Nanoscale Photonics

From single molecule nanofluidics to light-matter interaction in nanostructures

by
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NANOSCALE PHOTONICS

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Dedicated to

Bapidadu for teaching me to look at life differently, my family for always believing in me, my beloved best friend – Mou for always being aside and a lot had happened discussing with you, Satyajit Ray for writing ‘Professor Shonku’ series, Walter Lewin for writing ‘For The Love of Physics’, G. K. Ananthasuresh for allowing me to know what is academic research, my teachers of Patha Bhavan with whom my journey of learning ever started, and last but not the least, my brother and friend – Buba teaching whom enables me to think how to teach lucidly. I am sure, without these excellent minds this thesis would not have been materialised. Hence, I dedicate this thesis to all of you.

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‘My heart sings at the wonder of my place
In this world of light and life;
at the feel in my pulse of the rhythm of creation
cadenced by the swing of the endless time.

I feel the tenderness of the grass in my forest walk,
the wayside flowers startle me:
that the gifts of the infinite are strewn in the dust
wakens my song in wonder.

I have seen, have heard, have lived;
in the depth of the known have felt
the truth that exceeds all knowledge
which fills my heart with wonder and I sing.’

Rabindra Nath Tagore
1923, Calcutta.

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Abstract

Detecting single nanometre scale objects and studying them in a controlled manner in solution is still a difficult task due to diffusion. We demonstrate one-dimensional nanofluidic flows of single molecules by confining diffusion in two spatial dimensions. A high-throughput nanofabrication process was developed using electron-beam lithography, reactive ion etching, and shadow-angle-electron-beam deposition to prepare the required nanofluidic devices. We used the device to detect single organic dye molecules, nanodots, and small DNA molecules using two-foci fluorescence correlation spectroscopy.

Fluorescence is frequently exploited in single molecule detection. In this thesis, a non-toxic fluorescent nanomaterial called carbon nanodots (CNDs) is studied using a fluorescence and electron correlative microscopy approach. We find that CNDs are single photon emitters in the visible range. A strong electron-phonon coupling is observed in their photoluminescence. The light-matter interaction at atomic scale of CNDs is not well understood. We study the optical behaviour of CNDs using a time-dependent density-functional tight binding method. A type of CND studied in this thesis is graphene quantum dots. Their optical behaviour is strongly dependent on the edge properties, functional groups, and the total number of atoms.

We envision that single molecule nanofluidics will impact the biomedical research and diagnostics by enabling the study of protein-protein interactions and detecting single molecule level onset of any disease. The CNDs studied here have already been used in super-resolution microscopy with potential to replace the toxic quantum dots for labelling. Our theoretical findings on CNDs answer towards their optical and electronic properties. These are useful for the development of single photon quantum detectors and optoelectronic devices. Overall, the results presented here should have applications in biomedical sciences, optoelectronics, and quantum information technologies.

Published articles

During my doctoral research work, I have published some scientific articles which are directly and indirectly related to this thesis. Several results presented in this thesis are taken from those papers. They are:

- **S. Ghosh**, N. Karedla, I. Gregor, ‘High-throughput nanofluidic device for one-dimensional confined detection of single molecules and nanodots’, *arXiv*, 1702.03992, 2017 ([DOI](#)).
- **S. Ghosh**, M. Awasthi, M. Ghosh, M. Seibt, T. Niehaus, ‘Graphene quantum dots with visible light absorption of the carbon core: Insights from single-particle spectroscopy and first principles based theory’, *2D Materials* 3, 041008, 2016 ([DOI](#)).
- M. Ghosh*, **S. Ghosh***, H. Attariani, K. Momeni, M. Seibt, G. M. Rao, ‘Atomic defects influenced mechanics of II-VI nanocrystals’, *Nano Letters* 16, 5969-5974, 2016. ([DOI](#)) [*equally contributed]
- **S. Ghosh**, ‘Atomic structure of single dipole emitters: Scanning confocal fluorescence correlated HRTEM’, *Imaging & Microscopy*, 3, 2016, ([pdf](#)) Microscopy Conference 2015, Göttingen.
- **S. Ghosh**, M. Ghosh, M. Seibt, G.M. Rao, ‘Detection of quantum well induced single degenerate-transition-dipoles in ZnO nanorods’. *Nanoscale*, 8, 2632-2638, 2016, ([DOI](#)).
- M. Ghosh, **S. Ghosh**, M. Seibt, K.Y. Rao, P. Peretzki, G. M. Rao ‘Ferroelectric origin in one-dimensional undoped ZnO towards high electromechanical response’, *CrystEngComm*, 18, 622-630, 2016, ([DOI](#)).
- **S. Ghosh**, A. M. Chizhik, N. Karedla, M. O. Dekaliuk, I. Gregor, H. Schuhmann, M. Seibt, K. Bodensiek, I.A.T Schaap, O. Schulz, A.P. Demchenko, J. Enderlein, A.I. Chizhik., ‘Photoluminescence of Carbon Nanodots: Dipole Emission Centers and Electron-Phonon Coupling’. *Nano Letters* 14 (10), 5656-5661, 2014, ([DOI](#)).
- **S. Ghosh**, M. Kumbhakar, M. Platen, I. Gregor, J. Enderlein ‘Single-molecule fluorescence inside solid-state nanochannels’ *SPIE BiOS*, 895008-895008-7, 2014, ([DOI](#)).

Chapter 1

Motivation

1.1 Introduction to nanoscale photonics

When light interacts with nanometre scale objects or space several non-intuitive phenomena takes place such as fluorescence, surface plasmons, diffraction, and scattering. For example, soap bubbles frequently possess magenta colour due to its wall thickness of approximately 250 nm (i.e., half of the wavelength of green colour). A destructive interference of the inner and outer wall reflection (due to the wall thickness induced phase difference) eliminates the green colour. The absence of green colour in the reflected light exhibit the magenta colour (considering red-green-blue (RGB))

colour model, R+B produces magenta colour). Similar phenomena take place in the wings of the Morpho butterflies containing a periodic arrangement of nanostructures – giving rise to the iridescent blue colour. Another classic example is the colourful glass windows of churches which are photostable since ancient ages due to the presence of plasmonic nanoparticles. It was found that high-temperature treatment to incorporate metallic salts with the glasses results in these colourful glasses. Since decades it was unknown that the thermal treatment creates metallic nanoparticles within glasses. Depending on their sizes they produce different colours due to geometry and permittivity dependent plasmonic effect. Furthermore, if we place biological organisms or biomaterials under UV light, most of the time we see bright emission of different colours than the UV light due to fluorescence. For example, our nails and teeth are fluorescent under UV light similar to the starfish shown in the graphical abstract. On this note, organic molecules with bright fluorescent properties are artificially synthesised for several applications, such as cell labelling, biomolecular assays, and organic photovoltaics. With the advancement of nanoscience and nanotechnology, inorganic fluorescent nanomaterials are also discovered which have superior fluorescent properties than organic fluorophore molecules due to localised quantum wells generated from crystal defects. The localised quantum well is often found in crystalline materials where atomic defects frequently appear. Quantum wells can also be structural. Structural quantum wells – when crystalline materials are at nanometre length scale due to quantum confinement they become photoluminescent. An example of them is quantum dots. Quantum dots are interesting to a wide range of scientists. Some of the properties of quantum dots find applications in light emitting diodes [1], spin-based information processings [2], telecommunications [3], photovoltaics [4], super-resolution imaging [5], and biomedical diagnostics [6].

1.2 Scope and structure of the thesis

The research works presented in this doctoral thesis are motivated by the immediate requirements of early stage detection of life threatening diseases. Inabilities to detect early onset of diseases, like protein misfolding disorders are turning them into growing epidemics. The current human life expectancy is decreasing in rapid rate because of Alzheimer's, Parkinson's, prion diseases, amyotrophic lateral sclerosis, and type 2 diabetes. The World Alzheimer's Report of 2015 states that the current expenditure in the USA alone for dementia is USD 818 billion [7]. By 2018 this cost will rise to USD 1 trillion. Hence, it is a high time to perform scientific research on ultra-sensitive biomedical diagnostics. In biomedical diagnostics and biophysical research, ensemble measurements score poorly in contrast to contemporary single molecule level measurements.

To combat the aforementioned problem in the healthcare, single molecule sensitive detection has shown tremendous success in studying biomolecular interactions [8], genome sequencing [9], structural biology [10], nanoscale biomechanics [11], and super-resolution microscopy [12]. In

order to study a particular biomolecule in the presence of many interacting molecules, one needs to tackle the random motion or the diffusion of the biomolecule. Diffusion is the current technological bottleneck to study a particular single molecule for extended time. To overcome this problem we need a cost-effective, high-throughput, and ultra-sensitive fluidic platform. Besides, diffusion is the basis of most of the fundamental biomolecular interactions. It is required to be studied at single molecule level. This will enable us to detect and study scientific quests like protein misfolding, protein-aggregation, and inappropriate toxic interactions of oligomers at single molecule level. On that note, in this thesis, I present investigations of single molecule nanofluidics and light-matter interaction in nanostructures. The single molecule nanofluidic experiments presented in this thesis establish a path towards confining diffusion in two spatial dimensions and allowing molecular interactions in one dimension. The study shows an immense potential to use nanofluidics for single molecule detection where physiological conditions can be maintained. The light-matter interaction in nanostructure was studied to understand a novel nontoxic fluorescent nanomaterials – carbon nanodots with single photon emission aiming towards studying biomolecular interactions and imaging in future. A theoretical study of the nanodots investigated their excited state properties and suggested some of the optimal nanodots structures be fabricated in future.

The thesis contains four main chapters – *theoretical background*, *single molecule nanofluidics*, *carbon nanodots - single dipole emitters*, and *light-matter interaction in carbon nanodots*.

In chapter 2 – theoretical background, I address the theoretical background necessary to understand the thesis with brief reviews on each topic as well as their applications. The chapter is divided into four parts – nanofluidics, fluorescence, light-matter interaction in nanostructures, and single photon sources. The field of nanofluidics is motivated from the naturally existing nanopores in the cell membrane which can transport single molecules in a high-throughput manner. With brief review on biological nanopores, I discuss the fundamentals of molecular diffusion to present a general overview of the need of nanofluidics in biophysical research. Then, I discuss different strategies of realising nanofluidic flows. Self-assembled hollow nanostructures mimicking the biological nanopores are not difficult to synthesise. However, using them in a nanofluidic application is difficult. I discuss those difficulties in the chapter and give a brief review of the state-of-the-art nanofabrication and characterisation techniques which were used to create functional high-throughput nanofluidic devices. Further, to understand the study the single molecule fluid flow using fluorescence inside the nanochannels, I give a brief insight of fundamentals of fluorescence. Some of the topics I discuss in the chapter are basic quantum mechanics, quantum confinement, many-body interaction, photon absorption and desorption within the quantum mechanical framework, generation of electric dipoles and its coupling with phonons. Quantum mechanical modelling of fluorescence is not straightforward. So, I describe the light-matter interaction in the context of density functional theory (DFT) – the most accurate technique to handle the many-body interaction. I also discuss the time-dependent extension of DFT and linear response

theory to account the electromagnetic field of light within many-body formalism. Although DFT is accurate, the primary drawback is it is computationally heavy. I introduce a reasonable approximation of tight binding and coupling it to DFT – density-functional based tight-binding method (DFTB). DFTB turns out to be an excellent approximation where the computational load reduces hugely. Within the DFTB formalism, I describe how to obtain excited state dynamics of a finite molecule with many atoms. Next, I briefly discuss single-photon source since carbon nanodots are single-photon emitters. I also discuss topics like point spread function characterisation, de-focused imaging, single molecule photoluminescence spectroscopy, and fluorescence correlation spectroscopy – some of the essential fluorescence-based techniques to study single-photon source. To understand the nanoscale single-photon emitters, atomistic understanding is necessary where high resolution transmission electron microscopy (HRTEM) is required to be used. For that, I also discuss the instrumentation and fundamental insight of HRTEM.

Chapter 3 - single molecule nanofluidics presents how to perform single molecule experiments in fluid where the random motion of diffusion is confined. A high-throughput single molecule detection scheme is shown using a one-dimensional flow platform. The development of a simple and cost-effective nanofluidic device is presented here to achieve the one-dimensional flow of discrete single molecules. Shadow-angle-electron-beam deposition technique is introduced in this chapter in combination with electron beam lithography and reactive ion etching to fabricate nanofluidic devices with arrays of nanochannels with the diameter ranging from 30 nm to 100 nm. Short DNA molecules, carbon nanodots, and organic fluorophores are some of the nanoscale objects were studied using the nanofluidic devices. The one-dimension flow of discrete single molecules is identified with dual-focus fluorescence correlation spectroscopy (2fFCS). The fluid flow was generated using electroosmosis. Size distribution analysis of single molecule photon bursts due to single molecule transits through two foci of 2fFCS are also demonstrated in this chapter.

One of the key parameters of nanophotonic and single molecule experiments is fluorescence. To study biomolecular interaction one needs to use a fluorescent probe which is highly photostable as well as non-toxic. In the next chapter, carbon nanodots - single dipole emitters, I present newly identified carbon-based fluorescent nanocrystals, which are suitable fluorescent probes for biological cell labelling. Here, a fluorescence correlated electron microscopy approach was developed to investigate the origin of fluorescence. The size distribution of the nanodots is within 2 nm to 20 nm. They all possess single transition dipole. In other words, they are single photon emitters. A visible range emission and strong electron-phonon coupling are observed in the photoluminescence spectra of carbon nanodots. The phonon modes are calculated for respective structures of the nanodots. The calculated phonon frequencies match reasonably with the phonon energies obtained from the photoluminescence spectra. This infers that the origin of fluorescence is certainly coupled with the crystal structure of the nanodots. An elemental analysis of them shows a presence of oxygen atoms besides carbon. At the end of this study, it is also speculated that certain functional

groups in the nanodots could be the possible reason behind their visible range photoluminescence. Subsequently, in chapter 5, I address the light-matter interaction in carbon nanodots from a theoretical perspective considering the strong variability of edge topology and functionalisation in real nanodots. I concentrate on the small carbon nanodots which are within 2.5 nm diameter keeping in mind most of the nanopores in biological cells are within such dimension. I use an approximate time-dependent density functional theory. Here, several model structures are extracted using a high-resolution transmission electron microscopy simulation. In most cases, the nanodots smaller than 2.5 nm are found to be primarily two-dimensional. Hence, we categorise them as graphene quantum dots (GQD). We come up with several model structures of GQD. The model structures show excitation energies in the visible spectrum agreeing with the previous single CND level photoluminescence studies. Since in the previous chapter the presence of oxygen atoms in CNDs was found, hydroxyl and carboxyl functional groups are also considered to study their effect on excitation energies. Here, the size dependency on the optical properties of CNDs is also investigated. I conclude from this study that the carbon cores of GQDs are optically active. Introducing functional groups as chemical defects to GQDs show some change in the excitation energies, however, it is not the primary reason behind CNDs fluorescence.

Bibliography

- [1] Qingjiang Sun, Y. Andrew Wang, Lin Song Li, Daoyuan Wang, Ting Zhu, Jian Xu, Chunhe Yang, and Yongfang Li. *Bright, multicoloured light-emitting diodes based on quantum dots*. Nature photonics 1, no. 12 (2007): 717-722. [20](#)
- [2] David Press, Thaddeus D. Ladd, Bingyang Zhang, and Yoshihisa Yamamoto. *Complete quantum control of a single quantum dot spin using ultrafast optical pulses*. Nature 456, no. 7219 (2008): 218. [20](#)
- [3] Michael Graham Burt. *Optical fibre with quantum dots*. U.S. Patent 5,881,200, issued March 9, 1999. [20](#)
- [4] Yan Li, Yue Hu, Yang Zhao, Gaoquan Shi, Lier Deng, Yanbing Hou, and Liangti Qu. *An electrochemical avenue to green-luminescent graphene quantum dots as potential electron-acceptors for photovoltaics*. Advanced Materials 23, no. 6 (2011): 776-780. [20](#)
- [5] Keith A. Lidke, Bernd Rieger, Thomas M. Jovin, and Rainer Heintzmann. *Superresolution by localization of quantum dots using blinking statistics*. Optics express 13, no. 18 (2005): 7052-7062. [20](#)

- [6] Warren CW Chan, and Shuming Nie. *Quantum dot bioconjugates for ultrasensitive nonisotopic detection*. Science 281, no. 5385 (1998): 2016-2018. [20](#)
- [7] Martin James Prince. *World Alzheimer Report 2015: the global impact of dementia: an analysis of prevalence, incidence, cost and trends*. Alzheimer's Disease International, 2015. [20](#)
- [8] Rahul Roy, Sungchul Hohng, and Taekjip Ha. *A practical guide to single-molecule FRET*. Nature methods 5, no. 6 (2008): 507-516. [20](#)
- [9] David R. Bentley, Shankar Balasubramanian, Harold P. Swerdlow, Geoffrey P. Smith, John Milton, Clive G. Brown, Kevin P. Hall et al. *Accurate whole human genome sequencing using reversible terminator chemistry*. Nature 456, no. 7218 (2008): 53. [20](#)
- [10] Shimon Weiss. *Measuring conformational dynamics of biomolecules by single molecule fluorescence spectroscopy*. Nature Structural and Molecular Biology 7, no. 9 (2000): 724. [20](#)
- [11] Matthias Rief, Filipp Oesterhelt, Berthold Heymann, and Hermann E. Gaub. *Single molecule force spectroscopy on polysaccharides by atomic force microscopy*. Science 275, no. 5304 (1997): 1295-1297. [20](#)
- [12] Bo Huang, Mark Bates, and Xiaowei Zhuang. *Super-resolution fluorescence microscopy*. Annual review of biochemistry 78 (2009): 993-1016. [20](#)

Chapter 2

Theoretical background

This chapter introduces the necessary concepts required to understand this thesis. I divide them into four main parts – nanofluidics, fluorescence, light-matter interaction in nanostructures, and single transition dipole. In the first part, I explain the molecular diffusion and how to control it to perform single molecule or single nanoparticle level measurements in fluidic condition. The nanofluidic strategies are discussed from theoretical to experimental point of views. On that con-

text, the Brownian motion is described in three-dimensional and one-dimensional space. I discuss several nanofabrication techniques which we have used to develop nanofluidic devices. The second part describes the theory of fluorescence which is widely used in biophysical measurements and quantum optics. Fluorescence plays an important role in quantitative studies of single molecules and nanoparticles. I describe the physics of fluorescence starting from the particle in a box to electron-phonon coupling. After discussing fluorescence, I review the theoretical models to describe the light-matter interaction in nanostructures. Here, I emphasise on the development of density functional theory and its time-dependent extension.

2.1 Nanofluidics

When fluid flows through a narrow or confined cross-sectional area in the order of nm^2 , such fluidic systems are called *nanofluidics* [1]. In nature, proteins fold in different ways to produce nanofluidic channels for various purposes [2, 3, 4, 5], such as aquaporins, α -hemolysins, and porins as shown in figure 2.1 (black regions denote the nanofluidic openings). The primary function of aquaporins is to transport water in and out of cells [6]. Their pore diameters are in range of 2 Å to 10 Å (figure 2.1) [2, 7, 8, 9] allowing each subunit to transport up to three billion water molecule per second [10]. On the other hand, α -hemolysin monomers secreted from the bacterium *Staphylococcus aureus* binds to healthy animal cell membranes. The monomers oligomerise to form a transmembrane nanochannel facilitating the removal of important molecules, such as ATP. This can result in cell death and diseases such as hemostasis, thrombocytopenia, and pulmonary lesions [11, 12, 13]. The pore size of the α -hemolysin-based nanochannel is ~ 2.5 nm (figure 2.1) [12, 13]. Porins are another kind of transmembrane nanochannels composed of several antiparallel β -strands (figure 2.1) [5]. Fluidic openings in porins are ~ 3 nm [5], which is sufficient to allow passive diffusion of hydrophilic molecules [14]. Porins are found in the outer membranes of Gram-negative bacteria and some Gram-positive bacteria, the mitochondria, and the chloroplast [14, 15, 16].

