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Low-temperature spectroscopy of organic molecules in solid matrices: from the Shpol'skii effect to laser luminescent spectromicroscopy for all effectively emitting single molecules

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Contents

Abstract. Sixty years ago, in 1952, Prof. E V Shpol'skii and his colleagues were the first to see quasilinear spectra from complex organic compounds in specially selected solvents at low temperatures. Twenty years later, in 1972, a team headed by Prof. R I Personov discovered laser fluorescence line narrowing in the solid solutions of organic dyes. These two discoveries served as the basis for the field of laser selective spectroscopy of impurity centers in solids. The work in this field culminated in the techniques of spectroscopy and luminescence imaging (microscopy) of single molecules in condensed matter. Today, optical spectroscopy of impurity centers in solid solutions has become one of the most popular tools for solving a wide variety of interdisciplinary problems in physics, physical chemistry, optics and spectroscopy, biophysics, quantum optics, and nanotechnology. In this article, the development of this field is briefly reviewed, potentials of the developed methods are discussed, and some research results are highlighted.

1. Introduction

In 2012, the optical spectroscopy of impurity centers in condensed media celebrated several anniversaries at the same time. 120 years ago, Professor E V Shpol'skii, an outstanding Russian scientist, editor-in-chief of *Physics–Uspekhi*, head of the Theoretical Physics Department at the Moscow State Pedagogical Institute, and USSR State Prize Laureate, was born (see photo in Fig. 1). In 1932, Professor R I Personov, a well-known disciple of Shpol'skii, outstanding Russian spectroscopist, head of the Department of Molecular Spectroscopy at the Institute of Spectroscopy, Russian Academy of Sciences, USSR State Prize Laureate, was born. Two other jubilees are related to the outstanding discoveries of these scientists: 60 years ago, E V Shpol'skii with colleagues [1] observed for the first time the appearance of quasi-line spectra of complex organic compounds in specially selected solvents at low temperatures, and 20 years later, in 1972, researchers supervised by R I Personov discovered the effect of laser excitation of narrow-line luminescence spectra in solid solutions of organic dyes. These two discoveries have played the most important role in the advent and development of a new scientific field — selective laser spectroscopy of impurity molecules in solids.

In 1993, M Orrit, J Bernard, and R I Personov published a paper, "High-resolution spectroscopy of organic molecules in solids: from fluorescence line narrowing and hole burning to single-molecule spectroscopy," in the international *Journal of Physical Chemistry* [1]. They generalized the development of this scientific field: from the discovery of narrow inhomogeneously broadened lines in Shpol'skii matrices [2–4], then selective laser excitation of luminescence spectra [5] and

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[1] The original paper reporting the discovery of the effect is referred to as Shpolskii E V, Il'ina A A, Klimova A A, "Fluorescence spectra of coronene in frozen solutions" *Dokl. Acad. Nauk SSSR* **87** 935 (1952); see also review [140]. (*Editor's note to English proof.*)

Figure 1. E V Shpol'skii and R I Personov in the Theoretical Physics Department at the Moscow State Pedagogical Institute.

persistent spectral hole burning (HB) [6, 7], to, finally, single-molecule spectroscopy (SMS), which completely eliminates data averaging over the volume of a sample under study [8, 9].

All these methods proved to be extremely efficient tools for studying solids with different degrees of structural disorder (see, for example, Refs [1, 10, 11] and references cited therein). The methods are based on the detection of *zero-phonon lines* (ZPLs) corresponding to purely electronic transitions in impurity chromophore centers [12–23]. The ZPL parameters, such as their spectral position and width, depend on the surroundings of a corresponding chromophore molecule, allowing the use of chromophores as spectral nanoprobes to obtain information on dynamic processes in solids.

The nature of ZPLs observed in the spectra of impurity centers in crystals (the optical analog of the Mössbauer line) was extensively studied back in the 1950s–1960s (see papers [12, 13] and also reviews [17, 18] and references cited therein). In 1962, the appearance of narrow quasi-lines in Shpol'skii spectra was assigned to zero-phonon transitions between the electronic levels of impurity molecules [14] theoretically predicted in Refs [12, 13]. In 1966, experiments were performed in which the noticeable narrowing of the luminescence band of impurity ions in inorganic borate glass was observed upon resonance quasimonochromatic excitation by narrow emission lines from a mercury lamp [19]. Thus, it has been experimentally demonstrated that the spectra of impurity solids are inhomogeneously broadened and that this inhomogeneous broadening can be eliminated by using resonance (quasi-) monochromatic excitation of luminescence. Narrow lines have also been observed in the electronic–vibrational (vibronic) spectra of complex polyatomic organic molecules embedded in molecular crystals (see, for example, Refs [20, 21]), and it has been shown in paper [21] that the temperature dependences of the parameters of the narrow lines in quasi-line spectra are well described by the ZPL theory [22, 23] developed by that time, which confirmed the phononless nature of these lines.

The development of the selective spectroscopy of impurity centers in solid matrices has received a maximum of powerful impetus with the advent of monochromatic laser radiation sources providing the efficient to the utmost elimination of inhomogeneous broadening in the luminescence spectra of impurity solids. In 1970, laser-induced fluorescence-line narrowing (FLN) in an inorganic ruby crystal was demonstrated for the first time [24], and already in 1972 it was shown [5] that monochromatic laser excitation leads to the appearance of narrow-line luminescence spectra of many complex organic molecules in various solid solutions, in particular, molecular glasses and polycrystals with many defects. These papers generalized the technique of selective narrow-line spectroscopy to a very broad scope of objects.

Selective laser spectroscopy techniques [laser excitation of luminescence (or FLN) and HB] appeared in the early 1970s and remain in great demand now for studying crystals, glasses, polymers [25], laser materials [26], quantum dots [27], J-aggregates [28], nanostructures [29], proteins [30, 31], light-harvesting complexes [32], and bioanalytical compounds [33]. Lasers were also utilized in photon-echo experiments [34–36] for studying the internal dynamics of impurity condensed media with ultrahigh temporal resolution [37].

Despite their high spectral resolution, the FLN and HB techniques give data averaged over the volume of a sample under study, not revealing the individual features of single-molecule spectra. This disadvantage becomes critical in the studies of solids with a complex structure [38, 39]. A unique possibility of solving this problem is provided by single-molecule spectroscopy, which naturally eliminates averaging over an ensemble of impurity centers and, consequently, over the volume of the object under study. Single-molecule spectroscopy allows one to measure all the spectral parameters of the ZPL of a single emitting center (in particular, the frequency position within the inhomogeneous profile of the line, its width, and intensity), to observe slow spectral diffusion processes revealed as spectral jumps or drifts, to observe the interaction between impurity molecules in direct experiments, and to study the interaction of light with a chromophore at the single-photon level [40–50].

Single-molecule spectroscopy acquires qualitatively new capabilities when single-molecule luminescence imaging is performed with a scanning confocal microscope or a classical luminescence microscope [51, 52]. Because the emitter size is much smaller than the wavelength of light, the accuracy of determining single-molecule coordinates (for the radiation pattern, namely the instrumental function of a point source, known *a priori*) is not restricted by the diffraction limit but only depends on the stability of an experimental setup and the signal-to-noise ratio. The analysis of the instrumental function of a point light source [the point spread function (PSF)] allows the reconstruction of the coordinates of the emitting center with nanometer accuracy [53–56]. The modern methods of three-dimensional fluorescence imaging [57, 58] provide the measurement of all the three spatial coordinates of an emitter in a sample. Thus, the possibilities of completely reconstructing the arrangement of impurity centers in a solid matrix and recording the trajectories of motion of single fluorescing molecules in a medium have appeared.

One of the most important problems in SMS development is the search for fundamental rules relating microscopic single-molecule information to macroscopic properties of bulk samples. This problem can be resolved using the statistical analysis of spectral data obtained for numerous single molecules (see review [47] and references cited therein).

In 2011, researchers at the Institute of Spectroscopy, RAS (Yu G Vainer, A A Gorshelev, I Yu Eremchev, and A V Naumov) and the University of Bayreuth, Germany (L Kador and J Köhler) demonstrated in the international journal *Physical Chemistry Chemical Physics* that at present modern equipment makes it possible to perform qualitatively

new measurements in the spectroscopy of impurity centers — the recording of individual ZPLs and the measurement of spatial coordinates of *all efficiently emitting centers* in macroscopic volumes of solids (molecular crystals, organic glasses, and polymers) doped with fluorescing molecules at relatively low concentrations [59] and observing simultaneously their spectral dynamics [60]. This provides the possibility of following the development path of the spectroscopy of impurity centers as proposed in paper [1], approaching its logical culmination, i.e., measuring 'the true' spectrum of an impurity solid by detecting all impurity chromophore molecules in a macroscopic sample under study. Thus, it is possible to apply SMS for studying the formation of the inhomogeneous optical spectrum of the impurity medium averaged over a giant ensemble of chromophores, thereby relating local and spectral dynamic characteristics to the structure of the medium and its macroscopic photophysical properties. In this paper, a brief review of the development of this field is presented and the main possibilities and results of the developed methods are discussed.

2. Spectroscopy of an impurity center

The vibronic spectra of impurity centers (organic chromophores) in solid matrices contain important information on intra- and intermolecular interactions in impurity solids. An analysis of the spectra allows one to find the relationship between the spectral characteristics of a molecule and its physical and chemical properties and to study the photophysical properties of the impurity system. In turn, the embedment of impurity molecules into a solid matrix eliminates their rotational and translational degrees of freedom, thereby simplifying the recorded spectrum. As a result, the solution for direct and inverse spectroscopic problems is considerably facilitated. This circumstance becomes especially important in studies of the photophysical properties of complex organic molecules and molecular complexes. At the same time, the spectral properties of impurity molecules in solid matrices turn out to be highly sensitive to their interaction with the environment. This interaction can be minimized at low temperatures. Thus, investigations of complex organic molecules in solid matrices at low temperatures are closest to the studies of free molecules.

A plausible energy level diagram of a molecule in a solid matrix is schematically shown in Fig. 2. This diagram differs from that for a free molecule by the presence of 'phonon levels' corresponding to transitions with the creation or annihilation of matrix phonons. Phonon levels are arranged almost continuously because the phonon spectrum of the matrix is continuous.

The interaction of molecules with matrix phonons is represented in Fig. 2 by the 'spread' of vibrational sublevels. Singlet–singlet transitions give birth to absorption and fluorescence spectra, while triplet–singlet transitions are responsible for the emergence of phosphorescence spectra.

