Depolarization by high aperture focusing

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We introduce a method employing ferroelectric monomolecular layers, by which it is possible to measure the light field polarization in the focus of a lens. This method allowed us to noninvasively establish the perpendicularly oriented focal field that is anticipated at high apertures. For a numerical aperture 1.4 oil immersion lens illuminated with linearly polarized plane waves, the integral of the modulus square of the perpendicular component amounts to $(1.51\pm0.2)\%$ of that of the initial polarization. It is proven that depolarization decreases with decreasing aperture angle. Whereas for regular imaging conditions depolarization is largely negligible, it plays a significant role in microscopy of highest resolution, microspectroscopy, and single molecule studies.

Focusing with high apertures is important to all disciplines requiring highly localized fields of light. It is not only vital to optical microscopy, including its confocal variant, but also to optical spectroscopy in need of high sensitivity and localization. Therefore, high aperture objective lenses are also key elements for single molecule spectroscopy on surfaces and in solution. Nonlinear microscopy also relies on high aperture focusing. Likewise, high aperture lenses are used for collecting the fluorescence in near-field optical microscopy including polarization studies. Hence, a quantitative understanding of the focal field of high aperture lenses is of paramount importance.

Electromagnetic focusing theory is well developed. It is agreed that, while at small semiaperture angles $\alpha$ a scalar wave theory satisfactorily describes the focusing process, at higher angles the vectorial properties of light come into play. The differences are best explained for linearly polarized light, whose field points into say, the $x$ direction (Fig. 1). When focusing at low angles, the focal field $(E_x, E_y, E_z)$ is adequately described by a cylindrically symmetric function $[E(r,0,0)]$ with $r=\sqrt{x^2+y^2}$. However, at angles $\alpha>40^\circ$ theory predicts that the symmetry is broken and the field exhibits significant $E_y$ and $E_z$ components (Fig. 1). High aperture focusing is difficult to treat analytically. In a classical letter, Richards and Wolf\textsuperscript{b)} published an integral solution of the focal field that can be evaluated numerically. For a numerical aperture $\text{NA}=n \sin \alpha=1.4$ oil immersion lens featuring a semiaperture angle $\alpha=67.3^\circ$, the global maxima of the field components $E_y$ and $E_z$ in the focal plane are expected that amount up to $7.5\%$ and $40.6\%$ of the $E_x$ value at the focal point, respectively.

The focal field is of particular relevance in fluorescence microscopy since its orientation with respect to the molecular transition dipole determines the excitation rate. The relevance to single-molecule spectroscopy is even more evident. At low apertures, molecules with a linear transition moment $(0,0,\alpha)$ should absorb only weakly when the lens is illuminated with $x$-oriented linearly polarized light, however, at high apertures $E_y$ and $E_z$ could affect the interpretation of the molecular behavior. The vectorial structure of the focal field has been considered in microscopy theory, but in practice it has been largely ignored. The reason is that, to the best of our knowledge, no evidence for a perpendicular component in fluorescence imaging has been found, so that it is left open as to whether it is masked by the structure of the object, by noise, or by lens imperfections. Theoretical studies\textsuperscript{3,5,6} are not satisfactory because the real values may be codetermined by the lens coatings and the glass strain. For example, the refractive index steps occurring on the curved surfaces of the many individual lens components cause a slight rotation of the plane of polarization. This depolarization can be noticed as four patches separated by a dark cross in the light transmitted through two opposing objectives.

The direct measurement of the field components in a high aperture focus is difficult. Measurement by reflection or scattering does not readily disclose the focal field since the waves evolve as they propagate to the detector. A potential solution is the placement of a fixed, fluorescent molecule, into the focal region. Rotating the field allows the fluorescence to probe the modulus square of the field. However, the signal provided by a molecule may be insufficient for a measurement of the weak $E_y$ component. In this letter, we demonstrate that a solution is found through a fluorescent monomolecular Langmuir–Blodgett polydiacetylene film featuring ferroelectric domains. This method allows us to establish the ratio.