In this thesis, I present artificial nanofluidic channels using inorganic solid-state materials. Before going to the theoretical background of the preparatory methods of our nanochannels, let us briefly review the physics of nanofluidics. Later, this will also enable us to model nanofluidic flows. Diffusion induced random spatial motion of a single molecule reduces the probability of finding it at a given position. This can be suppressed by confining fluid flow in 2D or 1D which results to nanofluidics. In this part, I will briefly introduce diffusion, fluid flow, and the Fokker-Planck equation to model 1D flow. I also present the nanofabrication techniques (electron beam lithography (EBL), reactive ion etching (RIE), and electron beam evaporation (EBE)), which I used to develop nanofluidic devices. These devices were characterised using atomic force microscopy (AFM), focused ion beam (FIB), and scanning electron microscopy (SEM). The principles of these techniques are also briefly introduced in this section.

Figure 2.1: Some examples of biological nanochannels – aquaporin [9], α -hemolysin [12], and porin – mycobacterial outer-membrane channels [5] The insets show corresponding side views of the macromolecular complexes.

2.1.1 Diffusion

The general or lay definition of *diffusion* is ‘*spreading of something more widely*’ [17]. According to Fick’s laws of diffusion, it is a microscopic event in fluids and solids to reach the equilibrium concentration or steady state where the flux of atoms or molecules is proportional to the concentration gradient as shown figure 2.2. Here, a concentrated drop of watercolour is dropped on the surface of the water which starts diffusing across the volume. A magnified version shows after some time the colour molecules move randomly everywhere in the glass.

Figure 2.2: Diffusion of watercolour in water; captured with a Canon EOS 1100D camera and Tamron AF 18-200mm F/3.5-6.5 (IF) lens.

In 1827, Robert Brown observed certain agitations of pollen grains in water under a microscope. He could not explain the reason behind the motion of the pollen grains. 78 years later, in a seminal paper Albert Einstein described the physical reasoning behind the observation of Brown (figure 2.3) [18], which was experimentally verified by Jean Perrin in 1908 [19]. Einstein described that the pollens are continuously displaced from one position to other due to continuous collision with other molecules which are moving randomly in every other directions. This groundbreaking theory and measurement confirmed the existence of molecules and atoms. Einstein formulated the equation describing the Brownian or random motion of molecules. He came up to a constant called Diffusion coefficient (D) as a function of the mean squared displacement of molecule, $\langle d^2 \rangle = 2Dt$ where t is time. In liquid medium considering the *Stokes friction*, diffusion coefficient can be written as

$$D = \frac{k_B T}{6\pi r \eta} \quad (2.1)$$

where k_B is the Boltzmann constant, T is the temperature, r is the hydrodynamic radius of the molecule, and η is the viscosity of the medium. This enables us to calculate the diffusion coefficient of an argon atom in water at 25° as $2 \times 10^{-5} \text{ cm}^2/\text{s}$.

As observed in Perrin's drawing (figure 2.3), the primary concern of Brownian motion is the molecular collision induced perpetual irregular motion of a single molecule – *random walk*. A molecule in room temperature liquid collides 10^{21} times per second with surrounding molecules [20]. Each collision changes the path of molecule's time travel, as Subrahmanyam Chandrasekhar wrote, 'the details of the path are impossibly fine' [20]. In figure 2.4, traces of eight particles are simulated in toroidal space (if the molecule moves out of the frame, it appears back to the frame from opposite edge of the disappearing edge) where they are allowed to chose eight adjacent positions randomly (*Moore neighbourhood* criteria [21, 22, 23]). Here, the grid is 500×500 . In total, the simulation shows 5×10^4 steps.

Figure 2.3: Jean Perrin's drawing of Brownian motions [19].

The above Brownian motion can be described by the Langevin equation [20]

$$\frac{dv}{dt} = -\beta v + \Gamma(t) \quad (2.2)$$

where v is the velocity of the molecule. Here, $-\beta v$ and $\Gamma(t)$ represent friction and stochastic temporal (t) fluctuation resulting in Brownian motion, respectively. The frictional force, $-\beta v$ can be obtained from Stoke's law as $6\pi r \eta v/m$ where m is mass of the particle. Concerning the fluctuating Brownian part, the change of v is negligible within a time interval when $\Gamma(t)$ undergoes

Figure 2.4: Simulated trajectories of eight particles' random walk in a 2D space. The trajectory steps fade out exponentially over time.

several random positions. Hence, $\Gamma(t)$ is independent of v . Γ can be considered as a Gaussian white noise with correlations $\langle \Gamma(t)\Gamma(t') \rangle = \Delta\delta(t-t')$. Averages with respect to the noise Γ is performed to quantify physical quantities from the stochastic Langevin equation. But this is a computationally expensive method. Instead, one can use the equation of motion for time dependent probability distribution $p(x, t)$, which is well described by the *Fokker-Planck equation* [24] as:

$$\frac{\partial}{\partial t}p(x, t) = -\frac{\partial}{\partial x}[v^\dagger(x, t)p(x, t)] + \frac{\partial^2}{\partial x^2}[D(x, t)p(x, t)]. \quad (2.3)$$

where, x and t are the position of the particle and time, respectively, and D is the diffusion coefficient. In 1D with a constant drift velocity ($v^\dagger$), the probability distribution is given by

$$p(x, t) = \frac{1}{\sqrt{4\pi Dt}} \exp\left(-\frac{(x - v^\dagger t)^2}{4Dt}\right). \quad (2.4)$$

2.1.2 Strategies of suppressing diffusion

Diffusion can be suppressed to a large extent by increasing the η . The negative aspect of such condition is molecular interaction also decreases to a large extent [25]. This condition is useful to study *rotational diffusion** with significant application in structural biology [25, 26, 27, 25]. However, it is not useful to study biomolecular interactions at single molecule level. Another approach to suppress diffusion is by structurally confining the spatial freedom of molecules, similar to the fluidic behaviour in biological nanopores (figure 2.1). Inorganic mimics of biological nanofluidic channels are nanoporous alumina [31], carbon nanotubes [32] (figure 2.5), boron nitride nanotubes [33], silicon nanotubes [34], gallium nitride nanotubes [35], etc. However, using them

*Rotational diffusion coefficient is given by Stoke-Einstein-Debye equation where $D_{rot} = k_B T / (8\pi r_{rot}^3 \eta)$ [25, 29, 30]. Here, r_{rot} is the hydrodynamic radius of a Brownian molecule.

for single molecule nanofluidic studies are difficult because of two reasons. Firstly, aligning them in a periodic two-dimensional lattice for microscopic visualisation is difficult since they self-assemble [36, 37, 38]. Secondly, most of the time solid-state nanostructures are photoluminescent [39] [discussed in section 2] which reduces signal to noise ratio in fluorescence-based single molecule studies. The top-down approach enables us to prepare a confined nanofluidic channel with required structural geometries as well as with a choice of material to avoid the latter problem. The nanofluidic device presented in this thesis used a nanofabrication process called *shadow-angle-electron-beam-deposition* or *SAEBD* which is elaborated in chapter 3. To establish the SAEBD method we have used following techniques:

Electron beam lithography

Electron-beam (e-beam) lithography (EBL) is a nanofabrication technique that uses direct writing of electron beam on an electron-sensitive polymer (e-beam resist). In contrast to the optical lithography technique, EBL is able to produce structures at sub-10 nm dimension [40]. In EBL, an e-beam scans a surface of an e-beam-resist coated substrate and changes its solubility (by polymerising or monomerising the negative or positive e-beam-resist, respectively). During chemical development the exposed or unexposed region of a positive e-beam-resist gets removed, and the subsequent pattern is transferred to the substrate through an etching process (figure 2.6). Figure 2.7a shows a schematic of an EBL setup. It contains an electron gun source, electron optics with a sample stage, and a digital controller (computer controlled) to control the electron optics and sample stage movement. The e-beam is directed through an anode in vacuum and by proper controlling of its deflection through alignment coil, it is aligned with the electron optics. The selectable aperture is used to vary the final spot size of the e-beam. In order to obtain an optimal shape of the beam, the incident beam is directed to the sample through several magnetic lenses and polepiece. Depending on design of the required structure (passed to the sample stage through the computer controlled hardware interface), the respective area of the e-beam-resist is exposed to the e-beam and the corresponding design is written directly on the e-beam-resist. To generate

Figure 2.5: Single walled, double walled, and triple walled carbon nanotubes. The scale bar is 1 nm.

Figure 2.6: Process of electron beam lithography

a nanometre scale structure parameters like accelerating voltage of electrons, their exposure area

Figure 2.7: (a) A typical setup of electron beam lithography setup. (b) An array of nanochannels (40 nm width of silica on silicon) - mold for soft-lithography [43]

(controlled by aperture), exposure time, and e-beam current play crucial roles. By optimising the above parameters, this technique can produce high-resolution, densely packed arbitrary structure patterns depending on the electron optics, photoresist, substrate, developer and operating conditions. General precautions during EBL-based nanolithography to be taken care off are forward and back-scattering of electrons causing proximity effects [41], sudden change in the structural dimensions of the adjacent features [41] and collapse due to capillary forces [42] which play significant role in nanostructure development. I demonstrate an example of EBL in figure 2.7b – a soft-lithography mold of silica prepared for nanochannels of width 40 nm. The silica nanostructures were pattern-transferred on silicon substrate by using e-beam resist as a sacrificial layer. Here, the silica was evaporated using electron beam evaporation (discussed in the section: electron beam evaporation).

Reactive ion etching

Reactive ion etching (RIE) is a dry etching technique used in micro/nanofabrication process. It removes material from a substrate using a chemically reactive plasma. In this method plasma (from a composition of chemical gases) is created by an electromagnetic field under vacuum. This technology was first patented by IBM in 1981 [44], and subsequently, the inventor Linda Epharth reported the technique in the IEEE Transactions on Electron Devices in the same year where she named this technique as *reactive ion etching* [45]. The seminal paper showed the evidence of submicrometer

scale selective etching using RIE of silicon oxide, nitride, and/or oxynitride surface without using any liquid reactants [45]. In this technique, High-energy ions from plasma reacts with the material on the substrate surface and removes it. Figure 2.8 shows a typical RIE setup consists of a vacuum chamber with gas inlet/outlets and two electrodes. The substrate is positioned (upside down) at the top electrode and the chamber is filled with a choice of gases. A high electric field with a frequency around 13.56 MHz across the electrodes ejects secondary electrons from the bottom electrode. This electrons collide with the gas molecules (in between the electrodes) and produce reacting ions. The ions are accelerated towards the top electrode due to the applied electric field and react with the material on the substrate. Finally, the deposited material is etched away and the by-product gases are pumped out from the system. Plasma can be generated as a step function of high voltage, so etching can be stopped at any time during the process. Unlike solution based etching where diffusion plays a major role, RIE is a dry and ballistic etching process. In micro-/nanofabrication this is why RIE based etching is called *anisotropic etching*. An example of anisotropic etch profile is shown in figure 2.9. Here, an atomic force microscopy image of an 80 nm deep and 40 nm wide nanotrench profile is fabricated using RIE is shown.

Figure 2.8: An RIE setup. The purple colour between two electrodes represent a real-time plasma.

Figure 2.9: (a) Atomic force microscopy topography of a nanotrench etched using RIE. (b) A line profile from (a) showing width: 40 nm and depth: 80 nm of the nanotrench.

Atomic force microscopy

Atomic-force microscopy (AFM) is a high-resolution scanning probe microscopy technique. It maps sample topography with a cantilever tip and has resolution in the order of Angstroms [46, 47]. It was first introduced by Gerd Binnig, Calvin Quate, and Christoph Gerber in 1986 [46]. The concept of AFM came up immediately after scanning tunnelling microscopy (STM) was developed by Gerd Binnig and Heinrich Rohrer [48]. AFM is an extension of STM where a topography map

is generated by scanning a cantilever tip on a surface of interest and simultaneously, measuring the forces between the tip and the sample with respect to their separation. When the cantilever tip approaches a structure, the tip-sample separation changes (due to Van der Waal interaction, capillary force, electrostatic force and mechanical contact force). Hence, the force acting on the tip changes. This deflects the tip from its initial position. Change in tip deflection is detected by a laser that reflects from the cantilever top surface and acquired by a photodiode array. A piezoelectric feedback system is used in the AFM setup to control the cantilever movement. This technique is sensitive to cantilever stiffness and tip diameter. Figure 2.10 shows a typical

Figure 2.10: An atomic-force microscopy (AFM) setup. Tapping mode topography of a nanostructured PDMS surface.

AFM setup where a tapping mode scan is performed on a plasma induced wrinkled PDMS surface. The tapping mode scan is a non-contact mode AFM technique that maintains constant amplitude and frequency of the AFM cantilever throughout the measurement. Here, initially the cantilever tip is driven at a frequency (near to its resonance frequency) with constant amplitude while scanning the sample surface. When it approaches the wrinkled structure the amplitude changes and the cantilever deflects from its initial position. Displacement of the cantilever tip from its initial position is detected by the mirrors and position detectors. The feedback controller (piezoelectric actuator) adjusts the height of the cantilever to maintain the set amplitude and oscillation. An example of an AFM scan image of the PDMS nanostructures is shown in figure 2.10.

Electron beam evaporation

Electron-beam (e-beam) evaporation is a thin-film deposition technique that is widely used for electronic device fabrication [49], surface modification [50], and designing new reflective and transmissive optical elements [51]. In this technique atoms of the desired material are sputtered away from the material by bombarding high-energy e-beam in high vacuum (10^{-6} mbar). The disrupted atoms ballistically evaporate and solidify on the substrate of interest as a

coating. An e-beam evaporation system consists of a vacuum chamber, two electrodes (anode and cathode) and a source of electrons (thermoionic/field emission/anodic arc). The material to be deposited (target) is placed on the anode and the substrate is positioned with the cathode. The accelerating voltages of e-beam range from 3 kV to 40 kV. Thus the generated e-beam always has a high kinetic energy. The path of the e-beam is manipulated with a magnetic

Figure 2.11: Left panel: SEM image of a gold nanoring prepared with EBL and e-beam evaporation. Right panel: AFM topography of the same nanoring – evidence of non-conformal e-beam evaporation growth.

lens to bombard the target in high vacuum (a detail description is given in chapter 3). Once the high-energy electrons hit the target they rapidly lose energy [52]. The kinetic energy dissipates into thermal energy. The released heat is capable of sublimating the target material. The removed atoms from the target are therefore in gaseous phase. These atoms are then deposited ballistically. A constant slow growth rate over time results in a thin-film of target material on the substrate in non-conformal way [53]. Quality of the thin film deposited by e-beam evaporation technique is dependent on the pressure of the chamber and the e-beam energy. The non-conformal growth of e-beam evaporation is shown in figure 2.11. An array of gold nanorings were prepared for nanophotonic experiments where an e-beam resist is patterned with EBL and by a non-conformal growth of gold using e-beam evaporation. Here, the SEM image shows inner diameter of the nanoring as 70 nm, and an AFM topography shows the height of the nanoring as 50 nm (the final thickness of the e-beam evaporated thin-film). The inner and outer-edges of the nanorings are vertical – confirming the e-beam evaporation is a non-conformal ballistic physical evaporation process.

Focused ion beam (FIB)

Focused ion beam (FIB) is an imaging as well as nanofabrication technique for site-specific local characterisation [54], deposition [55], and ablation of materials [56]. At low current, it has an imaging resolution of 5 nm with gallium ions, Ga^+ [57]. This technique is similar to scanning electron microscopy (SEM) or EBL, instead of a focused e-beam FIB uses focused ion beam to image samples. Focused beam of ions operated at low beam currents are used for imaging whereas at high beam currents ions are used for site specific sputtering down to a nanometre scale. FIB is a direct lithography process without the need of photoresist for patterning. Here, a focused ion beam reacts with the material of interest and etches it. Scanning the sample surface with primary ion beam (mostly Ga^+) produces secondary ions or neutral atoms or secondary electrons. These

Figure 2.12: Focused ion beam setup where an ion beam source is aligned with an SEM. The highest eccentric angular separation between the ion beam and e-beam can be 52.4° .

sputtered ions or electrons are detected and used to form an image. Figure 2.12 shows a schematic of an FIB setup integrated with an SEM setup (details on SEM is given in EBL section). The FIB and SEM are aligned with an angular separation of 52.4° . If a sample is positioned in an eucentric height, it keeps a good correlation between FIB and SEM. The SEM is used to image and focus the region of interest to avoid ionic damage from FIB. In the setup shown in figure 2.12, the part of the FIB setup consists of a gallium ion source, ion optics, high vacuum chamber, and sample holder. The mirror electrode and condenser lens are used to focus the ion beam, and the aperture is used to select particular range of ion current (controlled by a group of selectable apertures). The deflector is used to deflect the ion beam in small angles, and the Einzel lens[†] is used to form a converging ion beam i.e., directed to focus towards the sample through the pole piece. An enclosed nanochannel prepared cannot be characterised using an AFM like technique. However, by milling with slow etch rate using an aperture of several pico-amperes, FIB produces a high-resolution cross-section of nanochannels, which can be visualised with the SEM since the sample can be eucentrically positioned. In figure 2.13 some of the FIB-based nanostructures ranging from 500 nm to 100 nm

[†]Einzel lens is used to focus charged particles without affecting the energy of the particles [58]. It uses a set of apertures producing a symmetric electrostatic potential. This enables the ions to come back to their initial energy while exiting the lens. Here, a velocity gradient is created along the radius of the beam to focus the ion.

are shown.

Figure 2.13: A range of different micrometre to nanometre sized structures etched with FIB. (a) Feature size 500 nm – calibration on 200 nm gold thin-film. (b) A channel with 280 nm width. (c) Nanoholes prepared using FIB on 200 nm gold. [d] FIB etched Nanoholes with a diameter of 100 nm.

2.2 Fluorescence

We often see glowing wristwatches in dark as shown in figure 2.14. They are generally coated with materials which emits lights after absorbing light. The materials used here are electronically excited when exposed to light. Electrons in such materials absorb certain wavelengths and attain their excited states. Now onwards, I will refer this event as *excitation*. The excited states are energetically not favourable, so the excited electron relaxes to equilibrium or electronic ground state by emitting a photon or as known as *emission*. Such photo-induced luminescence is called photoluminescence. When the electronic transitions related to forbidden energy states/interseem

crossings^{||} the process is called phosphorescence; a phenomena frequently observed in wristwatches for ‘*glow-in-the-dark*’ effect. When the excited state to ground state relaxation is from an excited singlet state[§] such photoluminescence is called fluorescence. Fluorescence is widely used in microscopic visualisation of biological organelles. Fluorescence microscopy is routinely used in biological research. Since past 25 years, the development of variety of methods in *super-resolution fluorescence microscopy* revealed many unknown and complex biophysical phenomena at nanometre length scale [59]. One of the key researches in super-resolution microscopy is the development of fluorescent probes. If one can precisely label biological organelles with high quality fluorophores, one can resolve nanometre scale structure using super-resolution techniques such as NSOM, 4Pi, SIM, STED, STORM, PALM, and SOFI [59, 60]. On that note in this thesis, we have used and studied new fluorescent probes and demonstrated their underlying quantum mechanical behaviour, which have potential application not only in super-resolution microscopy but also in quantum optics. So, in order to explain my work on nanoscale photonics, I will briefly introduce the fundamental atomic and molecular physics. I will start with the concept of energy levels and electronic transition and move to complex many-body interaction involves in molecules related to optical properties.

A free electron can have any arbitrary positive value of energy. The bound electron possesses negative energy values. The bound energies are restricted or defined by the atomic orbitals and known as energy levels. In ascending order the energy levels are denoted with E_0, E_1, E_2, E_3 . E_0 is the *ground state* or the *minimum energy state*, and the others are *excited states*. If an electron is situated at an excited state, it cannot sustain there forever. It relaxes back to the ground state by releasing energy as a photon emission. The frequency of the emitted photon is defined by the energy difference between the particular excited state and ground state. For example, if an electron was at E_3 and relaxed to E_1 , the emitted photon will have the frequency of

$$\omega_{31} = (E_3 - E_1)/\hbar \quad (2.5)$$

^{||}An example of forbidden mechanism/transition is luminescence decay involving a spin flip which is forbidden by electric dipole transitions considering the selection rule.

[§]Single state defines when all electrons are paired or the the net angular momentum is zero. If a quantum mechanical system contains one unpaired electron it is called a doublet state which shows splitting of spectral lines. Similarly, for a triplet state the system possess two unpaired electrons with threefold splitting in spectral lines.

