Exploring Fundamental Limits of Quantum Efficiency Measurements Using Quantum Electrodynamics

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Photoluminescence techniques play an important role for characterization of various functional fluorophores in the life and material sciences from biology to quantum technologies. A crucial and key parameter for comparing the performance of fluorophores is the photoluminescence quantum efficiency or quantum yield, which presents a direct measure of conversion efficiency of absorbed photons into emitted photons. This quantity characterizes the performance of emitters for applications in optical devices as single photon sources and in the biomedical sector. Several techniques such as optical and photothermal methods are used to measure the photoluminescence quantum efficiency of emitters in various environments and aggregation states. Quantum efficiency measurements can be challenging for fluorophores in solid matrix, in scattering systems and for highly diluted fluorophores embedded to thin films. The research described in this thesis overcomes these challenges by performing quantum efficiency measurement via modifying the spontaneous emission as a fundamental process of light-matter interaction.

In the first part of thesis, a Drexhage-type experiment was performed where a silver-coated millimeter-sized sphere is used to modify the local density of states. This provides a direct and reference-free approach to measure the quantum efficiency of various organic dyes. As a representative example for the potential of this method, we first observed the lifetime variation of Nile-Red doped polymer films as a function of the distance between emitter and sphere. After successful determination of the quantum efficiency through the modification of radiative rate, the pH-sensitive fluorescein isothiocynate (FITC) was covalently attached to a thin mesoporous silica film in order to observe the local quantum efficiency at different pH value. The local quantum efficiency decreases from 73 % to 25 % with the pH from 7.5 to 5.5, respectively. The corresponding quantum efficiencies obtained from the optical relative and absolute methods as well as results from literature were in very good agreement with each other.

In the second part of the work, it is shown that this approach can be extended to the single emitter level. The quantum efficiency of recently discovered defects in hexagonal boron nitride ($h$-BN) is measured absolutely and directly. A metal hemisphere attached to the tip of an atomic force microscope is employed to observe the lifetime variation of the single photon emitters as the tip approaches the $h$-BN. It is found that the solid state single photon emitter exhibited quantum efficiencies of around (52 ± 7) % at wavelengths of around 660 nm.

Our results presented in this thesis provide new insights for measuring quantum efficiency of fluorophores with the help of the miniaturized lab-on-chip devices.

Im ersten Teil der Arbeit wurde ein Drexhage-artiges Experiment durchgeführt, bei dem eine versilberte millimetergroße Kugel verwendet wurde, um die lokale Dichte der Zustände zu modifizieren, was einen direkten und referenzfreien Ansatz zur Messung der Quanteneffizienz verschiedener organischer Farbstoffe bietet. Als repräsentatives Beispiel für das Potenzial dieser Methode haben wir zunächst die Lebensdauervariation der Nil-Rot-dotierten Polymerfolie als Funktion des Abstandes zwischen Emitter und Kugel beobachtet. Nach erfolgreicher Extraktion der Quanteneffizienz durch Modifikation der Strahlungsrate wurde das pH-sensitive Fluorescein-Isothiocyanat (FITC) kovalent an einen dünne mesoporösen Siliziumdioxidfilm gebunden, um die lokale Quanteneffizienz bei unterschiedlichem pH-Wert zu beobachten. Die lokale Quanteneffizienz sinkt von 73 % auf 25 % mit dem pH-Wert von 7,5 bis 5,5. Die entsprechenden Quantenwirkungsgrade aus den optischen relativen und absoluten Methoden sowie die Ergebnisse aus der Literatur waren in sehr guter Übereinstimmung.

Im zweiten Teil der Arbeit wird gezeigt, dass dieser Ansatz bis auf die Ebene der Einzelemitter ausgedehnt werden kann. Die Quanteneffizienz der kürzlich entdeckten Defekte in hexagonalem Bornitrid (h-BN) wurde absolut und direkt gemessen. Eine an der Spitze eines Rasterkraftmikroskops befestigte Metallhalbkugel wurde verwendet, um die Lebensdaueränderung der einzelnen Photonenemitter zu beobachten, wenn sich die Spitze der Rasterkraftsonde dem h-BN nähert. Es wurde festgestellt, dass der Festkörper-Einzelphotonenemitter eine Quanteneffizienz um (52 ± 7) % bei Wellenlängen um 660 nm aufweist.

Unsere in dieser Arbeit vorgestellten Ergebnisse liefern neue Erkenntnisse für die zukünftige Messung der Quanteneffizienz von Fluorophoren mit Hilfe miniaturisierter Lab-on-Chip-Geräte.
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In the past years, photoluminescence techniques have become very useful for the characterization of various fluorophores such as organic dyes, semiconductor nanocrystals (quantum dots), nitrogen-vacancy centers, or upconversion nanocrystals [1–6]. Therefore, photoluminescence techniques play an important role as analytical and detection tools in life and material sciences [7–10]. The Nobel Prize in Chemistry 2014 was awarded for the development of super-resolved fluorescence microscopy which basically relies on specialized fluorophores and sophisticated techniques to analyze their emission [11]. The ultimate level of fluorescence is the emission from a single fluorophore. It emits individual photons one-by-one. Such fundamental light sources, or single photon sources [12] are a key resource for optical quantum information. The development of bright and stable fluorophores has also shown promising potential in a series of optoelectronic applications such as smartphones, laptops, lighting and automobile industry (Figure 1.1). The problem of a reliable performance of fluorescent material is also crucial for illumination applications. The Nobel Prize in Physics 2014 was awarded for the invention of efficient blue light-emitting diodes which has enabled bright and energy-saving white light sources, which leads the technological revolution of last 20 years [13]. In order to design, functionalize and characterize the suitability of fluorophores for an application area of interest, various spectroscopic parameters such as absorption and emission spectra, molar absorption coefficients, lifetime, emission anisotropy and quantum efficiency have to be determined [14–16].

A key parameter is the photoluminescence quantum efficiency $\Phi_{PL}$, which is a basic property to compare the emitter performance. It measures the efficiency of conversion of absorbed photons into emitted photons. Alternatively, it can be defined as the ratio of the radiative to the sum of radiative and non-radiative decay rates [1, 20–22]. The knowledge
of $\Phi_{PL}$ is mostly used to determine the suitability of fluorophores for the applications mentioned above, be it as single photon sources for quantum communication [23, 24], optical device for solar cells and lasers [25–27], in the biomedical sector for labeling and imaging of biological cells [16, 28, 29] and luminescent materials such as OLED/LED [30, 31]. $\Phi_{PL}$ of a fluorophore is sensitive to the local environment such as change in the polarity, viscosity, local temperature and pH [32–35].

Various methods for measuring $\Phi_{PL}$ are employed to inspect the limitations and potentials for intended applications. In 1924, Vavilo initiated the reliable method for quantitative measurement of $\Phi_{PL}$, where fluorophore emission was compared with scattering intensities [36]. Demas and Crosby reviewed the quantum efficiency measurement methods in 1970 [37]. In the past years, the photo-thermal and optical methods for determination of $\Phi_{PL}$ of transparent, liquid and solid samples have been utilized and new equipment has been steadily developed [38, 39]. The knowledge of $\Phi_{PL}$ can be obtained indirectly by using photothermal methods such as measuring dissipated heat by applying photoacoustic spectroscopy and thermal lensing techniques [40–42]. With the aid of an intense laser as an excitation light source, the part of photons absorbed by a sample, converted to heat and dissipated into the environment can be measured by using photoacoustic spectroscopy and thermal lensing techniques. This method requires a non-emissive reference with known absorption and thermal properties of the solvent or matrix matching with the sample. The radiant yield derived from the ratio of emitted to absorbed power is converted to the quantum efficiency by considering the absorption and emission spectra [43–45].

In contrast to the photothermal methods, $\Phi_{PL}$ of the fluorophore can be measured directly by counting the emitted photon output with a help of optical methods relatively and absolutely. In a relative measurement, the absorption and emission of fluorophore with unknown $\Phi_{PL}$ is compared to that of a standard with known $\Phi_{PL}$ [39]. This method is based on the comparison of integral emission and absorbance at the same excitation wavelength of a standard and an unknown fluorophore under identical conditions. As an alternative method, $\Phi_{PL}$ can be measured absolutely without the reference by using a calibrated integrating sphere setup. The sample is placed into the middle of a sphere in a commercial or homemade setup and excited directly to count the emitted photons with a single measurement [37, 46, 47].

Photothermal and optical methods are mostly used for ensemble studies where relatively high concentrations or number of emitters are required for signal detection [39, 40, 48–51]. At high concentrations, the dye-dye interaction and reabsorption prevent accurate $\Phi_{PL}$ measurement of the fluorophore due to quenching effects. To take this problem into account, measurements at various concentrations can be performed. However, this may hamper the concentration dependences of $\Phi_{PL}$ of luminescent nanocrystals with coordinatively bound surface ligands, which are prone to ligand desorption-induced fluorescence quenching [49, 52]. These types of studies are necessary to assess the quality of the surface functionalization and passivation of nanocrystals which are used at a very high dilution for molecular imaging [53]. This requires the development of new and advanced methods for $\Phi_{PL}$ measurements on the single emitter level [54–58] or at ultralow concentrations [59].
The importance of direct and accurate $\Phi_{PL}$ measurement even down to the single molecule level motivated researchers to explore alternative approaches. In one of them, the radiative emission rate is modulated and compared to the total decay rate, i.e. the sum of the radiative and non-radiative rate. The modification of the spontaneous radiative emission of an emitter in the vicinity of metal surface is a quantum electrodynamic effect related to the local density of states (LDOS) [20, 60–64]. The LDOS influences only the radiative rate and not the nonradiative decay rate [65]. This was first experimentally performed by Drexhage [66], who investigated the radiative decay rate of europium ions as a function of their distance to a silver mirror. By using time-correlated single photon counting techniques, photoluminescence lifetimes and thus the radiative rates can be measured relatively simply with high accuracy without influence from excitation power density and scattering, which can distort many optical methods for determining $\Phi_{PL}$ except for integrating sphere spectroscopy. Moreover, the total amount of emitted light does not have to be measured and setups can be quite small as compared in Figure 1.2. The analytical solutions of a dipole-like emitter in front of a reflecting plane in simple geometry can be obtained for the modification of the radiative rate [60, 67, 68]. This fundamental technique to measure the $\Phi_{PL}$ directly and absolutely has been used meanwhile by exploiting different geometry such as photonic crystals, nanocavity, scanning probes and spacer layers. There are several other configurations to modify the LDOS and to extract the $\Phi_{PL}$. The fluorophore can be placed into a tunable metallic nanocavity made of silver-coated glass substrate, which allows for the control of the LDOS by varying the cavity length [69–72]. On the other hand, sandwich-like structures consisting of spacer layer with calibrated heights are also used, which require deposition techniques or etching techniques to tune the distance between metal and rare earth ions [73] and nanocrystals [74, 75]. Moreover, this method is extended to measure the $\Phi_{PL}$ of a single emitter such nitrogen vacancy (NV) centers in nanodiamonds [76] and organic dyes [57] with a scanning mirror. In order to minimize the difficulties in the fabrication and calibration, the curvature of a sphere is used to measure the entire distribution of quantum dots [77] and silica nanoparticles [78].

![Figure 1.2. Quantum Efficiency Measurement Techniques](image)

Figure 1.2. Quantum Efficiency Measurement Techniques a) Schematic representation of the integrating sphere. (Taken from [79]) b) Schematic diagram of the nanomechanical tuning of LDOS to measure the quantum efficiency of defect centers in nanodiamonds. (Taken from [80])
The study of quantum electrodynamics not only enables the exploration of the limits of $\Phi_{PL}$ measurements as photoluminescence technique but also provides the control of light emission and amplification on the single photon level with the development of nanophotonic platform [81–83].

Outline of Thesis

The focus of this thesis is to explore the quantum efficiency of various fluorophores in a heterogeneous environment near planar interfaces. In order to perform such experiments, the photoluminescence techniques including the measurements of absorption and emission spectra are performed. New experimental techniques are tested and developed in detail to control and modify the light emission. The properties of various fluorophores from ensemble to single molecules are studied individually in a heterogeneous environment.

In chapter 2, the theoretical background of spontaneous emission near planar multilayer interfaces in an inhomogeneous environment is introduced for the determination of quantum efficiency of fluorophores. In addition to that, the optical methods for measuring quantum efficiency are mentioned with the focus on the electrodynamics approach through a modification of the LDOS. The concept of single photon detection and examples of quantum emitter are discussed at the end of this chapter.

In the following chapter 3, the experimental setup and techniques are acknowledged in detail with the focus of the home-built Time-Correlated Single Photon Counting (TCSPC) scanning confocal microscope with the fluorescence lifetime imaging (FLIM). Atomic force microscope (AFM), incorporated into confocal setup for the topography measurement, is also presented. At the end of this chapter, the instruments for measuring absolute and relative quantum efficiency are briefly explained.

In chapter 4, we investigate single colloidal quantum dots (CQDs) embedded in polymer films to evaluate their potential as a single photon emitter (SPE). The energy structure and type of CQDs are also discussed and compared to bulk semiconductor. Single CQDs are observed by using the TCSPC confocal microscope and single photon emission is verified with second order correlation measurements.

The main focus of chapter 5 is to measure the quantum efficiency of organic molecules through a modification of the LDOS by using the Drechsel-type method. To observe the influence of the environment, pH-sensitive dyes are embedded in a mesoporous silica thin film and the quantum efficiency as function of local pH of the surrounding liquid is determined. The results obtained using this technique are also compared with the absolute and relative quantum efficiency measurement.

Lastly, chapter 6 discusses two-dimensional atomic layer heterostructures and then introduces the atomic force microscope (AFM) with a metal AFM tip in order to measure the quantum efficiency of a single photon emitters (SPE) in few-layer hexagonal boron nitride ($h$-BN). Furthermore, the photophysical properties of a SPE of $h$-BN are investigated and the change in the lifetime of a SPE of $h$-BN is studied to extract the quantum efficiency of defects in $h$-BN by using metal AFM tip.
2

Theoretical Background and Quantum Emitters

In this chapter, the electrodynamic interaction between a fluorophore and its environment is discussed and related to the quantum efficiency. Then, the terminology and theoretical background of single photon experiments such as second order auto correlation and time correlated single photon counting measurements are mentioned. At the end of this chapter, the single photon emitters used in this thesis are listed with a brief information such as quantum dots, fluorescent molecules and defects in two-dimensional materials.

2.1. Spontaneous Emission

2.1.1. Simplified Model and Local Density of States

Spontaneous emission is a phenomenon, which requires a quantized light field [20]. In this thesis, we describe a fluorophore as a two-level system to define the spontaneous emission process in a simplified way. In this two-level fluorophore, the spontaneous emission can be described as a process in which a fluorophore (organic dye, quantum dots, defects in 2D materials) undergoes a transition from an excited energy state to a lower energy state and emits a quantized amount of energy in the form of a photon [84].

Jablonski Diagram

The relevant energy states and transitions processes are depicted by a Jablonski diagram as shown in Figure 2.1a for mostly organic dyes [85, 86]. A Jablonski diagram illustrates the energy levels of a fluorophore and possible transitions between them to illustrate various molecular processes after excitation with a light source [1, 86]. The singlet ground (S₀), first excited (S₁) and second excited (S₂) electronic states are shown. Additional indices indicate vibrational energy levels. Absorptions and radiative emissions are denoted by coloured arrows. Following light absorption, the fluorophore may undergo several radiative or non-radiative relaxations. It can relax from a higher excited state S₂ to the state S₁. There it rapidly relaxes to the lowest vibrational level on the time scale of $10^{-12}$ sec. An emission of a fluorescent photon then happens in time scales of $10^{-8}$ sec. This process is called internal conversion [1, 87, 88].

The radiative return to the ground electronic state occurs to a higher vibrational ground state level, labeled with $hν_F$, which quickly reaches thermal equilibrium in time scales of
Figure 2.1. a) Jablonski Diagram illustrating the process of light absorptions (excitation to the first or a higher excited state) and relaxation of the excited state by fluorescence and phosphorescence. b) Multiple vibronic states \( (v' = 1...4) \) exist in the ground and the upper potential \( (S_0, S_1) \). An excitation (green arrow) and fluorescence (red arrow) process is accompanied by fast relaxation processes that include interaction with phonons.

10\(^{-12}\) sec. Return to an excited vibrational state at the level of \( S_0 \) state causes the vibrational structure in the emission spectrum. Consequence of emission to higher vibrational ground states is that the emission spectrum is a mirror image of the absorption spectrum of the \( S_0 \) to \( S_1 \) transition. This process is known as Franck-Condon Principle [87, 89, 90], which describes the intensities of vibronic transitions for absorption and emission of a photon as shown in detail in Figure 2.1b.

Aside from fluorescence which occurs from \( S_1 \) excited state, emission from \( T_1 \) excited state can occur, which is called phosphorescence. This follows the spin-state conversion from \( S_1 \) singlet to \( T_1 \) triplet and is called intersystem crossing. Phosphorescence is strongly effected by factors such as solvents and quenching. Triplet emission rates are several orders of magnitude lower than those for fluorescence [1].

Local Density of States (LDOS)

Spontaneous emission occurs when the fluorophore emits the photon spontaneously and undergoes a transition from an excited state to a lower state as described in the previous section. This energy relaxation is a non-deterministic process with a probability per unit time, which is called decay rate \( \gamma \) of the fluorophore. According to Fermi’s Golden Rule [91], this decay rate can be derived to have the form

\[
\gamma = \frac{2\pi \omega}{3\hbar} |\mu|^2 \rho_\mu(\omega_0, r_0)
\]

where the expression separates into an atom and a field part. The atom part depends on the transition dipole moment \( \mu = \langle a | \hat{\mu} | b \rangle \) where \( \hat{\mu} \) is the dipole operator containing the electronic property of the fluorophore. This is the electronic property of the molecule and depends on the wavefunction overlap between the ground \( |a\rangle \) and excited state \( |b\rangle \). A field part given by the Local Density of States (LDOS) \( \rho_\mu(\omega_0, r_0) \) which gives the information of the density of photon states at the certain position \( r_0 \) for emitted photon frequency \( \omega_0 \).
and depends only on the environment of the fluorophore. This means that the spontaneous emission as well can be modified by the surroundings [20, 64].