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![Fig. 1. The focused $x$-polarized electric light field $E$ features non-negligible transverse ($E_x$) and longitudinal ($E_z$) components around the focal point $F$.](image-url)
which is a measure of the field depolarization. We refer to $\Pi$ as the integral depolarization giving the relative probability that a randomly oriented molecule is excited by the perpendicular field. We established $\Pi$ for the highest semiaperture angle available for oil immersion lenses: $\alpha = 67.3^\circ$. By reducing $\alpha$ with a diaphragm we followed its influence on the depolarization.

The setup (Fig. 2) featured a mode-locked Nd:Vanadate-laser (Time-Bandwidth Products, Zürich, Switzerland) emitting 7.1 ps pulses at 200 MHz which were frequency-doubled to yield $\lambda = 532$ nm; the pulsed mode had no relevance in our experiment. The frequency doubled beam was cleaned by a 10 $\mu$m pinhole. A tube lens of 300 mm focal length ensured nearly plane waves at the lens entrance pupil. The fundamental laser mode was stopped by a KG3 glass filter. The frequency doubled beam was polarized by a Glan-Thomson prism with a polarization ratio of 10$^{-4}$. A $\lambda/2$-plate mounted on a stepping motor was utilized to rotate the field. The distance of the diaphragm to the objective was 30 mm; in the case of the smallest aperture it was 2 mm. The lens transmission was 80% at 532 nm. The sample was mounted on an XYZ-piezo-stage enabling us to scan the layer with respect to the beam. Fluorescence was collected by a 1.2 NA with respect to the beam. Fluorescence was collected by a 10$^{-4}$.

To suppress stray light, the fluorescence passed a large pinhole placed in front of a photomultiplier. Depolarization through the collecting lens is irrelevant since our detection was insensitive to polarization. Without objective lenses the depolarization of the laser light by our setup was $<2 \times 10^{-4}$.

The fluorescence of the polydiacetylene LB layer arises from the delocalized $\pi$ bonds of the polymerized backbone, which is parallel to the cover slip. Therefore the transition dipoles should exhibit a negligible component along the Z axis and $E_z$ is expected to remain without influence. Although the orientation of the molecules might slightly vary over a distance of 50–60 $\mu$m, within the area of the Airy disk (radius <0.2 $\mu$m) we can expect that the transition dipoles are coherently oriented in a single direction. The measurement consists of rotating the orientation of the field with respect to that of the layer for various lens apertures. We denote their mutual angle with $\varphi$. The fluorescence signal obeys

$$
\Pi = \left( \iint |E_z|^2 \, dx \, dy \right) / \left( \iint |E_x|^2 \, dx \, dy \right),
$$

with $C$ being a constant. If the field is parallel to the transition dipole orientation, that is $\varphi = m \times 90^\circ$, with $m = 0, 2, 4$,.., the fluorescence is proportional to $\iint |E_z|^2 \, dx \, dy$. For the perpendicular case $m = 1, 3, 5$,.., only the contribution from $\iint |E_z|^2 \, dx \, dy$ and hence the strength of the depolarization is recorded. For reduced aperture angles we anticipate weak $E_x$ components and $\Phi$ be dominated by the cosine term. This is confirmed in the measurement shown in the upper left inset of Fig. 3, displaying the fluorescence as a function of the angle $\varphi$ at higher magnification. The depolarization $\Pi$ increases with increasing aperture.

$$
\Phi = C \iint (|E_x \cos \varphi|^2 + |E_y \sin \varphi|^2) \, dx \, dy
= C[\cos^2 \varphi + \Pi \sin^2 \varphi] \iint |E_x|^2 \, dx \, dy,
$$

with $C$ being a constant. If the field is parallel to the transition dipole orientation, that is $\varphi = m \times 90^\circ$, with $m = 0, 2, 4$,.., the fluorescence is proportional to $\iint |E_z|^2 \, dx \, dy$. For the perpendicular case $m = 1, 3, 5$,.., only the contribution from $\iint |E_z|^2 \, dx \, dy$ and hence the strength of the depolarization is recorded. For reduced aperture angles we anticipate weak $E_x$ components and $\Phi$ be dominated by the cosine term. This is confirmed in the measurement shown in the upper left inset of Fig. 3, displaying the fluorescence as a function of the angle $\varphi$ at higher magnification. The data is fitted by a squared cosine function.