Figure 2.14: ‘*Glow-in-the-dark*’. Two phosphorescent arms of a wristwatch. Two images were superimposed on each other. Images captured with a Canon EOS 1100D camera and Tamron AF 18-200mm F/3.5-6.5 (IF) lens. Image 1: 30 s exposure in complete darkness. Image 2: Fast exposure in ambient light. Image 2 was alpha composited with image 1 using 70% transparency.

where $\hbar$ is a reduced Planck's constant. Similarly, if an electron relaxed from E_1 to E_0 the emission frequency of a photon will be

$$\omega_{10} = (E_1 - E_0)/\hbar \quad (2.6)$$

According to the *Ritz combination principle*, the direct transition from E_3 to E_0 will be sum of the other intermediate transitions — ω_{31} and ω_{10}

$$\omega_{30} = \omega_{31} + \omega_{10} = (E_3 - E_0)/\hbar \quad (2.7)$$

With this, I will move to *quantum confinement* – a non-intuitive quantum property regularly encountered in the field of nanophotonics.

2.2.1 Quantum confinement

If an electron is confined within the length-scale of *de Broglie wavelength*^{*} of its wave function, such physical situation presents substantial difference in electronic properties from a bulk material [62, 63, 64]. This phenomenon is called quantum confinement. The confinement can be represented by potential energy – zero inside the region of confinement and suddenly increases at the edges. The configuration suggests a square well with infinite potential at the edges. Here, the energy of the particle is quantised due to the confinement, and its boundary conditions determine the allowed transition energies. The Hamiltonian of such a particle in a box system with confined region of space (length L) will be

$$H = -\frac{\hbar^2}{2m} \frac{d^2}{dx^2} + V(x) \quad V(x) = \begin{cases} 0 & \text{for } 0 \leq x \leq L \\ \infty & \text{otherwise} \end{cases} \quad (2.8)$$

where m is the mass of the particle and x is the position. The physical significance of the infinite potential energy at the walls suggests that the particle will not penetrate the walls. Outside the box where $x < 0$ or $x > L$ the wavefunctions are zero. This infers that at the walls the wavefunctions are also zero ($\psi(0) = 0$ and $\psi(L) = 0$). If these conditions are applied to a general solution of the form

$$\psi(x) = C \cos kx + D \sin kx; \quad k = \frac{\sqrt{2mE}}{\hbar} \quad (2.9)$$

we find $\psi(0) = C \cos 0 + D \sin 0$, when $x = 0$. Now, in order to get $\psi(0) = 0$, we need $C = 0$.

In case of $x = L$, $\psi(L) = D \sin kL$ by setting $C = 0$. Now, to make $\psi(L) = 0$, we can deduce the sine function to zero (since setting $D = 0$ is equivalent to never finding a particle within the box). If kL is an integral multiple of π , this condition can be achieved. Therefore,

$$k = \frac{n\pi}{L}; \quad \text{where, } n = 1, 2, 3, \dots \quad (2.10)$$

^{*}The de Broglie wavelength is the wavelength associated with a particle's momentum ($\lambda = h/p$ where λ is the wavelength, h is the Planck's constant, and p is the momentum of the particle).

Here, n represents a *quantum number* which labels each states of the system and thereby, energy of the system. From $E = \frac{k^2 \hbar^2}{2m}$, for any n state of a system will be

$$E_n = \frac{n^2 \hbar^2 \pi^2}{2mL^2} = \frac{n^2 h^2}{8mL^2} \quad (2.11)$$

So, the energy of the particle in a box is quantised. Now, the probability of finding a particle within the box can be represented by

$$\int_0^L \psi^* \psi dx = D^2 \int_0^L \sin^2 \left(\frac{n\pi x}{L} \right) dx = \frac{1}{2} LD^2 \quad (2.12)$$

Figure 2.15: A particle in a box: (a) Wavefunctions of first three energy levels; $n = 1, 2$, and 3. (b) Probability distributions at first three energy levels.

The norm of the wavefunction has to be 1 in order to find a particle in a box. In order to get that D should be $(2/L)^2$. In figure 2.15a, the wavefunctions for first three energy levels are plotted, where the energy gaps increase with increasing n . However, the amplitude of the wavefunction remains same at every energy levels. In figure 2.15b, the probability distributions (squares of the wavefunctions) of the same energy levels are plotted using equation 2.9. It is evident that the particle avoid the walls at $n = 1$. However, as the energy level increases the probability distribution becomes uniform in the x axis. At high energy levels, the distribution matches with the classically calculated distribution[‡].

Case 1, $n = 1$: At $n = 1$ a confined particle will hold the lowest energy level of the particle, where $E = \frac{h^2}{8mL^2}$. This is commonly known as zero point energy.

Hypothetically, this lowest energy can be removed if $h = 0$. However, the uncertainty of the particle's position is always finite (between 0 and L). This infers that uncertainty of particle's momentum can never be zero. In other words, the fundamental constant h in the real world is never zero.

[‡]As Niels Bohr formulated in 1920 the correspondence principle, quantum mechanically predicted behaviour should be reproduced by classical mechanics at large quantum numbers [65].

Case 2, $L \rightarrow \infty$: If the distance between two walls increases and the particle is more free than before, energy gaps among the neighbouring states reduces as

$$\Delta E = E_{n+1} - E_n = \{(n+1)^2 - n^2\} \frac{\hbar^2}{8mL^2} = (2n+1) \frac{\hbar^2}{8mL^2} \quad (2.13)$$

and approaches to zero when L is close to infinity at macroscopic length scale box. In other words, the particle at this scale is completely free and the quantisation is negligible. The same holds when m is large.

Case 3, 2D square well: Now, if we consider an infinite square well with two dimensions (x and y), the wavefunctions will behave like vibrating plates constrained at the edges. The Hamiltonian of such system will be

$$H = -\frac{\hbar^2}{2m} \left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) \quad (2.14)$$

Figure 2.16: Wavefunctions of a particle in a 2D square well where $\psi_{1,1}$ is for $n_1 = 1, n_2 = 1$, $\psi_{2,1}$ is for $n_1 = 2, n_2 = 1$, and $\psi_{2,2}$ is for $n_1 = 2, n_2 = 2$.

Figure 2.17: Probability distributions of a particle in a 2D square well, $\psi^* \psi_{1,1}$ is for $n_1 = 1, n_2 = 1$, $\psi^* \psi_{2,1}$ is for $n_1 = 2, n_2 = 1$, and $\psi^* \psi_{2,2}$ is for $n_1 = 2, n_2 = 2$.

and thereby, the Schrödinger equation of a confined particle inside the well with boundary condition of wavefunction as zero at the edges will be

$$\frac{\partial^2 \psi}{\partial x^2} + \frac{\partial^2 \psi}{\partial y^2} = -\frac{2mE}{\hbar^2} \psi \quad (2.15)$$

It can be solved by regular separation of variables technique for the Schrödinger equation. If we substitute $\psi(x, y)$ with XY as a trial solution

$$Y \frac{d^2 X}{dx^2} + X \frac{d^2 Y}{dy^2} = -\frac{2mE}{\hbar^2} XY \quad (2.16)$$

where X and Y are functions of x and y , respectively. Dividing this equation with XY we obtain

$$\frac{1}{X} \frac{d^2 X}{dx^2} + \frac{1}{Y} \frac{d^2 Y}{dy^2} = -\frac{2mE}{\hbar^2} \quad (2.17)$$

which can be separated into $\frac{d^2 Y}{dy^2}$ and $\frac{d^2 X}{dx^2}$ as

$$\frac{d^2 Y}{dy^2} = -\frac{2mE}{\hbar^2} Y \quad (2.18)$$

$$\frac{d^2 X}{dx^2} = -\frac{2mE}{\hbar^2} X \quad (2.19)$$

Here, $E_x + E_y = E$. The equations 2.18 and 2.19 resemble with the equation of one-dimensional system. Therefore, using $\psi = XY$, we can derive

$$\psi_{n_1, n_2}(x, y) = \frac{2}{\sqrt{(L_1 L_2)}} \sin\left(\frac{n_1 \pi x}{L_1}\right) \sin\left(\frac{n_2 \pi y}{L_2}\right) \quad (2.20)$$

$$E_{n_1, n_2} = \frac{\hbar^2}{8m} \left(\frac{n_1^2}{L_1^2} + \frac{n_2^2}{L_2^2} \right) \quad n_1 = 1, 2, 3, \dots \quad n_2 = 1, 2, 3, \dots \quad (2.21)$$

Here, we need two quantum numbers (n_1 and n_2) for two dimensions. Accordingly, in Figure 2.16 three wavefunctions for ψ_{n_1, n_2} are plotted. Considering the aforementioned boundary conditions, the shape of the eigenfunctions are shown. The corresponding probability distributions ($\psi^* \psi_{n_1, n_2}$) of finding the particle in two-dimensional confined space are plotted in Figure 2.17.

Case 4, Degeneracy: In the two dimensional square well $L_1 = L_2 = L$, so the energies at different levels can be deduced to

$$E_{n_1, n_2} = \frac{\hbar^2}{8mL^2} (n_1^2 + n_2^2) \quad (2.22)$$

Therefore, two states of reversible quantum numbers should have equal energy. In other word, a state of $|a, b\rangle$ with $n_1 = a$ and $n_2 = b$ should have same energy of $|b, a\rangle$ with $n_1 = b$ and $n_2 = a$, where $a \neq b$. This situation is widely known as *degeneracy of states*. An example of degenerate states are $|1, 2\rangle$ and $|2, 1\rangle$, where both have energy of $\frac{5}{8}(h^2 m^{-1} L^{-2})$, however, their wavefunctions are different as

Figure 2.18: Contour-plotted wavefunctions of two degenerate states. $\psi_{1,2}(x,y)$ corresponds to $n_1 = 1, n_2 = 2$ and $\psi_{2,1}(x,y)$ corresponds to $n_1 = 2, n_2 = 1$.

plotted in Figure 2.18. A symmetry transformation (90° about the centre $(0.5, 0.5)$) is observed with respect to each other wavefunctions, $\psi_{1,2}(x,y)$ and $\psi_{2,1}(x,y)$.

Degeneracy occurs due to high degree of symmetry. The degeneracy will be broken for the two state $|1, 2\rangle$ and $|2, 1\rangle$, if we consider a rectangle well (with $L_1 = L$ and $L_2 = 2L$) instead of a square well. Then, $E_{1,2} \neq E_{2,1}$.

Symmetry induced degeneracy can be explained with group theory. Considering an eigenfunction ψ_i of H with eigenvalue E ,

$$H\psi_i = E\psi_i \quad (2.23)$$

Multiplying equation 2.23 with an symmetry operation R from the left,

$$RH\psi_i = ER\psi_i \quad (2.24)$$

To obtain equation 2.25, $R^{-1}R$ is inserted for the identity in equation 2.24

$$RHR^{-1}R\psi_i = ER\psi_i \quad (2.25)$$

From the invariance of H , we cab obtain

$$HR\psi_i = ER\psi_i \quad (2.26)$$

So, ψ_i and $R\psi_i$ are of the same energy, E . One can formulate the maximum degree of degeneracy using the same approach. One can observe degeneracy in transition dipole due to overall structural symmetry of the crystal structure [61, 66] or localised symmetry in either valence or conduction band [67].

2.2.2 Many-body interaction

Till now I have only considered the cases of single particle or single electron systems. In reality, molecular photonics or nanophotonics deals with more than one particle. Therefore to establish

the fundamental reasoning behind photoluminescence of complex systems such as molecule or nanocrystal, many particle systems needs to be discussed where they all interact with each other as commonly called *many-body interaction*. Here, I present the standard model of a typical many particle system. The Hamiltonian of such system will be

$$H = \underbrace{\sum_{j=1}^M \frac{-\hbar^2}{2M_j} \vec{\nabla}_{\vec{R}_j}^2}_{E_{k,n}} + \underbrace{\sum_{i=1}^N \frac{-\hbar^2}{2m_e} \vec{\nabla}_{\vec{r}_i}^2}_{E_{k,e}} + \underbrace{\frac{1}{2} \sum_{i \neq j}^M \frac{e^2 Z_i Z_j}{4\pi\epsilon_0 |\vec{R}_i - \vec{R}_j|}}_{E_{s,n-n}} - \underbrace{\sum_{i=1}^N \sum_{j=1}^M \frac{e^2 Z_j}{4\pi\epsilon_0 |\vec{r}_i - \vec{R}_j|}}_{E_{s,e-n}} + \underbrace{\frac{1}{2} \sum_{i \neq j}^N \frac{e^2}{4\pi\epsilon_0 |\vec{r}_i - \vec{r}_j|}}_{E_{s,e-e}} \quad (2.27)$$

Here, charges of electrons, their masses, and spatial positions are denoted with $-e$, m_e , and $\vec{r}_j$, respectively, and the masses of nuclei, their atomic numbers, and spatial positions are denoted with M_j , Z_j , and $\vec{R}_j$. The first term, $E_{k,n}$ represents the kinetic energy of nuclei. The second term $E_{k,e}$, describes the kinetic energy of electrons. The third term, $E_{s,n-n}$ represents the electrostatic repulsion among the nuclei. The forth term, $E_{s,e-n}$ represents the electrostatic attraction among the electrons and nuclei. Finally, the last term, $E_{s,e-e}$ represents the electrostatic repulsion among electrons. There are certain deficiencies in the above model where necessary effects can be incorporated according to requirement. For example, in case of heavy elements with deep Coulomb potentials of the nuclei, *relativistic effects* cannot be ignored since the velocities of electrons near the nucleus is at relativistic domain [68]. One such relativistic effect is crucial for *magnetic anisotropy*. The photonic excitation can be incorporated in the model explicitly. [68].

Born-Oppenheimer approximation

In 1927 Max Born and Robert Oppenheimer wrote a seminal paper called *Zur Quantentheorie der Molekeln* (For the quantum theory of molecules) [69], where they had significantly simplified the many-particle interactions by segregating the nuclear and electronic coordinates. This simplification is known as *Born-Oppenheimer approximation*. Considering the large difference in masses of electrons and nuclei, dynamics of electrons are hugely dominated by the nuclei. Spatial displacement of nuclei affects electrons almost instantaneously. Therefore, instead of solving many-particle Schrödinger equation, the approximation starts by determining electronic eigenstates of fixed atomic positions. The Born-Oppenheimer Hamiltonian is constructed by removing the nuclear momenta dependent terms. So, the many-particle standard Hamiltonian model of equation 2.27 becomes

$$H_{BO}(\vec{R}_1, \dots, \vec{R}_M) = E_{k,e} + E_{s,n-n} + E_{s,e-n} + E_{s,e-e} \quad (2.28)$$

Here, H_{BO} is the Born-Oppenheimer Hamiltonian. In the Hilbert space, H_{BO} relates to electronic wavefunctions and parametrically dependent on the nuclear positions. So, the Hamiltonian is free from any gradients acting on the nuclear positions. The full Hamiltonian of equation 2.27 can be

obtained by adding the kinetic energy term for the nuclei to the equation 2.28 as $H = H_{BO} + E_{k,n}$. Now, the Born-Oppenheimer wavefunctions and surfaces can be obtained from

$$[H_{BO}(\vec{R}) - E_{BO}(\vec{R})] | \psi_{BO}(\vec{R}) \rangle = 0 \quad (2.29)$$

where, $E_{BO}(\vec{R})$ is the *Born-Oppenheimer surfaces* – position dependent energy eigenvalues of the H_{BO} .

In short, Born-Oppenheimer approximations suggests to use atomic wavefunctions. Therefore, a linear combination of atomic ground states – *molecular orbitals* (MO) – should be a starting point towards molecular calculations.

Let us now try to impose Born-Oppenheimer approximation on a simple molecule-ion, H_2^+ . The Hamiltonian for the molecule will be

$$H = -\left(\frac{\hbar^2}{2m_e} \vec{\nabla}^2 + \frac{e^2}{4\pi\epsilon_0 r_1} + \frac{e^2}{4\pi\epsilon_0 r_2} - \frac{e^2}{4\pi\epsilon_0 R_{12}} \right) \quad (2.30)$$

H_2^+ contains only one electron, therefore, in the equation 2.30 r_1 and r_2 are the distances of the electron from two nuclei (1 and 2), respectively and R_{12} is the distance between two nuclei. The term $e^2/(4\pi\epsilon_0 R_{12})$ represents the constant nuclear repulsive interaction within Born-Oppenheimer framework.

Density of states

One of the useful parameters in MO analysis is *density of states* (DOS). This function provides the total concentration of available states within an energy range dE .

$$N_{interval} = g(E)dE \quad (2.31)$$

Here, $N_{interval}$ is the number of density (carrier density) and $g(E)$ is the desired DOS.

2.2.3 Photon absorption and desorption/emission

For molecular spectra, the process of electronic transition is complex due to the structure of the molecules which produces many-body interactions due to electronic, vibrational, and rotational transitions. The interaction among them makes it impossible to observe an independent behaviour of them. It is dependent on the shapes and sizes of molecule, stiffnesses of bonds, etc. For optical emission, electronic transition plays the major role but the electronic transition is coupled with vibrational and rotational transitions. If a photon excites a ground state electron of a molecule to an excited state, at the excited the system vibrates near excited state equilibrium and the system thermalises by generating phonons [68]. After some time, the electron relaxes back to the ground state by emitting a photon. Due to this energy dissipation the emitted photon consist of

lower energy than the absorbed photon. The system once again vibrates near the equilibrium ground state structure and dissipates energy in the form of phonon emission. In figure 2.19, I have depicted the whole process in two ways - the simple Jablonski scheme and approximated Born-Oppenheimer scheme considering the Boltzman distribution at zero temperature. The excited and ground state equilibrium structure is attained by the electron's transition through the vibrational energy levels. At finite temperature, the system can already be at a vibrational excited state. Photon absorption is not an isolated optical transition matrix of the electronic subsystem induced process. The nuclear part of the wavefunction also plays a significant role.

Figure 2.19: Photon absorption and desorption using Jablonski scheme and Born-Oppenheimer approximation. Here, R is a spatial coordinates for example a bond distance and BO surfaces are the Born-Oppenheimer surfaces.

When an electromagnetic field of a particular frequency interacts with molecules, the electric component induces the transition from an initial state $|i\rangle$ to a final state $|f\rangle$. The intensity of the transition is proportional to the square modulus of the transition dipole moment $\vec{\mu}_t$. We can write $\vec{\mu}_t = \langle f | \vec{\mu} | i \rangle$. Here, $\vec{\mu}$ is the electric dipole moment operator. A particular transition occurs based on a selection rule by obeying the criteria of transition electric dipole moment being non-zero [70]. The transition dipole moment signifies a charge migration as shown in Figure 2.20. Molecular collision results to a wide variety of possible transitions obeying the selection rules [70]. The coupled effect of them equilibrates to thermal relaxation of rotational, vibrational, and electronic states. These phononic[†] interactions affect the emission spectra since the spectral intensities are based on density of states involved in the transition. This effect can predominantly be observed in the emission spectra of a crystalline nanostructures due to strong periodicity induced phonon modes [71].

Figure 2.20: Dipolar behaviour – An electric-dipole allowed transition. Here, ψ_i and ψ_f are initial and final wavefunctions, respectively.

[†]The term *phonon* came in analogy with photon. A phonon is the quantum of vibration, and a photon is the quantum of electromagnetic wave.

2.2.4 Phonon modes

Phonon modes of a periodic atomic arrangements or crystals can be described with a wavevector, $\vec{q}$. This wavevector has three vibrational modes in the primitive cell (figure 2.21). For a periodic structure, a Hessian matrix[§] of force constant can be constructed. The phonon frequencies ($\omega_{\vec{q}s}$) can be calculated from this dynamical matrix. The eigenvalues of the dynamical matrix are the phonon frequencies. Considering an atom t at equilibrium position ($R_l + t_l$) in a primitive cell l for α and β Cartesian coordinates, the dynamical matrix can be defined as

$$D_{s\alpha,t\beta}(\vec{q}) = \frac{1}{\sqrt{M_s M_t}} \sum_l F_{ls\alpha,l't\beta} \exp\{i\vec{q} \cdot (R_{l'} - R_l - \tau_t + \tau_s)\} \quad (2.32)$$

where M_s and M_t are the masses for atoms s and t , respectively, and $F_{ls\alpha,l't\beta}$ is the force constant matrix. The force constant matrix provides the relation between force and atomic displacements. Here, the force matrix depends on the atomic distances in primitive cell l and l' . The sum is over the number of primitive cells. Phonon frequencies ($\omega_{\vec{q}s}$) can be determined for any $\vec{q}$, if the complete $F_{ls\alpha,l't\beta}$ matrix is known. This can be generated with *ab initio* calculations such as density functional theory described in the next section.

