Complex Cylindrical Vector Beam Excludes the Orientation Dependence of the Intensity of Scanning Fluorescence Images of Single Molecules

S. V. Boichenko* and E. F. Martynovich

Irkutsk Branch, Institute of Laser Physics, Siberian Branch, Russian Academy of Sciences, Irkutsk, 664033 Russia

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A polarization inhomogeneous vector beam, which is the complex superposition of radially and azimuthally polarized beams, has been studied computationally and theoretically in application to scanning fluorescence microscopy of single molecules. It has been shown that its application makes it possible to almost exclude the dependence of the intensity maximum of the scanning fluorescence image of a single molecule on its orientation. It has been demonstrated that the scanning of one horizontal plane of a solid matrix containing impurity luminescent molecules provides images of all molecules located in a 1-μm-thick layer, replacing three-dimensional scanning. Recommendations on the technical implementation of the proposed beam have been given.

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The observation of single molecules and other quantum systems located on substrates and in media by confocal scanning luminescence microscopy is of interest for investigations in chemistry of high-molecular compounds [1], materials science [2], photonics [3], biotechnology and science of the genome [4], medicine [5], etc. Most of the quantum systems located in an homogeneous medium emit and absorb light as dipole oscillators. It is desirable in many applications and it is necessary in some of them to observe all single quantum systems irrespectively of the orientation of their transition dipole moments. For this reason, it is necessary to visualize arbitrarily oriented single molecules. A particular case is the problem of visualization of single molecules in an isotropic medium. Within the scanning luminescence microscopy method involving high-aperture objectives, this problem is solved through the spatial modulation of the electric vector of a light field in the cross section of an exciting beam.

One of the first mentions of solving such a problem can be found in [6]. However, the authors of that work aimed to observe single molecules with various orientations near the polymer–air interface, did not pretend to visualize arbitrarily oriented single molecules. A particular case is the problem of visualization of single molecules in an isotropic medium. Within the scanning luminescence microscopy method involving high-aperture objectives, this problem is solved through the spatial modulation of the electric vector of a light field in the cross section of an exciting beam.

A mathematical technique for calculating scanning confocal fluorescence images of single dipole emitters was described in our previous work [9]. Here, we present the main mathematical formulas necessary for such calculations. The electric vector of the light field at the point \( \mathbf{r} = (x, y, z) \) of the focal volume is calculated by the formula [9, 10]

\[
\mathbf{E}(\mathbf{r}) = \frac{-i}{\lambda} \int_{0}^{\theta_{\text{max}}} \int_{0}^{2\pi} \sin \theta \sqrt{\cos \theta} \exp(-ik\mathbf{r}) K(\theta, \varphi) E_{\theta}(\theta, \varphi) d\theta d\varphi,
\]
where $i$ is the imaginary unit, $f$ is the focal length of a microobjective, $\lambda$ is the wavelength, $k$ is the wavenumber of the exciting beam, $n = (\sin \theta \cos \varphi, \sin \theta \sin \varphi, \cos \theta)$ is the unit vector normal to the reference sphere, $K$ is the three-dimensional rotation matrix, $E_\theta$ is the electric vector of the light field of the input laser beam, integration is performed over the solid angle covered by the objective, $\theta_{\text{max}}$ is the angular aperture of the objective, and $\theta$ and $\varphi$ are the polar and azimuthal angles, respectively. The calculations are performed for the objective with $\theta_{\text{max}} = 64^\circ$. In [9], the distance between the point of the focal volume and the point on the reference sphere, rather than the scalar product, enters with negative sign into the argument of the exponential. However, since the characteristic $r$ values (hundreds of nanometers) are much smaller than the radius of the reference sphere (several millimeters), both formulas give almost the same result.)

