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Electric field allowed molecular transitions for one and two photon excitation microscopy

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Abstract We propose an excitation technique for observing single and two photon excitation in those molecules for which such transitions are forbidden by the selection rules. This is possible by the application of an external electric field that perturbs the molecular orbitals, thereby resulting in a significant shift of energy levels. Such a shift of energy levels may bring those levels in resonance with the radiation field which is normally forbidden by selection rules. Further, parity of the these states may significantly improve the emission process. The external electric field results in the mixing of excited (short lifetime) and metastable states (long lifetime), thus reducing the lifetime of metastable (or near metastable) states. This may provide an effective channel for allowing transition from the metastable states. An application of electric field may result in the excitation of poorly excitable biomolecules. This excitation technique may find applications in single- and multi-photon fluorescence microscopy, bioimaging and optical devices.

Since the theoretical prediction of two photon excitation (2PE) process by Goppert-Mayer (1931) and its first application in two-photon laser scanning fluorescence microscopy for 3D imaging of living cells (Denk et al. 1990), there is an increased interest in fluorescent materials and methods for obtaining a high 2PE signal. The applications of 2PE are expanding and very promising. Prominent application include high resolution 3D microscopy (Denk 1994; Diaspro et al. 2005) and high-density 3D optical data storage (Stickler and Webb 1991; Renn et al. 2002). The symmetric charge transfer in newly designed molecules is the basis for enhancing the 2PE cross-section (Albota et al. 1998). In this letter, we propose an enhancement of the 2PE signal by allowing forbidden transitions (excitation to selection rule forbidden excited states) using an external electric field.

Fluorescent molecules undergo one- or two-photon interaction based on the choice of wavelength, cross-section and intensity. A small values of two photon cross-section is responsible for a weak signal in 2PE microscopy. Selection rules differ for one- and two-photon electronic transitions. Deep understanding of absorption phenomena requires the Parity law. This law states that parity must change for a permitted atomic transition. Violation of this rule gives rise to “forbidden transitions”. The selection rules for electronic transitions for single photon interactions are not valid for two photon absorption (Hunt 2000; Shen 1984). The selection rules for electronic transitions can be summarized as follows: two-photon absorption is an even-parity transition and single-photon absorption is an odd-parity transition between the ground and excited electronic states (Masters and So 2004).

An electric field significantly shifts and remixes the molecular states. Such a technique has two fold advantage: (1) Application of an electric field can allow a molecule to absorb by lifting the barrier posed by the selection rules; (2) Enhancement of one- and two-photon excitation cross-section is possible by quenching the metastable states of the molecule. It is observed that forbidden states of even parity

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can be made Stark effect allowed in the presence of an electric field (Prince et al. 2006). Strong Stark mixing of the double excited states of He is reported by Harries et al. (2003). Increase in intensity with electric field strength is observed by Zitnik et al. (2006). The proposed technique can be easily combined to Confocal and TPE imaging system. A second laser source can be used to generate sufficient electric field at the geometrical focus. The field thus generated may result in efficient mixing of excited and metastable triplet states, thereby reducing the lifetime of triplet states. Short triplet lifetime minimizes the generation of reactive species such as, Oxygen. It should be noted that states with longer lifetime (such as triplet state) is a precursor for photobleaching reactions. Hence, this technique may result in appreciable reduction of in photobleaching and photodamage.

We propose a mechanism for possible increase of one/two photon cross-section by (1) tuning an appropriate parity-excited-state in resonance with the radiation field and (2) reducing the lifetime of metastable states thereby making them more likely candidates for emission. We proceed by describing the corrected Hamiltonian in the presence of an external electric field, followed by adding the Hamiltonian accounting the electromagnetic interaction between radiation field and the molecule.

The molecular Stark effect occurs when a high intensity radiation field is coupled to a nearly resonant fluorescent molecule, resulting in shifts of transition energies observed in absorption or fluorescence. The intensity (I) dependent absorption frequency shift for a two-level system (with transition frequency ω_{21}) is $\Delta\omega_{21} = \omega_p - \omega_{21}(I) + \delta$, where $\delta = \sqrt{(\omega_{21}(I) - \omega_p)^2 + \omega_{\text{rabi}}^2}$; ω_{rabi} and ω_p are respectively the Rabi frequency and pump frequency (Becker et al. 1988). For observing the Stark effect, a Rabi frequency comparable to the detuning is needed.