The recording and analysis of vibronic spectra of impurity molecules in solid matrices give information on the nature and characteristics of intramolecular processes, such as subtle spin–orbit and vibronic effects of electron–electron and electron–nuclear interactions in the excited electronic states of polyatomic molecules. On the other hand, the spectra of impurity centers are caused by transitions of outer-shell electrons and therefore are extremely sensitive to the environment parameters. For this reason, the spectra of impurity molecules carry important information about the structure and dynamics of the microscopic environment of chromophores. Thus, impurity centers can be used as spectral probes for studying processes in matrices. In particular, vibronic spectra of chromophores contain information on the interaction between electronic transitions in impurities and collective

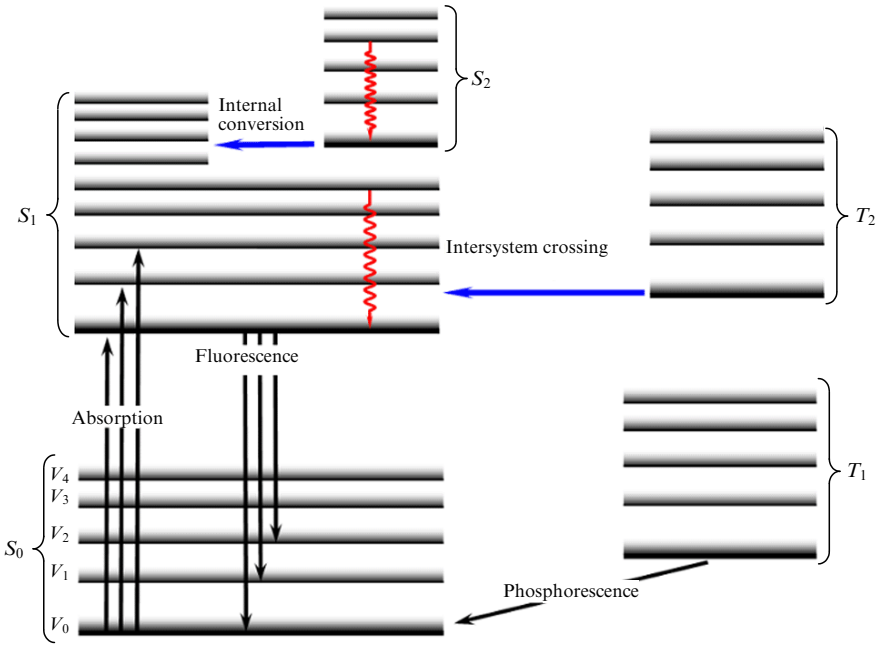

Figure 2. Simplified energy level diagram of complex organic molecules in a solid matrix [61]. The lifetime of the S_1 level is $\sim 10^{-9}$ s, the lifetime of highly excited states is $\sim 10^{-12}$ s, and the triplet-state lifetime is $\sim 10^{-4}$ and longer. The 'spread' of vibrational levels accounts for the interaction of a chromophore with matrix phonons.

vibrational excitations of matrices (electron–phonon coupling).

3. Shpol'skii effect

Under usual conditions, upon excitation by nonmonochromatic light sources at room temperature, the optical spectra of complex organic compounds exhibit broad bands which cannot really be utilized for obtaining information on the structure of molecules or the parameters of intra- and intermolecular interactions. In 1952, E V Shpol'skii and his collaborators L A Klimova and A A Il'ina discovered the appearance of quasi-line vibronic spectra of complex organic compounds dissolved in specially selected solvents at low temperatures [2]. To observe the Shpol'skii effect (which constitutes, in fact, an optical analog of the Mössbauer effect), a special solvent transparent in the absorption and emission spectral ranges of molecules under study is selected. Molecules are dissolved at low concentrations and cooled to temperatures below the crystallization point of the solvent (as a rule, to liquid-nitrogen (helium) temperatures). Under these conditions, molecules are isolated from each other and rigidly fixed in the solvent, resulting in the appearance of atomic-like quasi-line spectra (Fig. 3).

The emergence of quasi-line spectra of organic molecules in solid solutions was called the Shpol'skii effect in the foreign and domestic literature, and the corresponding solvents were termed Shpol'skii matrices.

An important step on the way to understanding Shpol'skii quasi-line spectra was the paper by K K Rebane and V V Khizhnyakov [14], in which the authors assumed that narrow quasi-lines are related to zero-phonon transitions between the electronic energy levels of impurity molecules, theoretically predicted a year earlier in Refs [12, 13].

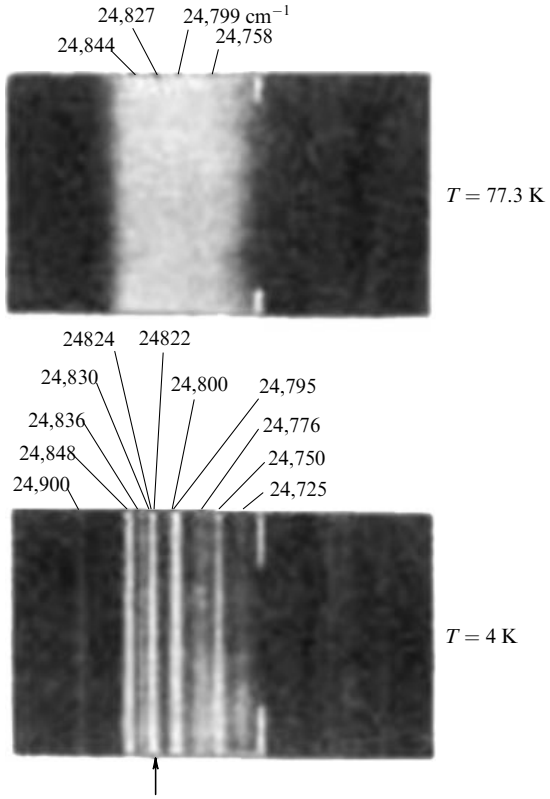

Figure 3. Illustration of the Shpol'skii effect: the appearance of the quasi-line vibronic spectrum of 3,4-benzpyrene in *n*-heptane will decreasing temperature (recorded on a photographic film). Taken (with changes) from review [62].

Such lines appear only upon specific transitions in an impurity molecule, which do not involve matrix phonons, similarly to the appearance of the Mössbauer γ-line. The calculation of the probabilities of electronic transitions in an impurity center from the initial state of a matrix (ideal crystal) to a final state shows that oscillator states exist among all their possible combinations, such that the internal state of the matrix does not change during the transitions between them [4, 14, 15]. During such transitions (between oscillator states with identical quantum numbers: 0–0, 1–1, etc.), the internal state of the crystal does not change, resulting in the emission of a narrow zero-phonon line. Electronic transitions involving matrix phonons give rise in turn to a comparatively broad *phonon sideband* (PSB). Because crystals are not ideal, different impurity centers are located in somewhat different environments, resulting in inhomogeneous spectral broadening. The inhomogeneous broadening in some impurity crystals is small, and therefore quasi-line spectra can be observed in experiments.

The study of the temperature dependences of spectral-line parameters became one of the most efficient methods for proving the zero-phonon nature of quasi-line spectra (see, for example, papers [20, 21]). The theories of electron–phonon coupling developed by that time [14, 22, 23] predicted specific temperature dependences of the ZPL width, spectral shift, and integrated intensity (see Section 5). In particular, the theories predicted the quasiexponential broadening of the ZPL and 'transfer' of the ZPL intensity to the PSB with increasing temperature. Thus, the agreement of the experimental temperature dependences of spectral quasi-line parameters with theoretical dependences could prove that quasi-lines in Shpol'skii spectra comprise ZPLs. In 1970, V A Kizel' and M N Sapozhnikov [21] stated for the first time, based on these grounds, that spectral lines observed in the luminescence spectra of polyatomic organic molecules in polycrystalline matrices in the temperature range from 77 to 225 K are zero-phonon lines. At the same time, theoretical predictions were not validated for some similar impurity media studied in a number of other experiments. In particular, a decrease in the quasi-line intensity with increasing temperature was not observed in paper [63] in this temperature range. This experimental result was notably interpreted by the fact that the ZPL and PSB cannot be separated at temperatures above a few dozen kelvins.

Beginning with the moment of its discovery and up to now, the Shpol'skii effect and Shpol'skii matrices have found wide applications is scientific studies [18, 21, 64–71]. This is explained by the fact that quasi-line spectra exhibit a distinct vibrational structure and thus can be used for determining vibrational frequencies of molecules in the ground and excited states, thereby studying the structure, photophysical, photochemical, and physicochemical properties of complex organic compounds. For example, the microscopic nature of quasi-line spectra and ZPLs was discussed already in Ref. [21]. By analyzing their experimental data, the authors of this work assumed that the emergence of ZPLs is due to single impurity centers (but not to their aggregates or conglomerates), single molecules being fixed during rapid crystallization in the regions of microcracks, defects, and dislocations, which gives rise to quasi-line spectra.

4. Selective laser spectroscopy

The employment of the technique for recording quasi-line spectra of organic molecules in Shpol'skii matrices was restricted because of a limited set of solvents providing appropriate impurity–matrix pairs for occurring an optical analog of the Mössbauer effect.

Because matrices contain local inhomogeneities, different impurity centers are located in different environments, resulting in inhomogeneous broadening effect. The inhomogeneously broadened spectrum (its width can reach several hundred cm^{-1}) is formed by numerous overlapping homogeneous lines frequency-shifted with respect to each other. The inhomogeneous broadening of spectral lines shows its worth especially in disordered solids such as glasses and polymers. The inhomogeneous broadening effect prevents the observation of quasi-line Shpol'skii spectra. Revolutionary changes in this field were brought on by the advent of monochromatic laser light sources in spectroscopy.

Laser spectroscopy methods provide selective excitation of a small ensemble of impurity molecules with close 0–0 transition frequencies. This can be used for studying both the homogeneous broadening of vibronic spectra and the formation mechanisms of the inhomogeneous absorption profile of an impurity medium. Thus, laser excitation was used to remove the inhomogeneous broadening of the ZPL (R line) in an inorganic ruby crystal [24]. However, in the case of broadband spectra of complex organic compounds in disordered solids (organic glasses, polymers, and biological media), the possibility itself of the existence of narrow ZPLs was doubted for a long time.

R I Personov and his colleagues (see photo in Fig. 4) found in their experimental studies on the nature of broad spectral bands in the spectra of impurity solid solutions of organic compounds (in particular, disordered impurity organic glasses) performed in 1972–1973 that these spectra are inhomogeneously broadened at low enough temperatures and contain a great number of narrow ZPLs masked by the inhomogeneous broadening. They showed that the fine structure can be resolved in such spectra upon selective laser excitation of luminescence (Fig. 5) [5].

Moreover, they argued that narrow-line spectra formed by ZPLs of impurity molecules can be observed upon excitation of luminescence by narrow-band monochromatic laser sources in almost any impurity solids, in particular, organic molecular glasses, polycrystals with many defects, and biological media. Thus, paper [5] became a generalization of a sort of selective fine-structure spectroscopy to a very broad scope of objects.

