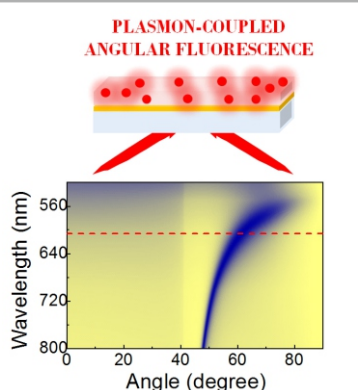


Fluorescence based Techniques

Studies on Surface Plasmon Coupled Fluorescence With a Custom-made Optical Setup for Measuring Angle-resolved Emission Spectra

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Plasmon-coupled angular fluorescence

ABSTRACT

Many applications in the chemical and biochemical sciences, like, sensing, imaging, or medical diagnostics, rely on fluorescence-based techniques. During the past decade, it has been realised that the near-field coupling of fluorescence with surface electromagnetic waves and guided electromagnetic modes in suitably designed plasmonic or photonic substrates can lead to interesting effects such as, increased brightness, collimation of the emission over a narrow angular range, colour routing, and selective emission polarization. Understanding these phenomena is of immense interest for improving the scope of fluorescence in practical applications. As the commercially available fluorescence spectrometers are not suitable for carrying out studies on plasmonic or photonic substrate coupled emission, a simple home built optical setup has been developed in Radiation & Photochemistry Division, BARC, which is compatible for investigations on surface wave coupled emission. In this setup, the excitation can be carried out in both the Kretschmann as well as the reverse Kretschmann configurations, and emission spectra can be recorded over a wide range of angles (~ 0 - 360°). This article describes the newly developed setup, as well as the observation of angle and polarization-resolved emission from fluorescent dyes that are coupled with surface plasmons and guided electromagnetic modes on Ag and Au thin film substrates.

KEYWORDS: Fluorescence, Surface plasmon, Kretschmann, Reverse Kretschmann, Directional emission

Introduction

Fluorescence is the phenomenon of light emission due to radiative relaxation of electronically excited species from their first singlet state to the ground state [1,2]. It is widely used in chemical sensing, optical devices, microscope imaging, biological research, and medical diagnosis. Traditional fluorescence applications rely on the spontaneous emission of fluorophores in an optically transparent medium and detection of emitted light in the far-field. In homogeneous solutions fluorescence is omnidirectional. Accordingly, conventional fluorescence spectrometers are designed to collect the emitted light perpendicular to the angle of excitation to avoid interference from the excitation source. This arrangement can typically collect about 1% of the total emitted light [3]. Novel methodologies are thus very much desirable to increase fluorescence collection efficiencies. The conversion of the usual isotropic emission into collimated emission over a narrow angular range that can be directly focused into the fluorescence detector is conceived to be one of the possible approaches. Directional emission is also desirable for optical devices, communications and single molecule detection [4,5].

The technique of surface enhanced fluorescence provides interesting opportunities for improvements in

fluorescence detection efficiency through modification and control of the local electromagnetic environment in the vicinity of the emitter [3,6,7]. Different kinds of plasmonic nanostructures are investigated as nanoantennas for directional light scattering and emission [8-12]. The nanoantennas basically act as transducers between far- and near-field light signals. Some varieties of plasmonic nanostructures that have been studied include Au V-antennas, aperture antennas, Yagi-Uda nanoantennas, Ag/Au nanowire junctions and nanohole arrays. The localized surface plasmon resonances that arise from the nonpropagating collective oscillations of free charge carriers in metal nanoparticles, and the propagating surface plasmons in metallic nanowires, nanoslits, and nanogaps, enable confinement of light in the nanoscale region and realization of directional light emission [8-12]. Compared to plasmonic nanostructures, dielectric nanostructures show very low dissipative loss and are emerging as an alternative approach for light manipulation at nanoscale [13].

