Dyes characterization for multi-color nanodiagnostics by phonon-less optical reconstruction single-molecule spectromicroscopy

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A B S T R A C T

The phenomenon of the zero-phonon spectral lines (ZPL), which corresponds to pure electronic transitions of impurity dye chromophore centers in solids, has already been the basis of high resolution spectroscopy for a few decades. The unique properties of the phonon-less emission in dye-doped solids (very narrow ZPLs and their extreme sensitivity to local environment) opens qualitatively new instrumental possibilities for sequentially-parallel separate spectromicroscopy of giant ensembles of single dopants. On this base the technique can be realized for multi-color far-field optical nanodiagnostics by phonon-less optical reconstruction single-molecule spectromicroscopy (PLORSM). Here we overview and systematize the already published data on photo-physical properties of a number of dyes (organic fluorescent molecules and quantum dots) in the context of their application in PLORSM in a broad range of temperatures. We show that some of dyes are suitable for realization of PLORSM technique at high (even room) temperatures.

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1. Introduction

Recent years have been marked by a rapid growth of techniques for far-field optical nanodiagnostics by imaging of single probe molecules or other point-like emitters (e.g. quantum dots) [1–4]. The single molecules (SM) emission photons form the image of a point-like light source. By analyzing its point spread function (PSF), the exact location (even all three spatial coordinates) of the emitter can be obtained with an accuracy limited only by the signal-to-noise ratio and stability of the setup.

In order to reconstruct an object structure it is necessary to achieve a sufficient spatial density of chromophores and, nevertheless, avoid overlapping of their images. For this purpose it is required to separate individual molecules located within the same diffraction-limited volume by an additional property. In the most popular techniques used so far, sequential imaging of SM is applied with the varying SM luminescence caused by random transitions between fluorescent and non-fluorescent states (blinking) [1–4]. The problems of using such methods in material sciences are an achievement of sufficiently high density of fluorophores and their controlled and deliberate activation.

One more problem relates to the emission quantum yield of chromophore centers (light absorption efficiency, bleaching processes etc.).

Recently [5] the technique was realized, which eliminates the above problems (see [6] for review). It is based on sequentially-parallel detection of spectra and images of single fluorescing molecules embedded in a solid at *low temperatures*, when narrow zero-phonon lines (ZPL) [7] are reachable for observation, which correspond to pure electronic transitions of dye centers. At cryogenic temperatures ZPLs are usually quite intensive and very narrow (see [6–8] and references therein). Furthermore a ZPL frequency is extremely sensitive to local environment that leads to the effect of inhomogeneous broadening – distributions of ZPLs of single chromophores within broad inhomogeneous absorption spectral band. As a result, the ZPL of single probe fluorophores can be excited in a controlled and deliberate way by frequency tuning of the narrowband excitation laser.

The number of resolved SMs within a diffraction limited spot depends on the ratio of a characteristic inhomogeneous width of the absorption spectral band and a homogeneous ZPL width, which at low temperatures achieves very high values. Thus, the frequency of ZPL can be used as an additional property for separation of SM images, which one can consider as a *"color" of probe molecule*. From this point of view, the phenomenon of narrow ZPL appearance in doped solids is a basis for real multi-color nanodiagnostics by phonon-less optical reconstruction single-molecule spectromicroscopy (PLORSM).

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One of the most important problems in PLORSM technique is the choice of probe dye-centers. In this case the fluorophores should suffer an observation of enough intensive and narrow ZPL in a broad temperature range (as ideally, up to room temperature that is interests of bio-science). A great number of scientific works have been published within last several decades, related to the study of ZPL phenomenon in different dye-matrix systems. The techniques which were used for this purpose are laser induced fluorescence line-narrowing (FLN) [9], persistent spectral hole burning (HB) [10], photon echo (PE) [11,12], and single-molecule spectroscopy (SMS) [13–15].

The present paper is aimed to overview the photophysical properties of the most popular fluorescent dyes (organic chromophores and semiconductor quantum dots) which are prospective for phonon-less optical reconstruction single-molecule spectromicroscopy as well as in other single-molecule detection experiments. The data about homogeneous linewidth, natural linewidth, the Debye–Waller factor, and highest temperature where ZPL was observed will be unified in the joint table which will be useful for future spectroscopic applications.

2. ZPL phenomenon, main properties of ZPL

The phenomenon of the zero-phonon spectral lines, which corresponds to pure electronic transitions of impurity dye molecules in solids, is the basis of high resolution spectroscopy of doped solids already for a few decades. The main reason for this is the extreme sensitivity of the ZPLs parameters to local environment of impurities in solids. Single-molecule (SM) spectroscopy and, especially, *highly parallel separate spectromicroscopy of giant ensembles* of single dopants opens qualitatively new possibilities for studying of different aspects of the ZPL phenomenon. The dynamical processes manifest themselves most clearly in broadening and evolution of narrow zero-phonon lines.

The luminescence spectrum (vibronic band) of an impurity molecule in an amorphous solid comprises a narrow ZPL corresponding to the purely electronic transition in the impurity without changing the number of phonons in the matrix, and a relatively broad phonon sideband (PSB) appearing due to radiative transitions in the impurity with the creation and annihilation of matrix phonons.