This method for obtaining narrow-line spectra was called laser fluorescence line narrowing and found wide applications. The effect of laser excitation of narrow-line spectra was also called in a number of publications [72] as the *Personov effect*. It was also revealed that selective laser excitation can cause various photochemical and photophysical transformations in impurity centers, which change the absorption spectra of transformed molecules. Such changes form the basis for another method of selective spectroscopy — persistent spectral hole-burning, which was also discovered at the Department of Molecular Spectroscopy at the Institute of Spectroscopy, RAS in 1974 [6]. Similar experiments were simultaneously performed at the Institute of Physics in Tartu (Esthonia) [7].

Figure 4. Laureates of the USSR State Prize 1986 (from left to right): E I Al'shits, L A Bykovskaya, B M Kharlamov, and R I Personov.

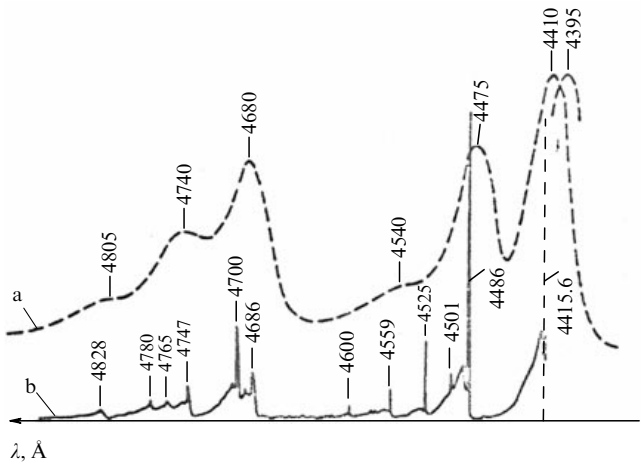

Figure 5. Illustration to the effect of the appearance of fine structure in the fluorescence spectrum of perylene in a frozen solution of ethanol at 4.2 K: (a) spectrum observed upon usual excitation; (b) spectrum observed upon laser excitation (taken from the paper by R I Personov, E I Al'shits, and L A Bykovskaya [5]).

Methods of selective laser spectroscopy can be applied to studying the nature of various intra- and intermolecular processes in impurity solids and can also shed light on the mechanisms of the impurity–matrix interaction and the role of the dispersion of parameters of this interaction in the formation of the spectral hole shape. The study of the temperature behavior of spectral-hole parameters also gives unique information on the internal dynamics of matrices. In particular, hole burning technique showed its worth in studying quantum size effects in impurity solids. For example, analysis of the temperature dependences of spectral-hole parameters in inorganic nanocrystals revealed a change in parameters of the electron–phonon interaction caused by the limited size of objects.

A popular and quite efficient method for studying impurity solids is hole burning experiments in external fields. Because of their small width, hole profiles are very sensitive to external perturbations, which makes it possible to study the distributions of local fields in matrices and to determine a number of parameters of impurity molecules [74–76]. For example, the weak contribution of the quadratic Stark effect was detected in papers [77–79] against the background of the quasi-linear Stark effect and the 'displacement' of a metal atom from the molecular plane was also studied.

Methods of selective laser spectroscopy have become a powerful tool for exploring processes in impurity solids and are successfully used by many research groups (see, for example, Refs [10, 25, 80] and references cited therein). Beginning in 1987 and up to the present days, the international conferences on hole burning have been held in different countries, with well-known worldwide research groups participating (the recent 11th International Conference on Hole Burning, Single Molecule and Related Spectroscopies: Science and Applications was held in Germany in 2012).

5. Zero-phonon spectral lines

Theories describing interaction of impurity centers with a matrix and the appearance of ZPLs in the luminescence and absorption spectra of impurity solids have been developed by many theoretical groups. These theories were originated from a description of the interaction of electronic transitions in impurity centers with collective vibrational excitations in crystals, viz. phonons (see Refs [12–16, 22, 23] and also [4, 17, 18, 21] and references cited therein). Later on, I S Osad'ko generalized the theory to the case of an arbitrary electron–phonon coupling (in particular, to the case of a strong coupling with a crystal lattice) (see papers [81–83], and monograph [44] and references cited therein). According to this approach, the electron–phonon coupling can be described by applying either the adiabatic or diabatic approximation. In the case of impurity polyatomic organic molecules, as a rule, the adiabatic approximation is used.

In the simplest case, the electron–phonon coupling is considered in the harmonic Franck–Condon approximation. The coupling of an impurity molecule with vibrational excitations of a matrix gives rise to a phonon sideband (PSB) in the spectrum and causes changes in the ZPL frequency, width, and shape. The ZPL position and width are governed by the electron–phonon coupling described by the operator **A** which depends on the nuclear coordinates q_j of matrix atoms and can be expanded into the series

$$\mathbf{A} = \sum_j V_j q_j + \sum_{jj'} W_{jj'} q_j q_{j'} + \ldots , \tag{1}$$

where the first term describes the linear electron–phonon coupling responsible for the ratio between the integrated intensities of the ZPL and PSB and the shift of the spectral band, while the second term describes the quadratic electron–phonon coupling producing the temperature-dependent homogeneous broadening of the spectrum and its additional shift.

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*

$$\alpha_{\mathrm{DW}} = \frac{I_{\mathrm{ZPL}}}{I_{\mathrm{ZPL}} + I_{\mathrm{PSB}}} . \tag{2}$$

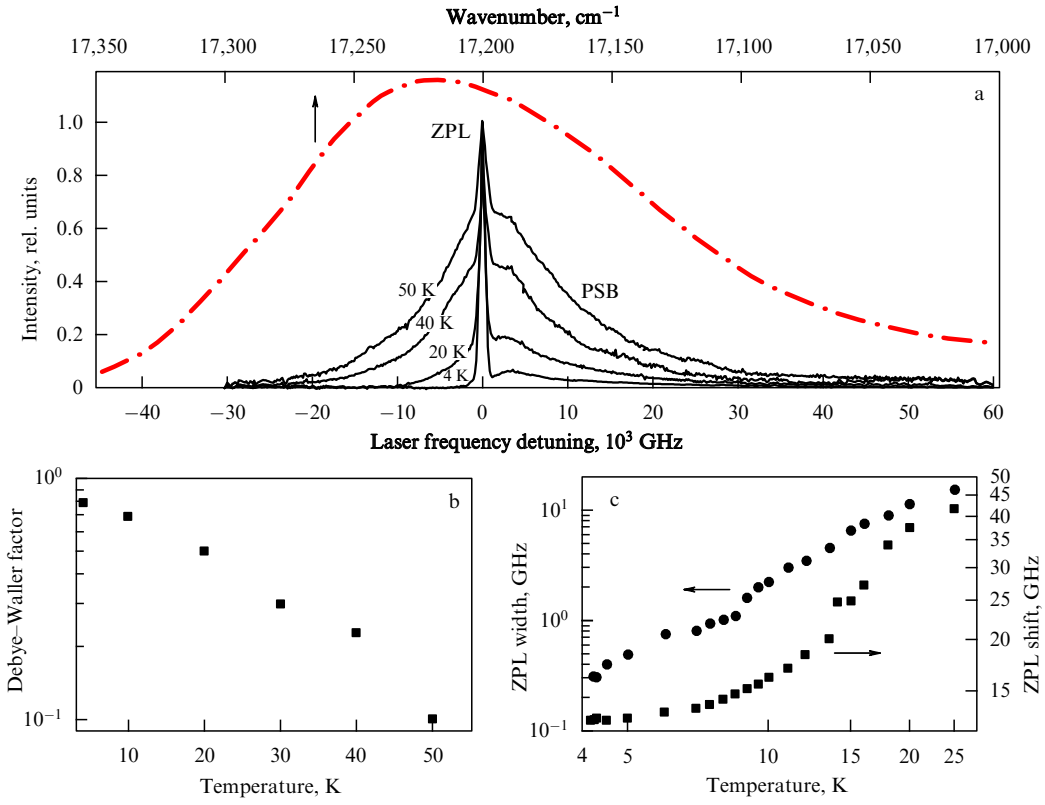

Figure 6. (a) Inhomogeneously broadened (dashed-dotted curve, 4 K) and homogeneously broadened (solid curves) narrow-line luminescence spectra of Mg-octaethylporphin in polystyrene recorded at 4, 20, 40, and 50 K. Initial data were taken from Ref. [84]. Notions are as follows: ZPL — zero-phonon line, and PSB — phonon sideband. (b) Temperature dependence of the Debye–Waller factor obtained by processing experimental data for Mg-octaethylporphin in polystyrene [84]. (c) Temperature dependences of the homogeneous ZPL width and shift for a single terrylene molecule in polyisobutylene are plotted for comparison [85].

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

$$\alpha_{\mathrm{DW}}(T) = \exp\left(-\int_0^{\infty} g(\nu)\left[2n(\nu)+1\right] \mathrm{d}\nu \right), \quad (3)$$

where $g(\nu)$ is the density of phonon states of the system, and $n(\nu)$ is the Bose factor:

$$n(\nu) = \frac{1}{\exp\left(h\nu/kT\right) - 1}. \quad (4)$$

One can see from formulas (3) and (4) that the ZPL intensity transfers to the PSB intensity with increasing temperature, while the integrated intensity of the vibronic band does not depend on temperature.

Most of the existing theories consider the interaction of impurity molecules with phonons and pseudolocal phonons or quasilocalized low-frequency vibrational modes only for highly ordered crystals. Approaches developed in the theories of electron–phonon interaction in impurity crystals are also applied for amorphous media, neglecting, however, the features of electron–phonon interaction in these media.

Thus, according to current concepts, the luminescence spectrum (vibronic band) of an impurity molecule in solid solutions comprises (a) a narrow ZPL corresponding to the purely electronic transition in the impurity without changing the number of phonons in the matrix, and (b) a relatively broad PSB appearing due to radiative transitions in the impurity with the creation and annihilation of matrix phonons (Fig. 6a).

In this case, the ratio of the width of the inhomogeneously broadened spectrum to the homogeneous spectral width of a monochromatic subensemble of impurity molecules in disordered solids can reach approximately $10^5 - 10^6$ at cryogenic temperatures (cf. the inhomogeneous width of the absorption profile with the homogeneous width of the ZPL in Fig. 6a, and with typical ZPL widths of single molecules in a polymer in Fig. 6c).