![Figure 2](image1.png)

**FIG. 2.** Setup for depolarization measurements. The rotating half-wave plate changes the orientation of the incoming field. The focused field excites a 0.2–0.7-$\mu$m-diam spot on a highly polarized LB layer whose fluorescence is registered in the photomultiplier.

![Figure 3](image2.png)

**FIG. 3.** Semilogarithmic plot of the fluorescence as a function of the angle between the orientation of the incoming field and that of the transition dipoles in the LB layer, as found for three different apertures in a typical measurement. $\varphi = 90^\circ$ and $270^\circ$ correspond to a crossed mutual orientation; the finite fluorescence reveals a transverse field component $E_x$. The upper left inset displays the fluorescence on a linear scale; the cosine behavior evidences the high degree of molecular orientation. The lower right inset depicts the region around $\varphi = 90^\circ$ at higher magnification. The depolarization $\Pi$ increases with increasing aperture.
focused, there is a "technical" cause for depolarization, such as refraction at the lens surfaces, scattering, and birefringence.

This is supported by the finding that the relative intensity of the depolarized transmitted light, here defined as \( \Pi \), exhibits a temperature \( (T) \) dependence [Fig. 4(a), dashed line]. We varied \( T \) from 21 to 32 °C as measured at the lens. We also found a \( T \) dependence of \( \Pi \) for both high and low apertures, that was less pronounced, though [Fig. 4(a), solid lines]. While the depolarization at the focus \( \Pi \) is influenced by a technical contribution \( \theta/2 \), it is not straightforward to disentangle it from vectorial defocusing, because for this purpose, the phase and magnitude of the technical component in the focal region are required. The latter could be calculated though, if the details of the lens were known.

Another cause for the depolarization could be a non-negligible dipole component orthogonal to the fluorescent backbone. Contributions from misaligned molecules had been excluded by photobleaching. For this purpose, we lined up the field perpendicular to the orientation of the domain, increased the power by a factor of 22, and scanned over the area for typically \( \sim 100 \) s. This precaution would have bleached the orthogonal molecules without affecting the properly oriented ones. In our measurements, however, we avoided photobleaching by keeping the excitation rate low and repeating the measurements of \( \Pi \) at several locations. Figure 4(b) displays a histogram of 53 and 43 measurements for \( \Pi = 0.375 \) and 1.4 respectively, performed at \( T = 22 \) °C. For the lower aperture we obtained \( \Pi = (0.34 \pm 0.12)\% \) and for the unobstructed lens \( \Pi = (1.51 \pm 0.2)\% \). Our results establish the experimentally relevant strength of the transverse depolarization in the focus irrespective of its cause.

In conclusion, we have quantified the relative integral intensity of the transverse component \( E_z \) of the field in the focus of a lens. We found that the depolarization is not solely connected with vectorial defocusing but also with technical limitations. Being stronger, the longitudinal component \( E_z \) will be less affected by technical causes of depolarization. Still this should be taken into consideration when comparing experimental findings with predictions by electromagnetic focusing theory. Depolarization is of lesser importance in standard imaging but in high resolution and spectroscopic studies it must be taken into account. If randomly dispersed molecules on a surface are excited through a 1.4 aperture oil immersion lens illuminated by plane waves, one out of \( \sim 66 \) excitations will be due to the depolarized field component.

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