By introducing the density of states of free space, an expression for spontaneous emission can be described:

\[
\gamma_0 = \frac{\omega_0^3 |\mu|^2}{3\pi\epsilon_0 \hbar c^3}
\]

(2.2)

However, as pointed out, when the density of states is modified, so is the spontaneous emission rate [20].

2.1.2. Modification of Spontaneous Emission Rate

As pointed out in the previous section, the spontaneous emission can be modified by changing the LDOS. Purcell in 1946 discovered that the spontaneous emission rate can be either enhanced or suppressed by changing its electromagnetic environment [65]. Hence, the modification of the spontaneous emission rate of the fluorophore due to change in its environment is called the Purcell Effect. It plays an important role in quantum optics and nanophotonics and has various applications for single-photon sources [92] and fluorescent microscopy [63]. This effect is also demonstrated by using microcavities [93], nanoantennas [94] and photonic crystals [95] in order to control the LDOS. In this thesis, we basically consider a modification of the LDOS by a planar metallic surface. Such a configuration was for the first time studied experimentally by Drexhage in 1960. He demonstrated that the decay rate of europium ion is modified near planar interfaces [66]. During the rest of the thesis we refer to such an experimental configuration as Drexhage-experiment.

2.1.3. Spontaneous Decay Rates near Planar Interfaces

As pointed out, Drexhage investigated the modification of spontaneous emission rate in the vicinity of a planar metallic interface. Drexhage measured the spontaneous emission rate (radiative rate) of europium rare-earth ions as a function of distance from a silver mirror as a metallic interface [66]. The oscillations of the spontaneous emission as a function of distance between metal and emitter was for the first time experimentally observed. Above a distance of about 20 nm, these oscillations result from constructive and destructive interference between virtually emitted and reflected photons at the silver interface. The period of these oscillations is given by the emission wavelength of the fluorophore. After the pioneering experiment of Drexhage, the LDOS calculation of fluorophores in front of interfaces were discussed by Amos and Barnes [68] and Chance, Prock and Silbey (CPS Model) [60].

In this thesis, the LDOS calculations for multilayer interfaces [30] are used based on the CPS Model. For each dipole orientation, perpendicular and parallel to the interface, the LDOS is derived as follows:

\[
\rho_\perp = \frac{3}{2} \operatorname{Im} \left[ \int_0^\infty dq \frac{q^3 (1 + r_{e,M}^p e^{-2l_e \delta})(1 + r_{e,-N}^p e^{-2l_e \delta})}{1 - r_{e,M}^p \rho_{e,M} e^{-2l_e \delta}} \right]
\]

(2.3)
Figure 2.2. The sketch of the numerical treatment of an emitter inside a planar multilayer medium. The central layer with permittivity \( \varepsilon_e \) contains emitters represented by perpendicular or parallel dipoles at a distance \( s \) and \( d \), respectively, from the neighbouring layers. The \( M \) top and \( N \) bottom layers are characterized by their permittivity \( \varepsilon_i \) and thickness \( d_i \). The simplified structure in blue curves is described by total reflection coefficient \( r_{e,M \rightarrow N}^{s/p} \). (Adapted from [30])

\[
\rho_\parallel = \frac{3}{4} \text{Im} \left[ \int_0^\infty dq \frac{q}{\varepsilon_e} \left( \frac{1}{1 - r_{e,M}^{s} r_{e,-N}^{s} e^{-2l_e d}} + (1 - q^2) \frac{1}{1 - r_{e,M}^{p} r_{e,-N}^{p} e^{-2l_e d}} \right) \right] \tag{2.4}
\]

where \( q = k_\parallel / k_e \) and \( l_i = -i \sqrt{\varepsilon_i / \varepsilon_e - q^2} \) are in-plane and out-of-plane wavevectors, normalized to the wavevector in the host medium of the emitter. In this formula, the emitter is placed with a distance \( \hat{s} = k_e s \) and \( \hat{d} = k_e d \) from \(-1^{th}\) and \(1^{th}\) layer and \( \hat{W} = k_e W \) is the thickness of the host layer as shown in Figure 2.2. The iteratively calculated total reflection coefficient of adjacent medium for both s- and p-polarized light can be expressed as

\[
r_{s/p}^{i,M \rightarrow N} = \frac{r_{s/p}^{i,M \rightarrow i \rightarrow 1} + r_{s/p}^{i,M \rightarrow i \rightarrow N} e^{-2l_{i \rightarrow 1} d_{i \rightarrow 1}}}{1 + r_{s/p}^{i,M \rightarrow i \rightarrow 1} r_{s/p}^{i,M \rightarrow i \rightarrow N} e^{-2l_{i \rightarrow 1} d_{i \rightarrow 1}}} \tag{2.5}
\]
where \( r^{s/p}_{M,M} = r^{s/p}_{-N,-N} = 0 \) is defined as \( M^{th} \) and \( N^{th} \) interfaces have an infinite thickness with 2-layer Fresnel reflection coefficients for \( s \)- and \( p \)-polarization in the following equation

\[
\begin{align*}
    r^s_{l,i,l+1} &= \frac{l_i - l_{i+1}}{l_i + l_{i+1}} \\
    r^p_{l,i,l+1} &= \frac{\varepsilon_i l_{i+1} - \varepsilon_{i+1} l_i}{\varepsilon_i l_{i+1} + \varepsilon_{i+1} l_i}
\end{align*}
\]  

(2.6)

\( \rho(d) \) is the modified LDOS normalized to the LDOS in the absence of the mirror. The LDOS \( \rho \) is calculated separately for upright standing \( (\rho_\perp) \) and flat lying \( (\rho_\parallel) \) dipolar emitters. The LDOS of a randomly oriented single dipole can be expressed as

\[
\rho(d) = \alpha \rho_\perp(d) + (1 - \alpha) \rho_\parallel(d)
\]

(2.7)

where \( d \) represents distance between emitter, and mirror and \( \alpha = \cos^2(\theta) \) is a free parameter with the dipole orientation angle \( \theta \) relative to the mirror. \( \alpha = 1/3 \) can be used for the ensembles of randomly oriented dipoles. In order to integrate this expression, \( q = 0 \) and \( q = \infty \) can be used as an integral boundaries. In the case that the integral runs from \( q = 0 \) to \( q \leq 1 \), the coupling of the dipole to radiation in the far field occurs. In the region of \( q > 1 \), the evanescent field couples to surface modes as either surface plasmon polaritons (SPPs) or lossy surface waves [61]. The normalized LDOS (normalized to the density of states in free space) away from the the mirror is calculated as a function of the distance between emitter and mirror for the perpendicular and parallel dipole in Figure 2.3. The normalized density of states with randomly oriented dipoles with \( \alpha = 1/3 \) is also plotted for a 5 layer system (silver, SiO\(_2\), vacuum, thin film, glass) where \( M = 4 \) and \( N = -1 \).

![Figure 2.3](image-url) 

Figure 2.3. The calculated normalized LDOS for the 5 layer system which is used in chapter 5 and material parameters as provided in A.1. The LDOS for emitters with a parallel (red) and perpendicular (black) orientation to the mirror and for randomly oriented dipoles LDOS (blue) are shown.
2.2. Quantum Efficiency

The photoluminescence quantum efficiency ($\Phi_{PL}$) as a fundamental property, is an important parameter for any type of fluorophore. The knowledge of $\Phi_{PL}$ is required for the characterization and application of photoluminescent materials as the $\Phi_{PL}$ of these materials may vary depending on the environment conditions such as solvent polarity and viscosity, temperature, pH and the concentration. According to Crosby [37], $\Phi_{PL}$ given as the ratio of the number of emitted photons ($N_E$) and absorbed photons ($N_A$):

$$\Phi_{PL} = \frac{N_E}{N_A} \quad (2.8)$$

In order to measure the $\Phi_{PL}$ of a fluorophore, different experimental techniques can be used. Photo-thermal methods rely on measuring the heat, which is caused by the absorbed photons. An example is a measurement of the thermal lensing effect [96, 97]. However, this technique requires a high concentration in order to achieve high accuracy. In the following section, optical methods and electrodynamics techniques are discussed with details.

2.2.1. Optical Methods

In this thesis, the different optical methods of $\Phi_{PL}$ measurements are described. The $\Phi_{PL}$ of transparent solutions of fluorophore can be measured by optical methods consisting of relative and absolute measurements.

Relative $\Phi_{R,PL}$ Measurement

In the relative $\Phi_{R,PL}$ measurement, the absorption and emission of a fluorophore solution of unknown $\Phi_{PL}$ are compared with those of known $\Phi_{PL}$ of standard fluorophore. Through a measurement of the absorption spectrum of both the unknown and standard fluorophores, the absorption factor $f$ at the excitation wavelength $\lambda_{ex}$ can be derived:

$$f(\lambda_{ex}) = 1 - 10^{-A(\lambda_{ex})} \quad (2.9)$$

where $A(\lambda_{ex})$ is the absorbance at the excitation wavelength. Under identical conditions, the integral photon flux $F$ is derived:

$$F = \int_{\lambda_{em}} I_c \cdot \lambda_{em} d\lambda_{em} \quad (2.10)$$

where $I_c$ is the blank-corrected spectrum of the sample. From this the quantum efficiency can be derived:

$$\phi_{PL,x} = \phi_{PL,st} \frac{F_x}{F_{st}} \frac{f_{st}(\lambda_{ex,st}) n_{st}^2}{f_x(\lambda_{ex,x}) n_x^2} \quad (2.11)$$

where the index $x$ denotes the unknown fluorophore and the index $st$ denotes the known standard. $n$ is the refractive index of the solvent and the terms with refractive index is applied when the standard and sample have the different solvents [39]. $\phi_{PL,st}$ can be either obtained from absolute quantum efficiency measurements or used from the literature.
values. In the section 3.3.2, the experimental setups and techniques are discussed with the details regarding relative quantum efficiency measurement.

Absolute $\Phi_{A,PL}$ Measurement

$\Phi_{PL}$ can be obtained absolutely as the method is named, with the aid of an integrating sphere in a single measurement without the need of a standard with a known quantum efficiency [98]. In the section 3.3.1, the working principles of integrating sphere will be explained.

In order to measure the quantum efficiency $\Phi_{A,PL}$, the absorbed and emitted photons of sample and blank solution are measured under identical conditions such as excitation wavelength and temperature. The $\Phi_{A,PL}$ of fluorophore is obtained by:

$$
\Phi_{A,PL} = \frac{\int_{\lambda_{em}}^{\lambda_{em2}} I_x(\lambda_{em}) - I_b(\lambda_{em}) \lambda_{em} d\lambda_{em}}{\int_{\lambda_{ex}-\Delta\lambda}^{\lambda_{ex}+\Delta\lambda} I_b(\lambda_{ex}) - I_x(\lambda_{ex}) \lambda_{ex} d\lambda_{ex}}
$$

(2.12)

where $\lambda_{ex}$ and $\lambda_{em}$ are excitation and emission wavelength, respectively. $s(\lambda_{ex})$ and $s(\lambda_{em})$ are the spectral responsivity of detection channel for excitation and emission wavelengths, respectively. To calculate the emitted photon flux, the difference between spectrally corrected $I_x(\lambda_{em})$ and blank corrected $I_b(\lambda_{ex})$ are integrated over $\lambda_{em}$. A similar calculation is used to determine the absorbed photon flux by calculating difference between blank $I_b(\lambda_{ex})$ and sample $I_x(\lambda_{ex})$ within the spectral region of the excitation bandpass $\Delta(\lambda)$ [38].

2.2.2. Deriving the Quantum Efficiency from Decay Rates

The quantum efficiency of fluorophores can also be derived by determining the radiative and non-radiative decay rates directly. In case of a very small number of fluorophores (down to the level of a single one), the time-correlated single photon counting techniques have to be applied. $\Phi_{PL}$ can be expressed as the ratio of radiative rate ($Y_r$) to the total decay rate (the sum of the radiative ($Y_{tot}$) and non-radiative rates ($Y_{nr}$)):

$$
\Phi_{PL} = \frac{Y_r}{Y_r + \sum_{nr} Y_{nr}} = \frac{Y_r}{Y_{tot}}
$$

(2.13)

where $\sum_{nr} Y_{nr}$ describes the sum of all possible non-radiative rates.

As described in section 2.1.2, the radiative rate can be altered by modifying the local density of states. Drexhage used a metal mirror for that purpose. With the modified local density of states $\rho$, the total decay rate can now be expressed as:

$$
Y_{tot} = \sum_{nr} Y_{nr} + Y_r V_{ac} \frac{\rho}{\rho_{vac}}
$$

(2.14)

where $Y_r V_{ac}$ and $\rho V_{ac}$ are the radiative rate and LDOS in vacuum respectively. Therefore, the $\Phi_{PL}$ can be extracted by measuring how much the total decay rate can be modified, e.g. by the presence of a nearby metal mirror.
Nowadays, there are several techniques based on this principle to modify the radiative rate of fluorophores, which have been used to determine $\Phi_{PL}$ by tuning the LDOS. The absolute $\Phi_{PL}$ of organic dyes (Rhodamine 6G, Atto 495), semiconductor nanocrystals (CdSe/ZnS) and a mixture of chromophores was determined directly by using a metal nanocavity consisting of two silver mirrors [70, 72, 99]. Owing to gray-tone optical lithography, a wedge shaped dielectric was fabricated to separate the layer of fluorophores from metallic layer in a controlled height profile in order to determine $\Phi_{PL}$ of fluorescent beads and dibenzoterrylene (DBT) molecules in anthracene (AC) [75]. A similar concept was implemented with changing the thickness of the SiO$_2$ spacer layer to control the distance between quantum dots and the metallic layer to measure $\Phi_{PL}$ [74].

2.3. Photon Correlations

Albert Einstein received the Nobel Prize in Physics in 1921 for his discovery of the law of the photoelectric effect. His consideration from his 1905 paper [100] introduced the concept of light as indivisible particle-like objects. Later the name photon was coined. Roy Glauber developed the theory of how to characterize light in terms of the statistical analysis of photon detection events [101].

2.3.1. First Order Auto Correlation

Following Glauber [102] the normalized first order coherence function is related to amplitude fluctuations of light and is expressed as:

$$g^{(1)}(r_1, t_1; r_2, t_2) = \frac{\langle E^*(r_1, t_1)E(r_2, t_2)\rangle}{\langle |E(r_1, t_1)|^2 \rangle \langle |E(r_2, t_2)|^2 \rangle^{1/2}}$$  \(2.15\)

where $E$ denotes a classical electric field at point $r$ with its complex conjugates ($^*$) and the brackets $\langle \ldots \rangle$ indicate the time averaging. This function describes the amplitude fluctuation of the light source at two points ($r_1$ and $r_2$) and two instances ($t_1$ and $t_2$). For a stationary field, $\tau = t_2 - t_1$ allows to express the simplified version of Equation 2.15 as:

$$g^{(1)}(\tau) = \frac{\langle E^*(t)E(t + \tau)\rangle}{\langle |E(t)|^2 \rangle}$$  \(2.16\)

The coherence time, $\tau_c$, is the characteristic time scale of the amplitude fluctuations. If $|g^{(1)}(\tau)| = 1$, the light is first-order coherent, otherwise the light is described as a chaotic light. Chaotic light has a finite coherence time and the amplitude correlations decay to zero ($g^{(1)}(\tau) \geq 0$) within the coherence time.

2.3.2. Second Order Auto Correlation

The classical second-order correlation function, $g^{(2)}(\tau)$, measures intensity correlations and can be expressed by:
With the negative and positive electric field operators $\hat{E}^-$ and $\hat{E}^+$, where the variance of the photon number is defined with

$$\langle n \rangle = \langle a\dagger a \rangle$$

work. In this case the light is called anti-bunched and a quantum mechanical treatment is necessary. In the quantum case the same functions to characterize first and second order correlation can be applied, but the field amplitudes have to be replaced by operators and the brackets $<>$ now denote the expectation value of a specific field state. The quantum operators do not commute and normal ordering with notation $: ... :$ has to be introduced. With the negative and positive electric field operators $\hat{E}^+$ and $\hat{E}^-$ which contain the creation $\hat{a}\dagger$ and annihilation $\hat{a}$ operators (see details at Appendix A.2 based on reference [84]) the second order correlation function reads:

$$g^{(2)}(\tau) = \frac{\langle \hat{I}(t)\hat{I}(t+\tau) \rangle}{\langle \hat{I}(t) \rangle^2} = \frac{\langle \hat{E}^-(t)\hat{E}^-(t+\tau)\hat{E}^+(t+\tau)\hat{E}^+(t) \rangle}{\langle \hat{E}^-(t)\hat{E}^+(t) \rangle^2} = \frac{\langle \hat{a}\dagger(t)\hat{a}(t+\tau)\hat{a}(t+\tau)\hat{a}(t) \rangle}{\langle \hat{a}\dagger(t)\hat{a}(t) \rangle \langle \hat{a}\dagger(t+\tau)\hat{a}(t+\tau) \rangle}$$

(2.19)

If the photons are emitted from a single quantum emitter, they are emitted one-by-one. Such a source is called a single photon source. The corresponding state of the light field is a number or Fock state. Fock states describe the electric modes of the quantized light with the defined number of quanta. A Fock state is an eigenstate of number operator $\hat{n} = \hat{a}\dagger\hat{a}$. From the Equation 2.19, the second-order function at $\tau = 0$ is for a Fock state with a photon number $n$ expressed as

$$g^{(2)}(\tau = 0) = \frac{\langle \hat{a}\dagger\hat{a}\dagger\hat{a}\hat{a} \rangle}{\langle \hat{a}\dagger\hat{a} \rangle} = 1 + \frac{V(n) - \langle \hat{n} \rangle}{\langle \hat{n} \rangle^2}$$

(2.20)

where the variance of the photon number is defined with $V(n) = \langle (\hat{a}\dagger\hat{a})^2 \rangle - \langle \hat{a}\dagger\hat{a} \rangle^2$.
It is clear that $g^{(2)}(\tau) = 0$ also gives the hint about the number of emitters. $g^{(2)}(0) = 0$ corresponds to light from a single quantum emitter. In order to define a "good" single photon source, the measurement value of $g^{(2)}(0)$ should give the value $g^{(2)}(0) < 0.5$. The intensity fluctuation and thus the second order correlation function $g^{(2)}(\tau)$ are relatively easy to measure. The measurement setup is a Hanbury Brown and Twist (HBT) setup explained in section 3.1.3.