Intramolecular processes and interactions, as well as the features of the local structure and processes proceeding in the matrix, are most pronounced in changes to the ZPL parameters; in other words, in the *spectral dynamics* of impurity centers [16]. It is for this reason that Shpol'skii spectra (exhibiting, in fact, the ZPLs of impurity centers) have become a source of information on both the photophysical properties of impurity molecules and impurity solid media as a whole. 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, Refs [10, 25, 85] and references cited 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:

$$\Gamma_{\mathrm{ZPL}}(T) = \Gamma_0 + \Gamma_{\mathrm{TLS}}(T, t_{\mathrm{m}}) + \Gamma_{\mathrm{V}}(T), \quad (5)$$

where Γ_0 is the natural linewidth determined by the excited-state lifetime of a molecule, Γ_{TLS} is the ZPL broadening caused by the interaction of electronic transitions in impurity molecules with tunneling excitations in the matrix, and Γ_{V} is the ZPL broadening caused by the interaction of impurity molecules with vibrational excitations (electron–phonon interaction).

The interaction of impurity molecules with tunneling type excitations gives rise to spectral diffusion resulting in the ZPL broadening depending on the recording time t_{m} of the spectrum. In disordered media (glasses, polymers, polycrystals with many defects), the contribution from tunneling excitations at ultralow temperatures ($< 1-2$ K) becomes dominant. The temperature dependence of this contribution in the standard model of low-temperature glasses, which is based on tunneling two-level systems (TLSs) [86, 87] and the stochastic sudden jump model [88], is quasi-linear in character:

$$\Gamma_{\mathrm{TLS}}(T) \sim T^{\alpha}. \quad (6)$$

At higher temperatures, ZPL broadening is mainly determined by the electron–phonon interaction. I S Osad'ko showed (see book [44] and references cited therein) and D Hsu and J L Skinner [89–92] later confirmed that 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

$$\Gamma_{\mathrm{V}}(T) = \frac{1}{4\pi}\int_0^{\infty} \mathrm{d}\omega \ln\left\{1 + 4n(\omega)\left[n(\omega)+1\right] \times W^2 g_{(0)}(\omega)\, g_{(1)}(\omega)\right\}, \quad (7)$$

where W is the dimensionless constant of quadratic electron–phonon coupling, and $g_{(0)}(\omega)$ and $g_{(1)}(\omega)$ are dimensionless weighted densities of phonon states in the ground and excited electronic states of the impurity, respectively. Here, $g_{(1)}(\omega)$ and $f_0(\omega)$ are given by the expressions

$$g_{(1)}(\omega) = \frac{g_{(0)}(\omega)}{\left[1 - W f_0(\omega)\right]^2 + W^2 g_{(0)}^2(\omega)},$$

where

$$f_0(\omega) = \frac{2}{\pi}\int_0^{\infty} \mathrm{d}\nu\, g_{(0)}(\nu)\, P\left(\frac{\nu}{\omega^2 - \nu^2}\right),$$

and P stands for the principal value of the integral.

The coupling of the electronic transition in the impurity with localized or quasilocalized vibrational excitations is considered, as a rule, by describing the density of phonon states by narrow functions such as Lorentzians or similar functions.

Thus, the temperature dependences of ZPL parameters give information on the impurity–matrix coupling and allow one to measure the characteristics of low-energy excitations (distributions of TLS parameters, and the density of vibrational states). On the other hand, low (cryogenic)-temperature ZPL measurements are the most informative, because α_{DW} drastically decreases with increasing temperature, while the homogeneous ZPL width increases [see formulas (3)–(7) and Figs 6b, c].

6. Single-molecule spectroscopy

6.1 Basic principles and the history of development

Despite their high selectivity, the laser-driven FLN and HB techniques average data over a great number of impurity

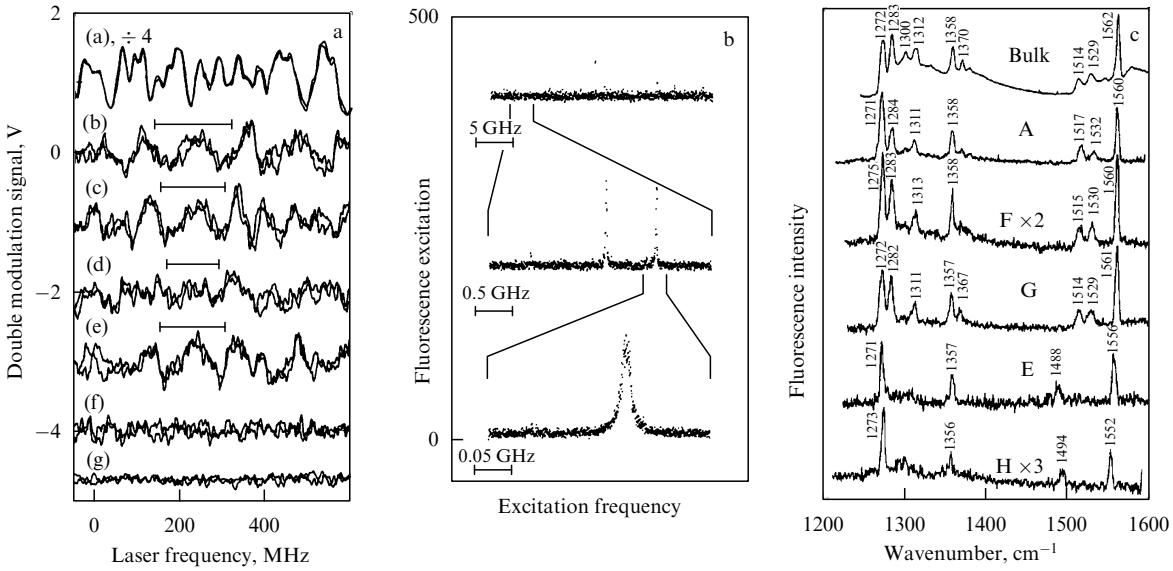

Figure 7. Absorption (a), fluorescence excitation (b), and fluorescence (c) spectra of single molecules in solid matrices (taken from pioneering papers [8, 9, 95] in single-molecule spectroscopy).

centers. In the case of disordered solid media, this averaging leads to a considerable loss of information about microscopic processes under study. This disadvantage is naturally removed in single-molecule spectrum detection.

The first optical studies of single quantum systems were performed in the late 1970s in a gas phase with rarefied atomic beams and single ions in electromagnetic traps [93]. The possibility of detecting emission from individual impurity molecules in condensed media appeared later. This is explained by at least two reasons: first, a surrounding matrix or a solvent is a powerful source of parasitic background emission complicating the detection of a weak signal from a single molecule, and second, the total number of photons that can be emitted by a molecule is usually restricted by photobleaching at room temperature or by spectral jumps at low temperatures (whereas the lifetime of a single ion is determined by its confinement time in a trap).

In 1976, T Hirshfeld reported the first experimental observation of fluorescence from ~ 100 biological macromolecules marked with fluorescein in a polymer matrix [94]. The advent of new highly sensitive photodetectors operating in the photon counting regime and the development of methods efficiently eliminating parasitic illuminations led to the detection of individual impurity molecules. In 1989, Moerner and Kador [8] observed for the first time the absorption spectra of single chromophores in a solid matrix (Fig. 7a), while in 1990 Orrit and Bernard observed fluorescence excitation spectra of individual molecules [9] (Fig. 7b).

This avenue of inquiry was extensively developed over the following twenty years and became one of the most topical in physics, physical chemistry, biophysics, and related sciences. On the one hand, interest in this technique is explained by the fact that it allows the study of the physical properties of a substance at the minimal possible level of individual molecules. On the other hand, as discussed above, the optical spectra of impurity chromophores (atoms and molecules) embedded into a transparent matrix under study as nanoprobes contain exclusively rich information on the parameters of the nearest environment.

Single molecules in a solid matrix were observed for the first time in paper [8] by recording a weak absorption signal (Fig. 7a). Because the absorption cross section of a single molecule is at least two–three orders of magnitude lower than the cross section of a focused laser beam, the absorption signal is very small compared to the noise level. This significantly complicates the application of absorption spectroscopy for the detection of the single-molecule spectrum, even invoking special techniques such as Stark frequency modulation.

Soon it was shown in work [9] that the detection of single-molecule spectrum using the laser fluorescence excitation drastically increases the signal-to-noise ratio compared to that in the case of absorption spectroscopy (cf. Figs 7a and 7b). This is explained by the fact that detecting signals against a low-intensity background is much simpler than distinguishing weak variations in the absorption coefficient against a high-intensity background. In the former case, the integrated signal of a Stokes component of fluorescence is detected by tuning a single-frequency laser. The dependence of this signal on the laser frequency coincides, in fact, with the single-molecule absorption spectrum, providing, however, unlike the latter, a high signal-to-noise ratio. Nevertheless, the fluorescence excitation technique also has its own disadvantages. First, the study of time-resolved spectra in a broad spectral range is complicated, because even modern single-frequency tunable lasers cannot be rapidly tuned in a broad spectral range. Second, it was found that only a small number of impurity–matrix systems exhibit narrow ZPLs even at cryogenic temperatures, whereas the observation of ZPLs in complex organic solids at room temperature is impossible, in principle.

Nevertheless, it is the fluorescence excitation spectroscopy technique that has become the most popular for studying the dynamics of complex solids by the spectra of impurity chromophore molecules.

Fluorescence spectra of single chromophore molecules in solid matrices were first recorded in 1994 [95] for pentacene molecules in p-terphenyl and terrylene molecules in polyethylene at cryogenic temperatures. Fluorescence spectra are

excited at a laser frequency resonant with the 0–0 electronic transition frequency of a molecule under study. Then, the vibrational structure of the spectrum is recorded with a high-resolution spectrometer. This technique provides the recording of fine structure in vibronic spectra of impurity molecules (Fig. 7c), thereby providing direct information on the vibrational energy levels of a single impurity center and the influence of the local environment of the matrix on their characteristics. The role of resonance Raman scattering in the formation of such spectra is still being debated. Fluorescence is usually treated as a process in which the absorption and emission of photons are independent and separated from each other, whereas resonance Raman scattering is a two-photon process in which photon emission and absorption processes cannot be principally separated. Thus, the contributions can be distinguished applying techniques in which emitted photons are detected with a time delay with respect to exciting light pulses. An important advantage of the measuring technique for single-molecule emission spectra is the possibility of the simultaneous recording of the total vibronic spectrum. However, for achieving the adequate signal-to-noise ratio in this case, a long enough signal accumulation time is required, which impairs the time resolution of this technique.

Another line of SMS inquiry related to the study of fluctuations (blinking) of fluorescence and the statistics of photons emitted by individual molecules has recently become increasingly topical [96–98]. The point is that fluctuations (intermittence) of fluorescence are inherent in almost all quantum emitters (impurity molecules in solid and amorphous matrices, quantum dots, emitting sites in individual molecules of conjugated polymers, light-harvesting biological complexes, fluorescing proteins, etc.). Blinking fluorescence is also observed during the low-temperature recording of narrow ZPLs from single organic molecules embedded in solid matrices ([99] and references cited therein).