The relation between the integrated intensity of the ZPL and the total intensity of the (ZPL+PSB) spectral band is determined by linear electron phonon coupling and called the Debye-Waller factor:

$$\alpha_{DW} = \frac{I_{ZPL}}{I_{ZPL}+I_{PSB}}. \tag{1}$$

The temperature dependence of the Debye–Waller factor in the general form is described by the expression:

$$\alpha_{DW}(T) = \exp\left[-\int_0^\infty f_0(\nu)\left(\frac{2}{\exp(h\nu/kT)-1}+1\right)d\nu\right], \tag{2}$$

where $f_0(\nu)$ is the density of phonon states of the system. Thus the ZPL intensity transfers to the PSB intensity with increasing temperature, while the integrated intensity of the vibronic band does not depend on temperature.

Intramolecular processes and interactions, as well as the features of the local structure and processes proceeding in the matrix, are the most pronounced in changes to the ZPL parameters; in other words, in the spectral dynamics of impurity centers [7]. In particular, analysis of the temperature dependences of ZPL parameters allows one to determine the characteristic frequencies of the collective vibrations of matrix atoms.

Numerous studies (see, for example, [6] and references therein) have shown that the homogeneous ZPL width Γ_{ZPL} in the

temperature range well below the melting/vitrification temperatures is determined in general by three main contributions:

$$\Gamma_{ZPL}(T, t_m) = \Gamma_0 + \Gamma_{TLS}(T, t_m) + \Gamma_V(T), \tag{3}$$

where Γ_0 is the natural linewidth determined by the excited-state lifetime of a molecule, $\Gamma_{TLS}(T, t_m)$ is the ZPL broadening caused by the interaction of electronic transitions in impurity molecules with tunneling excitations in the matrix, and $\Gamma_V(T)$ is the ZPL broadening caused by the interaction of impurity molecules with vibrational excitations (electron–phonon interaction), t_m is the characteristic (recording) time of a measurement.

One can study a temperature dependent part of a homogeneous ZPL as a general characteristic describing an optical dephasing in a different media, additional to the natural linewidth. This part can be expressed as:

$$\Gamma_{deph}(T) = \Gamma_{ZPL}(T) - \Gamma_0. \tag{4}$$

The interaction with tunneling type excitations gives rise to spectral diffusion resulting in the single-molecule ZPL frequency jumps. For example, within the standard tunneling two-level system (TLS) and stochastic sudden jumps models ZPL frequency for each molecule is determined by independent tunneling transitions in all nearby TLSs [16]:

$$\omega(t) = \omega_0 + \sum_j \zeta_j(t)\upsilon_j, \tag{5}$$

where ω_0 is the chromophore's transition frequency when all TLSs are in their ground states; and $\zeta_j(t)$ is the stochastic variable equal to -1, if j-th TLS being in its ground state, and $+1$, if j-th TLS being in its excited state. The value of υu_j depends on the parameters of SM, TLS and their interaction. Thus ZPL parameters depend (logarithmically) on the recording time t_m. In amorphous media, the contribution from tunneling excitations at ultralow temperatures ($T < 1-2$ K) becomes dominant. The temperature dependence of ZPL broadening due to this contribution within the standard model of low-temperature glasses [16] is quasilinear:

$$\Gamma_{TLS}(T) \propto T^\alpha. \tag{6}$$

At higher temperatures, ZPL broadening is mainly determined by the electron-phonon interaction. The spectral line broadening of an impurity center caused by the quadratic interaction of an electronic transition with the -phonon excitation spectrum can generally be described (for any electron-phonon coupling strength) by the expression [17]:

$$\Gamma_V(T) = \frac{1}{4\pi}\int_0^\infty d\omega \ln\{1+4n(\omega)[n(\omega)+1] \times W^2 g_{(0)}(\omega)g_{(1)}(\omega)\}, \tag{7}$$

where $n(\omega) = 1/[(\exp(\hbar\omega/kT)-1]$ is the Bose factor, W is the dimensionless constant of quadratic electron–phonon coupling, and $g_{(0)}(\omega)$ and $g_{(1)}(\omega)$ are dimensionless weighted phonon densities of states in the ground and excited states of the chromophore.

The temperature behavior of $\alpha_{DW}(T)$ and $\Gamma_{ZPL}(T)$ is much more complicated in the case of complex dye labels (e.g. semiconductor and dielectric quantum dots, ion-doped inorganic nanocrystals, metal–organic nanostructures). For example in the case of rare-earth ions-doped inorganic nanocrystals embedded into polymeric matrices the shell effect was observed which causes marked changes of dye–matrix interaction [18]. Thus there is a possibility to control the parameters of dye–matrix coupling on the stage of dye preparation.

3. Phonon-less optical reconstruction single-molecule spectromicroscopy

The unique properties of ZPLs can be used as a basis for a new technique for far-field optical nanodiagnostics of condensed matter [5]. The concept of this technique can be stated shortly as follows. The