2.4. Quantum Emitters

In this section, different quantum emitters including quantum dots, organic dye molecules and two dimensional (2D) materials are discussed briefly. As a single quantum emitter which emits a single photon at a time, colloidal quantum dots and 2D material hexagonal boron nitride [105] are used in order to measure the quantum efficiency and to investigate the photophysical properties. In order to develop devices for applications in quantum communication and cryptography, these types of quantum emitters are an important resource. Moreover, the photophysical properties and quantum efficiency of organic dye molecules are also investigated in ensemble levels [106].

2.4.1. Quantum Dots

Quantum dots are among the most promising single photon sources [107, 108], and are also known as "artificial atoms" due to discrete energy spectra formed by quantum confinement. Quantum dots can be excited electrically and optically [109, 110] (Figure 2.4). During the excitation, the electron jumps from valence to conduction band, which leads to recombination where electron-hole pairs are formed to relax to the lowest energy states [111]. The exciton, biexciton and multiexciton states are dependent on the population process and can be observed by using spectral filtering.

Figure 2.4. a) Schematic diagram of the device based on colloidal quantum dots. b) $g^{(2)}(\tau)$ function of a quantum dot. (Taken from [110])
Quantum dots are fabricated in different ways. The first type of quantum dots is produced using molecular beam epitaxy (MBE) by self-assembly where it is grown on various substrate. Epitaxially self-assembled quantum dots have a narrow and stable emission and can be integrated to cavities in order to direct its emission [107, 112, 113]. In this thesis, the second type, the colloidal quantum dots fabricated by chemical synthesis are used. The emission wavelength and size of these type quantum dots are tunable [114]. In the chapter 4, the experiment with the colloidal quantum dots are performed.

2.4.2. Fluorescent Molecules

Fluorescent molecules or organic dye molecules have an energy diagram with different transitions as depicted in a typical Jablonski diagram (Figure 2.1). Fluorescent molecules can be used as a single photon source in the anti-bunching experiment for applications quantum communication and information [115, 116] (Figure 2.5). In order to stabilize the emission of these type of aromatic molecules, the molecules are embedded in the host materials at either room [117] or cryogenic [118, 119] temperatures. When many molecules are embedded in the host materials at cryogenic temperatures, the zero-phonon line (ZPL), a transition from the lowest vibrational level of ground state to the lowest vibrational level of the excited state, can be observed [120]. However, the ZPL usually does not show as the molecules tend to have broader emission, which is not lifetime limited. The photophysical properties such as quantum efficiency, lifetime and spectrum play an important role also for the application in microscopy [121], biological labelling and imaging [8, 122]. However, these properties can vary at ambient temperature due to thermal fluctuations. In chapter 5, the photophysical properties of fluorescent molecules as an ensemble are discussed with the experimental results.

Figure 2.5. a) Confocal fluorescence image of single terylene molecules embedded in crystalline p-terphenyl. b) Second-order intensity correlation function of the emitted photon from single molecule. (Taken from [123])

2.4.3. Defects in 2D Materials

Atomically thin two-dimensional (2D) materials have emerged as a new type of luminescent material in the field of optoelectronics, photonics, and quantum technologies [124, 125]. Due to electronic and optical properties through a layered structure, the luminescent
property of 2D materials and van der Waals heterostructures are characterized in order to introduce these materials in the development of various applications such as photovoltaic devices and light emitting diodes [126, 127]. As a single photon source, defects in transition metal dichalcogenides (TMDs), molybdenum disulfide (MoS$_2$), tungsten diselenide (WS$_2$) and hexagonal boron nitride (h-BN) have been investigated in recent years [128–130]. More details of 2D materials are discussed in chapter 6 with theory and experimental results.

Figure 2.6. a) Simplified schematic of the PL setup showing the excitation and emission of a defect center in a h-BN lattice. b) Second-order intensity correlation function of the emitted photon from a defect center in h-BN. (Taken from [130])
Experimental Setup and Techniques

In this chapter, the experimental techniques and equipments are described in detail. A scanning confocal optical microscope capable to perform fluorescence-lifetime imaging (FLIM) was designed and built with diffraction-limited spatial resolution and single photon sensitivity in order to perform the Drexhage-type experiment with a metal coated sphere. The microscope is explained in detail regarding the choice of each individual component and with respect to its use in time-correlated single photon counting (TCSPC). Furthermore, atomic force microscopy (AFM) is briefly explained for the purpose of measuring the surface topography and its application in the Drexhage-type experiment. In the end of this chapter, the techniques for relative and absolute optical measurements of the quantum efficiency of fluorophores are presented. The fluorescence spectrometer and integrated sphere located at Bundesanstalt für Materialforschung und -prüfung (BAM) are explained in detail.

3.1. Scanning Confocal Optical Microscope

3.1.1. The Confocal Principle

A conventional wide-field fluorescence microscope measures the amount of scattered or emitted light at each point of the specimen simultaneously [131]. However, when the entire specimen was illuminated at the same time, every point of the specimen would have a large background noise from out of focus plane. In order to prevent the emission from above and below the focal plane of the light source and to increase the signal to noise ratio, Marvin Minsky [132] came up with a new approach in which only one point is excited at a time by using a microscope objective lens to focus the light on an aperture pinhole.

The basic principle of confocal fluorescence microscope is shown in Figure 3.1. The confocal microscopy is mostly established in a fluorescence microscope, where a laser is used as a light source to excite fluorophores. The laser beam is focused by a microscope objective lens and the emission of fluorophores located at the in-focus plane (red lines) is detected after a pinhole in the image plane. Figure 3.1 shows that the emitted light (orange and green dashed lines) from out-focus planes is blocked by the pinhole. For this matter, the size of pinhole plays an important role in determining how much background light is being suppressed.

In order to obtain an image, scanning of the specimen is required. The scanning can be done in two ways. A first method is to move the specimen along x-y plane, while the
The principle of confocal microscope. Light coming from out-of-focus planes (orange and green dash line) is suppressed by a pinhole whereas light coming from in-focus plane (red line) passes through a pinhole.

excitation beam is kept stationary. The second method is scanning with the laser while the specimen is kept stationary [133].

3.1.2. Optical Detection

Figure 3.2 shows schematically the different components of the home-built fluorescence confocal scanning microscope. As a light source, a pulsed or continuous wave (CW) laser is used to excite fluorophores on the specimen. The laser light propagating along the optical path is collimated to form the excitation beam with a help of a fiber coupler. The excitation beam is aligned with mirrors and its diameter is increased by using a telescope system to match with the numerical aperture (NA) of an objective lens. The telescope system consists of two coated lenses with 30 mm and 150 mm focal length (Thorlabs GmbH), which are placed between the light source and beam splitter. The excitation beam is directed with a beam splitter to the microscope objective lens with high NA which focuses the beam onto the specimen.

To focus the excitation beam onto the specimen, the z-axis is controlled by a translation piezo (MIPOS 100, Piezosystems Jena, Germany). To demonstrate a two-dimensional (2D) confocal scan, the specimen is moved with the x-y translation piezo stage (PXY D12, Piezosystem Jena, Germany). All translation stages for x-, y- and z-axis are controlled by the piezo controller amplifier (NV 40/3 CLE, Piezosystem Jena, Germany). The beam splitter (90:10) is used and 10% of the excitation beam reaches the specimen. 90% of the emitted
light coming from the specimen is collected by the same microscope objective lens to follow the same optical path. The emitted photons from the specimen are separated from the excitation beam with the suitable filters.

The emitted light collected from any point of the specimen is focused with a 100 mm focal length achromatic lens (Thorlabs GmbH) into a 50 μm confocal pinhole (Thorlabs GmbH) to block out-of-focus light as it is explained in the previous section. After a second 100 mm focal length achromatic lens (Thorlabs GmbH), the collimated beam is directed by a mirror to the single photon detectors, camera or spectrometer. In order to focus the excitation beam, the position of the beam along the z-axis and visual view of the specimen are viewed on the camera with the help of a flip mirror.

Figure 3.2. Sketch of the experimental setup. BS stands for beam splitter, NA for Numerical Apperture, FM for flip mirror, LP for long pass filter, APD for avalanche photo diode and HBT for Hanbury Brown and Twiss setup.

By using a second flip mirror and a 100 mm focal length achromatic lens, the emitted photons can be guided into a monochromator or a spectrometer (Acton 2500i, Princeton Instruments, USA). The spectral components are imaged onto a cooled CCD camera chip (Andor Newton). After the scan, when the specimen shows the region of interest, the specimen can be moved with a piezo controller for local spectral analysis. There are gratings with 1200 grooves per mm or 300 grooves per mm. The one with higher groove density
provides better resolution whereas the one with less grooves is better for dispersing weak signals.

For single photon detection, the emitted photons are guided by a 30 mm focal length achromatic lens into the small active area of each of the two avalanche photodiodes (APD) in the HBT setup. APD1 (Laser Component Blue Series) and APD2 (SPCM-AQR-14, PerkinElmer) are used with dark count rates 50 and 100 count/s respectively.

3.1.3. Time-Correlated Single Photon Counting

Time-correlated single photon counting (TCSPC) is a well established and a common technique to characterize fluorophores by measuring the temporal properties. Briefly, the main principle of TCSPC is based on the detection of single photons upon excitation by a laser pulse and the measurement of the arrival time statistics down to picosecond resolution. Therefore, TCSPC method requires a high repetitive light source (a pulse laser) and a single photon detector. APDs are mainly used in order to register the photon in time domain by converting the optical events to electrical pulses.

Figure 3.3a shows how the photon statistics is calculated for each cycle of incoming pulses. The repetitive short laser pulses excite the fluorophore which emits a photon and the time difference between incoming pulse and emitted photon is measured by electronics which can be compared to a fast stopwatch. The laser pulse can be defined as START and the emitted photon can be defined as STOP. The time difference between START-STOP sequences over many cycles in short time can be represented with a histogram which gives the decay behaviour of the fluorophore. The readings from the stopwatch are sorted into histograms with a range of time bins. The time bin width is equivalent to the resolution of the stopwatch in the range of picoseconds which can be changed as well.

Measurement of $g^{(2)}(\tau)$

In order to find single photon emitters, the second order correlation function $g^{(2)}(\tau)$ is determined by measuring all arrival times of the emitted photons and calculating the autocorrelation function. However, due to higher dead time of photon detectors than the lifetime of the emitters or the electronics limitation, the Hanbury Brown and Twiss (HBT) setup with two detectors is used to overcome those limitations. The 50/50 beamsplitter is used to split the beam into two different optical paths. A first APD (APD1) and a second APD (APD2) are used to determine the START and STOP events, respectively. Similar to TCSPC method, APD1 starts the measurement and APD2 stops the measurement when the photons are detected by each detector. The time intervals $\tau$ are then analyzed by a correlator (PicoHarp300, PicoQuant) and sorted into a histogram as shown in Figure 3.4.

Fluorescence Lifetime Imaging

In the previous section, it is explained how TCSPC is used to determine the lifetime of fluorophores. The lifetime of fluorophores depends on how long an excited atom remains in its
3.1. SCANNING CONFOCAL OPTICAL MICROSCOPE

![Diagram showing a) Measurement of start-stop times in time-resolved fluorescence measurement with TCSPC. b) Histogram of Start-Stop Measurements with TCSPC.](image)

...excited state before returning to the ground state by emitting a photon. The lifetime is an important parameter for each fluorophore which can vary due to the environments of fluorophores and external effects like pH, temperature or conjugating to the other structure. Therefore, in order to observe lifetime changes due to environment, one has to combine lifetime measurement and imaging, i.e. to perform Fluorescence Lifetime Imaging Microscopy (FLIM). To form the FLIM image of a specimen, the lifetimes of fluorophores are recorded and encoded as different colours for each point on the specimen.

In order to extend the TCSPC measurement, time tagged time resolved (TTTR) can be applied. In addition to measuring the time (t) of photon detection relative to the laser excitation pulse each detection event is tagged with the time (T) relative to the start of the experiment. This kind of measurements was carried out by using the time correlator (PicoHarp 300, PicoQuant). Moreover, it helps us to perform the FLIM measurement as the spatial origin of the photon is recorded as well. Furthermore, a TCSPC measurement should be completed for each pixel of specimen to create a FLIM map. To create a FLIM image, the time correlator is also fed by a piezo scanner with TTL signals (section 3.1.2) as markers (M) for spatial positions. These markers contain the information about the scanner’s position. Figure 3.5 shows how those signals are added in the TTTR data stream. The TTTR records with the position markers then allow creating a FLIM map. Hence, the emitted photons events are histogrammed at each pixel of scanned specimen and can be fitted a convenient exponential decay to construct a lifetime map.
EXPERIMENTAL SETUP AND TECHNIQUES

Figure 3.4. Sketch of a HBT setup. The photon pulse is split up into two arms and detected by two APDs. The detection events are sent to a time-correlator a time delay device is inserted into the signal channel from APD to time-correlator.

Figure 3.5. A principle of TTTR measurement. START-STOP times (t), arrival time of photon with respect to the beginning time of the experiment (T), channel information (CH) records the photon information, external marker characterizing the movement of the scanner (M).
3.2. Atomic Force Microscopy

The Atomic Force Microscope (AFM) is a well known instrument used for studying the surface topography of samples with a scale size ranging from microns even down to picometers with a very high resolution better than the optical diffraction limit [135, 136]. The AFM was invented and developed by Gerd Binning and Heinrich Rohrer at IBM Research in 1982 and first experiments were carried out by Binning, Quate and Gerber 4 years later [137]. Then, after the AFM was commercialized in 1989, it has played an important role for the application in the material science and nowadays in nanotechnology [138, 139]. In order to characterize the material surfaces, the adhesion strength, sample roughness, height profile and surface potential can be measured with AFM by measuring the force between a sample and AFM cantilever. The main components of an AFM are the cantilever with various tips, the scanning unit, the optical detection system and the feedback electronics.

3.2.1. Principles

The cantilever in the size of microns acts as a force sensor in the AFM. The bending of the cantilever indicates as a force between the surface and the AFM tip. Repulsive Coulomb or Pauli forces and attractive van der Waals forces push or pull the AFM tip in the direction of the surface when the tip approaches the sample [140]. During the approach, the attractive forces exceed the hardness of cantilever and pull the AFM tip away from or towards the surface. The resulting force depends on the distance of the tip to the surface and can be roughly described by a Lennard-Jones potential [141]. The amount of bending and thus the strength of the force can be measured by a laser. The laser hits the cantilever and the light is reflected to a quadrant photodiode. The reflected light on the photodiode can be utilized as a feedback for the piezo element, which determines the height of the cantilever (Figure 3.6a).

![Figure 3.6. Schematic representation of an Atomic Force Microscope b) An illustration of contact mode operation. The tip moves along a line of constant force. c) The tapping mode operation. The tip oscillates over the surface with a controlled amplitude.](image-url)
3.2.2. Imaging Modes

The AFM can be operated in different modes. The most often used modes are the contact mode and the intermittent contact (tapping) mode.

Contact Mode

In this mode of AFM operation, also known as static mode, the AFM tip is attached to the end of a cantilever with a low spring constant and the tip is continuously in contact with the sample. A constant force is maintained while the piezo regulates the height, which is adjusted in a way that the force and hence the distance to the sample remains constant. During scanning the sample in the lateral direction, the tip is moved across the surface as shown in Figure 3.6b. The contours of the surface are measured by using the feedback signal required to keep the cantilever at a constant distance from the surface [142].

Tapping Mode

The tapping or intermittent mode is the dynamic mode, which is used to prevent the tip from sticking to the surface and to protect the sample damage from the lateral forces [142]. In this mode, the tip is intermittently tapped to the surface with a frequency much faster than the lateral scan and the cantilever is vibrating close to its resonance. The amplitude and the phase of the oscillation are changed when the tip interacts with the sample as shown in Figure 3.6c. The amplitude of the oscillation varies on the order of hundreds of nanometers and is used as a control signal for the piezo, which changes the height of the sample [143].

3.3. Spectroscopic Analysis

3.3.1. Integrating Sphere

The photoluminescence quantum efficiency of fluorophore solution was absolutely measured at room temperature with a commercial integrating sphere setup (C9920-02, Hamamatsu Photonics K.K.). This commercial setup consists of a 150 W Xenon light source with full width half maximum (FWHM) 10 nm, a monochromator and a CCD spectrometer as shown in Figure 3.7. To excite the samples, a monochromatic light source coupled to a Xenon light source, was used and the excitation light was guided by using a bundled fiber to the integrating sphere with inner diameter 8.38 cm. The excitation wavelength from 250 nm to 800 nm is defined by the user. The inner surface of this integrating sphere is coated with Spectralon with spectral reflectivity almost 99% in the wavelength range from 350 nm to 1500 nm. The first reflection of the excitation light and the emission are prevented by Spectralon in front of the integrating sphere’s detection port. 10 mm x 10 mm long-necked quartz cuvettes with 10 cm neck length (Hamamatsu Photonics) were placed into the cuvette holder of the integrating sphere with the help of a positioning device. A CCD spectrometer operates in the spectral range from 250 nm to 950 nm. The sensor is
cooled to -15°C with an air cooled element. Therefore, thermal noise can be reduced and the signal-to-noise ratio is increased [144, 145].