Figure 2.21: Primitive cell (red rhombus) with primitive vectors ($\vec{a}$ and $\vec{b}$) and critical points (Γ , M , K , and K' of Brillouin zone) of a periodic atomic arrangement such as for graphene.

2.3 Light-matter interaction in nanostructures

Since I have started the discussion of fluorescence, to move forward I will establish the theoretical background of how to model excited state properties to understand light-matter interaction in nanostructures at atomistic level. In order to do that we need to solve time-dependent Schrödinger equation for a system. However, first lets review what could be the efficient approach to solve the time-independent Schrödinger equation to calculate the electronic structure of a large and complex many-particle system such as nanomaterial. It is sad that using Born-Oppenheimer approximation one can precisely solve only hydrogen like species. So, a method called *Hartree-Fock* (HF) was developed for an approximate solution where energy variational principle was utilised [72]. HF describes the many-electron wavefunctions using Slater determinant. Later, many modifications

[§]Hessian Matrix is a second order partial derivative matrix of energy with respect to the positions.

of HF came up to consider the electron correlation [73]. However, none of them could be considered as high-throughput methods because of their computational inefficiencies. To circumvent this problem an alternative efficient approach was developed by Walter Kohn. Later, he was awarded with a chemistry Nobel prize in 1998 ‘for his development of density-functional theory’ [74]. Till date density functional theory is the most efficient method to calculate the electronic properties of many-body systems.

2.3.1 Density functional theory

The energy of an electronic system can be defined using the probability density of electrons (ρ). This is the fundamental of *density functional theory* (DFT). If at a position vector $\vec{r}$ the total density of electrons is $\rho(\vec{r})$, the energy E will be a functional of the electron density and represented by $E[\rho]$ which is a function of $\rho(\vec{r})$.

Kohn-Sham Equation

Although the development of DFT is dated back to Thomas-Fermi method (1920s) [75] and Hartree-Fock-Slater method (1950s) [76], a formal proof of existence of energy as a functional of electronic density was given by Pierre Hohenberg and Walter Kohn in 1964 [77]. They gave the insight that ground-state electronic properties can be deduced from its electron density. However, still the exact form of the dependence was missing in Hohenberg-Kohn theorem. This major break through took place in 1965 when Walter Kohn and Lu Jeu Sham published a seminal paper on ‘Self-Consistent Equations Including Exchange and Correlation Effects’ [78]. It is considered as one of the most impacting and highly cited paper in Physics [79]. The credit also goes to John Pople who shared the 1998 Nobel Prize in Chemistry with Walter Kohn. His work on the ‘development of computational methods in quantum chemistry’ showed that Kohn-Sham or DFT is an efficient method to calculate complex electronic structures [74].

Kohn and Sham could show that the ground-state electron density can be obtained from a set of one-electron equations [78]. Therefore, they gave us the exact ground-state electronic energy $E[\rho]$, precisely $E[\rho(\vec{r})]$ of a system with n electrons as

$$E[\rho] = \underbrace{-\frac{\hbar^2}{2m_e} \sum_{i=1}^n \int \psi_i^*(\vec{r}_1) \vec{\nabla}_1^2 \psi_i(\vec{r}_1) d\vec{r}_1}_{E_{kin,e}} - \underbrace{j_0 \sum_{I=1}^N \frac{Z_I}{r_{I1}} \rho(|\vec{r}_1|)}_{E_{e,n}} + \underbrace{\frac{1}{2} j_0 \int \frac{\rho(\vec{r}_1) \rho(\vec{r}_2)}{|\vec{r}_1 - \vec{r}_2|} d\vec{r}_1 d\vec{r}_2}_{E_{Coulomb, \Sigma \psi_i}} + E_{xc}[\rho] \quad (2.33)$$

where ψ_i (one-electron spatial orbitals) are the *Kohn-Sham orbitals* (KS orbitals) with $i = 1, 2, 3, \dots, n$. The exact ground-state electron density is $\rho(\vec{r}) = \sum_{i=1}^n |\psi_i(\vec{r})|^2$. ρ can be deduced when

the sum over KS orbitals is computed. The term, $E_{kin,e}$ in the equation 2.33 denotes the kinetic energy of n number of fictitious non-interacting electrons[¶]; the term $E_{e,n}$ denotes the electron-nucleus attraction by summing over N nuclei with atomic number Z_I ; and the term $E_{Coulomb,\Sigma \psi_i}$ denotes the Coulomb interaction between the charge distributions at $\vec{r}_1$ and $\vec{r}_2$. Finally, $E_{xc}[\rho]$ is the *exchange-correlation energy* of the total system. It is a functional of electron density which considers all non-classical components of electron-electron interactions. The analytical form of E_{xc} is unknown, so an approximation is required in this part.

The KS orbitals can be determined if the Kohn-Sham equation can be solved by applying the variational principle on the electronic energy ($E[\rho]$) with charge density $\rho(\vec{r})$. The Kohn-Sham equation of single-electron orbitals ($\psi_i(\vec{r}_1)$) with orbital energy ϵ_i can be expressed as

$$\left\{ -\frac{\hbar^2}{2m_e} \nabla_1^2 - j_0 \sum_{I=1}^N \frac{Z_I}{\vec{r}_{I1}} + j_0 \int \frac{\rho(\vec{r}_2)}{\vec{r}_{12}} d\vec{r}_2 + V_{xc}(\vec{r}_1) \right\} \psi_i(\vec{r}_1) = \epsilon_i \psi_i(r_1) \quad (2.34)$$

where V_{xc} is the *exchange-correlation potential*. It is a functional derivative of E_{xc} :

$$V_{xc}[\rho] = \frac{\delta E_{xc}[\rho]}{\delta \rho} \quad (2.35)$$

The KS orbitals enables us to calculate the density from the Kohn-Sham equation (2.34). The KS equation is solved using self-consistent approach. Here, a superposition of atomic density-based electron density, ρ , is guessed and used to compute the functional E_{xc} . Later, V_{xc} is calculated as function of $\vec{r}$. The process is iterated to obtain the convergence of density and exchange-correlation within a tolerance. Now, the electronic energy can be computed from equation 2.33.

The approximate nature of E_{xc} is the primary source of error in DFT. The simplest way of expressing E_{xc} is with *local density approximation* (LDA) [78] as

$$E_{xc}[\rho] = \int \rho(\vec{r}) \epsilon_{xc}[\rho(\vec{r})] d\vec{r} \quad (2.36)$$

where $\epsilon_{xc}[\rho(\vec{r})]$ is the exchange-correlation energy per electron of a homogeneous electron density ρ . In reality, neither electronic nor positive charges are uniformly distributed, but surprisingly, usage of LDA produces realistic description of atomic structures, elastic, and vibrational properties. The accuracy decreases when the variation of electron density increases such as for many molecules. The inhomogeneity of electron density is considered by adding a non-local density gradient correction of $\vec{r}$ in equation 2.36. This approach is commonly called *generalized gradient approximation* (GGA) [80, 81]. It is an efficient method for calculations of d-metal complexes. Considering $\rho = \rho_{\uparrow} + \rho_{\downarrow}$, GGA exchange-correlation functional can be written using a diversified functional f as

$$E_{xc}^{GGA}[\rho_{\uparrow}, \rho_{\downarrow}] = \int f(\rho_{\uparrow}, \rho_{\downarrow}, \nabla \rho_{\uparrow}, \nabla \rho_{\downarrow}) d\vec{r} \quad (2.37)$$

[¶]This fictional system of non-interacting electrons generates the density of interacting electrons after solving the Kohn-Sham equation.

2.3.2 Time-dependent density functional theory

To address the light-matter interaction, in this section, I will discuss *time-dependent DFT* (TD-DFT). TD-DFT extends the ground-state DFT to time-dependent phenomena such as optical excitation. Runge-Gross (RG) theorem is the formal foundation of TD-DFT. It shows when a many-body system evolves from initial wavefunction, the time-dependent potential of the system, $V_{\text{ext}}(\vec{r}t)$ shows a bijective map with the time-dependent electron density $\rho(\vec{r}t)$; $V_{\text{ext}}(\vec{r}t) \leftrightarrow \rho(\vec{r}t)$. $\rho(\vec{r}t)$ can be used to determine the time-dependent Hamiltonian, $H(t)$ and the wavefunction, $\psi[\rho](t)$. In RG theorem a single-component system is considered within a time-dependent scalar field. Then, the Hamiltonian of the system will be

$$H(t) = \tilde{K} + V_{\text{ext}}(t) + \tilde{W} \quad (2.38)$$

where $\tilde{K}$ and $\tilde{W}$ are the kinetic energy operator and the electron-electron interaction, respectively. RG theorem introduces a principle of stationary action integral [82] ($A[\rho]$) to determine the $\rho(\vec{r}t)$ as

$$A[\rho] = \int_{t_0}^{t_1} \langle \psi[\rho](t) | i \frac{\partial}{\partial t} - H(t) | \psi[\rho](t) \rangle dt \quad (2.39)$$

This adds an arbitrary constant due to the presence of the phase factor and is a functional of density which is stationary about the true density of the system. The electron density can now be determined using the Euler equation:

$$\frac{\delta A[\rho]}{\delta \rho(\vec{r}t)} = 0 \quad (2.40)$$

This enables us to obtain the time-dependent Kohn-Sham (TDKS) equation,

$$H\psi_j(\vec{r}t) = \left[-\frac{1}{2}\nabla^2 + V_s(\vec{r}t) \right] \psi_j(\vec{r}t) = i \frac{\partial \psi_j(\vec{r}t)}{\partial t}. \quad (2.41)$$

Here, TDKS orbitals are represented with $\psi_j(\vec{r}t)$. This results to the time-dependent density as

$$\rho(\vec{r}t) = \sum_j n_j |\psi_j(\vec{r}t)|^2 \quad (2.42)$$

Now, the TDKS potential can be described as

$$V_s(\vec{r}t) = V_{\text{ext}}(\vec{r}t) + \underbrace{\int \frac{\rho(\vec{r}'t')}{|\vec{r} - \vec{r}'|} d\vec{r}'}_{\text{TD-Hartree potential}} + V_{\text{xc}}(\vec{r}t) \quad (2.43)$$

where the *TD-Hartree potential* term represents the time-dependent Hartree potential, and $V_{\text{xc}} = \delta A_{\text{xc}}/\delta \rho$. The unknown functional A_{xc} can be considered as a local approximation in time with an instantaneous density, ρ_t at time t and is given by

$$A_{\text{xc}} = \int_{t_0}^{t_1} E_{\text{xc}}[\rho_t] dt \quad (2.44)$$

Here, the external potential is varying slowly (infinitely) in time. This is called *adiabatic approximation* in TD-DFT. Using this approach the first derivative of A_{xc} can be written as

$$V_{xc}[\rho](\vec{r}t) = \frac{\delta A_{xc}[\rho]}{\delta \rho(\vec{r}t)} \cong \frac{\delta E_{xc}[\rho_t]}{\delta \rho_t(\vec{r})} = V_{xc}[\rho_t](\vec{r}) \quad (2.45)$$

The exchange-correlation potential is instantaneous – it evaluates the electron density at a particular time. Hence, the past information of density is completely disregarded. This is evident in the second derivative of A_{xc} or as known as *XC kernel*. It reflects the response of the exchange-correlation potential for certain density fluctuation. The XC kernel reads

$$\frac{\delta A_{xc}[\rho]}{\delta \rho(\vec{r}t) \delta \rho(\vec{r}'t')} = \frac{\delta V_{xc}[\rho](\vec{r}t)}{\delta \rho(\vec{r}'t')} \cong \delta(t-t') \frac{\delta[\rho_t](\vec{r}')}{\delta \rho_t(\vec{r})} \quad (2.46)$$

The XC kernel is within the adiabatic approximation. It is an important quantity because within the linear response function one can extract excitation energies using it.

2.3.3 Linear response

If the external perturbation is weak and the change in the Hamiltonian is in linear order, TDKS equation can be solved using linear response techniques – first developed by Mark Casida in 1995 [83]. In this approach a retarded correlation function represents the response of the system. The linear response function of a non-periodic or a finite system has discrete poles, which enables us to calculate the excitation energies. Using a density matrix formalism, I will show how to obtain excitation energies from linear response of a time-dependent ground state electron density due to a time-dependent external electric field. In this section, the linear response equation will be derived and transformed into an eigenvalue problem. Here, σ and τ will refer to spin variables. For MOs, i, j, k, l will refer to the occupied orbitals, a, b, c, d will refer to the virtual orbitals, and s, t, u, v will refer to the general orbitals. Here, fractional occupation and spin polarisation are allowed, so coupled indices such as $i\sigma$ and $a\sigma$ will be general for KS orbitals, then, $n_{i\sigma} > n_{a\sigma}$ or $n_{j\sigma} > n_{b\sigma}$.

Let us consider a system initially in a ground state, and an external linear ordered perturbation is introduced in the KS Hamiltonian. This can be expressed as,

$$\delta H^\sigma(\vec{r}t) = \delta V_s^\sigma(\vec{r}t) = \delta V_{\text{ext}}^\sigma(\vec{r}t) + \delta V_{\text{hxc}}^\sigma(\vec{r}t) \quad (2.47)$$

where $\delta V_{\text{hxc}}^\sigma$ is linear response of the Hartree-XC potential ($\delta V_{\text{H}}^\sigma + \delta V_{\text{xc}}^\sigma$). This is caused from the modified electron density:

$$\delta V_{\text{hxc}}^\sigma(\vec{r}t) = \sum_r \iint f_{\text{hxc}}^{\sigma\tau}[\rho_{\text{gs}}](\vec{r}t, \vec{r}'t') \delta \rho_\tau(\vec{r}'t') d\vec{r}' dt' \quad (2.48)$$

Here, $f_{\text{hxc}}^{\sigma\tau}[\rho_{\text{gs}}]$ is the sum of Hartree and XC kernels evaluated at the ground-state density. Within the adiabatic approximation it can be expressed as

$$f_{\text{hxc}}^{\sigma\tau}[\rho_{\text{gs}}](\vec{r}t, \vec{r}'t') = \delta(t-t') \left(\frac{1}{|\vec{r}-\vec{r}'|} + \frac{\delta V_{\text{xc}}^{\sigma}[\rho_{t,\uparrow}, \rho_{t,\downarrow}](\vec{r}')}{\delta \rho_{t,\tau}(\vec{r})} \bigg|_{\rho_{\text{gs}}} \right) = \delta(t-t') f_{\text{hxc}}^{\sigma\tau}[\rho_{\text{gs}}](\vec{r}, \vec{r}') \quad (2.49)$$

According to the RG theorem, bijective mapping can be used between the time-dependent density and the external potential. Hence, the density can be derived from linear density response to the external perturbation. The perturbation is characterised by a nonlocal susceptibility (χ) of the many-body system at the ground-state density:

$$\delta \rho_{\sigma}(\vec{r}t) = \sum_r \iint \chi^{\sigma\tau}[\rho_{\text{gs}}](\vec{r}t, \vec{r}'t') \delta V_{\text{ext}}(\vec{r}'t') d\vec{r}' dt' \quad (2.50)$$

Besides, the linear density response is same as the KS system for the perturbation of the KS Hamiltonian. So, it can be written as

$$\delta \rho_{\sigma}(\vec{r}t) = \sum_r \iint \chi_s^{\sigma\tau}[\rho_{\text{gs}}](\vec{r}t, \vec{r}'t') \delta V_s^{\tau}(\vec{r}'t') d\vec{r}' dt' \quad (2.51)$$

where χ_s denotes the KS susceptibility — the system's response function for non-interacting particles with unperturbed density, ρ_{gs} . Therefore, substituting equation 2.51 with 2.47 and equating with equation 2.50 gives a Dyson-like equation [84] for χ :

$$\chi^{\sigma\tau}(\vec{r}t, \vec{r}'t') = \chi_s^{\sigma\tau}(\vec{r}t, \vec{r}'t') + \sum_{\sigma'\tau'} \iiint \chi_s^{\sigma\sigma'}(\vec{r}t, \vec{r}t') f_{\text{hxc}}^{\sigma'\tau'}(\vec{r}t, \vec{r}'t') \chi^{\tau'\tau}(\vec{r}'t', \vec{r}'t') d\vec{r} dt' \quad (2.52)$$

It is the main equation of linear response TD-DFT. The χ_s can be determined from time-dependent perturbation theory. The true χ possesses important information related to the real interacting system. The excitation energies are the poles of the susceptibilities [83]. Further, dynamic polarisabilities can also be determined, and the residues of the mean polarisability enables to find the oscillator strength of the excitation energies.

2.3.4 Tight binding and density-functional theory

Conventional DFT approach is by far the most accurate method of calculating quantum mechanical properties of molecules. However, it lacks in efficiency. The time consumed in DFT is so large that it will be difficult to calculate a non-periodic molecular structure with hundreds of atoms in case of nanostructure. The efficiency came when it was combined with tight binding approach – *density-functional based tight-binding* (DFTB). In DFTB, electronic density is considered as a perturbed reference density, which is the superposition of atomic densities. The deviations are considered by atomic charge fluctuation. This assumption allows to reformulate all the expressions, therefore, the

computational load reduces a lot in DFTB. Here, parametrised expressions are used to determine the interacting charge distribution and their energies.