Apart from the nanoantenna structures, large area planar plasmonic and dielectric structures, that support surface waves or internal electromagnetic modes, are also capable of influencing the spontaneous emission rate and the angular patterns of emission by modifying the fluorophore surroundings [14-20]. The most commonly investigated structures in this regard are, thin metal films (mostly of Ag and

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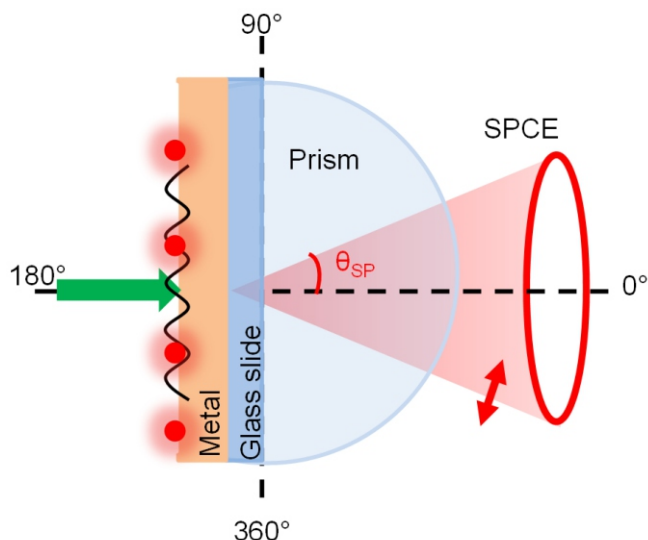


Fig.1: Schematic of surface plasmon coupled emission (SPCE) from fluorophores on a thin metal film (Ag/Au). The fluorophores (shown as red spheres) are directly excited from the air side (Reverse Kretschmann configuration). The fluorescence couples with surface plasmons on the metal/dielectric interface and appears toward the higher refractive index medium (prism side) as P-polarized emission at a characteristic angle (θ_{SP}).

Au) that support surface plasmon polaritons, 1D photonic crystals that support Bloch surface waves, and hybrid metal film/photonic crystal structures that can support Tamm plasmon polaritons.¹⁴⁻²⁰

Fig.1 shows a schematic representation of surface plasmon coupled emission (SPCE) for fluorophores placed on the surface of a thin metal film (Ag or Au) deposited on a glass slide. Excited fluorophores create surface plasmon oscillations on the metal film due to near-field interactions. The fluorescence couples with the plasmon mode, and radiates into the substrate at a characteristic angle, θ_{SP} , depending on the emission wavelength and the resonance condition of the surface plasmons. In order to extract the emission from the substrate, it is necessary to couple the substrate to a high refractive index medium like a glass prism, for wavevector matching [3,6,15]. In this schematic, the illumination geometry is in the Reverse Kretschmann (RK) configuration, where the fluorophores are excited directly by light incident from the air side. Alternatively, the fluorophores can also be excited by the evanescent field of the surface plasmons created by light incident through the glass prism at a definite angle that is proportional to the wavevector of the surface plasmon polaritons (Kretschmann configuration, KR) [3].

Since surface-plasmon resonance occurs exclusively for P-polarized light, it implies that SPCE is also P-polarized irrespective of the nature or orientation of fluorophores. Thus,



Fig.2: Optical setup for angle-resolved emission studies.

SPCE converts the isotropic fluorescence into a thin cone of P-polarized emission. SPCE has also been extended to include metal films coated with thick dielectric layers that support other electromagnetic waveguide modes in addition to the surface plasmon mode. This leads to the appearance of coupled emission at multiple angles, and with both S- and P-polarizations [21,22]. This article describes the SPCE from standard fluorescent dyes coupled to Ag and Au thin film substrates, recorded with the newly developed optical setup for angle-resolved emission studies in our laboratory.

Experimental Setup

The optical setup for recording angle-resolved emission spectra has been installed in room B-137, Mod Lab, RPCD (Fig.2). This custom made arrangement consists of a planoconvex cylindrical BK7 glass lens mounted on a height adjustable, precise rotational stage. The substrate containing the fluorescent material can be attached to the glass lens using a refractive index matching fluid. Excitation and observation of emission can be carried out at any angle relative to the vertical axis of the rotational stage. The emitted light after passing through suitable emission filters and polarizer, is collected with an optical fiber (core: 600 μm , NA: 0.22, wavelength: 400-2100 nm, from Ocean Optics) that is fixed on a holder at an adjustable distance from the substrate. The output of the fiber is connected to a miniature spectrometer (Ocean Optics, FLAME-S-XR1-ES, having wavelength range: 200-1025 nm, entrance slit: 50 μm , resolution (FWHM) \sim 2.0 nm), for recording the emission spectra. Analysis of the spectra is carried out with OceanView Cross platform spectroscopy operating software. Presently, the setup is equipped with two laser sources for excitation at 470 nm and 532 nm.