![Diagram of an integrating sphere](image)

**Figure 3.7.** The schematic diagram of an integrating sphere for measuring the absolute quantum efficiency.

In order to perform the measurement, an 8 mm diameter light spot illuminates the fluorophore solution filled cuvette. To determine the absolute quantum efficiency, the excitation peak and the emission spectrum of both the fluorophore solution (e.g. Rhodamine 6G in Ethanol) and a blank solution (e.g. only Ethanol) under the same excitation wavelength are measured for each scan. The wavelength accuracy of the setup, the linearity of the detection system, and the spectral responsivity of the integrating sphere-detector assembly (emission correction) were determined by the instrument manufacturer in Japan and controlled by us. The spectral responsivity (spectral emission correction) is implemented to obtain the number of emitted photons from the solution and these emission correction curves that were incorporated in the data evaluation software, were automatically applied to the measured data. The fluorescence quantum efficiencies were calculated from the spectra of the blank (solvent-filled cuvette) [47].

### 3.3.2. Absorption and Emission Measurement

In order to calculate relative quantum efficiency of fluorophore, the absorption and emission measurements were performed at a temperature of 25°C using 10 mm x 10 mm quartz cuvettes (Hellma GmbH).

**Absorption Spectrometer**

Absorption spectra of fluorophore solutions were recorded at room temperature on a calibrated Analytik Jena spectrometer at BAM. The accuracy of the intensity and wavelength scale of this instrument was regularly controlled. The absorbances of fluorophore solutions
used for quantum efficiency calculations were in the range of 0.05 to 0.1 in order to minimize the reabsorption and other effects which may influence the quantum efficiency [38].

**Fluorescence Spectrometer**

The emission measurements were performed with a fluorescence spectrometer (FLS-920, Edinburgh Photonics) at BAM. The emission spectra were recorded under magic-angle conditions where the excitation polarizer and emission polarizers were set to 0° and 54.7° respectively in order to render emission intensities independent of the emission anisotropy of the emitter. All emission spectra were corrected for blank emission and scattering (blank correction) and the wavelength dependence of the instrument’s spectral responsivity (spectral correction) [39].

**Time Resolved Measurements**

Fluorescence decay curves were recorded with a FLS-920 fluorescence lifetime spectrometer (Edinburgh Instruments) by using 10x10 mm quartz cuvettes. A super-continuum laser (Fianium SC400-2-PP) was employed for excitation at desired wavelength with a pulse repetition rate of 20 MHz using TCSPC without polarizers. The emission peak was detected at the emission maximum of each sample employing a spectral bandwidth of the emission monochromator of 6 nm. The calculated lifetimes represent the time after which the initial fluorescence intensity decreased to the 1/e part of its maximum value.
In this part of the thesis, the theoretical background information of the colloidal quantum dots is introduced with the basic concepts including the size-dependence of the energy level structure and their influence on the optical properties. In particular core-shell colloidal quantum dots are discussed with the main electronic properties described by modeling the excited electron and hole as particles in a spherical box using the effective mass approximation. The aim is to observe single quantum dots embedded in a polymer matrix at ambient temperature by using the homemade scanning confocal optical microscope based time correlating single photon counting. Fluorescence spectra and lifetime measurement are performed to characterize individual quantum dots and antibunching measurements show the quality of single quantum dots as single photon sources. The blinking behavior and stability of colloidal QDs under pulsed excitation are also discussed.

4.1. Colloidal Quantum Dots

Colloidal quantum dots (CQDs) are luminescent nanocrystals (NC) made from a semiconductor material in nanoscale dimensions (usually smaller than 20 nm in diameter) which determine their optical and electronic properties [146, 147]. Due to these small volumes, CQDs show strong quantum confinement effects which result in discrete energy levels similar to atomic energy levels. Hence, luminescent NCs are often referred to as quantum dots. CQDs consist of an inorganic core with 100 to 10000 atoms, surrounded by a shell of a different semiconductor as shown in Figure 4.1. In solution the CQDs are stabilized by organic ligand molecules. In order to design CQDs with different optical properties such tuning the energy level and emission wavelength across the visible spectrum range, the size, shape and chemical composition can be changed by using the size effect [148, 149].

The optical and structural properties of CQDs also depend largely on their surface due to large surface to volume ratio. In order to improve quantum efficiency and stability of the CQDs and change the optical properties, the passivation of the core via ligands might be required. On the other hand, the environment of CQDs such as solvent has to be considered as the organic ligands are soluble in chosen solvents. Various types of CQDs can be synthesized differing in size, shape and material yielding optical properties [149].
4.1.1. Heterostructures of CQDs

Regarding the core/shell heterostructure, the CQDs are categorized into two types based on the variation at the conduction and valence band as shown in Figure 4.1. The excited electrons and holes wavefunctions are confined in the same region of the structure, mostly the core region of the CQDs, in a Type-1 heterostructure. The radiative recombination probability is increased due to enhanced overlap of wavefunctions. On the other side, the excited state electrons and the holes are confined in a different region of crystals in a Type-2 heterostructure. Type-2 CQDs tend to have a reduced radiative recombination probability, longer lifetimes and lower exciton binding energy compared to Type-1 CQDs [150, 151]. Bawendi was the first, who demonstrated the Type-2 electronic structure of CQDs with the synthesis of CdTe/CdSe and CdSe/ZnTe core/shell heterostructures [149, 152, 153].

4.1.2. Energy Level Structure of CQDs

The peculiar optical and electronic properties of CQDs emerge from quantum confinement effects. The bulk semiconductor materials have an intrinsic band gap where electrons can be promoted to the conduction band after excitation with a light source to create a spatially correlated electron-hole pair or exciton [154]. QDs are nanocrystal semiconductor materials which are reduced below the size of the Bohr radius of the exciton ($a_B$). This exciton Bohr radius is material dependent and can be expressed as
where \( m_e \) and \( m_h \) are the effective mass of the electron and the hole, \( e \) the charge of electron and \( \epsilon \) is the material dielectric constant [155]. To simplify the expression, \( m_E \), the effective mass of the exciton is used. The Bohr radius of the exciton define the quantum confinement regimes for QDs. When the radius \( a \) of CQD is greater than \( a_B \), the weak confinement regime is given.

In the case that the size of spatial confinement in CQDs is close to the Bohr radius, the effects of quantum confinement become more dominant, and the energy levels in the valance and conduction bands start to split in electronic transitions with a tunable effective band gap energy, which can be controlled by simply changing the size and shape of the CQDs [156, 157]. The effective band gap energy of semiconductor nanocrystals increases with decreasing size of the CQDs as shown in Figure 4.2.

\[
a_B = \frac{\hbar^2 \epsilon}{e^2} \left[ \frac{1}{m_e} + \frac{1}{m_h} \right] = \frac{\hbar^2 \epsilon}{e^2} + \frac{1}{m_E}
\]

(4.1)

**Figure 4.2.** Energy bands in a bulk semiconductor and size-dependency of energy level in colloidal quantum dots (CQDs). In this simplified energy level diagram, the same bulk semiconductor material is compared with CQDs of various sizes. The bandgap and the difference of energy levels increases with decreasing size. The bandgap of a CQDs depends on its size which determines the wavelength of the emitted photon.

If the radius of CQDs is smaller than \( a_B \), the excitons are strongly confined in the three dimension. In order to explain what happens in the nanoscale size range, the spherical particle in a box model is assumed with physical boundaries [158]. The wavefunction for CQDs can be written as

\[
\psi(\mathbf{r}) = \phi_e(\mathbf{r}) u(\mathbf{r})
\]

(4.2)

where \( u(\mathbf{r}) \) is a cell periodic function of the conduction (valence) band and \( \phi_e(\mathbf{r}) \) is an envelope function. The form of the envelope function can be expressed as a plane wave
[\phi_e = e^{i k \cdot \hat{r}}] for Bloch wavefunctions in a bulk crystal. It is also assumed that the electronic bands are isotropic and parabolic. The confinement energy $E^c$ can be calculated by solving the particle in a spherical quantum box for the envelope function which obeys the Schrödinger equation:

$$
\left[ -\frac{\hbar^2}{2m_E} \nabla^2 + V(\hat{r}) \right] \phi_e(\hat{r}) = E^c \phi_e(\hat{r})
$$

(4.3)

where $m_E$ is the effective mass as mentioned above. $V(\hat{r})$ is the effective potential energy term which describes the size and shape of the CQDs:

$$
V(r) = \begin{cases} 
0, & r \leq a, \\
\infty, & r > a 
\end{cases}
$$

(4.4)

where $a$ is the radius of the CQD with infinite barriers. The envelope functions can be separated into a radial and an angular component and the wavefunction $\phi_e$ is expressed by

$$
\phi_e(r, \theta, \vartheta) = R_{n,l}(r) Y^m_l(\theta, \vartheta) = C_{n,l} j_l(k_{n,l} r) Y^m_l(\theta, \vartheta)
$$

(4.5)

where $C$ is a normalization constant, $j_l(k_{n,l} r)$ is the $l$th order spherical Bessel function and $Y^m_l(\theta, \vartheta)$ is a spherical harmonic. $k_{n,l}$ is defined as $\beta_{n,l}/a$ with $\beta_{n,l}$ the $n$-th zero of $j_l$. For the case of infinite barriers ($V(r) \to \infty$), the confinement energy of exciton ($E^c_{n,l}$) as sum of eigenenergies of the electron $E^e_{n,l}$ and hole $E^h_{n,l}$ is given by

$$
E_{n,l} = E_g + E^c_{n,l} \\
= E_g + E^e_{n,l} + E^h_{n,l} \\
= E_g + \frac{\hbar^2 \beta^2_{n,l}}{2m_e a^2} + \frac{\hbar^2 \beta^2_{n,l}}{2m_h a^2} \\
= E_g + \frac{\hbar^2 \beta^2_{n,l}}{2m_E a^2}
$$

(4.6)

with $E_g$ being the band gap of the bulk semiconductor. The $(2l+1)$ degenerate energy levels are shown and the 1s, 1p and 1d states have smaller eigenenergies than the 2s state. For a small sized particle, only the first states will be considered. Since $a < a_b$, the electron and hole interact with each other through the Coulomb attractions and this has to be taken into account. This can be added to the above expression as a Coulomb correction factor to the exciton energy $E_X(a)$. For a ground level, the exciton energy can be written as

$$
E_X(a) = E_g + E^c_{1,0} + E_C \\
= E_g + \frac{\hbar^2 \pi^2}{2m_E a^2} - 1.79 \frac{e^2}{4\pi \varepsilon a}
$$

(4.7)
where \( \epsilon \) is the dielectric constant of material. The number 1.79 is calculated from an integration of the Coulomb interaction over the position of electron and hole [153, 159, 160].

4.2. Experiments on Single Colloidal Quantum Dots

4.2.1. Cleaning and Spin Coating of CQDs

As a first step of the preparation procedure, microscope cover glasses (Menzel-Gläser) with the size of 24mm × 24mm were roughly cleaned by ethanol and acetone. Then, rinsed cover glasses were placed in a surface-functionalizing Hellmanex solution of 3% concentration in distilled water and sonicated for 30 minutes at 80 kHz with a temperature of 40 °C. The cover glasses were again rinsed with distilled water and then dried with nitrogen flow.

The CQD samples were prepared by spin coating a solution of CdSe/CdS suspended in hexane on the cleaned cover glasses. The highly photostable CdSe/CdS are used as they are one of the most studied and best developed core/shell QD systems. They have size-dependent wavelength tunable emission in the visible part of the spectrum and better photostability than single organic molecules. The quantum dot solution was further diluted with hexane to obtain lower concentration of quantum dots. Then, 50 µL of the final solution were dropped onto the middle of the cover glass which was placed on a spin coating machine. The quantum dot solution was finally spun on the cover glass at 3000 rpm for 1 minute. 0.6% w/w of Poly(methyl methacrylate) shortly PMMA (Sigma Aldrich) in a chloroform solution was later spin coated to keep the QDs from getting photo oxidized.

4.2.2. Observation of Single CQDs

In order to observe single QDs, the homemade fluorescence confocal microscopy based TCSPC (system in Figure 3.2) was used. A continuous wave (CW) laser operating at 532 nm was employed as an excitation source. The QDs were illuminated through an objective lens with a NA of 0.95. The emitted fluorescence photons were collected by the same objective lens and transmitted through a 575 nm long pass filter to suppress the excitation light. The fluorescence light was spatially filtered by focusing through a 50 µm pinhole and focused onto detectors (for lifetime or autocorrelation measurements), or alternatively to a spectrometer (for taking a spectrum) or charged coupled device (CCD) camera (to focus the laser beam) by using flipping mirrors.

In order to find single quantum dots on the cover glass, scanning was performed by moving the piezo stage. The power of the excitation light was 1 µW in front of an objective lens. Figure 4.3a is a 2D scan showing three different single QDs. The photoluminescence signal of each individual QD in each frame was measured as the maximal gray value in the analysis area of approximately 0.75 × 0.75 µm². If two neighboring QDs were far away from each other such that the distance between them was larger than 0.5 µm, the crosstalk or other effects could be eliminated between those QDs.

QD #1 was chosen to verify photon emission from a single emitter by measuring the second order correlation function \( g^{(2)}(\tau) \). Therefore, the emitted photons from QD #1 were
Figure 4.3. a) Photoluminescence images for single quantum dots covered by a PMMA thin film labelled as QD #1, QD #2 and QD #3. b) Autocorrelation function $g^{(2)}(\tau)$ of QD #1 measured with the Hanbury-Brown-Twiss setup. The red curve is a fit to the data with $g^{(2)}(\tau) = 0.14$.

directed to the Hanbury-Brown-Twiss setup as shown in Figure 3.4. Figure 4.3b demonstrates the $g^{(2)}(\tau)$ obtained from the single quantum dots. The signal-to-noise ratio in this measurement was satisfying as the QD #1 was stable over 3 minutes. The experimental data was fitted to the Equation 4.8.

$$g^{(2)}(\tau) = 1 - (1 - A)e^{-|x-x_0|^2/\tau_1} + Be^{-|x-x_0|^2/\tau_2}$$

where $A$ and $B$ are the bunching amplitudes for times $\tau_1$ and $\tau_2$ respectively. $\tau_1$ and $\tau_2$ are the antibunching time constants. $x_0$ is an offset parameter for the time $x$. From the fit parameters, the dip to zero at delay $\tau = 0$ was calculated as $g^{(2)}(0) = 0.14$ which shows that fluorescence is emitted predominantly by a single emitter. The photon statistics play an important role for characterizing a single photon source. Typically, $g^{(2)}(\tau)$ should be as low as possible. However, a commonly used criterion for a single photon source is $g^{(2)}(\tau)$ being below 0.5.

Figure 4.4. a) Emission spectra of the three individual QDs shown in Figure 4.3a. b) Lifetime measurements of three individual QDs.
After the emission from a single quantum dot was shown, the next important steps were to measure the lifetime and spectrum of each individual quantum dot. Fluorescence spectrum and fluorescence lifetime measurements of CQDs were then performed by using a pulsed laser (LDH-P-FA 530, PDL800-D, PicoQuant GmbH) operating at 532 nm with a repetition rate of 2.5 MHz or 5 MHz, an excitation power of around ~ 3 µW and a pulse width below 100 ps as an excitation source. In order to measure the spectrum, the emitted photons from individual QDs were directed to the spectrometer by using flipping mirrors. Therefore, the fluorescence spectra were taken from each individual quantum dot during 90 seconds as shown in Figure 4.4a. The emission maximum of each QD was measured as 630 nm (QD #1), 621 nm (QD #2) and 616 nm (QD #3). The emission peaks of each single QD range from 615 to 635 nm, with FWHM of 20-30 nm.

After the spectrum was taken, the emitted photons were directed to an avalanche photodiode (APD) from Laser Component Blue Series to perform the lifetime measurements. Single photon counting (TCSPC) was carried out using a time correlator. Figure 4.4b shows the fluorescence decay curves of each quantum dot. These fluorescence decays were measured by conventional TCSPC and their fluorescence lifetime were measured as 33.85 ns (QD #1), 40.95 ns (QD #2) and 42.05 ns (QD #3).

4.2.3. The $g^{(2)}$ Measurement and Blinking

The autocorrelation measurements of photons from individual CQDs were also performed under pulsed excitation. The pulse excitation source was employed at 532 nm with a repetition rate of 5 MHz for QD #1 and 2.5 MHz for QD #2 and QD #3. The left side of the Figure 4.5 shows the corresponding photon intensity correlation histograms. The suppression of the peak near zero time delay was clear evidence of the photon anti-bunching characteristics of light from a single quantum dot. The second order autocorrelation function $g^{(2)}_{\text{pulse}}(\tau)$ was fitted to Equation 4.9. From the fitting parameters, the dip to zero at delay $\tau = 0$ was calculated with $g^{(2)}_{1}(\tau) = 0.15, g^{(2)}_{2}(\tau) = 0.07$ and $g^{(2)}_{3}(\tau) = 0.03$ for QD #1, QD #2 and QD #3 respectively.

$$g^{(2)}_{\text{pulse}}(\tau) = A_{1}e^{-|x-(x_{0}-C)|\tau_{1}} + A_{0}e^{-|x-x_{0}|\tau_{0}} + A_{1}e^{-|x-(x_{0}+C)|\tau_{1}} + A_{1}e^{-|x-(x_{0}+2C)|\tau_{1}} + A_{1}e^{-|x-(x_{0}+3C)|\tau_{1}} + A_{1}e^{-|x-(x_{0}+4C)|\tau_{1}} + A_{1}e^{-|x-(x_{0}+5C)|\tau_{1}}$$

where $A_{0}$ and $A_{1}$ are the bunching amplitudes for time $\tau_{1}$ and $\tau_{2}$ respectively. $\tau_{1}$ and $\tau_{2}$ are the antibunching time constants. $x_{0}$ is an offset parameter for the time $x$. $C$ expresses the period between laser pulses.