The probability of the excitation of a molecule with the transition dipole moment $d = (\sin \theta_d \cos \varphi_d, \sin \theta_d \sin \varphi_d, \cos \theta_d)$ located at the point with the radius vector $r$ by the light field $E$ is

$$P_{\text{ex}}(r, d) \sim |E(r)d|^2. \quad (2)$$

The intensity at the point of the scanning confocal fluorescence image corresponding to the point of the objective, and other collecting optical elements. It is reasonable to assume that the transmission function is axisymmetric, i.e., independent of the angle $\varphi$ (it depends very slightly on $\varphi$ at least for qualitative implementation of collecting optics). Under this assumption, Eq. (3) can be rewritten as

$$I(r, d) \sim P_{\text{ex}}(r, d) \int_{\Omega_0} J(d, n) T(\theta) \sin \theta d\theta d\varphi, \quad (3)$$

where $\Omega_0$ is the angle covered by the objective, $J(d, n)$ is the radiation intensity from the dipole $d$ in the direction $n$ (the form of this function can be found in [11]), and $T(\theta)$ is the transmission function of the objective and other collecting optical elements. It is reasonable to assume that the transmission function is axisymmetric, i.e., independent of the angle $\varphi$ (it depends very slightly on $\varphi$ at least for qualitative implementation of collecting optics). Under this assumption, Eq. (3) can be rewritten as

$$I(r, d) \sim (1 - \alpha \cos 2\varphi_d)|E(r)d|^2, \quad (4)$$

where $\alpha$ is the light collection variation coefficient, which depends on the angular aperture of the objective and the transmission function of the optics. For the objective with $\theta_{\text{max}} = 64^\circ$ (such objectives are contained, e.g., in a MicroTime 200 (PicoQuant GmbH) microscope) at $T(\theta) = 1, \alpha = 0.25$. This coefficient is generally within the range from $-1/3$ to $1$.

As was mentioned above, the aim of this work is to theoretically analyze a complex cylindrical vector beam in order to use it to visualize arbitrarily oriented molecules in an isotropic medium. We specify the distribution of the electric vector of the light field in the cross section of the beam under study as follows:

$$E_\theta(\theta, \varphi) = E_\varphi(\varphi) + A \exp(i\Phi) E_{\varphi}(\varphi), \quad (5)$$

where $E_\varphi(\varphi) = (\cos \varphi, \sin \varphi)$ and $E_{\varphi}(\varphi) = (-\sin \varphi, \cos \varphi)$ are the radial and azimuthal polarizations, respectively; $\varphi$ is the angle of the polar coordinate system in the transverse cross section of the beam; and $A$ and $\Phi$ are the varying ellipticity parameters. Varying the former parameter from 0 to $\infty$ and the latter parameter from 0 to $2\pi$, we cover all possible complex superpositions of the radial and azimuthal polarizations. The ratio of the intensity maximum on the scanning confocal image of the minimally excited dipole to the respective value for the maximally excited dipole (the dipoles are in the focal plane) is used as the efficiency parameter [9]:

$$\varepsilon = \frac{\max(I_{\text{min.ex}})}{\max(I_{\text{max.ex}})}. \quad (6)$$

The aim of our study is to analyze the dependence of $\varepsilon$ on $A$ and $\Phi$ and to find its maximum achievable value.

Figure 1 shows the dependence of $\varepsilon$ on the parameters $A$ and $\Phi$ at $\alpha = 0.25$. The data for $\Phi = 180^\circ - 360^\circ$ are not shown because the numerical data satisfy the periodicity condition $\varepsilon(A, \Phi + \pi) = \varepsilon(A, \Phi)$. In the limit $A \to \infty$, $\varepsilon$ decreases to 0 (the beam becomes azimuthally polarized for which $\varepsilon = 0$). For this reason, $\varepsilon$ for $A > 2$ does not exceed 20% and its maximum achievable value is within the region shown in Fig. 1. The maximum value of the function under study is reached at $A = 0.76$ and $\Phi = 57.5^\circ$ and 122.5$^\circ$ and is 96%, i.e., almost achieving the maximum possible value of 100%. Under these conditions, $\varepsilon = 45\%$ for the radial field, $\varepsilon = 60\%$ for the generalized cylindrical vector beam and for the radial beam with the optimal diameter, and $\varepsilon = 47\%$ for the circularly inhomogeneous beam (i.e., much smaller).

We now estimate the computational error in $\varepsilon$. We use Eq. (4) for $z = 0$ (focal plane). The parameter $\varepsilon$ at fixed $A$ and $\Phi$ values was calculated as follows. The