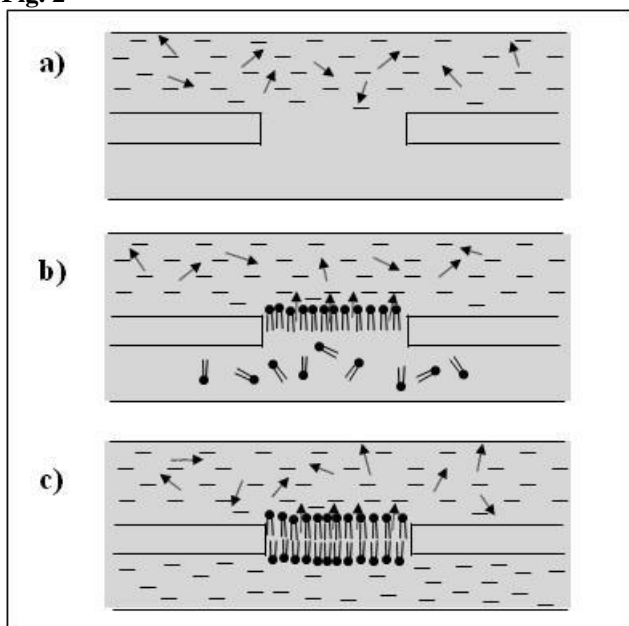


Fig. 3

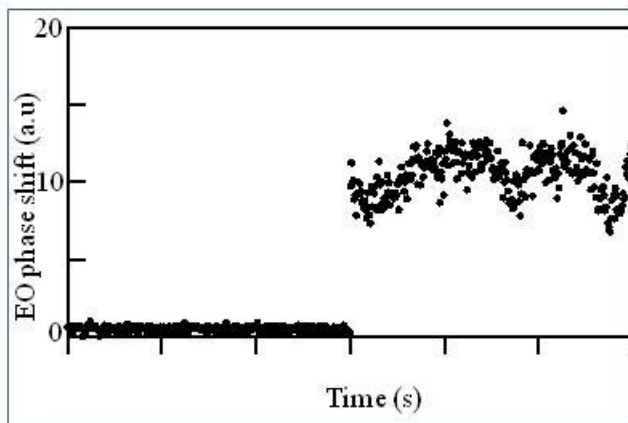


Fig. 4

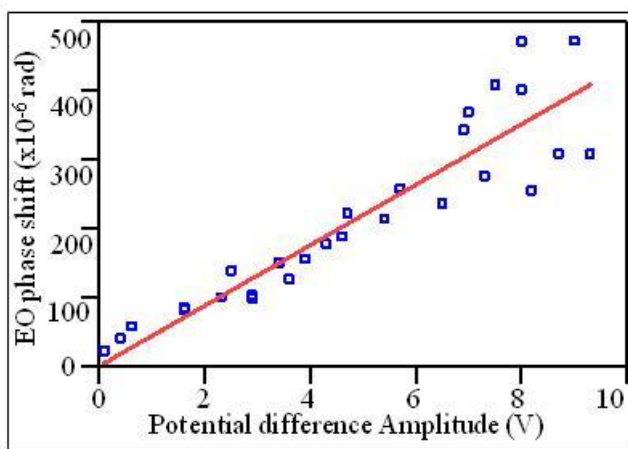


Fig. 5

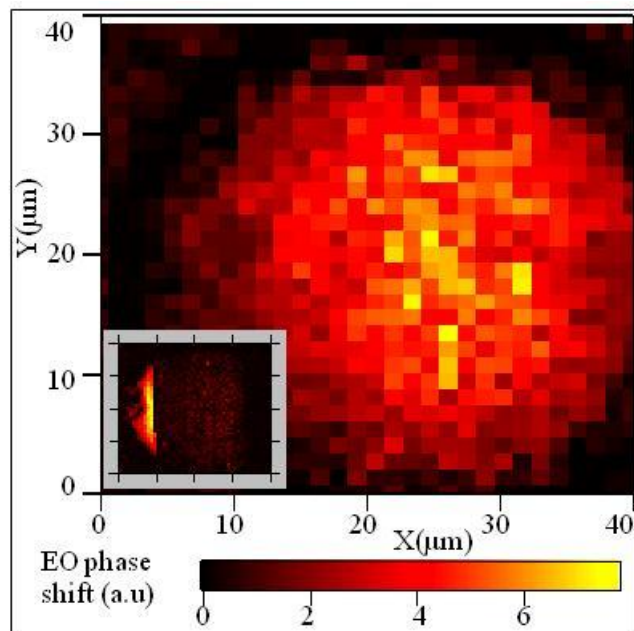


Fig. 6

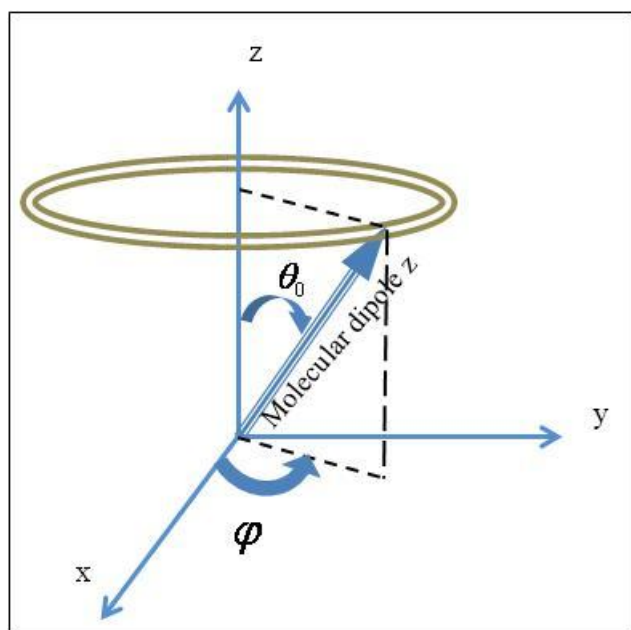


Fig. 7