Figure 4.5 shows blinking measurement of QD #1 (d) QD #2 (e) and QD #3 (f). The emission intensity for each of QD was recorded to show an on-off behaviour during transition between the bright and dark states of single QDs. The time trace could be interpreted as binary by setting every intensity value as ON (OFF) when it is above (below) the threshold. Although the emission intensity of each QD was recorded for 5 minutes, the number of
OFF states were less than ON states. Photobleaching was not observed demonstrating an excellent photostability of QDs.

Figure 4.5. Autocorrelation function $g^{(2)}(\tau)$ of a) QD #1 b) QD #2 c) QD #3 measured with the Hanbury-Brown-Twiss Setup. The red curves are fits to the data with $g^{(2)}(\tau)$ d), e) and f) show typical fluorescence blinking of each single QD.

4.3. Summary

In this chapter, colloidal quantum dots (CQDs) (CdSe/CdS) were characterized to test the measurement capability of the homemade confocal setup and verify the results. Samples with a low density of CQDs and very low intrinsic background fluorescence were prepared. It was possible to detect and characterize single quantum dots. Photon autocorrelation measurements proved single photon emission. Lifetimes, spectra and blinking dynamics were recorded from individual dots.
Measurement of Quantum Efficiency of Organic Dyes

This chapter deals with controlling the local density of optical states (LDOS) and measuring the quantum efficiency of the organic dyes in different nanoenvironments absolutely by using a metal coated sphere. By controlling the modification of the LDOS, the quantum efficiency is extracted without reference material as an alternative technique. The results with the pH-sensitive fluorophores in mesoporous silica thin films have been published in The Journal of Physical Chemistry C with the title Quantitative Measurements of the pH-sensitive Quantum Efficiency of Fluorophores in Mesoporous Silica Thin Films using a Drexhage-type Experiment [161]. This chapter is largely built on this reference.

5.1. The Mirror Method

5.1.1. Mirror Preparation

A high tolerance half-ball lens (Edmund Optics) with a diameter of 2mm with a diameter tolerance ±0.05 µm is used as inspired by Lunnemann’s method [77]. Due to the limitation of scanning range at our setup, we used the uncoated half-ball lens with a diameter of 2 mm instead of 4 mm. The half-ball lens is coated by sputtering of a 10 nm Titanium adhesion layer, followed by 100 nm Ag as a metal layer. Finally, as a protection against scratches and oxidation, a 35 nm layer of SiO$_2$ was sputtered as a spacer layer between the silver and emitters on the Ag-coated sphere. The coating procedure was carried out by the research group of Prof. Ronen Rapaport at The Hebrew University of Jerusalem in Israel. In order to simplify handling and prevent the rolling of spheres, the flat sides of three identical spheres were glued to 1cm × 1cm glass cover in tripod configuration [161].

5.1.2. Drexhage-type Setup

The setup used to perform the Drexhage-type experiment is shown in Figure 3.2. It is an inverted confocal microscope enabling time-correlated single photon counting (TCSPC) detection with single molecule sensitivity in order to conduct lifetime measurement, therefore FLIM, similar to what is described by P. Lunnemann et al. [77] for the determination of $\Phi_{PL}$ values of quantum dots (CdSe/CdS nanorods). Our setup was aligned and adapted to measurements of the dye-embedded thin films with various thicknesses up to 30 nm. On top of the transparent sample with the dye-embedded thin film, the previously described
millimeter-sized silver coated half spheres were placed in a tripod configuration. Owing to this configuration, the lateral position of any fluorophore in the measured film corresponds to a certain distance from the silver surface as shown in Figure 5.1. Fluorescence intensity and fluorescence lifetime imaging (FLIM) of the dye-embedded thin film were then performed using a pulsed laser as an excitation source. The laser was operated at wavelength of 532 nm and 470 nm with a repetition rate of 2.5 MHz or 5 MHz for different fluorophores. The excitation power was around ~3 µW and the pulse width below 100 ps. An objective lens with a numerical aperture of 0.95 (Olympus x100) was used to collect the photons emitted by the fluorophore. The collected fluorescence light was separated by a beam splitter and detected with single-photon avalanche diodes (APD) (Laser Component Blue Series) in confocal arrangements using various long pass filters to block the laser and other emission. (see section 3.1.2)

Figure 5.1. Schematics of the Drexhage-type experimental setup. A silver-coated sphere with a SiO$_2$ protective layer was placed on top of the transparent sample, namely the dye-labeled thin film. The zoom-in illustrates the geometry, which was locally approximated as flat layers.

The photon detection events define time tags, which were recorded using a PicoHarp 300 (Picoquant GmbH) data acquisition electronic as shown in 3.1.3. Markers for spatial coordinates obtained from the computer-controlled scanning-stage were also fed to the PicoHarp 300 by using a Labview-Code and were collected within the time tag stream to create the 2D lifetime map. The data in each pixel were histogrammed and fitted mono-exponentially to calculate the apparent fluorescence lifetime for each pixel using SymPhoTime software (PicoQuant GmbH).

5.2. Quantum Efficiency of Nile Red-Doped PMMA

5.2.1. Preparation of Nile Red-Doped PMMA Thin Film

Nile Red (NR) is a hydrophobic and fluorescent molecule which exhibits high solvatochromicity. The absorption and emission spectra of NR vary in different environments. Hence, the lifetime and quantum efficiency value of NR show variations by dissolving a solvent of varying polarity [162–164]. Briefly, the photoluminescence properties of NR are influenced by solvent polarity. NR is used as a probe by using FLIM technique to measure the spa-
tial variation in dielectric micro environment [165, 166], to monitor cell membrane phase transitions [167] and to study cells [168].

Poly[Methyl MethAcrylate] (PMMA) is used as a polymer layer for thin film formation. It provides a high transparency and a refractive index of 1.49. The photophysical properties of PMMA with fluorophores have been used recently for various applications from photonics to different optoelectronics devices [121]. Moreover, it has similar dielectric properties to SiO$_2$ (organic glass) which suggests its use in a Drexhage-type experiment. In order to form a thin film with Nile Red, PMMA is mostly dissolved in a volatile solution such as toluene and chloroform at room temperature. The volatile solvent evaporates during the spin coating, and a uniform thin film with high adhesions formed on top of the desired material on the substrate. It is expected that the film thickness is inversely proportional to the spin rate and proportional to the concentration and viscosity. Therefore, the spin rate and amount of PMMA was chosen to form thin films with a thickness less than 20 nm [169].

A fluorophore-doped polymer solution with 1.6 µM NR (Fluka Chemicals) and 0.6% w/w of PMMA (Sigma Aldrich, $M_W$ 120000) were dissolved in toluene were prepared together. After 20 minutes of sonication of this solution, 20 µL of this solution were dropped on the cleaned cover slips and spin coated for 1 minute at 3000 rpm. Then, a NR-doped PMMA film was formed with a thickness of 16 nm measured with ellipsometry. This concentration of NR was chosen in order to minimize interactions between the NR molecules [170].

5.2.2. Drexhage-type Experiment on Nile Red-Doped PMMA

The Drexhage-type experiment was then performed with the experimental setup described in the previous section. The setup was equipped with an excitation light source at 532 nm (LDH-P-FA 530, PDL-800) and ~3 µW power. An optical filter with 538 nm long pass filter was used to block the emission from the laser. Figure 5.2a shows the fluorescence lifetime imaging (FLIM) data extracted from measurements with a NR-doped thin film. The observed concentric rings are centered with respect to the contact point of the metal sphere and the NR-doped film surface. These concentric rings reflect the increasing radial distance $d$ between the NR molecules in the dye-doped thin film and the sphere surface, which can be regarded as locally flat in the µm range. Points of the FLIM image on the same ring correspond to the same distance $d$.

The real distance $d$ can be derived for any point in the FLIM image from the lateral distance $x$ to the contact point $x_0$ by using the Equation 5.1, where $R$ is the radius of the metal sphere.

$$d(x) = [R^2 + |x - x_0|^2]^{1/2} - R$$ (5.1)

After the real distance $d$ is calculated, the extracted decay rate can be plotted as a function of distance $d(x)$ from the sphere’s metal surface as shown in Figure 5.2b. This result reveals the typical oscillatory behavior as a function of the fluorophore-sphere distance.
In Figure 5.2b, a near-unity scaling factor for $d(x)$ was introduced in order to correct the piezo drifts.

The measured FLIM image depicted in Figure 5.2a demonstrates the local lifetime (decay rate) variations of the NR-doped PMMA thin film on a scale of micrometers with the visible concentric rings. Due to the circular symmetry of the decay rate images (FLIM) around the contact point of the sphere with the thin film, averaging the decay rate of fluorophores along entire rings where the data points correspond to the same NR-sphere distance, can be performed. As an alternative, in the case that either entire rings are not visible or only certain areas of the FLIM image are of interest, averaging can be restricted to data points inside the region of interest (ROI). This shows that the $\Phi_{PL}$ measurements by the Drexhage-type experiment do not necessarily require extremely smooth or large-area films. From the FLIM data shown in Figure 5.2a, $\Phi_{PL}$ can now be derived as shown in [161]. As described in section 2.2.2, $\Phi_{PL}$ can be defined as:

$$\Phi_{PL} = \frac{\gamma_r}{\gamma_r + \gamma_{nr}} = \frac{\gamma_r}{\gamma_{tot}}$$

where $\gamma_r$, $\gamma_{nr}$ and $\gamma_{tot}$ are radiative, nonradiative, and total decay rates, respectively. In front of the mirror only $\gamma_r$ is modified while $\gamma_{nr}$ remains constant as it is discussed in section 2.1.1. Near-field coupling to plasmon excitations is neglected in our calculation. Hence, the total $\Phi_{PL}$ of NR can be derived by fitting the modified fluorescence lifetime $\tau = (\gamma_r + \gamma_{nr})^{-1} = \gamma_{tot}^{-1}$ as a function of the distance $d$ to the theoretical curve, given in Equation 5.3:

$$\gamma_{tot}(d) = \gamma_{tot}^{(0)}[1 + \Phi_{PL}^{\text{NR}}[\rho(d) - 1]]$$

Figure 5.2. a) Fluorescence lifetime imaging (FLIM) map obtained by mono-exponential fitting of the measured fluorescence decay curves. A confocal scan of a 120 x 120 pixel array covering 60 µm to 60 µm was done with step sizes of 500 nm. The resulting concentric rings correspond to fluorophores that have the same distance to the metallized sphere surfaces. b) Decay rate as a function of distance between the mirror and the fluorophores in the NR-doped thin film. The dots represent the measured decay rates, extracted from the decay rate map shown in panel (a). The solid line is the theoretical fit according to Equation 5.3.
5.3. Quantum Efficiency of Dye-Doped Mesoporous Silica Thin Film

Here, $\rho(d)$ is the modified LDOS normalized to the LDOS in the absence of the mirror and $\Phi_{PL}^{\infty}$ is the fluorescence quantum efficiency in the absence of the mirror as explained in section 2.1.3. The LDOS $\rho$ is calculated separately for perpendicular ($\rho_{\perp}$) and parallel ($\rho_{\parallel}$) dipolar emitters corresponding to metal layer. This method allows us to use a free parameter $\alpha$ with $0 < \alpha < 1$. $\rho(d) = \alpha \rho_{\perp}(d) + (1 - \alpha) \rho_{\parallel}(d)$ can be written as a function of $\alpha$. As expected for random emitter orientations and equal probability of excitation for all orientations, $\alpha = 1/3$ gives accurate results for our calculation. Moreover, the radius of the silver sphere is quite large compared to the distance between its surface and the NR molecules in the PMMA thin film. Hence, the experimental configuration is approximated as a planar, layered system consisting of a semi-infinite glass, air, $\text{SiO}_2$ and an semi-infinite Ag layer as shown in Figure 5.1. For this 4-layer system we calculated $\rho(d)$ as described by Amos and Barnes [30, 61, 67, 171, 172]. The center wavelength is predicted based on the emission spectrum of the fluorophore. However, the approximate value of the center wavelength is chosen based on further LDOS calculations, which are discussed in Appendix A.1. The used dielectric permittivites and center emission wavelengths for all fluorophores are given in Table A.1 in Appendix A.1.

From these measurements, $\Phi_{PL}^{\infty}$ with 0.43±0.04 of NR-doped PMMA thin film is derived (average lifetime calculated as $\tau$=4.43 ns). A good fit to the mean decay rate is shown by assuming isotropically oriented dipole moments, in good agreement with earlier result from Blackett Laboratory [173]. In that work, $\Phi_{PL}^{\infty}$ was determined as 0.51 by using an integrating sphere. The authors of reference [173] measured a significantly higher $\Phi_{PL}^{\infty}$ than our result. $\Phi_{PL}^{\infty}$ of NR was highly influenced by its own environment [174] which may explain the deviation.

5.3. Quantum Efficiency of Dye-Doped Mesoporous Silica Thin Film

In order to observe the local $\Phi_{PL}$ as a function of temperature, pH or pressure as a sensor, a fluorophore which is influenced by its environment, has to be embedded in a structure where one can change the pH, temperature or pressure of the environment. As example we use the pH-sensitive molecule fluorescein isothiocyanate (FITC; Aldrich) [175, 176], covalently attached to a thin film of mesoporous silica and measure the local variation of the pH in the range of 5.5 to 7.5. Mesoporous materials like silica are ideal host matrices for analyte-responsive dyes for the fabrication of sensor devices because of their large surface area and pore volume.

5.3.1. Spectroscopic Characterization pH-sensitive Fluorescein

1 mg of FITC dye was dissolved in 1 ml of Milli-Q water as a stock solution. In order to change the pH of the environment of this dye, buffer solutions were used. 0.05 M citrate buffers were prepared by dissolving different amounts of sodium citrate and citric acid in distilled water to obtain pH values of around 5.5, 6.0, and 6.5. The pH of the solution was adjusted accurately through the addition of HCl or NaOH. The exact procedure was applied
again to prepare buffers at pH 7.0 and 7.5 by using trisodium citrate and disodium. In order to adjust the pH values, HCl or NaOH was added as explained above. 5 µL of FITC stock solution was added to the prepared 10mm × 10mm quartz cuvettes filled with different pH buffers.

**Decay Kinetics of FITC**

Lifetime measurements of solutions of the FITC at different pH buffers were performed with a FLS-920 fluorescence lifetime spectrometer by using the buffer filled cuvettes as explained in section 3.3.2. A super-continuum laser at 470 nm was employed with a pulse repetition rate of 20 MHz. The emitted photons were detected at 521 nm of the respective fluorescein species employing a spectral bandwidth of the emission monochromator of 6 nm. The instrument response function (IRF) was measured with a scatterer (a Ludox solution) where both excitation and detection were at 470 nm.

The fluorescence decay kinetics of the fluorescein solutions at various pH values were fitted as explained in reference [177]. The monoeponential and biexponential decay kinetics were used to demonstrate the various protonated emissive fluoresceins types that are the mono- and the dianion. The mono- and dianion show differences in their fluorescence quantum efficiencies and lifetimes. For the monoanion, an Acid Dissociation Constant ($pK_a$) of 4.31, a quantum efficiency of 0.37, and a lifetime of 3.0 ns were measured where as the dianion a $pK_a$ of 6.34, a quantum efficiency of 0.93, and lifetime of 4.1 ns were reported in [175]. Nonetheless, a monoexponential fit at the chosen pH values gave the satisfying $\chi^2$ values of typically less than 1.2 in our experiments even though both anionic fluorescein species should be excited at the chosen excitation wavelength of 470 nm. It is assumed that the monoanion has a noticeably lower quantum efficiency and its contribution to absorption and fluorescence are increasingly reduced at more basic pH values as shown its fluorescence decay curves in Figure 5.3a.

![Figure 5.3](image-url)
5.3. QUANTUM EFFICIENCY OF DYE-DOPED MESOPOROUS SILICA THIN FILM

Relative and Absolute Quantum efficiency Measurement

The fluorescence quantum efficiency of FITC solutions was determined by using the relative and absolute optical methods as mentioned in section 2.2.1. First, the relative quantum efficiency $\Phi_{R,PL}$ was performed. This method relies on the comparison of the absorption and emission spectra of the sample and the reference dye under same conditions. The instruments for measuring the emission and absorption spectra are described in detail in section 3.3.2. The absorption spectrum of FITC solutions at different pH-values were recorded at room temperature with a calibrated Analytik Jena spectrophotometer using the same cuvettes from the lifetime measurements, which were described in previous sections. Emission measurements with FITC solutions at different pH-values at room temperature were performed with a calibrated spectrophotometer FLS-920 from Edinburgh Photonics as described in section 3.3.2. For both the sample and standard, identical measurement settings and an excitation wavelength of 470 nm for FITC were used. Figure 5.3b shows both absorption and emission spectra of FITC solutions. The relative fluorescence quantum efficiencies $\Phi_{R,PL}$ were calculated according to the following Equation 5.4 using fluorescein in 0.1 M NaOH water (quantum efficiency of 0.89) as a quantum efficiency standard [39].

$$\Phi_{R,PL} = \Phi_{st} \frac{F_x}{F_{st}} \frac{f_{st}(\lambda_{ex, st})}{f_x(\lambda_{ex, x})} \frac{n_x^2}{n_{st}^2}$$  \hspace{1cm} (5.4)

where $F$ is the integral photon flux, $f$ is the absorption factor at the excitation wavelength $\lambda_{ex}$ (470 nm), and $n$ is the refractive index of the solvent. The index $x$ denotes the unknown sample, and the index $st$ denotes the known standard [39].