The individual properties of various quantum objects are also manifested in the statistics of emitted photons and of fluorescence blinking, as well as in their spectra [100–102]. However, at high (room) temperatures, which are most important for practical applications of the methods developed, the spectral selection of different molecules is significantly complicated. Thus, the analysis of statistical quantum properties of single-molecule emission can also give additional information about both the impurity molecule and its nearest environment, in particular, in cases when this information cannot be obtained from the analysis of molecular spectra.

6.2 Manifestation of the matrix dynamics in single-molecule spectra

The spectroscopy of single molecules allows one to detect the individual spectra of such molecules and to study the nature of the homogeneous broadening and time evolution of the spectrum of a single impurity center, thereby obtaining unique data on the microscopic properties of a solid matrix at the level of a separate impurity center and its nearest environment. Notably, the matrix dynamics, which are manifested for an ensemble of impurity molecules as spectral broadening (due to optical dephasing and spectral diffusion), in the case of individual spectra of single impurity molecules are manifested as frequency jumps, drifts, the broadening and shift of spectral lines.

The first SMS experiments [103] have already shown that the spectral parameters of individual molecules (the ZPL frequency, width and intensity, the characteristic fluorescence blinking time, and temporal behavior) in impurity solids have broad distributions (see paper [104] and references cited therein). It turned out that the parameters of these distributions depend on the spectrum measurement time. The increase in measurement accuracy has revealed a theoretically predicted fact that the emission frequency of an impurity molecule changes with time, which is manifested as spectral jumps of emission lines from single molecules. Thus, SMS experiments with amorphous polymers gave the first direct proof of the existence of low-energy elementary tunneling type excitations in disordered media — tunneling two-level systems (TLSs) [86, 87], which cause jumps of the spectral lines of impurity molecules.

According to modern concepts, a transition in a TLS interacting with a chromophore molecule leads to the frequency shift of the electronic transition in an impurity molecule and, hence, to a jump of the spectral line of this chromophore. Each impurity center interacts with numerous TLSs. Therefore, the frequency of a single molecule interacting with N independent TLSs will experience jumps between 2^N positions. Such jumps resulting in spectral diffusion, which has been investigated in detail by conventional methods of hole burning and photon echo with molecular ensembles, can also be observed in the spectra of single molecules. Thus, dynamic processes in glasses caused by transitions in TLSs will be manifested in the time variations in the spectra of single impurity molecules. The study of such variations can help to understand the microscopic nature of TLSs, which still remains a subject of discussion.

The above-discussed peculiarities of single-molecule spectra in low-temperature glasses point to appearance of certain methodological problems in the arrangement of the experiment on the recording of time-varying single-molecule spectra and in the development of a means for their representation. Moreover, the problem arises concerning he identification of complex in form and differing among each other individual spectra of various impurity centers.

One of the most informative and convenient methods for recording single-molecule spectra varying in time is the repeated many times recording of fluorescence excitation spectra in a chosen spectral range [105]. Such spectra can be conveniently represented by a two-dimensional image (2D plot) in which the marking intensity (shades of grey) or the color gamut of each spectral point put in correspondence with the intensity of the fluorescence excitation spectrum at a given frequency [106] (Fig. 8).

The tunable laser frequency is plotted on the horizontal axis of the 2D plot, and the scan number (or the time elapsed after the measurement onset) is plotted on the vertical axis. This two-dimensional picture gives a peculiar temporal topogram of measured single-molecule spectra. In some cases, the 3D plot, a three-dimensional analog of the two-dimensional representation, is also used. The analysis of the 2D plot allows one to uniquely assign spectral peaks to different single molecules [107].

A transition in one of the TLSs interacting strongly enough with a given molecule is manifested as simultaneous and identical jumps in all spectral peaks belonging to the spectrum of this individual molecule. At the same time, this transition either is not practically manifested in the spectra of other molecules because they are located, as a rule, far enough

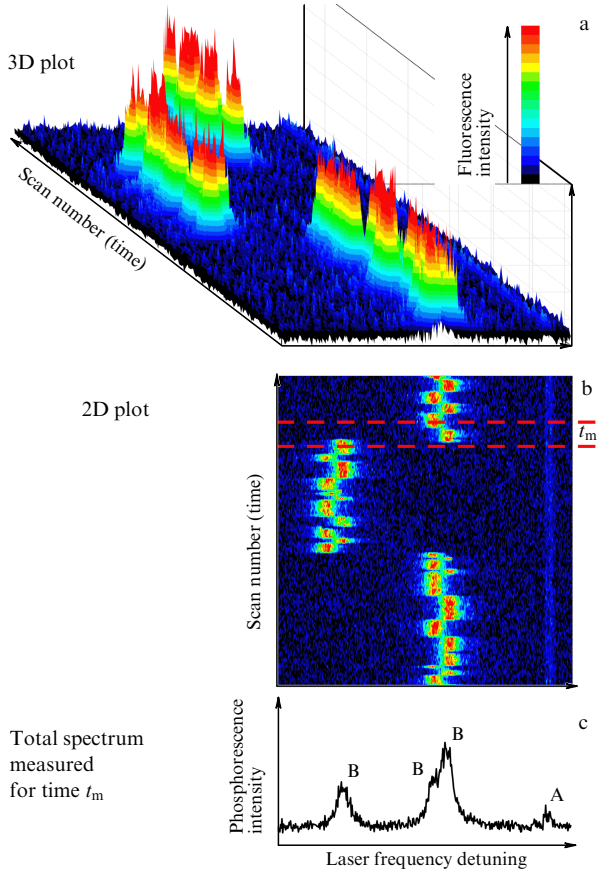

Figure 8. Time evolution of spectra of two single molecules (tetra-tert-butylterrilene) embedded in polyisobutylene and repeatedly recorded many times at $T = 7$ K (a, b). The results of measurements are presented in the form of three-dimensional (a) and two-dimensional (b) plots. The color gamut (shades of grey) of points in Figs 8a and 8b put in correspondence to the fluorescence intensity. The resulting integrated spectrum (c) is the sum of all spectra taken in some selected time interval $t_m = 120$ s, shown in Fig. 8b by solid horizontal straight lines. The total spectrum in Fig. 8c corresponds to two molecules: molecule A (one peak), and molecule B (three peaks).

from this TLS, or is manifested as shifts that differ from that mentioned above. Therefore, a spectral trail (also called spectral 'trajectory'), corresponding to the temporal evolution of the individual spectrum of the given molecule, can be distinguished in the 2D plot.

Thus, the 2D plot in Fig. 8b enables distinguishing the spectral trajectories of two molecules with different temporal evolutions of their spectra. Indeed, the spectrum of molecule A does not experience noticeable jumps during observation. The spectrum of molecule B exhibits jumps among four spectral positions (interaction with two close active TLSs). The resulting total spectrum in Fig. 8c is the sum of all spectra in the selected time interval $t_m = 120$ s, shown in Fig. 8b by solid horizontal straight lines. The total spectrum in Fig. 8c can be assigned to molecules A (one peak) and B (three peaks).

The recording of fluorescence excitation spectra has become a powerful tool for studying the low-temperature dynamics of disordered solid media. To date, the following studies have been performed:

(i) The technique of measuring spectra of the same molecule in a broad low-temperature range from about 1.5–2 K to a few dozen of kelvins has been developed (Fig. 6c). On its base (by analyzing the temperature dependences of parameters of single-molecule spectra), the local/individual parameters of low-frequency vibrational modes and tunneling TLSs have been measured in the vicinity of an impurity molecule under study [106, 108–112].

(ii) The zero-phonon lines of impurity chromophore molecules in solid matrices have been measured at ultralow temperatures (down to 30 mK), where the ZPL broadening caused by the coupling between the impurity and elementary excitations of the matrix is negligibly small and the natural linewidth can be directly measured. The dispersion of excited-state lifetimes in a polymer matrix has been revealed [113, 114].

(iii) The method has been proposed and devised for the correct quantitative description of spectral single-molecule lines of a complex shape, based on the concept of moments/cumulants of the distribution. It has been shown theoretically and confirmed experimentally [115–117] that parameters of single-molecule spectra in a polymer system at 2 K are governed by the Levi statistics.

(iv) Single-molecule spectra have been measured in molecular glasses — frozen glass-forming liquids. It has been found that the single-molecule spectral dynamics in low-molecular glasses and oligomers in the temperature range from 2 to 10 K and above is not consistent with conventional concepts about the internal dynamics of disordered solids and cannot be described by existing theories (the standard model of tunneling TLSs, the low-frequency mode model, etc.) [60, 118, 119]. This means that the features of the internal dynamics on the nanometer scale are directly related to the particular structure of a disordered solid.

(v) The technique has been developed for measuring the density of vibrational states in impurity disordered systems (polymers and glasses) on the basis of single-molecule spectra. It has been shown that the doping of amorphous solid matrices with chromophore molecules having a close chemical composition does not considerably change the low-temperature vibrational dynamics of the object. A direct linkage is demonstrated between quasilocal low-frequency vibrational modes and excitations determining the shape of a boson peak [120, 121].

(vi) Complex studies of the influence of external fields (electric, magnetic, external hydrostatic pressure) on ZPL parameters have been performed [122–126].

(vii) The nature of single-molecule fluorescence blinking has been studied in different conditions (see Ref. [127], and Refs [97, 99] and references cited therein).

At present, SMS is widely used in various fields of science and technology (see reviews and monographs [40–49]). The developed methods and approaches allow efficient application of single-molecule spectroscopy in fundamental studies and in a number of applications in physics, biophysics, nanotechnologies, and materials technology.

A principal task in the development of this line of inquiry is the search for fundamental regularities linking the unique microscopic single-molecule information with the macroscopic characteristics of bulk samples. The way to solve this problem involves the statistical analysis of spectral data obtained for many single molecules. Thus, a series of studies performed in 2000–2010 at the Institute of Spectroscopy, RAS in collaboration with the University of Bayreuth (Germany), have demonstrated that the involvement of original methods for statistical processing of individual spectral characteristics of single impurity centers can shed

light on the fundamental properties of low-temperature dynamic processes proceeding in disordered organic solids (see review [47] and references cited therein). This work was initiated by Yu G Vainer at the Institute of Spectroscopy, RAS, who proposed using SMS to study the dynamics of disordered media, applying the methods of statistical analysis [115, 128–130]. It is most important that such investigations preserve the unique information on the local dynamics of a matrix in the vicinity of impurity centers, down to single elementary excitations determining the dynamic characteristics of the material under study.