For the present SPCE studies, Ag and Au films (\sim 50 nm thick) were deposited on cleaned glass slides by vacuum evaporation. The surfaces of the films were then spin coated with poly vinyl alcohol (PVA) or poly methyl methacrylate (PMMA) solutions, containing the fluorescent dyes. The weight percent of PVA/PMMA in the solutions was varied to obtain PVA/PMMA films of different thicknesses. To correlate the observed emission patterns of the fluorophores with the surface plasmon mode and other waveguide modes present in the substrates, reflectivity calculations for the respective substrate geometries were carried out with TF Calc software package (Software Spectra, Inc., Portland, Oregon). The reflectivity plots correspond to energy conversion from far-field light to the optical modes present in the plasmonic structure, while the coupled emission is the conversion of near-field optical energy from the emitting molecules to the far-field, through these optical modes [4,23,24]. So essentially, the reflectivity plot simulates the expected angular distribution of the coupled emission.

SPCE with Au and Ag Thin Film Substrates

Fig.3A depicts the angle-dependent variation in the emission intensity of the fluorescent dye Nile Red embedded in a PMMA (0.5% PMMA) layer above an Au film deposited on glass slide. The excitation has been carried out in the RK configuration. The angular emission pattern shows that most of the emission from the fluorophore is channelized within a small angular range with the maximum at an angle of about 72° (and also 288° due to symmetry) from the normal. Furthermore, the emission is observed to be completely P-polarised. This is evident from the representative emission spectra of the dye (Fig.3B) recorded at an angle of 72°, but with two different orientations of the emission polarizer (S and P). The significantly weak fluorescence intensity of the dye when the polarizer is in the S-orientation clearly indicates

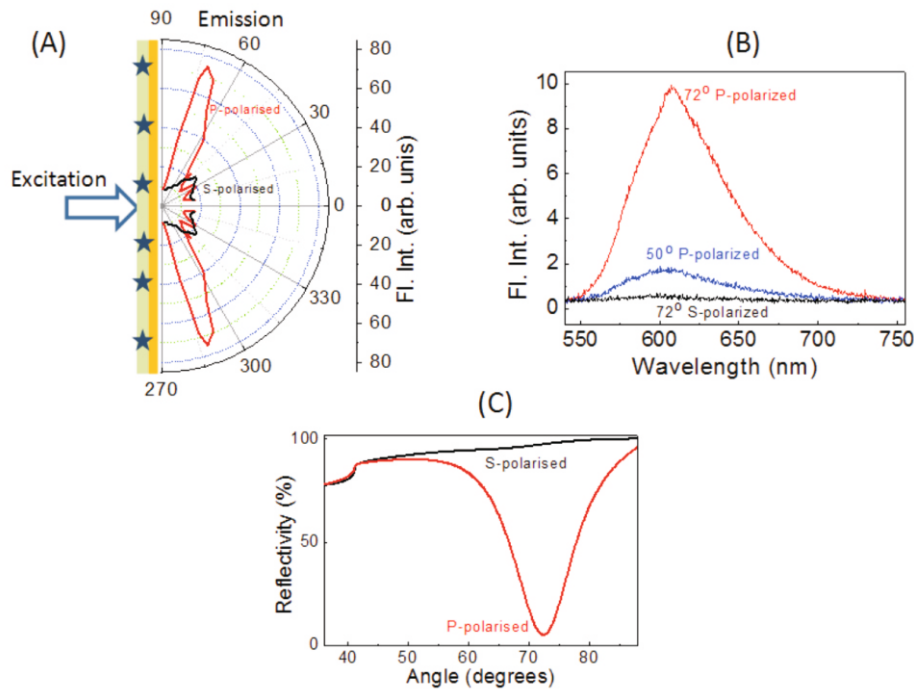


Fig.3: (A) Angular emission pattern of Nile Red on Au-PMMA substrate on excitation with a 532 nm laser; PMMA thickness ~ 70 nm. (B) Representative emission spectra of Nile Red on the Au-PMMA substrate at different angles and polarizer orientations. © Calculated reflectivity spectra for 605 nm light incident on the Au-PMMA substrate through a high refractive index medium (glass).