The fluorescence quantum efficiencies $\Phi_{A,PL}$ of FITC at different pH values were measured absolutely with the stand-alone integrating sphere setup C9929-02 from Hamamatsu Photonics K.K using $10\text{mm} \times 10\text{mm}$ long-necked quartz cuvettes employing an excitation wavelength of 470 nm for FITC as the details are described in section 3.3.1 [47]. The number of emitted and absorbed photons required for the determination of the quantum efficiency were obtained. The absolutely and relatively measured fluorescence quantum efficiencies are presented in Figure 5.8.

5.3.2. Mesoporous Silica Thin Film

Owing to properties of high surface-mass ratio and absorption capacity, porous materials play an important role in the life and science. As a host matrix for the organic materials, these porous materials lead to design devices in the catalysis of gas and liquid, and sensing in photonics [178, 179]. Depending on the type of material which is used, the surface area can change and the pore size and pore surface chemistry can be adjusted. Porous materials are categorized into three groups depending on the size of pores. Based on the description in reference [180], microporous materials have pores smaller than 2 nm in diameter and macroporous materials have pores larger than 50 nm in diameter. The pore diameter of mesoporous material is between 2 and 50 nm. In this thesis, mesoporous materials are used as host matrix to host the pH-sensitive fluorescein as a guest organic dye. The combination
of properties of the organic molecule as a guest and inorganic as a host matrix allows us to functionalize the properties [181].

The advantage of mesoporous silica materials is also to synthesize it in various forms such as thin/thick films, powders, microspheres with a thickness from nanometer to micrometer. Hence, these various forms, especially thin films, attract broad interest in the field of optics where one can perform the Drechsel-type measurement as demonstrated with polymer film (PMMA) in section 5.2.1. The mesoporous silica thin films are prepared parallel to aligned pores on a transparent substrate using a spin-coating process as shown in the next section, therefore the samples are studied with optical techniques. Thin mesoporous silica films are an important host for the organic dye in this thesis as they allow us to change the pH of the environment by using pH-sensitive fluorescein, whereas it was not possible to perform this change in polymer thin film.

Synthesis of FITC-Labeled Mesoporous Silica Film

Dye-doped mesoporous silica thin films were prepared by mixing 1.0 mg of FITC (for the control experiments 277.5 µL of pH-independent Atto532-NHS (ATTO-TEC) (10 mg/mL of anhydrous DMF) with 0.48 µL 3-aminopropyltriethoxysilane (APTES; Aldrich)) in 875 µL of absolute EtOH at room temperature in the dark overnight. Later, 1.105 mL of tetraethoxysilane (TEOS; Merck) were added to a mixture of 25 µL of HCl (10 mM) in 65 µL of Milli-Q water, and the solution was heated at 70°C for 1 hour under stirring. Simultaneously, a solution of 4.975 mL of absolute EtOH, 377 µL of Milli-Q water, 19.8 µL of HCl (1M), and 218.5 mg of hexadecyltrimethylammonium bromide (CTAB; Aldrich) was prepared and stirred at room temperature for 1 hour. Both solutions were mixed under stirring at ambient temperature and later the dye-APTES solution was added to the mixture. The resulting solution was further stirred for 30 minutes at room temperature. The final molar ratios in the solution were EtOH:H₂O:HCl:CTAB:TEOS:APTES:FITC/ATTO532-NHS=100:6.09×10⁻³:0.60:4.95:2.06×10⁻³:5.14×10⁻³. Next, the solution was further diluted by EtOH (1:9 v/v) in order to obtain a sol of the desired concentration and viscosity. 100 µL
of the solution were spin-coated onto glass coverslips at a spin speed of 6000 rpm. Immediately prior to spin coating, the glass substrates were pre-cleaned in 1 vol% Hellmanex solution and rendered more hydrophilic by leaving in piranha acid (H₂SO₄ :H₂O₂ = 3:1 (v/v)) for 2 hours, followed by cleaning with distilled water and blow-drying in a stream of compressed air. After spin-coating, the films were further aged at room temperature and ambient humidity for at least 24 hours to yield mesoporous silica thin films [161]. This process is illustrated in the flow chart in Appendix A.3. The resulting structure of the film is illustrated in Figure 5.4. Its thickness was measured by ellipsometry to be around 20 nm.

AFM Measurements

The resulting films were further characterized with Atomic Force Microscopy (AFM) (see section 3.2) in order to obtain information about their roughness and surface topology as shown in Figure 5.5a. AFM images were recorded in tapping mode using a NanoWizard 4 atomic force microscope from JPK Instruments with Multi75-G silicon AFM probes from BudgetSensors (75 kHz resonance frequency, 3 N/m force constant). The software Gwydion v2.50 was used to process and analyze the images. Figure 5.5b shows the large-area scan of the surface topography of the FITC-labeled mesoporous silica thin film. In order to obtain the roughness and thickness information, the film is scratched with a scalpel. Figure 5.5c and d show the height profile and the thickness of the thin film.

Figure 5.5. a) AFM image of the dye-labeled mesoporous silica thin film deposited onto a cover glass. The color bar represents the height calibration of the image. b) Large-area scan of the film shown in a). c) Scratch of the film shown in b) for determining the film thickness d) Height profile of along the line indicated in c). The film thickness was determined to be approximately 26 nm. (Taken from [161])
5.3.3. LDOS Change within pH Value

In order to observe and compare the variation of quantum efficiency as a function of pH with optical methods, the dye-containing mesoporous silica films were immersed in 50 mM citrate buffer solutions of varying pH and equilibrated for 20 minutes prior to each fluorescence measurement. The pH values were varied and measured in the following order: 7.5, 5.5, 7.0, 6.0 and 6.5. Hence, the reversibility from high pH to low pH or from low pH to high pH was observed with this order.

To observe how the local $\Phi_{PL}$ of FITC-doped mesoporous silica films changes with the pH of the environment, FLIM measurements were performed with different citrate buffer solutions (see Figure 5.6 at pH value of 7.5). To perform $\Phi_{PL}$ measurements, the same experiments were performed as described for Nile Red-doped PMMA thin film in section 5.2.2. For this experiment, the setup was equipped with a different excitation light source at 470 nm. (LDH-P-C-470 at 470 nm, PDL800-D). It was operated at 5 MHz repetition rates with a power of $\sim$3 $\mu$W and a long pass filter with 500 nm. The fluorescence decays of the FITC-labeled silica films derived at the different pH values were fitted with mono-exponential decay kinetics as similar to 5.3.1 and the fluorescence decay curves are shown in Figure A.4 (see Appendix A.4). In spite of the increased heterogeneity and defects of the dye environment in the mesoporous silica film as compared to the dye molecules in solution, the mono-exponential fits still provided a good match with the experimental curves. Here, the extracted lifetimes were presented as the apparent lifetimes. As expected and shown in Figure 5.8a, the lifetimes of FITC in mesoporous silica slightly differed from the lifetimes obtained in the solution.

![Figure 5.6. Fluorescence lifetime imaging (FLIM) map obtained by mono-exponential fitting of the measured fluorescence decay curve of FITC-doped mesoporous silica film at pH value of 7.5. (Taken from [161])](image)

As shown and described in section 5.2.2, the extracted decay rate can be plotted as a function of the distance $d(x)$ from the sphere’s metal surface. The results depicted in Figure 5.7 show the typical oscillatory behavior. The oscillations become less pronounced when de-
increasing the pH from 7.5 to 5.5, thereby reflecting the decreasing $\Phi_{PL}$. $\Phi_{PL}^0$, with $0.25\pm0.05$ of FITC-labeled silica thin film was derived (average lifetime calculated as $\tau=3.43$ ns) at pH 5.5, whereas $0.73\pm0.055$ ($\tau=3.95$ ns) was derived at pH 7.5. A good fit to the mean decay rate is shown by assuming isotropically oriented dipole moments, in good trends compared to ensemble results.

![Graph](image)

**Figure 5.7.** Decay rate as a function of distance between mirror and fluorophores in a mesoporous thin film at different pH value of the buffer solution. Solid lines are theoretical fits according to Equation 5.3. The direction of green arrow shows the increase in the pH value. (Taken from [161])

The results are summarized in Figure 5.8b. Figure 5.8a shows the changes in the lifetimes of FITC as a function of pH where the lifetime increased from 3.43 ns to 3.95 ns by increasing pH, as derived from the Drexhage-type experiment and conventional cuvette measurements of dye ensembles. The slight increase in lifetime was observed for increasing pH values in both types of experiments, that is attributed to the increasing contribution of the fluorescein dianion with its higher quantum efficiency and longer fluorescence lifetime, which was reported by Sjöback et al. [175] in the previous section. The corresponding quantum efficiencies achieved from the ensemble studies $\Phi_{A,PL}$, $\Phi_{R,PL}$, and $\Phi_{PL}^0$ data obtained from the Drexhage-type experiment and their dependency of the pH value are summarized in Figure 5.8b. They are in very good agreement and the increase in quantum efficiency with increasing pH reflects the enhanced contribution of the strongly emissive fluorescein dianion [161].
Figure 5.8. a) Measured lifetimes $\tau$ of FITC in mesoporous thin silica films and FITC at different pH values. b) pH dependence of the fluorescence quantum efficiencies derived from the Drexhage experiment ($\Phi_{PL}^{\infty}$), and obtained in the ensemble measurements (absolute quantum efficiencies $\Phi_{A,PL}$ and relative quantum efficiency $\Phi_{R,PL}$). (Taken from [161])

Control Measurements

A control experiment was performed in order to verify that the measured pH-dependence of lifetimes and quantum efficiencies in the mesoporous silica films were actually due to attached FITC and not caused by autofluorescence, contaminations, or morphological changes during the film preparation [161]. We performed experiments with the pH-insensitive dye ATTO532-NHS using the same setup and identical measurement conditions. NHS (N-Hydroxysuccinimide), an organic compound, conjugated to ATTO532, was used to bond the mesoporous thin films. Similar to the pH-sensitive dye FITC, the absolute and relative measurements of the quantum efficiency $\Phi_{A,PL}$ and $\Phi_{R,PL}$, as well as measurement with the Drexhage method providing $\Phi_{PL}^{\infty}$ were performed at different pH values.

Lifetime measurements of solutions of the ATTO532-NHS dyes were also done with a FLS-920 fluorescence lifetime spectrometer similar to FITC solution (see section 5.3.1). A super-continuum laser was employed at 500 nm with a pulse repetition rate of 20 MHz. The emitted photons were detected at 550 nm for ATTO532-NHS employing a spectral bandwidth of the emission monochromator of 6 nm. The instrument response function (IRF) was determined with a Ludox solution (excitation at 500 nm and, detection at 500 nm). Figure 5.9b shows the lifetime of the ATTO532-NHS at different pH values with slight changes. The fluorescence decays of the ATTO532-NHS at the different pH values were fitted with mono-exponential decay kinetics as shown in section 5.3.1.

In order to measure $\Phi_{R,PL}$, absorption and fluorescence measurements were performed under identical conditions as described for FITC solutions at different pH values (see details at section 3.3.2). The spectra are shown in Figure 5.9a. For both the ATTO532-NHS and Rhodamine 6G (Alfa Aesar) in ethanol (quantum efficiency of 0.91 [39]) as standard identical measurement settings and an excitation wavelength of 500 nm were used. The relative fluorescence quantum efficiencies $\Phi_{R,PL}$ were calculated according to Equation 5.4.
5.3. QUANTUM EFFICIENCY OF DYE-DOPED MESOPOROUS SILICA THIN FILM

Figure 5.9. Test experiments with the pH-insensitive dye ATTO532-NHS. a) Absorption (dashed lines) and emission (solid lines) spectra and (b) The fluorescence decay curves of ATTO532-NHS at pH values of 5.5, 6.0, 6.5, 7.0 and 7.5. c) Decay rates of the pH-insensitive dye ATTO532-NHS as a function of distance between mirror and fluorophores incorporated into the thin mesoporous silica film measured at pH values of 5.5 and 7.5. The dots represent the measured decay rate, extracted from the decay rate map, solid lines are theoretical fits. d) pH dependence of the fluorescence quantum efficiencies of ATTO532-NHS measured by the Drexhage experiment ($\Phi_{PL}$) and in the ensemble measurements $\Phi_{A,PL}$ and $\Phi_{R,PL}$. The lines in panel (d) present only a guide to the eye. (Taken from [161])

and shown in Figure 5.9d. As described in section 3.3.1, the absolute fluorescence quantum efficiencies $\Phi_{A,PL}$ of ATTO532-NHS at different pH values were measured by using the stand-alone integrating sphere setup C9929-02 with an excitation wavelength of 500 nm. Similar to $\Phi_{R,PL}$ measurement, $\Phi_{A,PL}$ wasn’t influenced by pH value.

Regarding Drexhage control experiments with the pH-inert dye ATTO532-NHS with red shifted absorption and emission bands (see section 5.3.2 for preparation of ATTO532-NHS-labeled silica thin films), the setup was equipped with a different excitation light source (LDH-P-FA 530 at 532 nm, 5 MHz repetition rates, $\sim$3 $\mu$W power) and a different optical filter (538 nm long pass filter). As shown in Figure 5.9c, the extracted decay rate can be plotted as a function of the distance $d(x)$ from the sphere’s metal surface at pH values with 5.5 and 7.5. Figure 5.9c shows the typical oscillatory behavior and $\Phi_{PL}^{ex}$ was almost same even at lowest and highest pH. All quantum efficiency results are shown in Figure 5.9d and no systematic change of the quantum efficiencies was observed within the measurement uncertainties.
5.4. Conclusion

In this chapter, a Drexhage-type experiment [161] using a silver coated millimeter-sized sphere to determine the local photoluminescence quantum efficiencies $\Phi_{PL}$ of immobilized fluorophores in both a polymer film and a mesoporous thin film was described. The measurement of the quantum efficiency relied on the modification of the Local Density of States (LDOS) [161]. First, as an example, Nile Red-doped PMMA was used to determine the $\Phi_{PL}$ for demonstrating reliability of the scanning mirror method. In the second part, $\Phi_{PL}$ of the pH-sensitive dye Fluorescein Isothiocyanate (FITC) doped silica film was measured as a function of pH. Although fluorophores in the silica film faced the heterogeneous environment, the extracted $\Phi_{PL}$ from Drexhage-type experiment were in good agreement with results from ensemble measurements ($\Phi_{R,PL}$ and $\Phi_{A,PL}$) with relative and absolute optical methods. The novel approach successfully demonstrated in this chapter is well suited for applications in fluorescence sensing using mesoporous thin films or alternative dye-doped spacer layers. Furthermore, the approach allows restricting quantum efficiency measurements to certain areas of the film in order to correlate it with possible defects on the surface. This type of experiment can open the door to measurement of spatially resolved quantum efficiency for samples containing very few emitters (or at the single emitter level) to study chemical or biological processes in especially mesoporous structures or in films containing a very small number of fluorophores.
Quantum Efficiency of Hexagonal Boron Nitride

This chapter presents the study of single photon emitters (SPEs) in two-dimensional (2D) materials. The quantum efficiency ($\Phi_{\text{SPE}}$) of SPEs in a few-layer hexagonal boron nitride ($h$-BN) is measured experimentally. A metal hemisphere, attached to the tip of an atomic force microscope, is employed to directly measure the lifetime variation of the SPEs as the tip approaches the $h$-BN. Owing to this technique, the direct and absolute measurement of the $\Phi_{\text{SPE}}$ of SPEs is performed. The results of this chapter are based on the publication in *Optica* with the title *Direct Measurement of Quantum Efficiency of Single-Photon Emitters in Hexagonal Boron Nitride* [182]. This chapter is oriented along this reference.

6.1. Van der Waals Heterostructures

As research on graphene has been expanded to many fields and seems to have its limit, alternative 2D materials have started to play an important role [183]. The 2D materials demonstrate unique optical, electrical and mechanical properties with bulk materials due to the confinement effect in one dimension. The first experiment was performed for 2D materials by using the Scotch-tape exfoliation method (few-layer of graphene) which shows the stability of those materials at ambient temperature [184]. Following this graphene exfoliation method, different types of 2D materials were also used by applying the same method. Molybdenum disulphide (MoS$_2$), tungsten diselenide (WS$_2$), other dichalcogenides, layered oxides and mono- and multi-layer of hexagonal boron nitride ($h$–$BN$) are the new candidates of graphene-like materials to broaden the research to various van der Waals material platforms [185]. Compared to graphene with zero bandgap, these mentioned 2D materials have non-zero bandgap which enable those to luminescence [186]. $h$-BN has a wide bandgap of nearly 6 eV with higher bandgap compared to 2D materials. In this chapter, the photophysical properties and quantum efficiency of $h$-BN as 2D material will be discussed in detail.

6.1.1. Hexagonal Boron Nitride

As a 2D material, hexagonal boron nitride, shortly $h$-BN, is used in this chapter. The different crystalline of $h$-BN can exist in cubic form or as a wurtzite. The hexagonal form, which is more stable and similar to graphite, was used in our experiment. Boron and nitrogen
atoms have the same honeycomb atomic lattice. In the single layer of h-BN (see schematic in Figure 6.1), the bond between boron and nitride are covalent and ionic to form the h-BN sheet. The bond length is 0.145 nm with lattice parameters $a = b = 0.246$ nm. Van der Waals bonds are more active at the out-of-plane interaction between the layers with inter-layer distance of 0.33 nm [187]. When the single layer of hexagonal structures form multilayer, the boron atom at the bottom layer is aligned with the nitrogen atom at the top layer or the nitrogen at the bottom layer is aligned with the boron atom at the top layer.