In the presence of an external electric field $\bar{E}$, we must add the Stark Hamiltonian H_s to the unperturbed Hamiltonian H_0. The Stark Hamiltonian H_s describes the interaction energy of the electric dipole moment $q\bar{r}$ of the atom with the external electric field $\bar{E}$. So, the modified Hamiltonian is given by,

$$H' = H_0 + H_s. \tag{1}$$

Quantum mechanically, it can be shown that the matrix element $(\langle\psi_i|H'|\psi_i\rangle)$ calculating the effect of the electric field to the first order $(-qE\langle i|\bar{r}|i\rangle)$ may be zero, depending upon the parity of the state. In that case, one needs to go beyond first order correction. It is always possible to find a state $|f\rangle$ whose parity is opposite to that of state $|i\rangle$. In the presence of a static electric field $\bar{E}$ parallel to $\bar{r}$, the states (both ground state and excited state) changes i.e, the ground state is no longer $|i\rangle$ but rather, $|i'\rangle$ and excited state $|f\rangle$ changes to $|f'\rangle$ (Stark Shift). In general, for single photon excitation, the state $|i\rangle$ transforms as $|i'\rangle$ i.e,

$$|i'\rangle = |i\rangle - qE|f\rangle\frac{\langle f|\bar{r}|i\rangle}{\xi_i - \xi_f} + \cdots \tag{2}$$

and for two photon excitation where both the ground and excited state have same parity, the state $|i\rangle$ transforms as $|i'\rangle$, given by,

$$|i'\rangle = |i\rangle - qE\sum_m |f\rangle\frac{\langle f|\bar{r}|m\rangle\langle m|\bar{r}|i\rangle}{\xi_{mi} - \frac{\hbar\omega}{2}} + \ldots \tag{3}$$

where, $\hbar\omega = |\xi_f - \xi_i|$ and $|m\rangle$ is virtual intermediate state between $|i\rangle$ and $|f\rangle$. (2) and (3) are true for all the states involved in the excitation process. The shift of molecular levels is expected to be linear when the molecule possesses a non-zero induced dipole moment and quadratic for symmetric molecules.

The corresponding resonance energy of the single photon transition $h\nu_0$ $(|i\rangle \rightarrow |f\rangle)$ shifts to a new position $h\nu = h(\nu_0 + \Delta\nu)(|i'\rangle \rightarrow |f'\rangle)$. The Stark shift $\Delta\nu$ is defined by the shifts of energy levels of the ground state and first excited states. Hence, the electric field induced shift in terms of resonance energy is given by,

$$\Delta\xi = h\Delta\nu = \Delta\xi_{f'} - \Delta\xi_{i'} \tag{4}$$

where, $\Delta\xi_{i'} = \xi_{i'} - \xi_i$ and $\Delta\xi_{f'} = \xi_{f'} - \xi_f$ can be calculated from the set of *Schrödinger* equations: $H|i\rangle = \xi_i|i\rangle, H|f\rangle = \xi_f|f\rangle, H'|i'\rangle = \xi_{i'}|i'\rangle$ and $H'|f'\rangle = \xi_{f'}|f'\rangle$ respectively, provided that the shifted states $|i'\rangle$ and $|f'\rangle$ are eigen states of the perturbed Hamiltonian H'.

Using Eq. 2 and 3 separately for the single and two photon excitation scheme, the resonance energy shift $\Delta\xi$ can be rewritten in terms of external electric field E as (Latychevskaia et al. 2002),

$$\Delta\xi = C_1E + C_2E^2 + \cdots \tag{5}$$

where, C_1, C_2, ... are expressed in terms of $\Delta\xi_{i'}$ and $\Delta\xi_{f'}$.

The new Hamiltonian is H' [see, (1)] and let the time dependent perturbation which defines the interaction of radiation field with the molecule be $H_e(= V(t) = Ce^{-i\omega t})$, and $k = \frac{\omega}{c}$ is the wave number. Perturbation theory states that the modified Hamiltonian for one/two-photon excitation in the presence of electric field is given by,

$$H = H' + H_e. \tag{6}$$

Consider that, $|i'\rangle$ and $|f'\rangle$ are eigen states of the new Hamiltonian (H). These are the eigen states which are in resonance with the radiation field. The transition probability between the modified eigen states is given by Goppert-Mayer (1931),

$$W_{i'\rightarrow f'} = \begin{cases} |\langle f'|U(t)|i'\rangle|^2\delta(E_{f'} - E_{i'} - \hbar\omega), & \text{for 1 PE;} \\ |\langle f'|U(t)|i'\rangle|^2\delta\left(E_{f'm'} + E_{m'i'} - 2\hbar\frac{\omega}{2}\right), & \text{for 2 PE.} \end{cases} \tag{7}$$