DFTB is a second-order expansion of total KS energy functional with respect to the charge-density fluctuations $\Delta\rho$ based on a given reference density, ρ_0 . In 1988, Mathew Foulkes and Roger Haydock first attempted to couple tight-binding method and DFT [85] by dividing the total-energy functional into kinetic energy of noninteracting electrons and electrostatic interactions. If we follow the derivation by Foulkes and Roger [85], $E[\rho]$ can be expanded using the initial density ρ_0 as

$$E[\rho] = E_{\text{Harris}}(\rho_0) + \frac{1}{2} \iint f_{\text{hxc}}[\rho_0](\vec{r}, \vec{r}') \Delta\rho(\vec{r}) \Delta\rho(\vec{r}') d\vec{r} d\vec{r}' + \dots \quad (2.53)$$

The $\Delta\rho$ represents the difference between ρ and ρ_0 and f_{hxc} can be expressed as

$$f_{\text{hxc}}[\vec{r}, \vec{r}', \rho_0] = \frac{1}{|\vec{r} - \vec{r}'|} + \left. \frac{\delta^2 E_{\text{xc}}}{\delta\rho(\vec{r}) \delta\rho(\vec{r}')} \right|_{\rho_0} \quad (2.54)$$

The Harris functional, E_{Harris} , is a significant functional because it relates to the $E[\rho]$ at the ground state density. This is stationary about the ground state density and does not vary with minor fluctuations around its ground state value, $(\delta E_{\text{Harris}} = E_{\text{Harris}}[\rho_{\text{gs}} + \delta\rho] - E_{\text{Harris}}[\rho_{\text{gs}}])$. The advantage is, it does not depend on the output density, ρ . It can be expressed as

$$E_{\text{Harris}}[\rho] = \sum_i n_i \varepsilon_i - E_{\text{H}}[\rho] + E_{\text{xc}}[\rho] - \int V_{\text{xc}}[\rho] \rho(\vec{r}) d\vec{r} \quad (2.55)$$

Here, the solutions of a non-self-consistent Schrödinger equations are the eigenvalues, ε_i . The effective potential is only dependent on ρ_0 . Hence, solving the stationary solution of E_{Harris} is same as solving the KS equations in an efficient manner. Here, we simply need to identify the input density, ρ_0 . Foulkes and Haydock mentioned that ρ_0 can be defined by various available ways [85]. One can consider ρ_0 as the superposition of spherical atomic-like densities, $\rho_0(\vec{r}) = \sum_S \rho_S(\vec{r})$. If we evaluate the Harris functional using this density, we can obtain:

$$E_{\text{Harris}}[\rho_0] = \sum_i n_i \varepsilon_i - \sum_A E_{\text{H}}[\rho_A] - \frac{1}{2} \sum_{S \neq M} \iint \frac{\rho_S(\vec{r}) \rho_M(\vec{r}')}{|\vec{r} - \vec{r}'|} d\vec{r} d\vec{r}' + E_{\text{Harris}}^{\text{xc}} \left[\sum_S \rho_S \right] \quad (2.56)$$

The inter-atomic Hartree energy of atom S is given by $E_{\text{H}}[\rho_A]$. The spherical symmetry of ρ_S enables to deduce the third term by the sum of pairwise potentials. Additionally, if the atomic density undergoes to certain constraint and the nucleus-nucleus repulsion energy is included, the two-body potential turns out to be short-ranged because for large interatomic distances electron-electron and nucleus-nucleus interactions are cancelled. Due to the non-linear property of the exchange-correlation functional, $E_{\text{Harris}}^{\text{xc}}$, it cannot be separated as pair-wise potentials. One usual practice is to expand it in a cluster series:

$$E_{\text{Harris}}^{\text{xc}} \left[\sum_S \rho_S \right] = \sum_S E_{\text{Harris}}^{\text{xc}}[\rho_S] + \frac{1}{2} \sum_{S \neq M} (E_{\text{Harris}}^{\text{xc}}[\rho_S + \rho_M] - E_{\text{Harris}}^{\text{xc}}[\rho_S] - E_{\text{Harris}}^{\text{xc}}[\rho_M]) + \dots \quad (2.57)$$

We can neglect the expansion after the two centre terms because of negligible overlapping densities from three or more atoms [85]. Finally, the Harris functional can be expressed in a tight-binding form:

$$E_{\text{Harris}}[\rho_0] = \sum_i n_i \varepsilon_i + \sum_S (E_{\text{Harris}}^{\text{xc}} \rho_S - E_{\text{H}}[\rho_S]) + \frac{1}{2} \sum_{S \neq M} U_{SM}(|\vec{R}_S - \vec{R}_M|) \quad (2.58)$$

U_{SM} provides the repulsion potentials by including Hartree and XC pairwise contribution. Using a saddle point finding method (dimer calculation ^{††}) one can evaluate these potentials. The eigenvalues, ε_i , can be found from the one-electron Schrödinger equation:

$$H_0 \psi_i(\vec{r}) = \left[-\frac{1}{2} \vec{\nabla}^2 + \sum_S V_S(\vec{r}) + U(\vec{r}) \right] \psi_i(\vec{r}) = \varepsilon_i \psi_i(\vec{r}) \quad (2.59)$$

V_S is the sum of $V_{\text{ext},S}$, $V_{\text{H}}[\rho_A]$, and $V_{\text{xc}}[\rho_S]$; U is additional potential to consider the non-linearity of exchange-correlation functional, $U = V_{\text{xc}}[\sum_S \rho_S] - \sum_S V_{\text{xc}}[\rho_S]$. The ε_i can be obtained by reducing the equation 2.59 (into $|H - \varepsilon I|$ general form). To perform that a non-orthogonal atomic-like functions are used, and the overlapping matrices are evaluated.

DFTB method enables us to realise the above theory using a series of practical approximations beyond the exchange-correlation functional.

Input density: At the first step, an atomic-like density is defined — a critical step to achieve a successful model structure. The neighbour atomic interactions is an important factor to simulate such density. A modified KS equation (2.60) is used to define the confined atomic structure by introducing an extra parabolic potential, $(r/r_0)^2$, where r_0 is the radius of confinement.

$$\left[-\frac{1}{2} \vec{\nabla}^2 + V[\rho_S] + \left(\frac{r}{r_0} \right)^2 \right] \psi_v(\vec{r}) = \varepsilon_v \psi_v(\vec{r}) \quad (2.60)$$

r_0 is based on the given element in order to obtain an ideal transferability in different reference systems.

Basis sets: DFTB uses a minimal set of compressed atomic orbitals. One of the approaches is to use equation 2.60 to compute them self-consistently. Another approach to improve DFTB results is by individual optimization of the basis set. Here, atomic densities and orbitals are calculated in separate steps. The optimal value of confinement radius is shown to be 1.85 times the covalent radius of the corresponding element. A pseudo-atomic wave functions are constructed using a linear combination of *Slater-type orbitals* (STO) [87].

Hamiltonian and overlap matrices: Once the atomic orbitals and densities are calculated, the Hamiltonian and overlap matrices (OM) can be obtained by

^{††}Dimer method: A min-mode method where from any initial configuration one can search for surrounding saddle points [86].

$$H_{\text{STO},v} = \langle \psi_{\text{STO}} | -\frac{1}{2}\vec{\nabla}^2 + V \left[\sum_S \rho_S \right] | \psi_v \rangle \quad (2.61)$$

and

$$OM_{\text{STO},v} = \langle \psi_{\text{STO}} | \psi_v \rangle, \quad (2.62)$$

respectively. Although, in equation 2.59 the effective potential, V , was decomposed into atomic-like contributions, currently it is evaluated at superposed atomic densities as a regular practice [88]. The non-linearity of the XC functional is taken into account by this approach. DFTB simplifies the scheme and reduces the computational load by evaluating the V in the matrix element $H_{\text{STO},v}$ for the contributing atoms of the orbitals ψ_{STO} and ψ_v . Now, considering $\text{STO} \in S$ and $v \in M$ where $S \neq M$, the Hamiltonian matrix can be written as

$$H_{\text{STO},v} = \langle \psi_{\text{STO}} | -\frac{1}{2}\vec{\nabla}^2 + V[\rho_S + \rho_M] | \psi_v \rangle \quad (2.63)$$

By this DFTB completely neglects the higher terms. A diagonal matrix ($H_{\text{STO},v} = \delta_{\text{STO},v} \epsilon_{\text{STO}}$) represents the atomic blocks of the Hamiltonian matrix. ϵ_{STO} denotes the eigenvalues of the Hamiltonian matrix for an isolated atom (are obtained using self-consistent field (SCF) DFT calculation). Now, the Hamiltonian and OM can be determined based on the distance of the atom pairs. Hence, DFTB does not evaluate any integration operation during the geometry optimisations.

Repulsive potential: To evaluate the Harris functional (equation 2.58) the final task is to evaluate the one and two-body potentials. All constant monomer contributions are ignored to confirm the large distance repulsive energies become zero. Instead of calculating the pairwise potentials by directly evaluating at ρ_0 , they are fitted to *ab-initio* results for a reference system, and then, a polynomial or spline is used to represent every elemental pair (up to an interatomic distance limit) [89]. The threshold distance is considered as 1.5 to 2 times the equilibrium bond length.

Finally, the DFTB total energy is:

$$E = E_{\text{Harris}}[\rho_0] = \sum_i n_i \epsilon_i + E_{\text{rp}} \quad (2.64)$$

where E_{rp} represents repulsion energy as the sum of the fitted pairwise potentials.

Using the above approach of DFTB many systems were successfully investigated and modelled [90, 91, 92, 93, 94]. However, for charged atoms, heteronuclear molecules, and polar semiconductors the error is significant for considering neutral density. Therefore, energy functional up to second term is considered (equation 2.53) – the second-order in the density difference, $\Delta\rho$, leading to a self-consistent redistribution of Mulliken charges. Here, the multipole expansion of the square of the basis functions is truncated after the monopolar term. With this condition, the electron density is iteratively updated until the system reaches to its convergence. This extension of DFTB is termed as *self-consistent-charge DFTB* (SCC-DFTB) [95]. Additionally, if a third order expansion

of the total energy is considered, undoubtedly accuracy will increase especially for highly charged molecules [96, 97, 98].

Time dependent DFTB: In nanophotonics TD-DFT is a useful technique to investigate light-matter interaction. However, due to its large computational load for a finite system with many atoms it is disadvantageous for nanostructures. On the contrary, the time-dependent extension of DFTB (TD-DFTB) gives a contrasting efficiency in such cases. TD-DFTB was first developed by Niehaus and coworkers in the year 2001 by extending ground-state DFTB to account for excited state properties [99]. The concept behind TD-DFTB was following:

- (i) in density-functional response theory beside the KS eigenpairs the coupling matrix is also evaluated;
- (ii) by expanding the KS orbitals into the basis set of atomic orbitals one can express the coupling matrix as a sum of multicentre integrals;
- (iii) this can be applied to evaluate the multicentre integrals used in second-order DFTB and monopole truncation of a MO multipole expansion.

These enable TD-DFTB to utilise the inexpensive and integration-less approach of DFTB by using pre-calculated two-center parameters. Within the DFTB framework to consider linear response theory no additional approximation and neglect is required.

DFTB uses a density matrix formulation [100, 101] where the energy functional and KS Hamiltonian are expressed as KS density matrix fluctuations – for more details see [101]. This is useful to derive the TD-DFTB extension. At first, an SCF calculation is performed to obtain the single-particle KS orbitals (ψ_i) and its corresponding KS energies (ϵ_i). Next, a coupling matrix is constructed to obtain the response of the SCF potential with respect to electron density fluctuation. Considering the adiabatic approximation, this coupling matrix can be written as,

$$K_{ij\alpha,kl\tau} = \iint \psi_i(\vec{r}) \psi_j(\vec{r}) \times \left(\frac{1}{|\vec{r} - \vec{r}'|} + \frac{\delta^2 E_{xc}}{\delta \rho_\sigma(\vec{r}) \delta \rho_\tau(\vec{r}')} \right) \times \psi_k(\vec{r}') \psi_l(\vec{r}') d\vec{r} d\vec{r}' \quad (2.65)$$

where σ and τ are spin indices. Here, only closed shell systems are considered. Now, the excitation energy (ω_l) can be found by solving the following eigenvalue problem:

$$\sum_{ij\sigma} [\omega_{ij}^2 \delta_{ik} \delta_{jl} \delta_{\sigma\tau} + 2\sqrt{\omega_{ij}} K_{ij\alpha,kl\tau} \sqrt{\omega_{kl}}] F_{ij\sigma} = \omega_l^2 F_{kl\tau} \quad (2.66)$$

where ω_{ij} is the energy difference between ϵ_j and ϵ_i ; i, k are occupied and j, l are unoccupied KS orbitals, and F denotes a normalised spherical density fluctuation. The transition density obtained from the coupling matrix is decomposed between two different orbitals into atom-centred contributions. The transition density is then subjected to a multipole expansion and a monopole approximation considering the Mulliken atomic transition charges [99]. This simplified approach yields acceptable agreement with experiential optical spectra and first-principles calculations where the computational cost is reduced to a great extent [99, 102] – for example, the newest version of TD-DFTB can calculate the low lying excitation energies (of 15 singlet-singlet and singlet-triplet) for

Thiel's set of 28 molecules [103] within 96 s using an Intel Xeon processor E3-1225 at 3.10 GHz and a memory of 8 Gb. In contrast to that PBE0 [104, 105] and CAM-B3LYP [106, 107] take 12.7 h, 20 h to 30.2 h, respectively [102].

2.4 Single-photon source

If a molecule or nanostructure is a single photon emitter, it finds potential application in super-resolution microscopy [108, 110, 109] to quantum information processing [111, 112, 113]. A single photon emitter generates light as single photons where the time delay between every two successive photons are detectable. This event takes place due to presence of a single transition dipole. In nanophotonics and super-resolution imaging most of the time single molecules have single dipole centres [114, 115, 117, 118], for example, the organic dyes molecules. Organic dye molecules have low photostability and frequently show dark states. Later, inorganic quantum dots (QDs) were discovered which also possess single photon emission behaviour with better photostability compare to organic dye molecules. The principle behind single photon emission of QDs are based on quantum confinement (see section 2.2.1). The optical behaviour of them can be tuned by varying their sizes. QDs can be categorised as (a) structural QDs, where the optical behaviour is dependent on their global structural geometry and (b) localised narrow-gap QDs, where a heterostructure or crystal defect creates a localised quantum well and generates single photon emission. Among variety of other structural QDs, II-VI semiconductor-based QDs are widely researched and used by many research groups [119, 120, 121, 122, 123, 124]. Most of the II-VI semiconductors are highly toxic [125, 126, 127]. For environmental safety as well as for biological application non-toxic single photon emitters are currently needed. In this section, I will briefly address several techniques to recognise single photon emitter such as *defocused optical imaging*, to understand their atomistic organisation such as *high-resolution transmission electron microscopy*, and to use them for practical application such as *fluorescence correlation spectroscopy*.

2.4.1 Point spread function

Defocused optical imaging is based on the structure of *point spread function* (PSF). For an infinitely small point emitter, PSF defines the distribution of light at and near the image plane. As shown in figure 2.23, let us consider photon emission from a point source in a diverging spherical wave form. Using an objective lens the spherical wave from the source can be trans-

Figure 2.22: Two high resolution PSFs captured using a widefield microscope. Here the colour scale bar denotes photon counts.

formed into a plane wave, which can be focused into a point by using a tube lens. Instead of creating a point resembling the point source emitter, PSF is a complex pattern around the image plane. This can be understood by Huygens wavelet approach. In 1678 Christiaan Huygens proposed that each wavefront is composed of infinite number of wavelets [128]. I demonstrate this in figure 2.23a by considering two in-phase wavelets of the spherical waves after the tube lens. Here, we chose the image plane in a distance where those two in phase wavelets create a constructive inference at the optical axis. Now, if we reconstruct the inferences pattern of these two wavelets at different positions on the image plane we will create a stripped pattern or diffraction pattern as shown in figure 2.23a. If all the wavelets of the same wavefront are considered, we can obtain the

Figure 2.23: Formation of a PSF. Inset (a) Huygens wavelet approach using two wavelets. Inset (b) PSF formation using many number of wavelets.

pattern similar to figure 2.23b. This pattern represents the PSF of the point source emitter. The shape of a PSF depends on parameters, like wavelength, refractive index of the medium, numerical aperture of the objective lens, and the orientation/position of the point source. The principle of Huygens-Fresnel describes this phenomenon of wave propagation [129].

Before measuring PSF of a single photon source using a microscope, one needs to calibrate it to avoid misleading conclusion from a defocused pattern. To calibrate a fluorescence microscope one should have uniformly distributed stable point emitters within a complete field of view of a microscope. A stable point source provide high photon counts and thereby PSFs of high signal to noise ratio (Figure 2.22). There are two problems in this technique: (i) How to obtain uniformly distributed point source emitters within the complete field of view? (ii) How to make a point emitter stable for a long duration to capture many photons? The solution is to prepare an

Figure 2.24: (a) SEM image: FIB etched array of nanoholes on gold coated cover slip for PSF characterisation of a microscope. The holes contain no gold and rest is the gold thin-film. Scale represents $50\text{ }\mu\text{m}$. (b) Fluorescent widefield microscopy image: Array of nanoholes filled with fluorescent dye molecule covering nearly $90\text{ }\mu\text{m}$ field of view of a wide field microscope with xz and yz view of the PSFs.

array of nanoholes (with diameters below the diffraction limit) on a metal thin-film (grown on glass coverslip) where the nanoholes are optically transparent. These nanoholes array are filled with liquid solvents containing high concentration of fluorescent molecules (in the mM) to avoid photobleaching of dye molecules. In this situation, the dye molecules are freely diffusing in and out of the molecules. Due to the high concentration of dye molecules and their free diffusions there is no interruption of fluorescence emission over time. The metallic regions will quench the fluorescence of molecules, and playing with the thickness one can also avoid any optical transmission through the metallic thin-film. The molecules present within the nanoholes will be fluorescent due to dielectric interface of the glass. The molecules at the edge of the nanoholes will be non-fluorescent due to quenching effect of the metals. Diffraction limited high photon counting spots can be obtained by optimising the diameter of the nanoholes. Overall, one can avoid the problem of fluorescence-bleaching as well as distributing the point emitters uniformly over the complete field of view by using a nanofabricated glass-metal coverslip with nanoholes. Figure 2.24a shows array of nanoholes on gold thin-film where the black holes contains no gold instead only transparent glass and rest of the region is covered with a 200 nm gold thin-film. A schematic drawing shows the profile of a nanohole. The nanoholes are filled with solution of highly concentrated fluorescent molecules (for example, a micromolar concentration of Atto-488 fluorophores in isopropanol – isopropanol helps to avoid air-bubble formation within the nanoholes due to its low surface tension). In figure 2.24a the diameter of the holes are 300 nm . Similar arrays with diameter of nanoholes from 80 to 300 nm were also prepared using an FEI Nova NanoLab 600 FIB

(Appendix E). A fluorescence image of an array of nanoholes with 165 nm diameter is shown in figure 2.24b. We captured stack images along the height (z axis) using a widefield fluorescence microscope. The xy image shown here is from a z position where the optics is focused. The xz and yz image of a row and a column, respectively shows the size distribution of PSFs. From the cross-sectional images we can identify if the PSFs are distorted at any position within the field of view and further, quantify the distortions by fitting them with the Zernike polynomials.

Defocused imaging

A single photon source can be identified by analysing the structure of its PSF. Instead of capturing the total PSF, analysing any of the optical plane of a PSF is sufficient for this. Such image is equivalent to a defocused image of an emitter in a fluorescence microscope. By calculating the electromagnetic radiation pattern at the exact experimental optical plane of a single oscillating dipole we can identify the position and structures of the emitter [61]. Figure 2.25 schematically demonstrate the optics working behind defocused imaging. Here, a degenerate dipole is considered by super position of three dipoles (x, y and z). By displacing the optics, one can capture the defocused image of the transition dipole on the EMCCD plane. In short, the focal point has to be displaced along the optical axis as shown by displacing the EMCCD plane. However, the easiest approach is to displace the objective lens. The angular distribution of dipole depends on the electrodynamic interaction with the interface as shown in figure 2.25(2), where a vertical component of the 3D dipole is interacting with a air/glass interface. The angular distribution of the oscillating dipole is simply defined by two particular angles, θ and ϕ . The amplitude of the electric field will be:

Figure 2.25: Geometric schematic of defocused imaging. A 3D transition dipole is imaged onto a EMCCD plane by moving the optics along the optical axis in the direction away from the dipole. The defocused pattern containing the orientation information. A vertical dipole's electrodynamic interaction with glass-air interface is shown from the side view (2), where the angular distribution of emission is dependent on two particular angles θ and ϕ .

$$E(\theta, \phi) = \sin v [t_s E_s^{\parallel}(\theta) \sin \phi + t_p E_p^{\parallel}(\theta) \cos \phi] + t_p E_p^{\perp}(\theta) \cos v \quad (2.67)$$

Here, ν is the polar angle and t_p and t_s are orthogonal unit vectors. Considering transmission coefficient of Fresnel for p and s waves, distance of the dipole from the glass/air interface, and the refractive indices of all the medium, one can easily define the $E_s^{\parallel}(\theta)$, $E_p^{\parallel}(\theta)$, and $E_p^{\perp}(\theta)$ as shown by Enderlein et al. [61].

A degenerate or 3D dipole can be approximated by the superposition of three dipoles at a particular position [61]. When we are considering superposition of three dipoles, it is important to define their intensity distributions. Considering their intensity as I_x , I_y , and I_z for x , y , and z dipoles, respectively, and their ratio of intensities as κ and η , the coupled intensity distribution [61] is proportional to P :

$$P = \kappa I_z + (I - \kappa) \left[\frac{I_y(1 + \eta)}{2} + \frac{I_x(1 - \eta)}{2} \right] \quad (2.68)$$

2.4.2 Single molecule fluorescence spectroscopy

One of the key measurements in light-matter interaction in nanostructure is photoluminescence (PL)/fluorescence spectroscopy. In single molecule fluorescence spectroscopy, the fluorescence emission from an emitters is divided by a beam splitter to be recorded in a single photon avalanche diode (SPAD) detectors and in a spectrometer as shown in figure 2.26. The SPAD collected photons are used to identify the position of the emitter. In our case, this is based on a scanning confocal fluorescence microscopy. The spectrometer collected photons are diffracted using a diffraction grating to identify the energies of the emitted photons. To prepare the sample, single molecules are sparsely spin coated on a coverslip. Using a piezoelectric positioning stage the coverslip is scanned with respect to the excitation laser. Once they are localised at low excitation power (to avoid photobleaching) using an SPAD, we can measure the emission spectra of the localised single molecules using a single photon sensitive imaging spectrometer.