The band gap of h-BN is nearly 6 eV, which is greater than the bandgap of most other 2D materials. Therefore, this material is able to host stable fluorescent defects which makes h-BN a good candidate for a single photon source [188, 189]. Nonetheless, there are still many unanswered questions to understand optical properties of h-BN [189]. The defects in h-BN demonstrate very stable and robust emissions and provide a wide range of emissions covering the whole visible spectrum together with rather narrow zero phonon line (ZPL) [130].

6.2. Quantum Efficiency of Single Photon Emitter in h-BN

6.2.1. Synthesis of Hexagonal Boron Nitride

h-BN was fabricated by using a low pressure chemical vapor deposition (LPCVD) technique as explained in [190] in detail. h-BN multilayers were grown on a copper foil by using ammonia borane ($\text{H}_3\text{NBH}_3$) as a precursor which was stored at high temperature. By using a PMMA assisted wet transfer process, the as-grown multi-layer h-BN films were spin-coated with PMMA to transfer to a glass coverslip and baked on hot plate to increase adhesion between h-BN and the PMMA. After this process, the substrate was placed in

![Schematic of an h-BN monolayer and its crystalline hexagonal structure with alternating boron (red), nitrogen (blue) and hydrogen (white) atoms.](image)
6.2. QUANTUM EFFICIENCY OF SINGLE PHOTON EMITTER IN H-BN

A solution of ammonium to etch away the metal. PMMA/h-BN was washed then with HCl and Milli-Q water. The transferred film was then placed on a hot plate to increase the adhesion of the h-BN to the glass substrate. After that, the PMMA was removed by soaking the sample in warm acetone overnight on a spin coater, before cleaning by exposure to a UV-Ozone atmosphere for 20 min.

6.2.2. Measurement of Photophysical Properties

The schematic of the experimental setup to measure the photophysical properties of single photon emitters in h-BN is shown in Figure 3.2. Similar measurements were also performed in section 4.2 for the spectroscopic characterization of colloidal quantum dots. In order to perform the measurement with h-BN flakes, a wavelength tunable pulsed laser (SOLEA, PicoQuant) was employed as an excitation source and focused through the cover glass with h-BN flake by using an oil immersion objective lens (NA 1.4, 100x). The fluorescence of single defect in h-BN was collected by the same objective lens and guided through a confocal setup into a Hanbury-Brown and Twiss setup as described in section 3.4 with polarizing beam splitter (PBS). Before the emitted photon arrived to PBS, the fluorescence polarization is determined with a λ/2-plate.

\( g^{(2)} \) Measurement

In order to detect single defects in h-BN for studying optical properties at room temperature, a \( g^{(2)} \) measurement was first performed. One single defect was chosen and its emission was analyzed at an excitation power of 56 µW.

![Figure 6.2](image_url)

**Figure 6.2.** Typical antibunching curves from a single defect in a h-BN. The pulsed \( g^{(2)} \) function with correlation times of 100 ns and 10 µs and resolutions of 0.25 ns and 25 ns in a) and b). Bunching correlations on the order of micro seconds are shown. (Adapted from [182])

A normalized second order correlation function \( g^{(2)} \) was measured as shown in Figure 6.2. In order to observe the single photon characteristic of the emitted light, the zeroth peak has to be below 0.5.
Dipole Orientation Measurement

After the single defect center in $h$-BN was verified with the autocorrelation measurement, the orientation of a single emitter was determined. In order to measure the two angles in the plane $\phi$ and out-of-plane $\theta$, a polarimetric measurement was performed as described in references [191, 192]. To perform this measurement, the emitted photons from single defect center in $h$-BN were guided through the $\lambda/2$ plate turned by an angle $\alpha/2$. The vertical and horizontal polarization components of single emitters were split by a polarizing beam splitter (PBS). The emission from each output of PBS was guided to the different APDs (APD1 and APD2). $I_{APD_1}$ and $I_{APD_2}$ are the intensities at each detector. Figure 6.3 shows the relative intensity detected at the one port. This was calculated by the following expression:

$$I_{Norm}(\alpha) = \frac{1}{2} \left( \frac{I_{APD_1}}{I_{APD_1} + I_{APD_2}} \right)^{(\alpha)} \left( \frac{I_{APD_1}}{I_{APD_1} + I_{APD_2}} \right)$$

Figure 6.3. The high contrast of the shown polarization measurement of SPE in $h$-BN indicates for a horizontally polarized dipole. The corresponding polarization measurement of the detected light and fit function are represented by the black dots and red line, respectively.

$$I(\alpha) = I_{min} + (I_{max} - I_{min}) \cos^2(\alpha - \phi)$$

where $\phi$ is a fitting parameter. The degree of polarization is determined by $\delta = (I_{max} - I_{min})/(I_{max} + I_{min}) = 0.797 \pm 1$, which is related to $\theta$. The result shows an orientation of horizontally aligned dipole. In order to verify the polarization measured, $\delta$ as a function
of $\theta$ was simulated. The degree of polarization was determined for a dipole located in the center of a 10 nm thick $h$-BN layer on top of a glass cover slide and observed with a NA 1.4 objective lens [182].

Spectrum and Lifetime of $h$-BN

To determine another parameter for the calculation of the quantum efficiency, a representative photoluminescence spectrum was recorded from one single emitter in the $h$-BN flakes as shown in Figure 6.4a. This defect shows a pronounced ZPL at $\approx 660 \pm 4$ nm and a phonon sideband at ambient temperature. To relate the measurement with local density of states (LDOS), time-resolved fluorescence measurements were also performed to directly measure the excited state lifetime of the single emitter in the $h$-BN flake. Figure 6.4b. shows the decay of the signal from a single quantum emitter with a ZPL at 660 nm.

![Figure 6.4](image)

Figure 6.4. a) Representative photoluminescence spectra of a typical defect in $h$-BN showing a pronounced ZPL at $\approx 660$ nm and a phonon sideband at room temperature. b) The lifetime measurement of single defect center in $h$-BN. The fit represented by the red line is given by a circular convolution of a double exponential decay with the IRF.

The excited state lifetime was determined by recording time differences between the applied laser pulse and the photon arrival time at the avalanche photodiodes (APDs) as described in section 3.1.3. The data (black) points were fitted, represented by the red line is given by a circular convolution of a double exponential decay with the instrument response function (IRF). The lifetimes are calculated as $\tau_1 = 4.89 \pm 0.01$ ns and $\tau_2 = 0.5 \pm 0.01$ ns.

6.2.3. Calculations of Quantum Efficiency

In order to determine the quantum efficiency of the single photon emitter (SPE) in the $h$-BN flakes, $\Phi_{SPE}$, measurement by using the similar concept from section 5.2, the atomic force microscope was employed to this setup as shown in Figure 6.5. To measure the $\Phi_{SPE}$ of the SPE in $h$-BN by modifying local density of states, a hemispherical gold coated AFM tip with a radius of 2.75 $\mu$m was used to modify the lifetime of emitters by moving the AFM tip vertically with respect to the emitter. The position of the AFM tip and focus of the excitation laser were matched by scanning the tip over a large area while recording laser
light reflected by the tip. Once matched, the vertical position of AFM tip was changed accurately by using the build-in AFM piezo which allows to measure lifetime as a function distance between AFM tip and defect.

![Diagram](image)

**Figure 6.5.** An AFM equipped with a gold-coated hemispherical tip with a diameter of 5.5 µm is aligned with a defect center in h-BN with a distance d. An oil immersion objective lens excites defects in h-BN and collects its emission from below the glass substrate.

After the single emitter in h-BN was found and the photophysical properties of this emitter were investigated as shown in previous subsections, the distance-dependent lifetime measurements were performed for various distances between tip and emitter. The gold coated AFM tip is fixed to emitter distance d approximately 1.2 µm. At this distance, the lifetime measurement was performed for 1-2 seconds. Then, the tip was approached to defect center in h-BN with 15 nm steps. For each step, the absolute photon arrival times were recorded with laser pulse arrival times, which allows us to extract the decay dynamics of the single emitter. This process was repeated until the tip reaches the surface. In order to minimize drifts in the AFM and confocal setup, the lifetime measurements at higher tip distances \( \tau_1 \) were not performed. Therefore, the maximum distance was set to 1.2 µm, where only low amplitude variations from \( \tau_1 \) are expected. By this method a correct fit of \( \tau_1 \) was obtained.

The built-in AFM laser points reflected to a built-in four quadrant photo diode at the end of the cantilever. A bending of the cantilever caused a change in position of the laser on the photo diode. The \( \Phi_{SP_E} \) measurement was stopped when AFM tip was completely approached to the surface. Due to the unknown nearest distance emitter to AFM tip, the small offset \( d_0 \) was used as an additional fit parameter.

The exponential fit to obtain the corresponding decay rate of the emitter for each mirror position is plotted in Figure 6.6. Here, the black points represent the experimental data, which is the fitted decay rate corresponding to a defined mirror position. As expected, the distinct oscillation of the lifetime as a function of distance is observed and varied between 4.4 and 5.3 ns. Hence, varying LDOS in front of the mirror is observed for the single defect in h-BN flakes.

To calculate the \( \Phi_{PL}^{\infty} \), a horizontally polarized emitter hosted in the center of a 10 nm h-BN flakes with refractive index of 1.65 on the cover glass (see Figure 6.5) is simulated by comparing the change in LDOS. A gold coated AFM tip is centered at a distance d from
6.2. QUANTUM EFFICIENCY OF SINGLE PHOTON EMITTER IN H-BN

Figure 6.6. Measured lifetime as a function of the position of the AFM tip above a single the h-BN defect. The red line display theoretical fits.

the dipole acting as a mirror and changes the LDOS $\rho(d)$. The LDOS is expressed \cite{182} by

$$\frac{\rho(d)}{\rho(\infty)} = \frac{p(d)}{p(\infty)}$$

with the distance-dependent emitted power $p$. Then, Equation 6.4 is fitted to the distance-dependent lifetime measurements.

$$\tau(d) = \frac{\tau_{\infty}}{1 + \Phi_{SPE}^{\infty} \left( \frac{p(\lambda, d+d_0)}{p(\lambda, \infty)} - 1 \right)}$$

The values of $\tau = 4.89 \pm 0.07$ ns, $\lambda = 660 \pm 4$ nm and $d_0 = -36 \pm 3$ nm are used and $\Phi_{SPE}$ of single emitter in h-BN flakes is calculated to be $\eta = 0.52 \pm 0.07$, with a corresponding fit. 10 identical quantum efficiency measurements were performed with same emitter in order to calculate the error margins of $\Phi_{SPE}$ as shown in paper \cite{182}. The average and standard deviation of the measurement was calculated as 6.6 %. In addition to this, the lifetime and $\Phi_{SPE}$ of single defect might be varied due to other effects such as moving AFM tips and time.

In order to observe the effects on $\Phi_{SPE}$ and lifetime measurement, the variation of the temporal lifetime and the effect of a AFM tip were investigated. The measurement took around 400 s, the time trace first was recorded when the AFM tip was 7 $\mu$m away from the emitters. When the AFM approached the emitters irregularly, a higher count rate was observed as the collection efficiency increased due to reflection from the metal coated AFM tip. Second, the lifetime measurement was performed during 400 s and no significant variation of lifetime was observed opposed to the count rate as shown in \cite{182}.
6.3. Summary

To summarize, a Drexhage-type method is introduced using an Au sphere bound to the tip of an AFM cantilever to measure the ΦSPE of a SPE in h-BN absolutely and directly. The one emitter with ZPL around 660 nm of h-BN was chosen and the ΦSPE of SPEs in h-BN was calculated with the standard deviation obtained from 10 different measurements. As the crystallographic origin and spectroscopic properties of the defects are unknown, the methodology can be used to help revealing the nature of defects in h-BN or even in other 2D materials [193].
Conclusion and Outlook

In this thesis, the photophysical features of various fluorophores were studied. The herein described novel approach allowed for measuring the fluorescence quantum yield as a key and important spectroscopic parameter of fluorophores. The quantum efficiencies of different fluorophores were studied in detail from ensemble of emitters down to single emitters.

7.1. Conclusion

In chapter 4, single colloidal quantum dots (CQDs) were used in order to verify single photon sensitivity of the newly established experimental setup. Statistical properties of single CQDs were studied with the homemade scanning confocal optical microscope using time correlating single photon counting (TCSPC). In addition, measurements of the emission spectra, lifetimes and second order-correlation of various single quantum dots were performed. Single photon generation from CQDs at room temperature with a second-order temporal correlation function at zero delay $g^{(2)}(0) \leq 0.15$ was demonstrated.

In chapter 5, as a novel approach the Drexhage-type method that employs a metal coated sphere was implemented to the homemade confocal setup in order to measure directly the quantum efficiencies of organic dyes. Owing to this method, the quantum efficiency was measured through a control of the local density of optical states (LDOS) in the vicinity of a metallic surface. The method allows for measurements of the quantum efficiency of small ensembles down to the level of single fluorophores. For the first time this approach was utilized for fluorophores in mesoporous films. Nile Red and pH-sensitive fluorescein were embedded in PMMA and mesoporous silica thin films, respectively. First, the quantum efficiency ($\Phi_{PL}^{\text{PL}}$) of Nile Red was estimated as $0.43 \pm 0.04$. Second, the local quantum efficiency variation of pH-dependent fluorescein isothiocyanate bound to a mesoporous silica thin film was observed by varying the pH. The local $\Phi_{PL}^{\text{PL}}$ decreased from 75% to 25% by changing the pH from 7.5 to 5.5, respectively. The corresponding quantum yields obtained from the relative and absolute quantum yield measurements were compared [161].

In chapter 6, the Drexhage-type method was used for the first time to measure the absolute quantum efficiency of single defects as emitters in 2D materials, i.e. in hexagonal Boron Nitride ($h$-BN), by modifying the spontaneous emission in front of a metal coated AFM tip. The single emitter from one family of defects in $h$-BN was chosen and the quantum efficiency $\Phi_{SPE}$ of a single emitter in $h$-BN was derived as $0.57 \pm 0.07$ [182]. The approach can
be applied to shine light on the photophysical properties of other emitters in 2D materials and may eventually relate optical to structural properties.

7.2. Outlook

The developed experimental schemes and results are expected to encourage numerous future experiments and progress in the life and material science. This includes some examples which are further detailed in the following.

7.2.1. Quantum Dots

Further experiments with single CQDs can be envisioned, i.e. observing the quantum efficiency while modifying the external parameters. The quantum efficiency of a single CQD can be determined by using the metal AFM technique as introduced in chapter 6 while changing the temperature or applying electrical or magnetic fields (via the tip) at the same time. The modification of quantum efficiency of various of single CQDs will then reveal information about their photophysics or blinking behaviour [58, 74].

7.2.2. LDOS in Microfluidics and Lab-on-a-Chip Devices

The Drexhage-type method has been used to measure quantum efficiency of organic dyes for the application in the fluorescence sensing as our results are shown in the mesoporous silica thin films. In order to observe local photochemical processes in mesopores or electrochemical processes, this method allows to measure the localized quantum efficiency. By increasing the experimental abilities, this method will be extended to measure the quantum efficiency at the certain area of the film to observe the effect of in homogeneity and surface topography of the structure.

Figure 7.1. Schematic representation of Drexhage-type experiment with metal sphere (gold) on microfluidic platform which provides the control and manipulation of the small volumes of low concentrated fluorophores (red) through the microfluidic channels (light blue).
Furthermore, a conceptual study is performed in Appendix A.5 to pave the road for implementing the Drexhage-type method into a liquid or microfluidic platform. However, the proposed experiment in Appendix A.5 has technical challenges when the sphere is placed into liquid. This approach might make it difficult to observe oscillations of lifetime to extract localized quantum efficiency as the position of each fluorophore fluctuates due to Brownian motion. In order to overcome this problem, a microfluidic platform may be designed with precisely fabricated very thin channels. Such an approach may lead to a novel lab-on-a-chip (LOC) technology for quantum efficiency measurements in a liquid environment. In contrast to absolute and relative quantum yield measurements which require high concentrations, this approach can also result in accurate and precise quantum efficiency measurement in liquid environment at very low concentrations of the analyte.
A.1. Parameters and Approximations for Local Density of States Calculation

In the numerical calculation of the LDOS $\rho(d)$, the parameters in Table A.1 are used for the fluorophores described in chapter 5.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nile Red</th>
<th>FITC</th>
<th>ATTO532-NHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Wavelength (nm)</td>
<td>532</td>
<td>470</td>
<td>532</td>
</tr>
<tr>
<td>Emission Wavelength* (nm)</td>
<td>607.7</td>
<td>521</td>
<td>550</td>
</tr>
<tr>
<td>RI of Host Material</td>
<td>1.49 (PMMA)</td>
<td>1.23 (Silica)</td>
<td>1.23 (Silica)</td>
</tr>
<tr>
<td>RI of Glass [194]</td>
<td>1.47</td>
<td>1.47</td>
<td>1.47</td>
</tr>
<tr>
<td>RI of SiO$_2$ [195]</td>
<td>1.46</td>
<td>1.46</td>
<td>1.46</td>
</tr>
<tr>
<td>RI of Silver [196]</td>
<td>0.0572+i4.0097</td>
<td>0.0500+i3.3249</td>
<td>0.0596+i3.5974</td>
</tr>
<tr>
<td>Thickness of Film (nm)</td>
<td>16</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Thickness of SiO$_2$ (nm)</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Thickness of Ag (nm)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table A.1. Parameters used for the calculations of the LDOS for the utilized fluorophores. RI denotes the Refractive Index. The parameter marked with asterisk(*), the central emission wavelength, is further discussed in this section.