where, $E_{f'm'} = E_{f'} - E_{m'}$ and $E_{m'i'} = E_{m'} - E_{i'}$; and the time evolution operator $U(t)$ can be expressed as,

$$U(t) = U_0U_D(t,0) = \mathrm{e}^{-i(H'/h)t} \times \left[1 + \sum_{n=1}^{\infty}\left(\frac{1}{i\hbar}\right)^n \int_0^t \mathrm{d}t_n \int_0^{t_n} \mathrm{d}t_{n-1} \ldots \int_0^{t_2} \mathrm{d}t_1 \prod_{j=1}^{n} V(t_j)\right] \tag{8}$$

The transition probability can be estimated at different orders: The zeroth order correction leading to zero probability of transition between $|i'\rangle$ and $|f'\rangle$. The first order correction ($W^{(1)}_{i'\rightarrow f'}$) corresponding to single-photon absorption is proportional to $|\langle f'|V|i'\rangle|^2$. The second-order transition probability relevant to 2PE is given by,

$$W^{(2)}_{i'\rightarrow f'} = \frac{1}{\hbar^4}\left|\int_0^t \int_0^{t_2} dt_1 \langle f'|U_0(t,t_2)V(t_2)U_0(t_2,t_1)V(t_1)U_0(t_1)|i'\rangle\right|^2 \times \delta(E_{f'm'} + E_{m'i'} - \hbar\omega) \tag{9}$$

Now, the second order transition probability for $t \rightarrow \infty$ is,

$$w^{(2)}_{i'\rightarrow f'} = \lim_{t\rightarrow\infty} W^{(2)}_{i'\rightarrow f'} = \frac{2\pi}{\hbar^4\omega^2}\sum_{m'}|C_{f'm'}C_{m'i'}|^2\delta(\omega_1 - \omega) \tag{10}$$

where, the operator C in Coulomb gauge is given by, $C = \frac{e\bar{p}}{mc}.\hat{\epsilon}A_0\mathrm{e}^{i\bar{k}.\bar{r}_{mo}}$; $\hat{\epsilon}$ and A_0 being the polarization vector and vector potential respectively (Esposito et al. 2004). $\bar{k}$, δ and $\bar{r}_{mo}$ are respectively wave vector, Kronecker's delta function and dimension of the fluorophore molecule.

Under the dipole approximation that fluorophore is very small compared to the wavelength of light i.e, $(\lambda \gg \bar{r}_{mo})$:

$$w^{(2)}_{i'\rightarrow f'} = 8\alpha^2\pi^3|\langle f'|\hat{\epsilon}.\bar{r}_{mo}|m'\rangle\langle m'|\hat{\epsilon}.\bar{r}_{mo}|i'\rangle|^2\delta(\omega_1 - \omega)\left(\frac{I}{\hbar\omega}\right)^2 \tag{11}$$

and two-photon cross-section is obtained by summing over all the possible excitable intermediate levels (m'):

$$\sigma^{(2)}_{i'\rightarrow f'} = 8\alpha^2\pi^3\sum_{m'}|\langle f'|\hat{\epsilon}.\bar{r}_{mo}|m'\rangle\langle m'|\hat{\epsilon}.\bar{r}_{mo}|i'\rangle|^2\delta(\omega_1 - \omega) \tag{12}$$

where, $C_{f'm'} \propto |\langle f'|\hat{\epsilon}.\bar{r}_{mo}|m'\rangle|I$ and $C_{m'i'} \propto |\langle m'|\hat{\epsilon}.\bar{r}_{mo}|i'\rangle|I$.

The corresponding single-photon absorption cross-section is given by Sakurai (1985),

$$\sigma^{(1)}_{i'\rightarrow f'} \approx 4\alpha\pi^2|\langle f'|\hat{\epsilon}.\bar{r}_{mo}|i'\rangle|^2\omega\delta(\omega_1 - \omega). \tag{13}$$

Equations (12) and (13) are the general expressions for the single- and two- photon cross-section of a molecule subjected to external static electric field. The modified single and two- photon cross-section have respectively square and fourth power dependence on the matrix element $(|\langle\ldots|\hat{\epsilon}.\bar{r}_{mo}|\ldots\rangle|)$ in the presence of an electric field. The states in the matrix elements $(|\langle\ldots|\hat{\epsilon}.\bar{r}_{mo}|\ldots\rangle|)$ are the modified molecular states which are given by (2) and (3) for the single and 2PE scheme respectively. It should be noted that it is very difficult to calculate single and two photon cross-sections even for simple molecules due to the complex nature of matrix elements in the practical situations.