Figure 2.26: Experimental setup of single molecule photoluminescence spectroscopy.

2.4.3 Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy (FCS) is a correlative analysis of fluorescence intensity fluctuation over time. Some of the practical application of FCS are measuring concentration of

emitters in a solution, their diffusion coefficients, and flow velocities of emitters [134, 135, 136]. These have potential application in micro- and nanofluidics. FCS is also useful to identify size distributions and mono/heterodispersity of emitters. A typical FCS setup is based on a standard confocal fluorescence microscope (figure 2.27). Here, a high numerical aperture (NA) objective lens is used to focus the excitation laser at the sample plane. The emission due to fluorescence is collected from the same objective lens. Using a dichroic mirror it is diverted to the detectors from the same path of excitation laser. On the emission path, a pinhole is used to remove the unwanted photons from the below or above the focal plane. The detectors used to detect the emission from an FCS measurement are SPADs. The post-processing electronic units are capable to record simultaneous single photon time traces from several SPADs. Hence, correlation of detected wavelength or polarisation sensitive photon counts from the SPADs are possible. If we autocorrelate the fluorescence intensity trace $I(t)$ by varying delay times (Δt) for the total time (t) we get:

$$g(\Delta t) = \langle I(t)I(t + \Delta t) \rangle_t \quad (2.69)$$

$g(\Delta t)$ gives the probability of detecting a photon at time, $t + \Delta t$. In case of statistically independent event, if more than one emitters emit photons the autocorrelation function gives a constant offset. As shown in figure 2.27, at short delay times - within few nanoseconds, $g(\Delta t)$ represents fluorescence anti-bunching [137]. If an emitter is single photon source, at this domain a dip should be observed. The dip represents the excited state time of each emitter. From the nanosecond to microsecond domain is the decay time due to the rotational diffusion of the emitter. The triplet state property of an emitter (if it is present) will be observable in the microsecond domain. The decay in the microsecond time domain is due to the diffusion of the emitter within the detection volume.

Dual focus FCS

Dual focus FCS (2fFCS) is an extension of FCS where instead of a single focus two closely placed foci are used to detect molecules (figure 2.28). If the size of the detection volume of an FCS setup is known, diffusion coefficient can be quantified^{||}. The detection volume for a confocal fluorescence microscope can be determined by the aforementioned technique of measuring PSF. The shape of the PSF of a diffraction limited point source can be considered as the detection volume. The other way to measure the size of the detection volume is by calibrating the setup using a fluorescent molecule whose diffusion coefficient is known. The problem with conventional single focus FCS is that if the setup is not calibrated or the refractive index and coverslip thickness are not known, it may lead to significant error in the correlation analysis. 2fFCS can resolve these problems. Here, the two foci are generated using a Normaski prism [138]. Normaski prism is placed before

^{||}The time taken for a molecule to diffuse through the detection volume is proportional to the size of the detection volume.

Figure 2.27: A typical confocal FCS setup [139]. Inset: A schematic FCS curve showing all the domains.

the back-focal plane of the objective lens as shown in figure 2.28. The prism divides the lasers based on their polarisations. If one uses two lasers (orthogonally polarised from each other), the Normaski prism generates two spatially separated partially overlapping foci as shown in figure 2.28. The cross-correlation for a concentration of molecule, C , moving through these two foci with inter-focal distance δ can be expressed as

$$g(\Delta t) = g(\infty) + I_1 I_2 C \iint \frac{1}{(4\pi D \Delta t)^{3/2}} \exp \left[-\frac{(\vec{R}_1 - \vec{R}_2 - \hat{x}\delta - \vec{v}\Delta t)^2}{4D\Delta t} \right] U(\vec{R}_1) U(\vec{R}_2) d\vec{R}_1 d\vec{R}_2 \quad (2.70)$$

where $g(\infty)$ is a constant offset, I_1 and I_2 are detection efficiency describing excitation intensities and molecular brightness at each focus, $\vec{R}_1$ and $\vec{R}_2$ respectively denote initial and displaced position of a molecule, $\hat{x}$ is the unit vector for a particular axis x , δ is the distance between two foci, $U(\vec{R})$ represents the molecular detection functions (MDF) of the detection volumes and $\vec{v}$ is the flow

Figure 2.28: Two foci created with a Normaski prism and a time travel of a molecule from one focus to another focus is detected with photon emission due to excitation from each focus.

vector. The constant offset $g(\infty)$ is given by

$$g(\infty) = \left[I_{BG} + I_1 C \int U(\vec{R}_1) d\vec{R}_1 \right] \left[I_{BG} + I_2 C \int U(\vec{R}_2) d\vec{R}_2 \right] \quad (2.71)$$

where I_{BG} is the background intensity. $g(\infty)$ gives the position dependent probability of detecting a photon from an emitter. It includes the excitation intensities (with spatial dependence) and detection efficiency. Hence, it is important to find a good model for MDF. Dertinger et al. came up with a suitable MDF [139]. It reads

$$U(\vec{R}) = \frac{k(z)}{w^2(z)} \exp \left[-\frac{2}{w^2(z)} (x^2 + y^2) \right] \quad (2.72)$$

In each plane perpendicular to the optical axis the MDF can be approximated using a Gaussian distribution. The width and amplitude of the Gaussian is represented by $w(z)$ and $k(z)$, respectively. The shape, $w(z)$, along the optical axis can be described by a hyperbolic function dependent on the excitation wavelength (λ_x),

$$w(z) = w_1 \sqrt{1 + \left(\frac{\lambda_x z}{\pi w_1^2 n} \right)^2} \quad (2.73)$$

It is scalar approximation of a diverging laser beam with w_1 as the beam waist radius. The amplitude, $k(z)$ represent PSF of a confocal microscope. It can be described as

$$k(z) = 2 \int_0^a \frac{\Upsilon}{R^2(z)} \exp\left(-\frac{2\Upsilon^2}{R^2(z)}\right) d\Upsilon = 1 - \exp\left(-\frac{2a^2}{R^2(z)}\right) \quad (2.74)$$

This non-trivial term hinders the $U(\vec{R})$ to be considered as a Gauss-Lorentz profile. The function $R(z)$ in the equation 2.74 has similar expression as $w(z)$ and is dependent on emission wavelength (λ_m):

$$R(z) = R_1 \sqrt{1 + \left(\frac{\lambda_m z}{\pi R_1^2 n}\right)^2} \quad (2.75)$$

In the above equations, the refractive index of the immersion medium is denoted with n , a is the confocal aperture divided by the magnification, and w_1 and R_1 are two unknown model parameters. Now, for numerical simplicity as well as for easy understanding, let us consider two substituting variables, ζ and η :

$$\zeta = \frac{z_2 - z_1}{2\sqrt{D\Delta t}}, \eta = \frac{z_2 + z_1}{2} \quad (2.76)$$

Using these two variables and the MDF described in the equation 2.72, the cross-correlation can be written as

$$g(\Delta t) = g(\infty) + \frac{I_1 I_2 C}{2} \sqrt{\pi} \iint_0^\infty \frac{k^2\{\eta^2 - (\sqrt{D\Delta t}\zeta)^2\}}{8D\Delta t + w^2(\eta - \sqrt{D\Delta t}\zeta) + w^2(\eta + \sqrt{D\Delta t}\zeta)} \times \exp\left[-\left(\zeta - \frac{v_z}{2}\sqrt{\frac{\Delta t}{D}}\right)^2 - \frac{2(\delta - v_x\Delta t)^2 + 2(v_y\Delta t)^2}{8D\Delta t + w^2(\eta - \sqrt{D\Delta t}\zeta) + w^2(\eta + \sqrt{D\Delta t}\zeta)}\right] d\zeta d\eta \quad (2.77)$$

The infinite integration limit can be approximated by numerically evaluating the equation 2.77 within a finite 2D strip, $|\eta \pm \sqrt{D\Delta t}\zeta| < Q$. Q is the value of truncation. An ideal Q is such that the numerical integration result should not change when Q is increased further – can be found using a finite element method-based simulation. The simulation converges when the result do not change upon refining the finite element sizes and increasing the Q values. A least-square fitting approach is used to fit the equation 2.77. The distance between two foci, δ , should be precisely known. The fit parameters in the data fitting are w_1 , R_1 , $I_1\sqrt{C}$, $I_2\sqrt{C}$, D , v , and offset values. The cross-correlation suggests that 2fFCS is an efficient approach to measure flow velocities besides diffusion coefficient.

Using time-correlated single photon counting (TCSPC), the time tagged photons can be used to identify the temporal position of the molecule in the detection volumes. The inset in figure 2.29 a TCSPC histogram is shown for Atto-488 molecules were dispersed in pure ethanol. The measurement was performed inside a nanochannel. The two decay curves are because of two pulsed interleaved lasers at two detection volumes. Typically, between two successive laser pulses the time gap used is 25 ns (can be varied). This time window of 25 ns is large enough to avoid

Figure 2.29: Two auto-correlations and two cross-correlations of a 2fFCS measurement. Here, the single molecules are Atto 488 fluorophores. The measurement was performed within a nanochannel. $L1 \times L1$ and $L2 \times L2$ represent autocorrelation of 1st focus and 2nd focus. The cross-correlations between two foci are represented by $L1 \times L2$ and $L2 \times L1$. The small difference between two cross-correlations is an evidence of flow velocities. Inset: Time-correlated single photon counting histograms. The photon counts in the first time window (<25 ns) is due to the first laser (first focus ($L1$)), and the second time window (>25 ns) is due to the second laser (second focus ($L2$)). In both the windows the other curves $D1$ and $D2$ corresponding to two SPADs.

detecting a photons from past excitations. In case of emitters with long lifetimes, to avoid any bleed through of photon counts from prior pulses, the pulsing frequency can be reduced. In the figure 2.29(inset), $L1$ and $L2$ are first and second laser, respectively and $D1$ and $D2$ are the detectors. The auto-correlations of 1st focus and 2nd focus are also shown in figure 2.29 (1×1 and 2×2). The cross-correlation between them are also shown (1×2 and 2×1). In a flow measurement, cross-correlation curves are fitted with a suitable Fokker-Planck equation to determine the flow velocities. If we notice, in figure 2.29 the 1×2 and 2×1 are slightly different from each other within 10^3 s to 10^4 s. This proves presence of unidirectional flow. For a qualitative understanding a schematic diagram of two cross-correlations analysis is depicted in figure 2.30 under a unidirectional flow where similar shape difference can be observed clearly.

Figure 2.30: A unidirectional flow condition: Schematic curves of two cross-correlations between (1×2) and (2×1) showing the difference in the correlation amplitudes due to flow component.

2.4.4 High resolution transmission electron microscopy

The origin of a single transition dipole can be understood from the atomistic structure of an emitter. A technique to investigate atomic level structural configuration of nanostructure is high-resolution transmission electron microscopy (HRTEM). It is an imaging mode [130] used in transmission electron microscopy (TEM). This technique can resolve atomic details of materials with a resolution of 1 Å to 0.1 Å [131, 132]. In this method highly energetic electron beam is directed towards an electron-transparent sample at high accelerating potential (60 kV to 300 kV). The electron beam interacts with the atoms of the nanostructure. During the interaction some portions of the electrons get transmitted through the sample, some get elastically and inelastically (generate X-ray) scattered, and some portions get absorbed by the sample. The transmitted and elastically scattered electrons are detected by the detectors and projected on the phosphor screen. An HRTEM setup

Figure 2.31: An HRTEM setup

Figure 2.32: Left: HRTEM captured atomic level information of a localised quantum well associated with a basal stacking fault in II-VI nanostructure by Ghosh et al. [67]. Right: The electron intensity profile in red-yellow denotes stacking fault containing lattice distance and black denotes regular periodicity of wurtzite ZnO as shown in the corresponding crystal structure at the top.

consists of an electron source, electron optics along with projection screen and digital controllers to control the electron optics. The electron optics consist of several lenses, apertures, detectors, and imaging systems (figure 4.3). The sample is placed in between the condenser and objective

lenses. In HRTEM, transmission and scattering of electrons are material composition and thickness dependent. To obtain high-resolution image thickness of the sample should be less than 80 nm. Resolution of HRTEM is also dependent on accelerating voltage of the electrons. In bright field HRTEM imaging, comparatively dark places or low signals of an HRTEM image indicate thick region of a sample. In figure 2.32, a localised quantum well of ZnO nanorod is shown at atomic resolution. The lattice distance is also plotted. The quantum well is located at a stacking fault shown by overlapping the lattice distances. For an elemental analysis of HRTEM observed structure, energy dispersive X-ray spectroscopy is used together with TEM as an add-on tool.

Bibliography

- [1] Reto B. Schoch, Jongyoon Han, and Philippe Renaud. *Transport phenomena in nanofluidics*. Reviews of modern physics 80, no. 3 (2008): 839. [26](#)
- [2] Kazuyoshi Murata, Kaoru Mitsuoka, Teruhisa Hirai, Thomas Walz, Peter Agre, J. Bernard Heymann, Andreas Engel, and Yoshinori Fujiyoshi. *Structural determinants of water permeation through aquaporin-1*. Nature 407, no. 6804 (2000): 599-605. [26](#)
- [3] Michelle Montoya, and Eric Gouaux. *β -Barrel membrane protein folding and structure viewed through the lens of α -hemolysin*. Biochimica et Biophysica Acta (BBA)-Biomembranes 1609, no. 1 (2003): 19-27. [26](#)
- [4] Lukas K. Tamm, Heedeok Hong, and Binyong Liang. *Folding and assembly of β -barrel membrane proteins*. Biochimica et Biophysica Acta (BBA)-Biomembranes 1666, no. 1 (2004): 250-263. [26](#)
- [5] Dirk Grüninger, Nora Treiber, Mathias OP Ziegler, Jochen WA Koetter, Monika-Sarah Schulze, and Georg E. Schulz. *Designed protein-protein association*. Science 319, no. 5860 (2008): 206-209. [26](#), [27](#)
- [6] Bert L. de Groot, and Helmut Grubmüller. *Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF*. Science 294, no. 5550 (2001): 2353-2357. [26](#)
- [7] David F. Savage, Pascal F. Egea, Yaneth Robles-Colmenares, Joseph D. O'Connell III, and Robert M. Stroud. *Architecture and selectivity in aquaporins: 2.5 Å X-ray structure of aquaporin Z*. PLoS Biol 1, no. 3 (2003): e72. [26](#)
- [8] Tamir Gonen and Thomas Walz. *The structure of aquaporins*. Quarterly reviews of biophysics 39, no. 04 (2006): 361-396. [26](#)

- [9] Anna Frick, Michael Jaerva, and Susanna Toernroth-Horsefield. *Structural basis for pH gating of plant aquaporins*. FEBS letters 587, no. 7 (2013): 989-993 (PDB structure 4IA4). 26, 27
- [10] Peter Agre, Melanie Bonhivers, and Mario J. Borgnia. *The aquaporins, blueprints for cellular plumbing systems*. Journal of Biological Chemistry 273, no. 24 (1998): 14659-14662. 26
- [11] Aleksij Aksimentiev, and Klaus Schulten. *Imaging α -hemolysin with molecular dynamics: ionic conductance, osmotic permeability, and the electrostatic potential map*. Biophysical journal 88, no. 6 (2005): 3745-3761. 26
- [12] Langzhou Song, Michael R. Hobaugh, Christopher Shustak, Stephen Cheley, Hagan Bayley, and J. Eric Gouaux. *Structure of staphylococcal α -hemolysin, a heptameric transmembrane pore*. Science 274, no. 5294 (1996): 1859-1865 (PDB structure 7AHL). 26, 27
- [13] Yoshikazu Tanaka, Nagisa Hirano, Jun Kaneko, Yoshiyuki Kamio, Min Yao, and Isao Tanaka. *2-Methyl-2, 4-pentanediol induces spontaneous assembly of staphylococcal α -hemolysin into heptameric pore structure*. Protein Science 20, no. 2 (2011): 448-456. 26
- [14] Jean-Marie Pages, Chloë E. James, and Mathias Winterhalter. *The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria*. Nature Reviews Microbiology 6, no. 12 (2008): 893-903. 26
- [15] Roland Benz. *Permeation of hydrophilic solutes through mitochondrial outer membranes: review on mitochondrial porins*. Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes 1197, no. 2 (1994): 167-196. 26
- [16] Bettina Bölter and Jürgen Soll. *Ion channels in the outer membranes of chloroplasts and mitochondria: open doors or regulated gates?*. The EMBO journal 20, no. 5 (2001): 935-940. 26
- [17] Oxford Dictionary, Oxford, UK; definition obtained on 24.05.2016. DOI: <http://www.oxforddictionaries.com/definition/english/diffusion> 27
- [18] Albert Einstein *The theory of the Brownian movement*. Ann. der Physik 17 (1905): 549. 28
- [19] Jean Perrin, *Brownian movement and molecular reality*. Dover Phonenix editions. Translated from The Annales De Chimie De Et De Physique, 8th Serie, Sep. 1909, . 28
- [20] Subrahmanyam Chandrasekhar *Stochastic problems in physics and astronomy*. Reviews of modern physics 15, no. 1 (1943): 1. 28
- [21] Eric W. Weisstein. *Moore Neighborhood*. MathWorld – A Wolfram Web Resource (<http://mathworld.wolfram.com/MooreNeighborhood.html>) on 25.05.2016. 28

- [22] N. J. A. Sloane. *Sequence A016754*. The On-Line Encyclopedia of Integer Sequences. 28
- [23] Lawrence Gray. *A mathematician looks at Wolfram's new kind of science*. Notices-American Mathematical Society 50, no. 2 (2003): 200-211. 28
- [24] Hannes Risken. *Fokker-Planck equation*. Springer Berlin Heidelberg, 1984. 29
- [25] Rob Zondervan, Florian Kulzer, Gregorius CG Berkhout, and Michel Orrit. *Local viscosity of supercooled glycerol near T_g probed by rotational diffusion of ensembles and single dye molecules*. Proceedings of the National Academy of Sciences 104, no. 31 (2007): 12628-12633. 29
- [26] Shimon Weiss. *Measuring conformational dynamics of biomolecules by single molecule fluorescence spectroscopy*. Nature structural biology 7, no. 9 (2000): 724-729. 29
- [27] Laura A. Deschenes and David A. Vanden Bout. *Heterogeneous dynamics and domains in supercooled o-terphenyl: A single molecule study*. The Journal of Physical Chemistry B 106, no. 44 (2002): 11438-11445. 29
- [28] Anastasia Loman, Ingo Gregor, Christina Stutz, Markus Mund, and Jörg Enderlein. *Measuring rotational diffusion of macromolecules by fluorescence correlation spectroscopy*. Photochemical & photobiological sciences 9, no. 5 (2010): 627-636. 29, 80, 88
- [29] Marco G. Mazza, Nicolas Giovambattista, H. Eugene Stanley, and Francis W. Starr. *Connection of translational and rotational dynamical heterogeneities with the breakdown of the Stokes-Einstein and Stokes-Einstein-Debye relations in water*. Physical Review E 76, no. 3 (2007): 031203. 29
- [30] R. G. Gordon. *On the rotational diffusion of molecules*. The Journal of Chemical Physics 44, no. 5 (1966): 1830-1836. 29
- [31] S-Z. Chu, Kenji Wada, Satoru Inoue, Masafumi Isogai, and Atsuo Yasumori. *Fabrication of Ideally Ordered Nanoporous Alumina Films and Integrated Alumina Nanotubule Arrays by High-Field Anodization*. Advanced Materials 17, no. 17 (2005): 2115-2119. 29
- [32] Gerhard Hummer, Jayendran C. Rasaiah, and Jerzy P. Noworyta. *Water conduction through the hydrophobic channel of a carbon nanotube*. Nature 414, no. 6860 (2001): 188-190. 29
- [33] Dmitri Golberg, Yoshio Bando, C. C. Tang, and C. Y. Zhi. *Boron nitride nanotubes*. Advanced Materials 19, no. 18 (2007): 2413-2432. 29
- [34] Jian Sha, J. Niu, X. Ma, J. Xu, X. Zhang, Q. Yang, and D. Yang. *Silicon nanotubes*. Advanced Materials 14, no. 17 (2002): 1219-1221. 29