6.3 Single-molecule luminescence microscopy

Single-molecule spectroscopy acquires radically new possibilities upon recording the spectra of a great (statistically confident) number of emitting centers in a macroscopic volume of a sample. This method can be realized by combining SMS and far-field luminescence microscopy with narrowband tunable laser sources (Fig. 9a).

The first experiments in which fluorescence excitation spectra of single molecules in solid matrices were recorded at cryogenic temperatures using a luminescence microscope and a multichannel 2D detector were performed in the middle of the 1990s (see, for example, papers [51, 52]). These experiments demonstrated the unique capabilities of single-molecule spectromicroscopy for studying processes in solid media. In particular, the simultaneous recording of the emission spectra of many single molecules in the total field of a luminescence microscope allows one to synchronously control dynamic processes proceeding at different places of a sample, the possibility of achieving a high statistical confidence of the measurement results opens up, and the coordinates of single molecules are detected simultaneously with their spectra.

The last circumstance is all the more important because the accuracy of determining coordinates of single molecules (which constitute, in fact, point-like light sources that are considerably smaller than the wavelength of emitted electromagnetic waves) is not restricted by the diffraction limit but only depends on the stability of an experimental setup and the signal-to-noise ratio for the spectrum recorded [54, 55]. As was shown in the first papers [53], the accuracy of measuring transverse coordinates for most bright molecules can reach 4 nm, and that for the longitudinal coordinate can reach 100 nm. The latest study [56] shows that the accuracy of determining coordinates of an emitting center can reach 0.9 nm.

To achieve the subdiffraction accuracy of determining the coordinates of a radiator, it is necessary to take into account the instrumental function of a point radiation source (point spread function) [131].

In the first approximation, we can consider an individual fluorophore as a point emitter with the ideal radiation pattern in a total solid angle of 4π steradian. In this case, a fluorescent image is an Airy disc (Fig. 10a), and transverse coordinates are determined either by calculating (in the simplest case) 'the center of gravity' of the molecule image (Fig. 10b1) or by approximating the registered radiation intensity distribution within the fluorescent image of a single molecule by the two-dimensional Gaussian

$$f(x,y) = f_0 + \frac{A}{\Gamma}\exp\left(-\frac{(x-x_c)^2}{\Gamma^2}\right)\exp\left(-\frac{(y-y_c)^2}{\Gamma^2}\right), \quad (8)$$

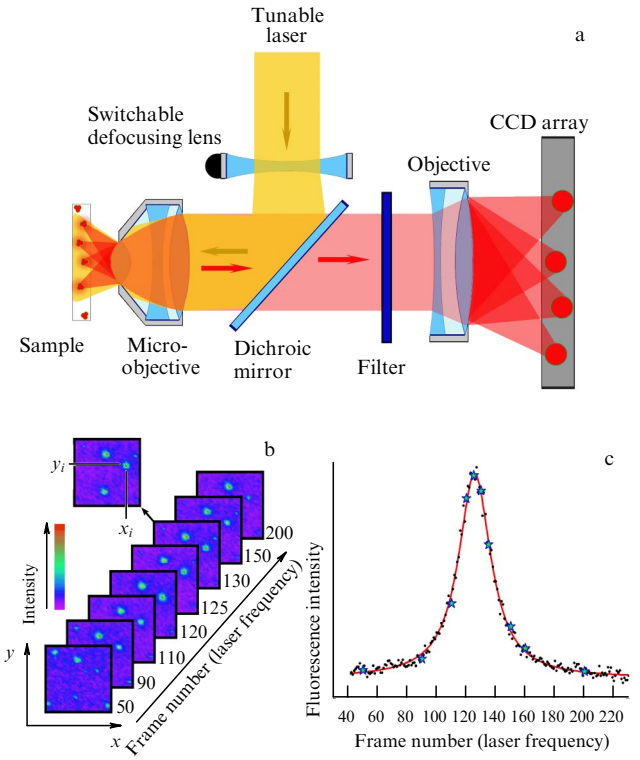

Figure 9. (a) Schematic of the experiment. (b, c) Illustration of the procedure of single-molecule imaging, recording, and recognition of a single-molecule spectrum from a sequence of video frames. (b) Identification of luminescent images of the same single molecule upon laser frequency tuning. (c) Fluorescence excitation spectrum of the selected ith molecule with coordinates (x_i, y_i) (points) and its approximation by a Lorentzian (solid curve). Asterisks correspond to the video frames shown in Fig. 9b.

where f_0 is a noise pedestal including the detector noise, parasitic illuminations, nonresonantly excited fluorescence, etc.; A is the signal amplitude; Γ is the width of the point spread function, and x_c and y_c are the desired coordinates of the center of the point source image (Fig. 10b2).

A more accurate analysis requires accounting for the radiation pattern of an emitter with a nonzero electronic transition moment (Fig. 10c) [131, 132]. Finally, fluorescent images should be analyzed taking into account the change in the radiation pattern of the emitting dipole near the interface of media with different refractive indices [131].

Modern methods of the 3D visualization of fluorescent images (for example, the 'double helix point spread function' technique [57, 58]) can provide the required modification of the point spread function and allow one to measure all three spatial coordinates of an emitter in a sample with a nanometer accuracy, i.e., to completely reconstruct the map of locations of impurity centers in a matrix.

The development of efficient algorithms and software packages for the automatic recognition and identification of images of emitting centers with different instrumental functions plays a key role in experiments (and subsequent data processing) on the optical reconstruction of optical images of single quantum objects. The open source rapidSTORM software package, which is supported at the University of Würzburg (Germany) [133, 134], can be recommended for practical applications.

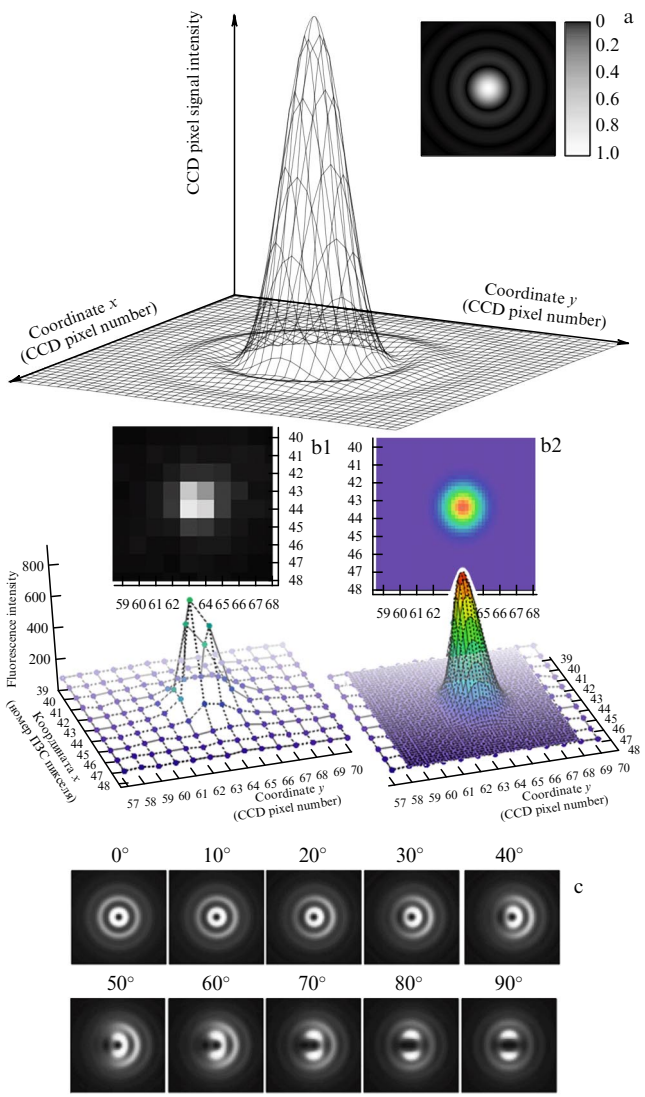

Figure 10. (a) Instrumental function of an ideal point light source (surface) and its appropriate Airy disc (topogram in the shades of grey) [taken with changes from a paper at http://en.wikipedia.org/wiki/airy_disk]. (b1) Typical fluorescent image of a single tetra-tert-butylterrylene molecule in amorphous polyisobutylene observed upon resonance excitation at the ZPL frequency in the 575-nm region at 4.3 K. The relative transverse coordinates of the molecule can be calculated by finding the 'center of gravity' $x_c = 22.3$ μm, $y_c = 32.3$ μm of the image. (b2) Determination of the transverse coordinates of the molecule by approximating the fluorescent image by two-dimensional Gaussian (8) with $x_c = 22.24 \pm 0.04$ μm; $y_c = 32.35 \pm 0.03$ μm. (c) Point spread function in the emitting dipole approximation: fluorescent images of a single emitting center with the dipole transition moment calculated in paper [132] depending on the direction of the dipole moment with respect to the optical axis of a luminescence microscope.

The efficient employment of the synchronous recording of both the coordinates and fluorescence excitation spectra for a great number of single molecules with a luminescence microscope was complicated over many years for several reasons: the low sensitivity of detector arrays, difficulties with the accumulation and fast processing of giant data arrays, and the absence of optical algorithms for the fast automatic recognition of fluorescent images of quantum objects. The development of computer technologies and new high-sensitivity detector arrays initiated the practical realization and systematic application of this technique.

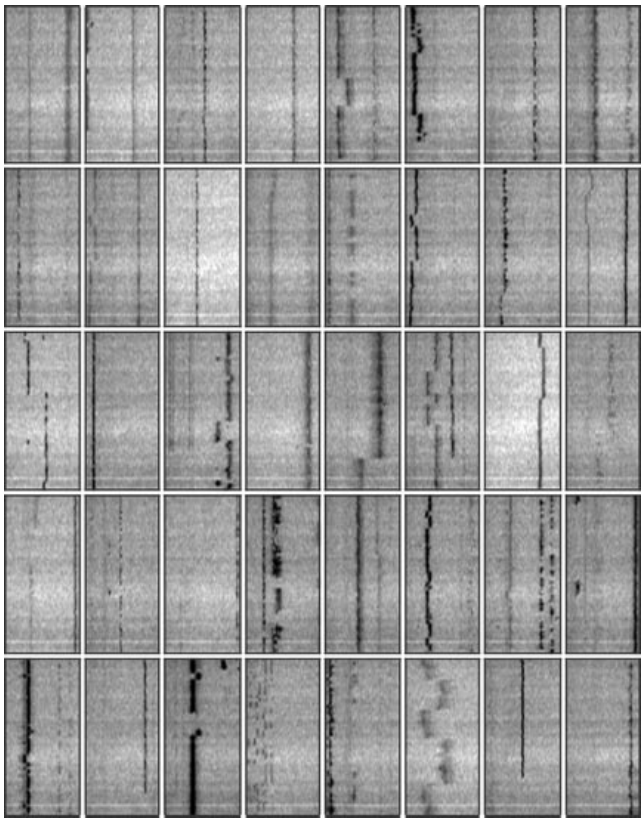

Figure 11. Spectral trails of numerous single molecules recorded using a new technique (tetra-tert-butylterrylene molecules in polyisobutylene, $T = 4.5$ K, the laser tuning range is 30 GHz, the number of points in the scan reaches 1000, the time per point is 40 ms, and the number of scans equals 100). Each panel corresponds to a certain (diffraction-limited) region of the sample in the view field of a luminescence microscope. In some cases, several clearly distinguishable spectra trails with different time dynamics can be observed on one panel, which corresponds to the situation when a selected diffraction-limited volume contains several luminescing impurity molecules with their ZPLs having close frequencies.