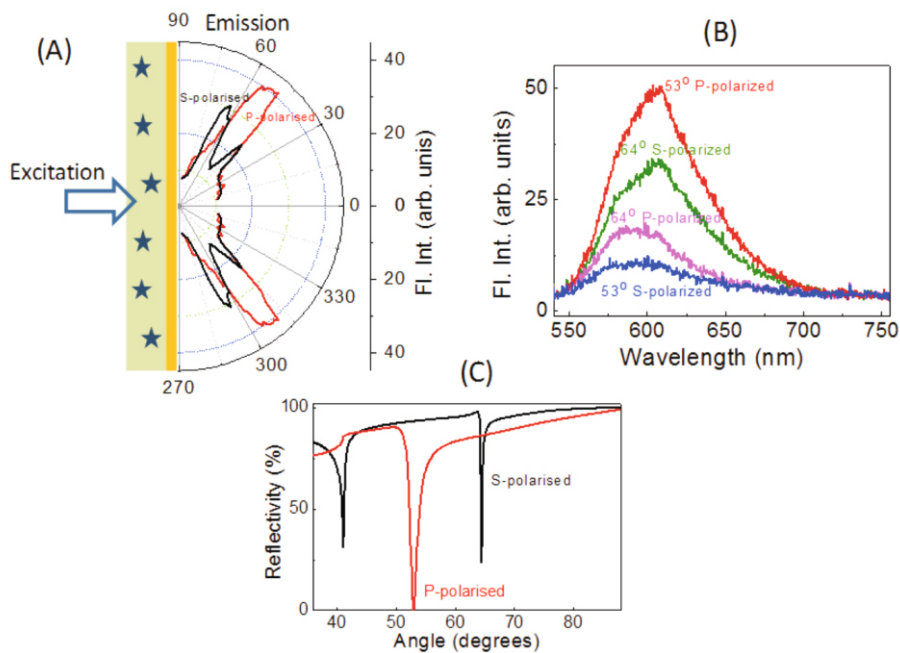


Fig.4: (A) Angular emission pattern of Nile Red coupled with Au-PMMA substrate, on excitation with a 532 nm laser; PMMA thickness ~ 375 nm. (B) Representative emission spectra of Nile Red on the Au-PMMA substrate at different angles and polarization orientations. (C) Calculated reflectivity spectra for 605 nm light incident on the Au-PMMA substrate through a high refractive index medium (glass).

that the coupled emission from Nile Red is P-polarized. The emission spectra at two different angles (72° and 50°) are also shown in Fig.3B to highlight the variation in the emission intensity with respect to the observation angle. The appearance of P-polarized emission from Nile Red at specific angles is characteristic of the surface plasmon coupling effect. To further emphasize this aspect, the calculated reflectivity spectra of light having a wavelength of 605 nm (corresponding to the emission maximum of Nile Red) and incident on the Ag-PVA substrate at different angles through a high refractive index medium (glass) is shown in Fig.3C. A dip in the reflectivity is observed for a PMMA thickness of about 70 nm when the

incident angle is 72° . Further, the reflectivity dip appears for P-polarized light but not for S-polarized light. This suggests that incident light of wavelength 605 nm is capable of exciting surface plasmon oscillations in the Ag-PMMA substrate under certain incidence angle and polarization conditions. Accordingly, the emission from Nile Red dye (having emission maximum at 605 nm) that is coupled with the surface plasmon mode appears as P-polarized light at an angle of 72° .

The angular emission pattern of Nile red on the Au-PMMA substrate changes when the thickness of the PMMA layer is increased (Fig.4A). For a PMMA layer thickness of about 375 nm (2% PMMA solution), the P-polarized emission from Nile