- [35] Joshua Goldberger, Rongrui He, Yanfeng Zhang, Sangkwon Lee, Haoquan Yan, Heon-Jin Choi, and Peidong Yang. *Single-crystal gallium nitride nanotubes*. Nature 422, no. 6932 (2003): 599-602. [29](#)
- [36] Taeseup Song, Jianliang Xia, Jin-Hyon Lee, Dong Hyun Lee, Moon-Seok Kwon, Jae-Man Choi, Jian Wu et al. *Arrays of sealed silicon nanotubes as anodes for lithium ion batteries*. Nano letters 10, no. 5 (2010): 1710-1716. [30](#)
- [37] Dan-Dan Zhou, Wang-Yu Li, Xiao-Li Dong, Yong-Gang Wang, Cong-Xiao Wang, and Yong-Yao Xia. *A nitrogen-doped ordered mesoporous carbon nanofiber array for supercapacitors*. Journal of Materials Chemistry A 1, no. 29 (2013): 8488-8496. [30](#)
- [38] Lin Qiu, Xiaotian Wang, Guoping Su, Dawei Tang, Xinghua Zheng, Jie Zhu, Zhiguo Wang, Pamela M. Norris, Philip D. Bradford, and Yuntian Zhu. *Remarkably enhanced thermal transport based on a flexible horizontally-aligned carbon nanotube array film*. Scientific reports 6 (2016). [30](#)
- [39] Michael J. O'connell, Sergei M. Bachilo, Chad B. Huffman, Valerie C. Moore, Michael S. Strano, Erik H. Haroz, Kristy L. Rialon et al. *Band gap fluorescence from individual single-walled carbon nanotubes*. Science 297, no. 5581 (2002): 593-596. [30](#)
- [40] Wei Chen and Haroon Ahmed. *Fabrication of 5-7 nm wide etched lines in silicon using 100 keV electron-beam lithography and polymethylmethacrylate resist*. Applied physics letters 62, no. 13 (1993): 1499-1501. [30](#)
- [41] M. A. McCord and T. H. Newman. *Low voltage, high resolution studies of electron beam resist exposure and proximity effect*. Journal of Vacuum Science & Technology B 10, no. 6 (1992): 3083-3087. [31](#)
- [42] Siddharth Ghosh and G. K. Ananthasuresh. *Single-photon-multi-layer-interference lithography for high-aspect-ratio and three-dimensional SU-8 micro-/nanostructures*. Scientific reports 6 (2016). [31](#)
- [43] Siddharth Ghosh, Manoj Kumbhakar, Mitja Platen, Ingo Gregor, and Jörg Enderlein. *Single-molecule fluorescence inside solid-state nanochannels*. In SPIE BiOS, pp. 895008-895008. International Society for Optics and Photonics, 2014. [31](#)
- [44] Linda M. Ephrath, *Reactive ion etching*. U.S. Patent 4,283,249, issued August 11, 1981. [31](#)
- [45] Linda M. Ephrath, *Reactive ion etching for VLSI*. Electron Devices, IEEE Transactions on 28, no. 11 (1981): 1315-1319. [31](#), [32](#)

- [46] Gerd Binnig, Calvin F. Quate, and Ch Gerber. *Atomic force microscope*. Physical review letters 56, no. 9 (1986): 930. [32](#)
- [47] Yanyan Wang, Xiaodong Hu, Linyan Xu, and Xiaotang Hu. *Improving the scanning speed of atomic force microscopy at the scanning range of several tens of micrometers*. Ultramicroscopy 124 (2013): 102-107. [32](#)
- [48] Gerd Binnig and Heinrich Rohrer. *Scanning tunneling microscopy*. Surface science 126, no. 1 (1983): 236-244. [32](#)
- [49] Coskun Kocabas, Hoon-sik Kim, Tony Banks, John A. Rogers, Aaron A. Pesetski, James E. Baumgardner, S. V. Krishnaswamy, and Hong Zhang. *Radio frequency analog electronics based on carbon nanotube transistors*. Proceedings of the National Academy of Sciences 105, no. 5 (2008): 1405-1409. [33](#)
- [50] Wei Gao, Xiaomiao Feng, Allen Pei, Yonge Gu, Jinxing Li, and Joseph Wang. *Seawater-driven magnesium based Janus micromotors for environmental remediation*. Nanoscale 5, no. 11 (2013): 4696-4700. [33](#)
- [51] N. R. Aghamalyan, I. A. Gambaryan, E. Kh Goulanian, R. K. Hovsepyan, R. B. Kostanyan, S. I. Petrosyan, E. S. Vardanyan, and A. F. Zerrouk. *Influence of thermal annealing on optical and electrical properties of ZnO films prepared by electron beam evaporation*. Semiconductor science and technology 18, no. 6 (2003): 525. [33](#)
- [52] J. George. *Preparation of thin films*. Marcel Dekker, Inc., New York (1992), p.13-9. [34](#)
- [53] Christiane Becker, Tobias Sontheimer, Simon Steffens, Simone Scherf, and Bernd Rech. *Polycrystalline silicon thin films by high-rate electronbeam evaporation for photovoltaic applications-Influence of substrate texture and temperature*. Energy Procedia 10 (2011): 61-65. [34](#)
- [54] L. Holzer, F. Indutnyi, P. H. Gasser, B. Muench, and M. Wegmann. *Three-dimensional analysis of porous BaTiO₃ ceramics using FIB nanotomography*. Journal of Microscopy 216, no. 1 (2004): 84-95. [34](#)
- [55] Reo Kometani, Takahiko Morita, Keiichiro Watanabe, Takayuki Hoshino, Kazushige Kondo, Kazuhiro Kanda, Yuichi Haruyama et al. *Nanomanipulator and actuator fabrication on glass capillary by focused-ion-beam-chemical vapor deposition*. Journal of Vacuum Science & Technology B 22, no. 1 (2004): 257-263. [34](#)
- [56] L. A. Giannuzzi and Frederick A. Stevie. *A review of focused ion beam milling techniques for TEM specimen preparation*. Micron 30, no. 3 (1999): 197-204. [34](#)

- [57] V. Castaldo, C. W. Hagen, B. Rieger, and P. Kruit. *Sputtering limits versus signal-to-noise limits in the observation of Sn balls in a Ga⁺ microscope*. Journal of Vacuum Science & Technology B 26, no. 6 (2008): 2107-2115. [34](#)
- [58] A. Adams and F. H. Read. *Electrostatic cylinder lenses II: Three element einzel lenses*. Journal of Physics E: Scientific Instruments 5, no. 2 (1972): 150. [35](#)
- [59] Bo Huang, Mark Bates, and Xiaowei Zhuang. *Super resolution fluorescence microscopy*. Annual review of biochemistry 78 (2009): 993. [37](#)
- [60] Thomas Dertinger, Ryan Colyer, G. Iyer, S. Weiss, and J. Enderlein. *Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI)*. Proceedings of the National Academy of Sciences 106, no. 52 (2009): 22287-22292. [37](#)
- [61] Digambara Patra, Ingo Gregor, Jörg Enderlein, and Markus Sauer. *Defocused imaging of quantum-dot angular distribution of radiation*. Applied Physics Letters 87, no. 10 (2005): 101103. [42](#), [59](#), [60](#)
- [62] Y. Arakawa and Hiroyuki Sakaki. *Multidimensional quantum well laser and temperature dependence of its threshold current*. Applied Physics Letters 40, no. 11 (1982): 939-941. [38](#)
- [63] Brian P. Anderson, and Ma A. Kasevich. *Macroscopic quantum interference from atomic tunnel arrays*. Science 282, no. 5394 (1998): 1686-1689. [38](#)
- [64] Feng Chen, Edwin B. Ramayya, Chanan Euaruksakul, Franz J. Himpsel, George K. Celler, Bingjun Ding, Irena Knezevic, and Max G. Lagally. *Quantum confinement, surface roughness, and the conduction band structure of ultrathin silicon membranes*. ACS nano 4, no. 4 (2010): 2466-2474. [38](#)
- [65] Paul Tipler, Ralph Llewellyn. Modern Physics (5 ed.). W. H. Freeman and Company. pp. 160-161. ISBN 978-0-7167-7550-8 (2008). [39](#)
- [66] S. A. Empedocles, R. Neuhauser, and M. G. Bawendi. *Three-dimensional orientation measurements of symmetric single chromophores using polarization microscopy*. Nature 399, no. 6732 (1999): 126-130. [42](#)
- [67] Siddharth Ghosh, Moumita Ghosh, Michael Seibt, and G. Mohan Rao. *Detection of quantum well induced single degenerate-transition-dipoles in ZnO nanorods*. Nanoscale 8, (2016): 2632-2638. [42](#), [66](#)
- [68] Peter E. Blöchl. *Advanced Topics of Theoretical Physics I: The electronic structure of matter*. pp. 6-7 and pp. 25 Institute of Theoretical Physics, TU Clausthal, 2013 (Source: <http://orion.pt.tu-clausthal.de/atp/phix.html> [\[pdf\]](#)). [43](#), [44](#)

- [69] Max Born and Robert Oppenheimer. *Zur Quantentheorie der Molekeln*. Annalen der Physik 389, no. 20 (1927): 457-484. [43](#)
- [70] Peter W. Atkins, and Ronald S. Friedman. Molecular quantum mechanics. 4 ed. Oxford university press, 2011 pp. 343. [45](#)
- [71] Siddharth Ghosh, Anna M. Chizhik, Narain Karedla, Mariia O. Dekaliuk, Ingo Gregor, Henning Schuhmann, Michael Seibt, Kai Bodensiek, Iwan A. T. Schaap, Olaf Schulz, Alexander P. Demchenko, Jörg Enderlein, and Alexey I. Chizhik. *Photoluminescence of Carbon Nanodots: Dipole Emission Centers and Electron–Phonon Coupling*. Nano letters 14, no. 10 (2014): 5656-5661. [45](#)
- [72] Charlotte Froese Fischer *Hartree–Fock method for atoms. A numerical approach*. (1977). [46](#)
- [73] Takao Tsuneda. *Density functional theory in quantum chemistry*. New York: Springer, 2014, 35-63. [47](#)
- [74] "The Nobel Prize in Chemistry 1998". Nobelprize.org. Nobel Media AB 2014. Web. 6 Jun 2016. (http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1998/) [47](#)
- [75] J. C. Slater and H. M. Krutter. *The Thomas-Fermi method for metals*. Physical Review 47, no. 7 (1935): 559. [47](#)
- [76] E. J. Baerends, and P. Ros. *Evaluation of the LCAO Hartree-Fock-Slater method: Applications to transition-metal complexes*. International Journal of Quantum Chemistry 14, no. S12 (1978): 169-190. [47](#)
- [77] Pierre Hohenberg, and Walter Kohn. *Inhomogeneous electron gas*. Physical review 136, no. 3B (1964): B864. [47](#)
- [78] Walter Kohn and Lu Jeu Sham. *Self-consistent equations including exchange and correlation effects*. Physical review 140, no. 4A (1965): A1133. [47](#), [48](#)
- [79] Sidney Redner. *Citation statistics from more than a century of physical review*. arXiv preprint physics/0407137 (2004). [47](#)
- [80] John P. Perdew. *Accurate density functional for the energy: Real-space cutoff of the gradient expansion for the exchange hole*. Physical Review Letters 55, no. 16 (1985): 1665. [48](#)
- [81] John P. Perdew, J. A. Chevary, S. H. Vosko, Koblar A. Jackson, Mark R. Pederson, D. J. Singh, and Carlos Fiolhais. *Atoms, molecules, solids, and surfaces: Applications of the generalized gradient approximation for exchange and correlation*. Physical Review B 46, no. 11 (1992): 6671. [48](#)

- [82] R. F. W.Bader. *Principle of stationary action and the definition of a proper open system*. Physical Review B 49, no. 19 (1994): 13348. [49](#)
- [83] M. E. Casida. Recent Advances in Density Functional Methods:(Part I). Vol. 1. Chapter - *Time- dependent Density Functional Response Theory for Molecules*, pages 155-192. World Scientific, 1995. [50](#), [51](#)
- [84] M. G. U. J. Petersilka, U. J. Gossmann, and E. K. U. Gross. *Excitation energies from time-dependent density-functional theory*. Physical review letters 76, no. 8 (1996): 1212. [51](#)
- [85] W. Matthew C. Foulkes and Roger Haydock. *Tight-binding models and density-functional theory*. Physical review B 39, no. 17 (1989): 12520. [52](#), [53](#)
- [86] Daniel Sheppard, Rye Terrell, and Graeme Henkelman. *Optimization methods for finding minimum energy paths*. The Journal of chemical physics 128, no. 13 (2008): 134106. [53](#)
- [87] John C. Slater, *Atomic shielding constants*. Physical Review 36, no. 1 (1930): 57. [53](#)
- [88] Marye Anne Fox and Maria T. Dulay. *Heterogeneous photocatalysis*. Chemical reviews 93, no. 1 (1993): 341-357. [54](#)
- [89] B. Grundkötter-Stock. *Density Functional based Tight Binding parameters for boron and sublattice symmetry broken adatom adsorption on graphene*. PhD thesis, University of Bremen, 2013. [54](#)
- [90] Th Frauenheim, F. Weich, Th Köhler, S. Uhlmann, D. Porezag, and G. Seifert. *Density-functional-based construction of transferable nonorthogonal tight-binding potentials for Si and SiH*. Physical Review B 52, no. 15 (1995): 11492. [54](#)
- [91] M. Haugk, J. Elsner, and Th Frauenheim. *A density-functional based tight-binding approach to GaAs surface reconstructions*. Journal of Physics: Condensed Matter 9, no. 35 (1997): 7305. [54](#)
- [92] Rafael Gutierrez, Thomas Frauenheim, Thomas Köhler, and Gothard Seifert. *Stability of silicon carbide structures: from clusters to solid surfaces*. Journal of Materials Chemistry 6, no. 10 (1996): 1657-1663. [54](#)
- [93] F. Weich, J. Widany, and Th Frauenheim. *Paracyanogenlike structures in high-density amorphous carbon nitride*. Physical review letters 78, no. 17 (1997): 3326. [54](#)
- [94] P. K. Sitch, Th Frauenheim, and R. Jones. *A density functional tight-binding approach for modelling Ge and GeH structures*. Journal of Physics: Condensed Matter 8, no. 37 (1996): 6873. [54](#)

- [95] Marcus Elstner, Dirk Porezag, G. Jungnickel, J. Elsner, M. Haugk, Th Frauenheim, Sandor Suhai, and Gotthard Seifert. *Self-consistent-charge density-functional tight-binding method for simulations of complex materials properties*. Physical Review B 58, no. 11 (1998): 7260. [54](#)
- [96] M. Elstner, *The SCC-DFTB method and its application to biological systems*. Theoretical Chemistry Accounts 116, no. 1-3 (2006): 316-325. [55](#)
- [97] Yang Yang, Haibo Yu, Darrin York, Qiang Cui, and Marcus Elstner. *Extension of the self-consistent-charge density-functional tight-binding method: third-order expansion of the density functional theory total energy and introduction of a modified effective coulomb interaction*. The Journal of Physical Chemistry A 111, no. 42 (2007): 10861-10873. [55](#)
- [98] Michael Gaus, Qiang Cui, and Marcus Elstner. *DFTB3: extension of the self-consistent-charge density-functional tight-binding method (SCC-DFTB)*. J. Chem. Theory Comput 7, no. 4 (2011): 931-948. [55](#)
- [99] T. A. Niehaus, S. Suhai, F. Della Sala, P. Lugli, M. Elstner, G. Seifert, and Th Frauenheim. *Tight-binding approach to time-dependent density-functional response theory*. Physical Review B 63, no. 8 (2001): 085108. [55](#)
- [100] S. R. White, *Density matrix formulation for quantum renormalization groups*. Physical Review Letters 69, no. 19 (1992): 2863. [55](#)
- [101] Balint Aradi, Ben Hourahine, and Th Frauenheim. *DFTB+, a sparse matrix-based implementation of the DFTB method*. The Journal of Physical Chemistry A 111, no. 26 (2007): 5678-5684. [55](#)
- [102] A. Dominguez, B. Aradi, Th Frauenheim, Vitalij Lutsker, and Thomas A. Niehaus. *Extensions of the time-dependent density functional based tight-binding approach*. Journal of Chemical Theory and Computation 9, no. 11 (2013): 4901-4914. [55](#), [56](#)
- [103] Marko Schreiber, Mario R. Silva-Junior, Stephan PA Sauer, and Walter Thiel. *Benchmarks for electronically excited states: CASPT2, CC2, CCSD, and CC3*. The Journal of chemical physics 128, no. 13 (2008): 134110. [56](#)
- [104] Carlo Adamo, and Vincenzo Barone. *Toward reliable density functional methods without adjustable parameters: The PBE0 model*. The Journal of chemical physics 110, no. 13 (1999): 6158-6170. [56](#)
- [105] Matthias Ernzerhof, and Gustavo E. Scuseria. *Assessment of the Perdew-Burke-Ernzerhof of exchange-correlation functional*. The Journal of chemical physics 110, no. 11 (1999): 5029-5036. [56](#)

- [106] Takeshi Yanai, David P. Tew, and Nicholas C. Handy. *A new hybrid exchange-correlation functional using the Coulomb-attenuating method (CAM-B3LYP)*. Chemical Physics Letters 393, no. 1 (2004): 51-57. [56](#)
- [107] Miquel Huix-Rotllant, Michael Filatov, Samer Gozem, Igor Schapiro, Massimo Olivucci, and Nicolas Ferre. *Assessment of density functional theory for describing the correlation effects on the ground and excited state potential energy surfaces of a retinal chromophore model*. Journal of chemical theory and computation 9, no. 9 (2013): 3917-3932. [56](#)
- [108] Ute Resch-Genger, Markus Grabolle, Sara Cavaliere-Jaricot, Roland Nitschke, and Thomas Nann. *Quantum dots versus organic dyes as fluorescent labels*. Nature methods 5, no. 9 (2008): 763-775. [56](#)
- [109] Anna M. Chizhik, Simon Stein, Mariia O. Dekaliuk, Christopher Battle, Weixing Li, Anja Huss, Mitja Platen et al. *Super-Resolution Optical Fluctuation Bio-Imaging with Dual-Color Carbon Nanodots*. Nano letters 16, (2016): 237-242. [56](#)
- [110] Wesley Wei-Wen Hsiao, Yuen Yung Hui, Pei-Chang Tsai, and Huan-Cheng Chang. *Fluorescent Nanodiamond: A Versatile Tool for Long-Term Cell Tracking, Super-Resolution Imaging, and Nanoscale Temperature Sensing*. Accounts of chemical research 49, no. 3 (2016): 400-407. [56](#)
- [111] Brahim Lounis and W. E. Moerner. *Single photons on demand from a single molecule at room temperature*. Nature 407, no. 6803 (2000): 491-493. [56](#)
- [112] P. Michler, , A. Kiraz, C. Becher, W. V. Schoenfeld, P. M. Petroff, Lidong Zhang, E. Hu, and A. Imamoglu. *A quantum dot single-photon turnstile device*. Science 290, no. 5500 (2000): 2282-2285. [56](#)
- [113] A. Kiraz, M. Atatuer, and A. Imamoglu. *Quantum-dot single-photon sources: Prospects for applications in linear optics quantum-information processing*. Physical Review A 69, no. 3 (2004): 032305. [56](#)
- [114] Th Basche, W. E. Moerner, M. Orrit, and H. Talon. *Photon antibunching in the fluorescence of a single dye molecule trapped in a solid*. Physical review letters 69, no. 10 (1992): 1516. [56](#)
- [115] Brahim Lounis and W. E. Moerner. *Single photons on demand from a single molecule at room temperature*. Nature 407, no. 6803 (2000): 491-493. [56](#)
- [116] Digambara Patra, Ingo Gregor, and Jörg Enderlein. *Image analysis of defocused single-molecule images for three-dimensional molecule orientation studies*. The Journal of Physical Chemistry A 108, no. 33 (2004): 6836-6841. [56](#)