6.4 Sequential–parallel recording of the spatial coordinates, spectra, and spectral histories of single molecules in solid matrices

Starting in 2004, our team at the Institute for Spectroscopy, RAS have been developing original algorithms and computer software for the rapid automatic recognition and analysis of fluorescent images, spectra, and spectral histories of the huge number of individual quantum objects detected with a laser luminescence microscope [135]. The use of low-noise electron-multiplying CCD cameras and high-performance modern computers, along with the possibility of the rapid recording and processing of mass data applying original algorithms and software provided the synchronous recording of spectra and spectral histories of individual molecules. The automatic recognition of the spectral trajectories of different single molecules (Fig. 11) is performed here by a comparative analysis of coordinates of single-molecule images on a CCD array and of the complete set of their spectral parameters (the ZPL peak position, linewidth, etc.). Thus, the developed technique provided the synchronous monitoring of dynamic processes at different points of a solid sample with retention of information on the spatial arrangement of molecular probes. In this case, the accuracy of determining single-molecule coordinates can approach a few nanometers. This allows one to find the relation between the local dynamic

properties of an impurity solid system under study and its structure and macroscopic characteristics.

It is important that this technique eliminates the necessity of a spatial search for single molecules, and it is necessary in experiments to perform only the quest of single molecules by the position of their spectra in the inhomogeneous spectral contour. This advantage acquires a fundamental importance when recording single-molecule spectra in impurity media with nonreproducible spectral dynamics. Indeed, with the single-channel detection system utilized in a confocal microscope, the spectral position of a given single molecule can irreversibly change during the time interval required for a spatial search for this molecule, which will make the recording of its spectrum impossible. This advantage was conclusively demonstrated already in the first experiments utilizing this technique: single-molecule spectra were recorded for the first time in low-molecular-weight glasses and oligomers [60, 118]. It was shown that, unlike high-molecular-weight polymers, the spectral single-molecule dynamics in such media are nonreproducible and random. Most likely that this circumstance prevented the recording of single-molecule spectra in these media using a confocal microscope with a single-channel detector.

6.5 Spectromicroscopy of all efficiently emitting single molecules in a macroscopic sample volume

Thus, the modern experimental base makes it possible to perform qualitatively new spectral measurements of impurity centers, namely, the recording of individual spectra (ZPLs) and spatial coordinates of all efficiently emitting centers in the macroscopic volume of a solid sample doped with fluorescing molecules at low concentrations.

This raises the important question of the relation between the distributions of local spectral parameters observed in SMS experiments and photophysical characteristics of a bulk macroscopic sample, in particular, measured by the FLN and HB techniques. This problem can be solved by the statistical processing of data files containing information about the individual spectral parameters of all efficiently emitting single molecules in the macroscopic volume of an impurity sample under study. Fluorescence excitation spectra in such measurements (see details in papers [59], 136]) are recorded by tuning the laser frequency within the inhomogeneous absorption band (a few hundred cm^{-1} in width) of an impurity sample. The total tuning range is divided into 'segments' with the selected interval $\Delta\tilde{\nu}$ (several GHz). During the tuning of the exciting laser frequency within the segment interval $\Delta\tilde{\nu}$, a sequence of video frames is recorded (up to a few thousand frames, depending on the conditions). The fluorescent images of single molecules are recorded by acquiring data from a high-sensitive CCD array. The individual fluorescence signals from all efficiently emitting single molecules located in the observed area of the sample with ZPL frequencies falling within the laser tuning range are simultaneously detected in each spectral segment.

All the recognized single-molecule spectra are stored in PC ROM together with the parameters obtained by approximating a ZPL by a Lorentzian: $I_{\min}$ is the background intensity, ω_0 is the frequency of the ZPL maximum, γ is the ZPL full width at half-maximum, A_{fluo} is the integrated single-molecule fluorescence signal minus the noise pedestal, and $I_{\max}$ is the peak single-molecule fluorescence intensity (fluorescence intensity at the signal maximum). For each segment of width $\Delta\tilde{\nu}$, the number N_{SM} of single molecules recognized within the segment can also be easily determined. The data obtained can be utilized to construct different 'artificial' distributions (dependences), for example (see details in papers [59, 136, 137]:

(i) The dependence $N_{SM}(\tilde{\nu})$ giving the number of single molecules detected in a spectral segment beginning from the frequency $\tilde{\nu}$. $N_{SM}(\tilde{\nu})$ shows the distribution of ZPL frequencies for individual impurity molecules within the inhomogeneous absorption band. Physically, $N_{SM}(\tilde{\nu})$ is related to the inhomogeneously broadened absorption (fluorescence excitation) spectrum of a macroscopic volume of a sample measured by classical spectroscopic techniques, but is not identical to it. The difference consists in the fact that the widths, intensities, and even shapes of individual single-molecule spectra can be quite different. In addition, the inhomogeneous absorption spectrum contains a contribution from nonresonantly excited centers, various parasitic contributions, etc. Thus, $N_{SM}(\tilde{\nu})$ is, in fact, *the spectral density of single molecules*.

(ii) $A_{\text{sum}}(\tilde{\nu})$ stands for the dependence of the sum of integrated ZPL intensities (areas) for all the recognized single molecules in the given scanned segment $\Delta\tilde{\nu}$ on the spectral position of the segment. The identification of single-molecule fluorescent images involves the separation of signals from narrow intense ZPLs exceeding the hum noise level. Thus, $A_{\text{sum}}(\tilde{\nu})$ is related to the fluorescence excitation spectrum of the macroscopic volume of a sample (but does not coincide with it) because $A_{\text{sum}}(\tilde{\nu})$ does not contain signals from molecules excited nonresonantly through PSBs. Consequently, $A_{\text{sum}}(\tilde{\nu})$ can be called the '*phononless absorption band*'.

Studies have shown that the spectral density of single molecules and the phononless absorption band are profoundly more informative than the conventional high-resolution fluorescence excitation spectrum. They exhibit a more distinct structure which is not smeared out by fluorescence signals from nonresonantly excited single molecules, thus allowing the study of a fine (for example, subsite) structure in the spectra of complex organic compounds.

The recording of a complete databank containing information on all individual emitting centers in a bulk sample opens up qualitatively new possibilities for the *postexperimental formulation* of and solution to a broad scope of unique spectroscopic, analytic spectral, and applied problems, such as:

(i) Studies of the photophysical properties of single emitting quantum objects (enhancement, quenching, blinking, interaction with each other and the local surrounding). In particular, the most important one is the question of which part of the total number of the impurity molecules emits efficiently enough for their spectra (images) to be sufficiently intense (bright) for recognition at the single-emitter level. It is for this reason that the title of this review contains the term '*all efficiently emitting single molecules*'.

(ii) The search for and investigation of rare events (for example, tunneling three-level systems in disordered solid media).

(iii) The detection and analysis of anomalies and artifacts [for example, parasitic chemical inclusions (contaminations) at the single-molecule level].

(iv) The construction of artificial distributions of spectral parameters having a physical meaning (for example, the density of vibrational states in solids [47, 120, 121]).

(v) The comparison of distributions of spectral parameters for a great number of single molecules with data

obtained by classical experimental techniques averaged over molecular ensembles (FLN, HB, neutron scattering, and thermodynamic techniques [47, 120, 121]).

(vi) The search for various correlations between characteristics measured in experiments (for example, spectral–spatial correlations [59, 136]). In particular, investigations of the influence of quantum-size and surface effects on single-emitter spectra seem quite promising.

(vii) The spectral–spatial nanodiagnostics (nanotomography) of thin films (for example, the far-field optical visualization of defects and cracks in polycrystals with characteristic sizes starting from several nanometers [136]).

(viii) The detection of dielectric, semiconductor, and metal micro- and nanoparticles, and the inspection of surfaces (for example, the visualization of defects and contaminations, the detection and recognition of viruses).

Therefore, it has become possible at present to employ the spectroscopy of impurity centers to analyze at the microscopic level (single-molecule impurity center) the process and understand the formation nature of inhomogeneously broadened absorption and luminescence bands averaged over a giant ensemble of chromophores, thereby relating local spectral and dynamic characteristics to the structure of a material and its macroscopic photophysical properties [59, 136].

7. Nanodiagnostics of solid media by the spectroscopy and fluorescent imaging of giant ensembles of single impurity molecules

Optical microscopy is one of the oldest experimental methods retaining its importance in many scientific fields. Beginning from Abbe's studies in 1873 [138], it has become clear that the spatial resolution of a microscope based on focusing optics has its limit, which is governed by diffraction and equals approximately 1/2 of the wavelength of light.

The development of high-resolution techniques based on fluorescence microscopy has led to the overcoming of the classical diffraction limit in far-field optical microscopy [55, 139]. Some of these techniques are based on single-molecule spectroscopy.

The concept of these techniques is as follows. As discussed above (Section 6.3), luminescence photons from single molecules form the image of a point-like light source. The analysis of the single-molecule image taking the point spread function into account allows one to determine the position (coordinates) of single molecules with an accuracy restricted only by the fluorescence signal-to-noise ratio and the stability of an experimental setup, and can considerably exceed the diffraction limit.