In order to take into account the spectral width of the investigated emitters, LDOS calculations for emitters next to a metallic surface were performed by averaging over wavelength. These LDOS oscillations as a function of the distance of the emitter to metal surface for each wavelength were weighted with the corresponding spectrum of the fluorophore in order to find the effective LDOS. In this calculation, FITC was used as a fluorophore.

Three LDOS were calculated and are shown in Figure A.1 for the wavelengths 521 nm (black solid), 505 nm (orange) and 555 nm (black dashed), respectively. They were then weighted equally 1/3 in Figure A.2. The effect of the spectral width is exaggerated by using procedure. In order to average more accurately, weights for each wavelength according to its contribution to the spectrum of the fluorophore have to be used. However, for simplicity the difference of the relative LDOS ($\rho=LDOS$ next to mirror/LDOS no mirror) curves using an equally-weighted average are compared to curves with a single-peaked spectrum [161].
Figure A.1. Normalized LDOS curves for three wavelengths (orange 505nm, black solid 520nm and black dashed 555nm). (Taken from [161])

Figure A.2b shows that even in this case the difference is subtle, i.e. a few per mille of the relative LDOS. Based on the Equation 2.13, it can be seen that the radiative rates ($\gamma_r$) contribute only partially to the total decay rate ($\gamma_{tot}$). Consequently, the difference between a theoretical curve assuming one wavelength or including a broader spectrum are reduced even further. The chosen peak wavelength only for the LDOS calculation and fitting is well justified.

Figure A.2. a) Normalized LDOS for 521 nm (orange, solid) and averaged normalized LDOS (black, dashed). b) Difference between normalized LDOS curves at 520 nm and equally-weighted wavelength as shown in Fig. 3. (Taken from [161])
A.2. Quantization of Electromagnetic Fields

In this part of the appendix, the quantized of the electromagnetic field in free space is derived by beginning from the Maxwell equations:

\[
\nabla \cdot E = 0 \quad (A.1)
\]

\[
\nabla \cdot H = 0 \quad (A.2)
\]

\[
\nabla \times E = -\mu_0 \frac{\partial H}{\partial t} \quad (A.3)
\]

\[
\nabla \times H = \epsilon_0 \frac{\partial E}{\partial t} \quad (A.4)
\]

with \(E\) and \(B\) representing the electric and magnetic fields, respectively. \(\epsilon_0\) is the electric permittivity and \(\mu_0\) is the magnetic permeability in free space. The speed of light is \(c = \frac{\epsilon_0 \mu_0^{-1}}{2}\) in the vacuum. Taking the curl of Equation A.3 and using the Maxwell’s equations, the wave equation for the electric field is derived.

\[
\nabla^2 E - \frac{1}{c^2} \frac{\partial^2 E}{\partial t^2} = 0 \quad (A.5)
\]

In a cavity with length \(L\) and volume \(V\), electric field linearly polarized in \(x\)-direction is assumed which satisfies the Equation A.5 can be described as

\[
E(r, t) = E_x(z, t) = \sum_j A_j q_j(t) \sin(k_j \cdot z) \quad (A.6)
\]

where \(k_j = j\pi/L\) with \(j=1,2,3,\ldots\) and the coefficient \(A_j\) is defined as

\[
A_j = \left( \frac{2\omega_j^2 m_j}{\epsilon_0 V} \right)^{1/2} \quad (A.7)
\]

where \(m_j\) is a constant with dimension of a mass and \(q_j\) is an amplitude with the dimension of length. Both terms are introduced to establish the analogy to a mechanical oscillator. The magnetic field component can be obtained from Equation A.6

\[
H(r, t) = H_y(z, t) = \sum_j A_j \left( \frac{\dot{q}_j(t) \epsilon_0}{k_j} \right) \cos(k_j \cdot z) \quad (A.8)
\]

The classical Hamiltonian of the field is given

\[
\mathcal{H} = \frac{1}{2} \int_V [\epsilon_0 E_x^2 + \mu_0 H_y^2] dV \quad (A.9)
\]

where the integration is calculated over the cavity volume. Substituting Equation A.6 and A.8 for \(E_x\) and \(H_y\), Hamiltonian becomes
\[ \mathcal{H} = \frac{1}{2} \sum_{j} \left( m_{j}\omega_{j}^{2}q_{j}^{2} + m_{j}\omega_{j}^{2}q_{j}^{2} \right) \]

\[ = \frac{1}{2} \sum_{j} \left( m_{j}\omega_{j}^{2}q_{j}^{2} + \frac{p_{j}^{2}}{m_{j}} \right) \quad (A.10) \]

where \( p_{j} = m_{j}\dot{q}_{j} \) is defined as a canonical momentum of the \( j \)th mode. This equation is equivalent to the Hamiltonian of a sum of harmonic oscillators. For each mode, the light field can be quantized as the mechanical harmonic oscillator by associating \( p_{j} \) and \( q_{j} \) operators.

\[ [q_{j}, q_{j}'] = [p_{j}, p_{j}'] = 0 \quad (A.11) \]

\[ [q_{j}, q_{j}'] = i\hbar\delta_{j,j'} \quad (A.12) \]

By using a canonical transformation, \( p_{j} \) and \( q_{j} \) operators are replaced by \( a_{j} \) and \( a_{j}^{\dagger} \) operators

\[ a_{j} e^{-i\omega_{j}t} = \frac{1}{\sqrt{2m_{j}\hbar\omega_{j}}} (m_{j}\omega_{j}q_{j} + ip_{j}) \quad (A.13) \]

\[ a_{j}^{\dagger} e^{i\omega_{j}t} = \frac{1}{\sqrt{2m_{j}\hbar\omega_{j}}} (m_{j}\omega_{j}q_{j} - ip_{j}) \quad (A.14) \]

The annihilation in terms of \( a_{j} \) and creation \( a_{j}^{\dagger} \) operators obey the commutator relations

\[ [a_{j}, a_{j}'] = [a_{j}^{\dagger}, a_{j}'] = 0 \quad (A.15) \]

\[ [a_{j}, a_{j}^{\dagger}] = \delta_{j,j'} \quad (A.16) \]

By using \( a_{j} \) and \( a_{j}^{\dagger} \) operators, the Hamiltonian of the free electromagnetic field becomes

\[ \mathcal{H} = \frac{1}{2} \sum_{j} \hbar\omega_{j} \left( a_{j}^{\dagger}a_{j} \right) \quad (A.17) \]

Therefore, in terms of \( a_{k} \) and \( a_{k}^{\dagger} \) operators, electric and magnetic fields can be expressed with

\[ E_{x}(z, t) = \sum_{j} \beta_{j} \left( a_{j} e^{-i\omega_{j}t} + a_{j}^{\dagger} e^{i\omega_{j}t} \right) \sin(k_{j} \cdot z) \quad (A.18) \]

\[ H_{y}(z, t) = -i\varepsilon_{0}c \sum_{j} \beta_{j} \left( a_{j} e^{-i\omega_{j}t} - a_{j}^{\dagger} e^{i\omega_{j}t} \right) \cos(k_{j} \cdot z) \quad (A.19) \]
where the expression
\[ \beta_j = \sqrt{\frac{\hbar \omega_j}{\epsilon_0 V}} \]  
(A.20)
is the dimension of an electric field. The quantization of the electromagnetic field is defined in a finite one-dimensional cavity. This result can be generalized to a field in unbounded free space. In this case the volume \( V \) represents a volume for which periodic boundary conditions are established. The quantized electric and magnetic fields take the form as shown in [84]

\[ E(r, t) = \sum_k \hat{\xi}_k \beta_k \hat{a}_k e^{-i(\omega_k t - k \cdot r)} + \text{H.c.} \]  
(A.21)

\[ H(r, t) = \frac{1}{\mu_0} \sum_k \frac{\hat{k} \times \hat{\xi}_k}{\omega_k} \beta_k \hat{a}_k e^{-i(\omega_k t - k \cdot r)} + \text{H.c.} \]  
(A.22)

where \( \hat{\xi} \) is a unit polarization and H.c. is Hermitian conjugates. The positive and negative part of the given field can be written separately with following expression

\[ E(r, t) = \hat{E}^+ + \hat{E}^- \]

\[ = \sum_k \hat{\xi}_k \beta_k \hat{a}_k e^{-i\omega_k t + ik \cdot r} + \sum_k \hat{\xi}_k \beta_k \hat{a}_k^* e^{i\omega_k t - ik \cdot r} \]  
(A.23)
A.3. The Flow Chart of Sample Preparation

The preparation of dye-doped silica thin film was explained in detail in section 5.3.2. Figure A.3 shows the flow chart of the this sample preparation in order to visualize it step by step.

<table>
<thead>
<tr>
<th>Solution A</th>
<th>Solution B</th>
<th>Solution C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.48 μL APTES</td>
<td>1.105 TEOS</td>
<td>4.975 mL EtOH</td>
</tr>
<tr>
<td>1 mg FITC</td>
<td>25 μL HCl (10 mM)</td>
<td>377 μL Milli-Q Water</td>
</tr>
<tr>
<td>875 μL EtOH</td>
<td>65 μL Milli-Q Water</td>
<td>19.8 μL HCl (1 M)</td>
</tr>
<tr>
<td>1.105 TEOS</td>
<td>218.5 mg CTAB</td>
<td>218.5 mL Milli-Q Water</td>
</tr>
</tbody>
</table>

- Stir overnight (24 h) at room temperature in the dark
- Stir 1 h at 70 °C (timing is critical) (clear and colorless solution)
- Stir 1 h at room temperature (timing is not critical) (clear and colorless solution)
- Add Solution B to Solution C under stirring
- Add Solution A to mixture of Solution A and B under stirring
- Stir mixture of all solution 30 min at room temperature (timing is critical)
- Diluting with EtOH (1:9 v/v)
- 100 μL of solution for spin coating on cleaned substrate
- Cleaning glass substrate with 1 vol% Hellmanex in distilled water for 30 minutes
- Cleaning glass substrate in Piranha Acid H₂SO₄ : H₂O₂ (3:1 v/v)
- Rinsing with distilled water and blowing with air
- Aging thin films for 24 h at room temperature and dark room

Figure A.3. Preparation of dye-labeled mesoporous silica thin films.
A.4. The Lifetime Measurement of FITC-labelled Thin Films

Lifetime measurements of FITC-labelled silica thin film at varying pH buffers were performed with the confocal setup before the quantum efficiency measurements were performed. Figure A.4 shows the fluorescence decay curves of FITC-labeled silica thin films at different pH.

![Fluorescence decay curves](image)

Figure A.4. The fluorescence decay curves of FITC-labeled silica thin films obtained at different pH values. IRF stands for instrument response function.
A.5. Experiments with Fluorophores in Liquids

The results of the test experiments with solvatochromic dye Nile Red (NR) are shown in this section. The test experiment was performed to verify the result from the literature whether hydrogen bonding exerts significant change on the quantum efficiency and the lifetime of NR for the purpose of the future Drexhage-type experiment.

First, the fluorescence lifetimes of NR in the mixture of ethanol (EtOH) and methanol (MeOH) were measured as the quantum efficiency and fluorescence lifetime of NR are related and both of them are sensitive to polarity of environment. In order to observe the small changes of lifetime in the mixture of two solvents, NR is dissolved in both EtOH ($\tau = 3.65$ ns and $Q.E. = 0.75$) and MeOH ($\tau = 2.8$ ns and $Q.E. = 0.40$) separately.

Figure A.5a shows the variation of the lifetime NR by changing the polarity of the mixture solution. The solvent polarity is sensitive to the amount of EtOH and MeOH in this measurement. The methanol was added to NR in ethanol, which caused the decrease in the lifetime of the fluorophore as expected (Black curve). A similar experiment was performed by adding the EtOH to NR in MeOH. Simultaneously, the shifts in the emission spectra were also shown in Figure A.5b with the peak wavelengths. The results from Figure A.5 show that the polarity of solvent changes the fluorescence lifetime of NR, therefore the quantum efficiency of NR changes as measured in reference [163].

Figure A.6 shows the schematic of Drexhage-type experiment in aqueous solution where the metal coated sphere is placed in liquid solution. The main idea of using this method is to observe the change of the quantum efficiency of a low concentrated fluorophore in aqueous solution by modifying the spontaneous emission rate of NR. To modify the lifetime of a fluorophore on the local optical surrounding, the sphere should be stabilized in the liquid against flow for the precise calculation of distance between the emitter and sphere. Even though the sphere is stabilized, the liquid measurement brings new challenges to excite the fluorophores as the fluorophores diffuse in or out of confocal volume. It is assumed...
that the mechanics of the confocal system cancel out this effect as the collection volume is smaller than the excitation volume.

In the planned experiment, the focus of the laser is stabilized on the interface between cover glass slip and liquid interface in order to determine accurate position instead of using two metal interfaces similar to the experiment in [70]. To show the change in quantum yield of NR by the polarity of environment, the similar method could be used as shown in Figure A.5. In this configuration, the sphere was placed on the cover glass and NR (green dots) in aqueous solution of ethanol was added into polydimethylsiloxane (PDMS) cell as a reservoir. It was attempted to scan the liquid sample in order to observe the lifetime’s modifications, however, the actual distance between the sphere and emitters could not be accurately determined similar to experiment in section 5.2.2. In addition to that, it is found that fluorophores tend to make a cluster in the close proximity to surface of the sphere as the emission intensity was higher near the sphere. This prohibited the accurate analysis of lifetime at the certain distance between sphere and fluorophore. Therefore, this current system shows that the measurement of quantum yield in liquid was ultimately hampered by the technical difficulties. The large collection volume makes the data too sensitive to non-linear distributions of fluorophores in the collection volume and prevents the clear oscillatory shape of lifetime modification in a thin layer. Hence, new geometries should be served in order to overcome these problems and perform quantum yield imaging. The transparent thin film in the microfluidic systems could be used to separate the sphere and liquid interface to measure the distance accurately.
References


A. Jablonski. 'Efficiency of Anti-Stokes Fluorescence in Dyes'. In: *Nature* 131.3319 (June 1933), pp. 839–840. doi: 10.1038/131839b0 (cit. on p. 5).


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Letztendlich bin ich meiner Familie und meinen Freunden dankbar, die mich zu diesem Erfolg begleitet haben. Sie gaben mir moralische Unterstützung, Ermutigung und Motivation um meine persönlichen Ziele zu erreichen.
Publication List

Related to this thesis/During my PhD Thesis

   
   The first author led the research. He prepared the samples and performed the experiments, analyzed the data and wrote the manuscript.

   
   The third author was responsible for the theoretical conception and contributed to the manuscript.

Not Related to this thesis/During my Master Thesis


Scientific Contributions

Talks

*Measuring Quantum Yield of Organic Dyes by Lifetime Modifications Using a Metal Ball*
- DPG Frühjahrstagung, Erlangen, 2018

*Determining pH-dependent Quantum Efficiency of Emitters by Using a Metal Sphere*
- DPG Frühjahrstagung, Rostock, 2019

Poster

*Measuring Quantum Yield of Perylene Bisimide Dyes by Lifetime Modifications Using a Metal Ball*
- NANOP Nanophotonics and Micro/Nano Optics Conference, Barcelona, 2017

*Determining Quantum Efficiency of the pH-sensitive Dye in Mesoporous Thin Films Using a Metal Sphere*
- OSA Frontiers in Optics/Laser Science, Washington D.C., 2019
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<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen Peroxide</td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>Sulfuric Acid</td>
</tr>
<tr>
<td>H₃NBH₃</td>
<td>Ammonia Borane</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric Acid</td>
</tr>
<tr>
<td>MoS₂</td>
<td>Molybdenum Disulfide</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>SiO₂</td>
<td>Silicon Dioxide</td>
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<tr>
<td>h – BN</td>
<td>Hexagonal Boron Nitride</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscope</td>
</tr>
<tr>
<td>APD</td>
<td>Avalanche Photodiode</td>
</tr>
<tr>
<td>APTES</td>
<td>3-aminopropyltriethoxysilane</td>
</tr>
<tr>
<td>CDD</td>
<td>Charged Coupled Device</td>
</tr>
<tr>
<td>CPS Model</td>
<td>Chance, Prock and Silbey Model</td>
</tr>
<tr>
<td>CQDs</td>
<td>Colloidal Quantum Dots</td>
</tr>
<tr>
<td>CTAB</td>
<td>Hexadecyltrimethylammonium Bromide</td>
</tr>
<tr>
<td>CW</td>
<td>Continuous Wave</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescin Isothiocyanate</td>
</tr>
<tr>
<td>FLIM</td>
<td>Fluorescence Lifetime Imaging</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full Width Half Maximum</td>
</tr>
<tr>
<td>HBT</td>
<td>Hanbury Brown and Twiss</td>
</tr>
<tr>
<td>IRF</td>
<td>Instrument Response Function</td>
</tr>
<tr>
<td>LDOS</td>
<td>Local Density of States</td>
</tr>
<tr>
<td>LPCVD</td>
<td>Low Pressure Chemical Vapor Deposition</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical Aperture</td>
</tr>
<tr>
<td>NC</td>
<td>Nanocrystals</td>
</tr>
<tr>
<td>NR</td>
<td>Nile Red</td>
</tr>
<tr>
<td>PBS</td>
<td>Polarizing Beam Splitter</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polymethylsiloxane</td>
</tr>
<tr>
<td>pKₐ</td>
<td>Acid Dissociation Constant</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>SPP</td>
<td>Surface Plasmon Polariton</td>
</tr>
<tr>
<td>TCSPC</td>
<td>Time Correlated Single Photon Counting</td>
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<td>TEOS</td>
<td>Tetraethoxysilane</td>
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