External electric field significantly mixes the excited states with the metastable states thereby shortening the lifetime of metastable states and hence resulting in a modified single and two photon absorption cross-sections. The lifetime of excited state defines the emission probability. For example, metastable states (ψ_M) which have longer lifetimes are less likely to emit photons due to competing non-radiative processes as compared to excited states (ψ_f) with short lifetime. If the Stark Hamiltonian has non-zero elements between two possible excited states (i.e, ψ_M and ψ_f), any electric field may significantly mix the excited states. This reduces the lifetime of the metastable states, thus making them a more likely candidate for photon emission. The phenomenon is called metastable quenching. Hence, the application of an electric field may increase single and two photon cross-section of molecules, thereby mimicking transitions from metastable or near metastable states.

Application of electric field significantly shifts the molecular states and then undergoes remixing with other molecular states as evident from the expansion coefficients of eqn. (2) and (3). This shift-and-remix mechanism of molecular states may bring certain excited states possessing appropriate parity which are relevant to two-photon-absorption, in resonance with the radiation field. For two photon absorption, this mechanism mimics the possibility of allowing normally forbidden transitions that require same parity for both ground and excited state. This could lead to an absorption spectrum starting at longer wavelengths than typical for 2-photon absorption, which does not start at double the 1-photon wavelength. Classically, a finite molecule could deform its original structure anisotropically in accordance with the direction of the applied electric field, particularly for biomolecules which include locally polarized parts and groups. Consequently, the point group symmetry of the deformed molecules should change and the irreducible representation of the resulting molecular orbitals (MOs) should change. Hence, molecules that are not two-photon excitable can be brought in resonance in the presence of an external electric field. A similar argument is also true for a forbidden single photon excitation in which selection rules require parity flip for ground state and excited state.

Experimental demonstration of the Stark shift was observed in several molecules. The Stark shift on single molecules of Terrylene and 2.3.7.8-di-(peri-naphthylen)-pyren (DPNP) has been reported by T Yu Latychevskaria et al. (2002). For example, application of an electric field upto 20 kV/cm on three single molecules of Terrylene and DPNP has shown a first order coefficient (C_1) of 2.275, -11.819 and -19.087 MHz cm/kV, respectively. The corresponding second order coefficient (C_2) is reported as, -0.169, -0.618 and 1.266 MHz $\mathrm{cm}^2/\mathrm{kV}^2$, respectively. Effects of the electric field on other single molecules such as, Dibenzanthanthrene and *n*-hexadecane were reported in Ref. (Brunel et al. 1999). While the excitation technique could be useful, but one needs to take into account the fundamental limitation connected with dielectric breakdown process occurring at high field strengths especially associated with TPE process. In case of dielectric breakdown, molecular integrity of the fluorescent molecules may be destroyed, resulting in white light generation (Shen 1984; Boyd 1992). Dielectric breakdown of for Water with a picosecond pulse at 1.06 m is measured to be 10^{16} W/m^2 (Shen 1984). Maximum permitted intensity suggested for imaging is in the range 10^{15}–10^{17} W/m^2 (Brakenhoff et al. 1996).

A possible application of the proposed excitation technique could be to study the photo-physics of fluorescent molecules in photo-bleaching experiments. 2PE could be preferred over one-photon excitation so as to reduce fluorescence from off-focus planes and for selective localized excitation of fluorescent molecules. Moreover, 2PE reduces the complexity of the study especially in mobile samples, by point localization ($\approx 10^{-15}$ l) of the excitation volume. In this case, the decrease of radiative lifetime of the fluorescent molecules due to the shift-and-remix mechanism decreases photo-bleaching and photo-damage because short lifetime minimizes the generation of reactive oxygen species during the excitation process.

In this letter, we predict the possibility of excitation from both selection rule forbidden states and metastable states. This is achieved by the application of electric field along the polarization direction of the radiation field. The external electric field perturbs the molecular orbitals, thereby bringing those levels in resonance with the radiation field which are normally forbidden by the selection rules. Significant mixing of molecular orbitals is predicted which results in a reduced lifetime of metastable states thus making them more likely candidate for emission. Such an effect also raises the possibility of dynamically switching molecular transitions on and off, which may find application in fluorescence microscopy and optical data storage.

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