At the same time, the diagnostics of extended objects require, as a rule, the detection of a great number of single molecules and their high spatial density. In this case, the simultaneous emission of thousands of molecules prevents the images of single point sources from being distinguished, and therefore the identification and determination of the exact positions of single molecules become impossible. This difficulty can be eliminated by distinguishing molecules located within the common diffraction limit by invoking some additional feature or property. This idea can be realized by several methods (see the special issue of the international journal *Nature Photonics* [139] and references cited therein). For example, stochastic optical reconstruction microscopy (STORM) [54] based on the blinking fluorescence

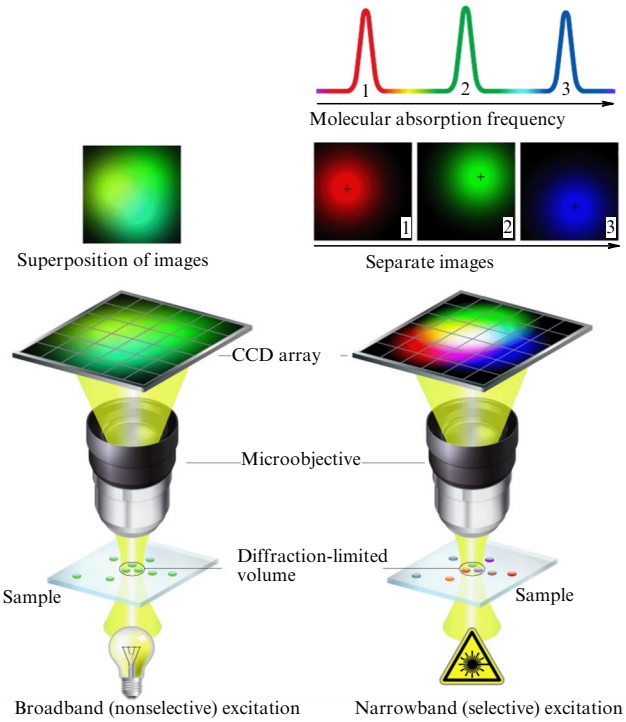

Figure 12. Schematic of the experimental approach to the diagnostics of solid films by recording narrow ZPLs and the corresponding fluorescence images of impurity single molecules. On the left: upon broadband (nonselective) excitation, all the molecules are simultaneously excited and emit indistinguishable signals, resulting in a diffraction-limited image. On the right: selective excitation can be performed by tuning the wavelength of a narrowband single-mode laser to the resonance with individual molecules. A macroscopic image is recorded by parallel data acquisition from a CCD array. In this case, no more than one molecule is resonant with the laser in any diffraction-limited volume at each instant of time [136].

of individual quantum objects has recently become more and more popular and was already realized in commercial microscopes. Random interruptions of single-molecule fluorescence occur at different time moments. Thus, if the concentration of impurity molecules is low enough, only one molecule within the diffraction-limited volume of a sample luminesces at each instant of time, thus allowing one to detect its image and, as a consequence, coordinates.

We distinguished such properties of single molecules in our experiments [59, 136] as their individual spectral characteristics, namely, the ZPL position, width, and intensity (Fig. 12). The number of molecules whose images can be separated within the diffraction-limited volume of a sample is determined by the ratio of the inhomogeneous width of the absorption band of the impurity medium to the homogeneous ZPL width. This ratio can reach approximately $10^5 - 10^6$ at cryogenic temperatures.

Similarly to the STORM abbreviation, we may formulate the basic principle of this technique as 'phononless optical reconstruction single-molecule spectromicroscopy (PLORSM)'.

The PLORSM technique naturally eliminates two principal problems of the already developed techniques of far-field optical nanodiagnostics by using the detection of fluorescent images of single emitting centers:

(i) deliberate (rather than stochastic) excitation of single-molecule emission;

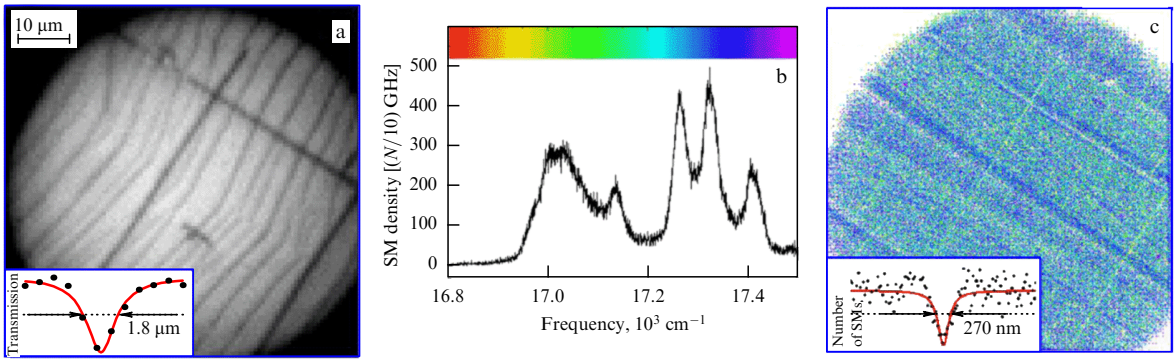

Figure 13. (a) White-light photograph of a sample. The inset shows the procedure for measuring the width of cracks from the transmission coefficient of a sample. (b) Spectral density of single impurity molecules — the ZPL frequency distribution within the inhomogeneous absorption band. (c) Color (grey level) images of 286,931 single molecules [each single molecule (SM) is shown by a dot with color corresponding to the position of a single molecule within the inhomogeneous absorption band (see color palette in Fig. 13b)]. The inset shows the crack profile obtained by analyzing the spatial distribution of single-molecule images (taken with changes from Ref. [136]).

(ii) the achievement of unprecedentedly high spatial density of molecules being recognized within the diffraction-limited volume of a sample (up to 10^5-10^6).

In point of fact, the new technique makes possible the detection of all efficiently emitting fluorescing molecules in the field of view of the microscope by preserving complete information on the individual spectral parameters and coordinates of each molecule. All this allows us to determine the relation between the local spectral properties of objects under study and their structure, as well as macroscopic characteristics of the material.

The potential of this technique was tested in studies [59, 136] of a number of impurity solid media, in particular, a Shpol'skii n-hexadecane matrix, and an ortho-dichlorobenzene molecular crystal doped with terrylene molecules (see a microphotograph of a sample in white light in Fig. 13a). The microphotograph in transmitted light clearly demonstrates the polycrystalline structure of the sample. One can see that an analysis of the transmission curve in the vicinity of a crack cannot give its width with an acceptable accuracy (Fig. 13b).

Experiments [59, 136, 137] have shown that this impurity molecular crystal at cryogenic temperatures (1.5 K) exhibits unique photophysical and spectral properties: the Debye–Waller factor close to unity, the high photostability of impurity molecules, the absence of noticeable spectral diffusion, the absence of fluorescence blinking in the observation time range from ms to minutes, a considerable inhomogeneous broadening ($\gamma_{\text{inhom}} \sim 10^3$ cm^{-1}), and a small homogeneous ZPL width (ZPL widths γ_{hom} are distributed in the range from 30 to 300 MHz ($\sim 10^{-2}-10^{-1}$ cm^{-1}), the maximum of the ZPL width distribution being at ~ 62 MHz ($\sim 2\times10^{-2}$ cm^{-1}). Thus, the ratio $\gamma_{\text{inhom}}/\gamma_{\text{hom}}$ reaches approximately 10^4-10^5, which allowed us to perform the sequential–parallel detection of fluorescent images and spectra of a huge number of individual emitting centers within the diffraction-limited volume of a sample using additional selection by the ZPL frequency. Because the tuning of an exciting narrowband laser in a broad spectral range and recording of the entire volume of spectral information requires a certain time, also of great importance are the acceptable photostability of molecules and the absence of the spectral dynamics.

Thus, fluorescence excitation spectra of all (286,931) efficiently emitting single molecules were recorded in the macroscopic volume (the region of a ~ 1 μm thick film ~ 70 μm in diameter) for the time of ~ 4 h by preserving complete information on individual spectral ZPL parameters and coordinates of each emitting impurity molecule. Figure 13b illustrates the ZPL frequency distribution for impurity single molecules along the inhomogeneous contour, which is, in fact, the single-molecule spectral density — the number of individual molecules per chosen spectral range.

The subdiffraction accuracy of measuring single-molecule coordinates allows us to construct the pattern of the arrangement of molecules in the sample plane (Fig. 13c). Inhomogeneities in the spatial single-molecule distribution near cracks are distinctly discernible. In addition, a strong correlation was found between the position of a single-molecule spectrum in the inhomogeneous band and the spatial location of a single molecule in the sample's structure. The high accuracy of determining molecular coordinates allows us to visualize the nanostructure (defects, cracks) with subdiffraction spatial resolution (inset to Fig. 13c).

In summary, it should be emphasized that the most important advantage of phononless optical reconstruction single-molecule spectromicroscopy (PLORSM) over other methods of far-field optical diagnostics [139] is the fact that it gives the relation between the structure of the object under study and other single-molecule spectral parameters (the ZPL frequency, width, and intensity, the spectral dynamics, parameters of temperature dependences, and many others), which considerably expands the scope of scientific and applied problems solved by this method.

8. Conclusions

Having come a long way from the observation of broadband structureless spectra to the recording of individual spectra and fluorescent images of all efficiently emitting single impurity molecules in a macroscopic volume of a sample, the optical spectroscopy of impurity centers in solid solutions has now become one of the most demanded tools for solving a broad scope of interdisciplinary problems in physics, physical chemistry, optics and spectroscopy, biophysics, medical physics, quantum informatics, and nanotechnologies. The key role in the development of this field belongs to the discovery of the Shpol'skii effect (1952) (the history of this discovery is described in review [140]); the discovery and

theoretical understanding of the nature of zero-phonon spectral lines (1962); the discovery and development of selective laser spectroscopy (1972, 1974); the development of techniques for the high-sensitive recording of single-molecule spectra (1989, 1990), and, finally, the development of fluorescence microscopy (detection of images and determination of coordinates with a subdiffraction accuracy) of individual molecules in solid matrices (1994).

The techniques and approaches that have been developed can be used to study photophysical and photochemical processes in impurity materials at the single-emitter level, to investigate the matrix dynamics at the level of single elementary excitations and single charge carriers, to perform the nanodiagnostics of the sample structure, to find the fundamental rules determining the relation between the photophysical and dynamic characteristics of the sample with its local structure and chemical composition, to determine the relationships between the local properties of the object under study and macroscopic (functional) characteristic of the material, and to perform real-time diagnostics and high-sensitivity spectral analysis of complex molecular systems and nanoobjects. Notice that developments in this field are very important for practical applications, opening up unprecedented possibilities for solving diagnostic and other related problems in materials technology, biophysics, nanotechnologies, physical chemistry, and quantum informatics.

Similarly to the development of approaches to the processing of intense data flows in astrophysics [141, 142], the selective spectroscopy of impurity centers in solid matrices has come to the stage at which a large open source-distributed database can be created which will contain complete experimental information about the photophysical properties of various impurity solids in the form of spectromicroscopic data about all efficiently emitting single impurity centers in macroscopic volumes of various materials. This will allow the realization of the 'data mining' concept, thus combining the efforts of many specialists in the processing of a giant body of information, the development of methods for analyzing this information (in particular, the recognition of images of emitting objects and the reconstruction of the material structure), a search for fundamental regularities, the development of corresponding models and theories, and practical applications of the findings.

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