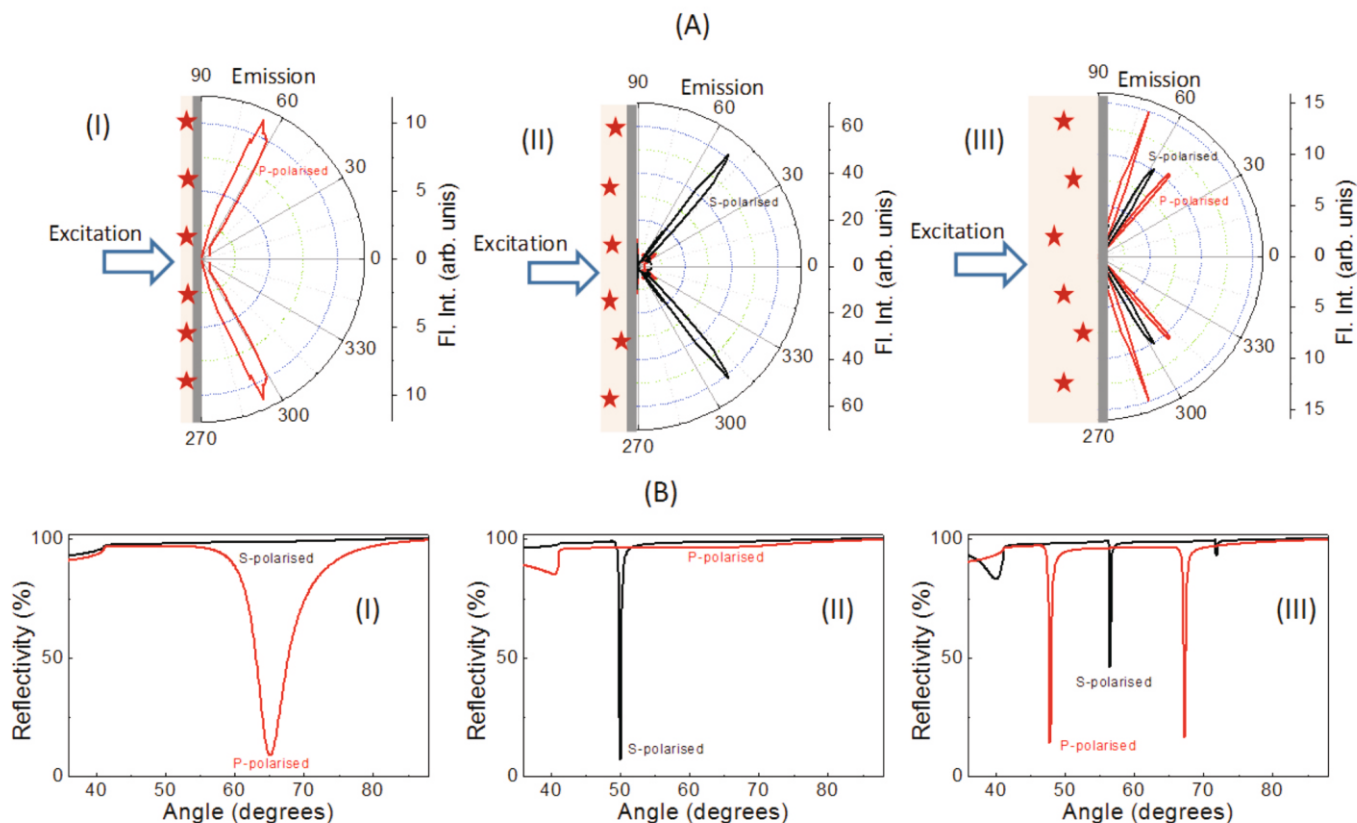


Fig.5: (A) Observed angular emission patterns of Fluorescein dye coupled with Ag-PVA substrates having different PVA thickness; excitation is with a 470 nm laser. (B) Calculated reflectivity spectra for 520 nm light incident on Ag-PVA substrates through a high refractive index medium (glass); PVA thicknesses are 50 nm (I), 160 nm (II) and 540 nm (III).

Red appears at have maximum intensity at an angle of 53° (and 307°), whereas the S-polarized emission appears to have maximum intensity at 42° and 64° (318° and 296°). The emission of Nile Red at multiple angles and having different polarizations arise due to the coupling of the fluorescence with the surface plasmon mode as well as the waveguide modes that are present in the Au-PMMA substrate with thick PMMA film. Representative emission spectra of Nile Red dye on the Au-PMMA substrates at different observation angles and polarizer orientations are depicted in Fig. 4B. The calculated reflectivity spectra (Fig. 4C) for 605 nm light incident on the Au-PMMA substrate with PMMA thickness of 375 nm shows reflectivity dips at 53° (for P-polarization), and around 42° and 64° for S-polarization) which nicely corresponds with the observed SPCE for Nile red on the studied substrate.

The observed angle-resolved emission patterns of the dye Fluorescein (emission maximum 520 nm) coupled with Ag-PVA substrates with different PVA thicknesses, on excitation with a 470 nm laser source are shown in Fig.5A. For PVA concentration $\sim 1.5\%$ w/v, the emission is completely P-polarized and appears at an angle of around 65° . When the PVA concentration is increased to 5% w/v, the emission from Fluorescein is completely S-polarized and appears at about 65° . With further increase in the PVA concentration to $\sim 7\%$ w/v, the emission from Fluorescein is channelized at multiple angles and with both S- and P-polarizations.

The experimentally observed results correlate well with the calculated reflectivity patterns (Fig.5B) for light having wavelength of 520 nm that is incident on Ag-PVA substrates having PVA thicknesses about 50 nm, 160 nm and 540 nm, respectively. This clearly suggests the occurrence of SPCE from the fluorescence in dyes coupled with the Ag-PVA substrates.

Overall, our study demonstrates the capability of the developed optical setup to measure angle and polarization resolved emission spectra of fluorophores coupled with different metal-dielectric layered substrates. Studies on SPCE are important not only for a fundamental understanding of light manipulation in the nanoscale but also for their prospects in efficient fluorescence sensing applications. The developed setup is compatible for fluorescence studies with many types of photonic and plasmonic substrates and can also be upgraded for fluorescence lifetime measurements.

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References

- [1] J. R. Lakowicz, Principles of Fluorescence Spectroscopy, Springer, 2006.
- [2] B. Valeur, Molecular Fluorescence: Principles and Applications, Wiley-VCH, 2001.
- [3] J. R. Lakowicz, Anal. Biochem., 2004, 324, 153.
- [4] S. Dutta Choudhury, R. Badugu, J. R. Lakowicz, Acc. Chem. Res., 2015, 48, 2171.

- [5] N. Li, Y. Lai, S. H. Lam, H. Bai, L. Shao, J. Wang, *Adv. Optical Mater.*, 2021, 9, 2001081.
- [6] E. Fort, S. Grésillon, *J. Phys. D: Appl. Phys.*, 2007, 41, 013001.
- [7] A. Sinibaldi, N. Danz, E. Descrovi, P. Munzert, U. Schulz, F. Sonntag, L. Dominici, F. Michelotti, *Sensors and Actuators B*, 2012, 174, 292.
- [8] H. Aouani, O. Mahboub, E. Devaux, H. Rigneault, T. W. Ebbesen, J. Wenger, *Nano Lett.*, 2011, 11, 2400.
- [9] A. Dasgupta, D. Singh, R. P. N. Tripathi, G. V. P. Kumar, *J. Phys. Chem. C*, 2016, 120, 17692.
- [10] V. Giannini, A. I. Fernandez-Dominguez, S. C. Heck, S. A. Maier, *Chem. Rev.*, 2011, 111, 3888.
- [11] Y. Wang, L. Wu, T. I. Wong, M. Bauch, Q. W. Zhang, J. L. Zhang, X. H. Liu, X. D. Zhou, P. Bai, J. Dostalek, B. Liedberg, *Nanoscale*, 2016, 8, 8008.
- [12] H. M. Shen, R. Y. Chou, Y. Y. Hui, Y. B. He, Y. Q. Cheng, H.-C. Chang, L. M. Tong, Q. H. Gong, G. W. Lu, *Laser Photonics Rev.*, 2016, 10, 647.
- [13] A. Vaskin, J. Bohn, K. E. Chong, T. Bucher, M. Zilk, D.-Y. Choi, D. N. Neshev, Y. S. Kivshar, T. Pertsch, I. Staude, *ACS Photonics*, 2018, 5, 1359.
- [14] J. A. Polo, A. Lakhtakia, *Laser Photonics Rev.*, 2011, 5, 234.
- [15] I. Gryczynski, J. Malicka, Z. Gryczynski, J. R. Lakowicz, *Anal. Biochem.*, 2004, 324, 170.
- [16] R. Badugu, K. Nowaczyk, E. Descrovi, J. R. Lakowicz, *Anal. Biochem.*, 2013, 442, 83.
- [17] S.-H. Cao, W.-P. Cai, Q. Liu, Y.-Q. Li, , *Annu. Rev. Anal. Chem.*, 2012, 5, 317.
- [18] R. Badugu, E. Descrovi, J. R. Lakowicz, *Anal. Biochem.*, 2014, 445, 1.
- [19] Y. Chen, D. Zhang, L. Zhu, Q. Fu, R. Wang, P. Wang, H. Ming, R. Badugu, J. R. Lakowicz, *Phys. Chem. Chem. Phys.*, 2014, 6, 25523.
- [20] S. Dutta Choudhury, Y. Xiang, D. Zhang, E. Descrovi, R. Badugu, J. R. Lakowicz, *J. Opt.*, 2021, 23, 035001.
- [21] I. Gryczynski, J. Malicka, K. Nowaczyk, Z. Gryczynski, J. R. Lakowicz, *J. Phys. Chem. B*, 2004, 108, 12073.
- [22] S. Dutta Choudhury, R. Badugu, K. Ray, J. R. Lakowicz, *Chem. Commun.*, 2014, 50, 9010.
- [23] J. Enderlein, T. Ruckstuhl, S. Seeger, *Appl. Opt.*, 1999, 38, 724.
- [24] N. Calander, *J. Phys. Chem. B.*, 2005, 109, 13957.