- [117] B. Sick, B. Hecht, and L. Novotny. *Orientational imaging of single molecules by annular illumination*. Physical Review Letters 85, no. 21 (2000): 4482. [56](#)
- [118] Narain Karedla, Simon C. Stein, Dirk Hähnel, Ingo Gregor, Anna Chizhik, and Jörg Enderlein. *Simultaneous Measurement of the Three-Dimensional Orientation of Excitation and Emission Dipoles*. Physical review letters 115, no. 17 (2015): 173002. [56](#)
- [119] Paul A. Alivisatos. *Semiconductor clusters, nanocrystals, and quantum dots*. Science 271, no. 5251 (1996): 933. [56](#)
- [120] Jinwook Lee, Vikram C. Sundar, Jason R. Heine, Mouni G. Bawendi, and Klavs F. Jensen. *Full color emission from II-VI semiconductor quantum dot-polymer composites*. Advanced Materials 12, no. 15 (2000): 1102-1105. [56](#)
- [121] W. William Yu and Xiaogang Peng. *Formation of high-quality CdS and other II-VI semiconductor nanocrystals in noncoordinating solvents: tunable reactivity of monomers*. Angewandte Chemie International Edition 41, no. 13 (2002): 2368-2371. [56](#)
- [122] X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. J. J. L. Dooze, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, and S. Weiss. *Quantum dots for live cells, in vivo imaging, and diagnostics*. science 307, no. 5709 (2005): 538-544. [56](#)
- [123] Paola Borri, Wolfgang Langbein, Stephan Schneider, Ulrike Woggon, Roman L. Sellin, Dongxun Ouyang, and Dieter Bimberg. *Ultralong dephasing time in InGaAs quantum dots*. Physical Review Letters 87, no. 15 (2001): 157401. [56](#)
- [124] Warren CW Chan, Dustin J. Maxwell, Xiaohu Gao, Robert E. Bailey, Mingyong Han, and Shuming Nie. *Luminescent quantum dots for multiplexed biological detection and imaging*. Current opinion in biotechnology 13, no. 1 (2002): 40-46. [56](#)
- [125] Ron Hardman. *A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors*. Environmental health perspectives (2006): 165-172. [56](#)
- [126] Alireza Valizadeh, Haleh Mikaeili, Mohammad Samiei, Samad Mussa Farkhani, Nosratalah Zarghami, Abolfazl Akbarzadeh, and Soodabeh Davaran. *Quantum dots: synthesis, bioapplications, and toxicity*. Nanoscale research letters 7, no. 1 (2012): 1-14. [56](#)
- [127] Jasmina Lovric, Hassan S. Bazzi, Yan Cuie, Genevieve RA Fortin, Francoise M. Winnik, and Dusica Maysinger. *Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots*. Journal of Molecular Medicine 83, no. 5 (2005): 377-385. [56](#)
- [128] Christiaan Huygens, *Traité de la lumiere* (Leiden, Netherlands: Pieter van der Aa, 1690), Chapter 1 pp. 18. [57](#)

- [129] Huygens-Fresnel principle (Mathematical expression of the principle), Wikipedia.Org (source) URL taken on 01.08.2017. [57](#)
- [130] D. B. Williams and C. B. Carter. *Transmission electron microscopy a text book for materials science* Springer 2009. [66](#)
- [131] Michael A. O'keefe, E. C. Nelson, Y. C. Wang, and Andreas Thust. *Sub-Ångström resolution of atomistic structures below 0.8 Å*. Philosophical Magazine B 81, no. 11 (2001): 1861-1878. [66](#)
- [132] Niels Fischer, Piotr Neumann, Andrey L. Konevega, Lars V. Bock, Ralf Ficner, Marina V. Rodnina, and Holger Stark. *Structure of the E. coli ribosome-EF-Tu complex at <3 Å resolution by Cs-corrected cryo-EM*. Nature 520, no. 7548 (2015): 567-570. [66](#)
- [133] J. C. Russ. *Fundamentals of Energy Dispersive X-ray Analysis* Butterworths, London 1984.
- [134] Douglas Magde, Elliot L. Elson, and Watt W. Webb. *Fluorescence correlation spectroscopy. II. An experimental realization*. Biopolymers 13, no. 1 (1974): 29-61. [61](#)
- [135] Jörg Enderlein, Ingo Gregor, Digambara Patra, Thomas Dertinger, and U. Benjamin Kaupp. *Performance of fluorescence correlation spectroscopy for measuring diffusion and concentration*. ChemPhysChem 6, no. 11 (2005): 2324-2336. [61](#)
- [136] Tyler J. Arbour, and Jörg Enderlein. *Application of dual-focus fluorescence correlation spectroscopy to microfluidic flow-velocity measurement*. Lab on a Chip 10, no. 10 (2010): 1286-1292. [61](#)
- [137] H. J., M. Dagenais Kimble, and L. Mandel. *Photon antibunching in resonance fluorescence*. Physical Review Letters 39, no. 11 (1977): 691. [61](#)
- [138] R. D. Allen, G. B. David, and Gf Nomarski. *The zeiss-Nomarski differential interference equipment for transmitted-light microscopy*. Zeitschrift fur wissenschaftliche Mikroskopie und mikroskopische Technik 69, no. 4 (1969): 193-221. [61](#)
- [139] Jörg Enderlein, Ingo Gregor, Digambara Patra, Thomas Dertinger, and U. Benjamin Kaupp. *Performance of fluorescence correlation spectroscopy for measuring diffusion and concentration*. ChemPhysChem 6, no. 11 (2005): 2324-2336. [62](#), [63](#)

Chapter 3

Single molecule nanofluidics

Ensemble averaging experiments may conceal many fundamental molecular interactions. To overcome that, a high-throughput detection of single molecules or colloidal nanodots is crucial for biomedical, nanoelectronic, and solid-state applications. One-dimensional (1D) discrete flow of nanoscale

objects is an efficient approach. The development of simple and cost-effective nanofluidic devices is a critical step to realise 1D flow. This chapter shows a nanofabrication technique using shadow-angle-electron-beam-deposition for a high-throughput preparation of parallel nanofluidic channels. These were used to flow and detect 11 nm long DNA, 2 nm sized carbon-nanodots, and organic fluorophores of 1.5 nm. The 1D flow behaviour was identified and analysed using dual-focus fluorescence correlation spectroscopy. The 1D molecular mass transport was performed using electro-osmotic flow. A range of flow velocities of single molecules was achieved from 15 $\mu\text{m/s}$ to 290 $\mu\text{m/s}$. The transitions of single molecules or nanodots through the two foci were quantitatively analysed using confocal scanning imaging, correlative photon detection, and photon burst size distribution analysis. The results suggest an efficient nanofabrication technique is developed to prepare nanofluidic devices.

3.1 Introduction

The study of nanofluidics has become popular for wide scientific applications [1, 2, 3, 4]. With the advancement of nanotechnologies, nanofluidic devices comprising arrays of nanochannels with diameters less than hundred nanometres are important for bioanalytical diagnostics applications, such as DNA optical mapping [5, 6, 7, 8], single virus and nanoparticle detection [9, 10, 11, 12]. Nanochannel is an ideal tool where systematic studies of entities from single molecule to single viruses are possible. It can induce 1D fluidic confinement by suppressing the thermal motions in two directions.

The primary problem is to establish a simple and efficient way of nanofabricating 1D nanofluidic channels. Different methods have been published, which described the fabrication of such nanofluidic devices [16, 17, 18, 19, 20]. However, majority of them are technically challenging, costly, and not easily applicable for high-throughput production. Hence, we have developed a cost-effective and simple nanofabrication technique to produce nanochannels within short duration for solid-state materials. The technique is based on electron beam lithography (EBL) and shadow-angle-electron-beam-deposition (SAEBD).

The nanochannels were used to detect flow of photoluminescent nanomaterials, such as small DNA molecules labelled with single organic fluorophores, carbon nanodots (CND) [19], and pure organic fluorophores. Using 2fFCS [22, 23, 24, 25]. We recorded the transits of single molecules or nanodots through the nanochannels and quantitatively analysed their flow velocities. Here, the detection regions were two diffraction limited focal spots (figure 2.28). When a molecule is not lost between these two foci due to diffusion, we consider the flow is 1D. To meet this condition, diameters of the nanochannels should be smaller than the detection region. The nanochannels presented here have diameters ranging from 30 nm to 100 nm, which are smaller than the focal diameters of the confocal optical microscope which are the detection region. The 1D flow velocities of single molecules were quantified by correlating the time-tagged photons detected and fitting the correlation curves with the 1D Fokker-Planck equation. The photon bursts were also analysed to confirm if they were from single molecules or from aggregates.

3.2 Fabrication of nanochannel

The ballistic path of the electron beam (e-beam) assisted evaporation [26] is the working principle behind SAEBD. When a straight beam of depositing material hits onto an open nano-trench at shallow incident angles, no deposition occurs in the shadowed region [27, 28]. Such deposition can enclose a large number of parallel nanochannels (depending on the e-beam diameter) leaving the shadowed region as the fluidic path of interest. The process is unaffected by nanometre sized

leftover residues of the e-beam resists and does not require an atomically clean surface, unlike any wafer bonding process [29]. To achieve high signal to noise ratio (SNR) for fluorescence based detection the nanochannels were prepared using silicon dioxide/silica. The process steps to create an enclosed nanochannels involve – nanofabrication of open nanochannels using EBL and RIE and enclosing them using SAEBD. Being an insulator silica contributes to surface charging under e-beam, which is problematic for EBL and SEM. As a proof of principle experiment, SAEBD technique was first established on semiconducting wafers (silicon) using metallic deposition (titanium). Later, we used silica where EBL and SEM induced surface charges were compensated using a thin-film of gold.

3.2.1 Nano-trenches

Figure 3.1a shows a schematic flow-chart of creating nanochannels on [100] silicon wafers. The width of the nano-trenches (i.e. the final width of the enclosed nanochannels) can be varied by the e-beam exposure of the EBL to the positive e-beam resist. Different widths of nano-trenches were created ranging from 30 nm to 100 nm. In figure 3.1b, we observe such nano-trenches. The nanolithographed e-beam resist acted as a mask for RIE to etch the final nano-trenches on silicon. The depth of the nano-trenches was investigated with AFM as 35 nm to 40 nm. All the parameters related to nanofabrications are detailed in the method section (placed at the end of this chapter).

3.2.2 Nanochannels – enclosing nano-trenches

In the next step, SAEBD was used to enclose the nano-trenches to create enclosed nanochannels. Figure 3.1c schematically explains the concept of the SAEBD process. A high-energy e-beam (bent with magnet) sublimates the material which deposits onto the substrate of interest. Here, the substrate was silicon wafers containing arrays of nano-trenches. Figure 3.1d schematically explains the role of deposition angle (θ). The shadowed region is created due to the angular depositions. This region is unexposed to the depositing material. The deposited region and shadowed region are colour coded as red and yellow, respectively. In the time evolution schematic the SAEBD leads to enclosing the nano-trenches leaving apart a void.^{††} By decreasing θ one can increase the hypotenuse. At an acute angle close to 0° , the hypotenuse becomes nearly parallel to the base as shown in figure 3.1e-h. Figure 3.1e schematically represents a high angle deposition which was experimentally obtained at 80° in figure 3.1f. Here, an array of 5 mm long nano-trenches were cross-sectioned using a wafer sawing instrument to observe the intermediate steps of SAEBD. Figure 3.1g schematically represents a low angle deposition which was realised at 45° in figure 3.1f. Designing the deposition stage is difficult for an acute angle close to 0° . Nevertheless, satis-

^{††}The e-beam assisted growth produces an amorphous film, unlike the exaggerated scheme in figure 3.1d.

Figure 3.1: Nanochannel fabrication using SAEBD. *a.* Fabrication of nano-trenches on silicon with EBL and RIE. *b.* SEM of silicon nano-trenches with 62 nm and 100 nm width. *c.* SAEBD at angle θ . *d.* Shadows of electron beam – arrows represent ballistic path of e-beam evaporation. *e.* Schematic of high angle deposition and *f.* SEM of deposition at 80° . *g.* Schematic of low angle deposition and *h.* SEM of deposition at 45° . *i.* FIB cross-section of the enclosed nanochannels. Two layers of platinum were used to protect the nanochannel from high energy ions. *j.* Magnified view of the enclosed nanochannel, where Si-1 is the silicon substrate on which nano-trenches were fabricated, Ti-2 is the 50 nm thick titanium layer which was used in SAEBD, Pt-3 and Pt-4 were platinum deposited inside FIB. *k.* Further magnified view of the nanochannel where ‘1’ represent silicon (Si-1) and ‘2’ represent titanium (Ti-2).

factory results were obtained using $\theta = 45^\circ$ as shown in figure 3.1i-k. Here, 60 nm titanium was SAEBDed on the open nano-trenches at an angle of 45° with a deposition rate of $1 \text{ \AA/s}$ at a pressure of 2×10^{-6} mbar. Preparing a sample holder with less than 45° angle is challenging. We predict that at an angle 30° one can prepare high quality flat edge.

After SAEBD, we used an FIB to cross-section the SAEBD produced nanochannels for high resolution characterisation. To avoid ion beam induced damage the region to be milled was protected with metallic thin-film layers (see section 2.1.11 for theoretical understanding). As noticeable, we deposited two thin-films on the top surface of the enclosed nanochannels. Figure 3.1i - k show SEM images of the milled regions from low to high magnification. In figure 3.1j the first layer

(Si-1) is the silicon substrate on which nano-trenches were fabricated. The second layer was the 45° SAEBDed titanium (Ti-2) layer. The third and fourth layers — Pt-3 and Pt-4 are platinum layers of 100 nm and 450 nm, respectively. They acted as protective layers to avoid FIB damage. Pt-3 and Pt-4 were deposited using FIB, which have no relation to the SAEBD process. In figure 3.1k, we observe the magnified cross-section of an enclosed nanochannel. Here, ‘1’ and ‘2’ refer to Si-1 and Ti-2 layers, respectively. As predicted, the non-conformal SAEBD growth of titanium thin-film produces a well defined flat encloser of the nanochannel. A 60 nm of titanium SAEBD at 45° angle should produce a vertical thickness of 51 nm. In figure 3.1j, the vertical thickness of the titanium film is 47.8 nm. Considering the uncertainty of few nanometres deposition thickness, this agrees well with our expectation.

After this proof of principle experiment with titanium, fused silica based nanofluidic devices were prepared. Here, 5 nm of gold thin-film was sputter coated prior to spin-coating e-beam resist on the silica wafer. Monte-Carlo simulations were performed to optimise the gold thin-film thickness in order to reduce charging of silica under EBL. Fused silica based nano-trenches were enclosed with SAEBD using silicon dioxide at 45°. They were used to study 1D flow of single molecules and nanodots using widefield microscopy and 2fFCS.

3.3 Nanofluidic device and 2fFCS detection scheme

The design of the silica based nanofluidic device to perform single molecule experiments is schematically illustrated in figure 3.2a. It has two reservoirs with gradual decrease of length-scale from milli-scale to microscale and finally, the nanometre sized channels. Two milli-scale reservoirs were used as inlet and outlet. These two inlets were prepared using sand blasting method on silica wafers using 70 μm silica particles prior to the nanofabrication process. The microscale reservoirs with the same thickness of nanochannels and micrometre width were etched with RIE. In the figure 3.2a, the white regions and blue regions are milli-scale and microscale reservoirs, respectively (see appendix B for microscopic images). The red stripes correspond to nanochannels. They are connected to the microscale reservoirs. An SEM image of these silica nanochannels is also shown in the right inset of figure 3.2a. We filled these nanochannels with a solution of fluorescent probes. They were unidirectionally transported with electro-osmotic flow [30, 31, 32, 33] as shown in figure 3.2b. After filling one reservoir with fluorescent molecules (diluted in PBS buffer - see methods - electro-osmotic flow), capillary force transported the fluid to the other reservoir. A relaxation time of 30 s was given (to avoid development of trapped air bubble) before the second reservoir was filled. Two 100 μm thick platinum electrodes were immersed into the PBS filled reservoirs (see appendix B), and an electric field was applied along the nanochannels (figure 3.3 the experimtnal setup of the electrode integration with nanofluidic device). In figure 3.2c, single Alexa-647 fluorophore molecules (purchased from Thermo Fisher, Massachusetts, USA) are hori-

zonally lined up in all the parallel nanochannels. The pixel to photon counts profile plot shows an average SNR of 90. This image was captured with a wide-field optical microscope by exciting the molecules with a 640 nm continuous wave laser (Coherent Laser Systems GmbH, Göttingen, Germany). Beside the evidence of FIB and SEM, this also infers that the nanochannels are properly enclosed. No leakage of single molecules is observed among nanochannels (from one channel to other [29]).

3.4 1D flow

Fluid flow inside a nanochannel is 1D when $D_f \gg d_{nc}$, where D_f and d_{nc} are the diameters of the detecting region and the nanochannels, respectively (figure 3.2a). This condition is satisfied because in our case D_f is 300 nm to 500 nm and d_{nc} is 30 nm to 100 nm.

The time-tagged photons from flowing single molecules were correlated using two APDs of 2fFCS setup (figure 3.2b) [23]. The diffraction limited two foci of 2fFCS were 40 MHz pulsed interleaved excitation. The two foci were partially overlapping with each other. They were generated using a Nomarski prism, a $100\times$ 1.49 NA of oil immersion objective (see appendix F), and two linearly polarised lasers perpendicular to each other. An x - y - z piezo-scanner was used to acquire scan images and point measurements of photon counts. [see section 2.4.4 for theoretical insight.]

3.4.1 EMCCD based detection

Before going to 2fFCS based detection, I demonstrate the EMCCD based detection approach. If the SNR is high, single molecules are visible in a widefield microscope. The nanofluidic device as shown in figure 3.2 was investigated under widefield microscope with a continuous wave excitation. Videos of figure 3.4a and b show that keeping a track on a single molecule from one frame to next frame is difficult due to the fast diffusion of molecules. The gif files of the videos can be obtained from the GWDG cloud storage [34, 35]^{‡‡}. The videos were captured at 50 ms (figure 3.4a) and 100 ms (figure 3.4b) EMCCD camera exposure time (Andor iXon3 897, Oxford Instruments Ltd, Oxford, UK). The molecules shown in figure 3.4 are Atto 488 (Atto-tec GmbH, Siegen, Germany). They were excited with 488 nm continuous wave laser (Coherent Laser Systems GmbH, Göttingen, Germany), which was triggered by the exposure time of the camera. Other problem with EMCCD based detection was non-specific adsorption of single molecules to the nanochannel-walls. The fluorescence signal from the adsorbed molecules are much higher compare to the flowing molecules. Thus, the relative signals from flowing molecules are sometimes

^{‡‡}10 $\times$ magnification was introduced while recording the video file of figure 3.4a. So, a change of magnification is observed in the video [34].

Figure 3.2: Nanofluidic device in a 2fFCS setup. *a.* Schematic top view of nanofluidic device together with an SEM image of real nanochannels (scale bar is 30 μm). *b.* Side view schematic of the complete experimental set up where electric field is applied through two reservoirs along the nanochannels using platinum electrodes. Two foci of 2fFCS were aligned with nanochannel using a 100×1.49 NA oil immersion objective lens. Two different linearly polarised pulsed interleaved lasers were used in the foci as excitations. The emission from flowing single molecules were detected with two APDs. *c.* A wide-field of image frame showing the presence of single molecule (scale bar is 8 μm).

not detectable, and quantitative analysis of flowing molecules (with low SNR) become difficult at fast exposure time using EMCCD.

Figure 3.3: Integrating electrodes with the nanofluidic device.

3.4.2 APD based detection

To restrict unwanted surface adsorption of single molecule, we used fluorescent molecules carrying the same charge as the nanochannels' wall. Pure silica is negatively charged above its isoelectric point ($\text{pH(I)} = 2$) [36, 37]. A buffer of pH 8.5 was used to increase the negative charges on the walls of silica nanochannels. The condition was suitable to flow negatively charged molecules

Figure 3.4: **EMCCD based detection under widefield optical microscope.** *a.* 1D flow captured at low magnification. Continuity of single molecules forming horizontal lines correspond to the individual nanochannels – video file [34]. *b.* Flow captured at high magnification. The red lines correspond to equally spaced nanochannels – video file [35].

Figure 3.5: **1D Flow of CNDs.** *a.* Schematic top-view of nanochannels along which a y-x scan was performed. *b.* Confocal scan image of nanochannels filled with CNDs. *c.* Schematic cross-sectional side view of nanochannels along which y-z scans were performed. The dashed blue arrow represent the optical excitation path – direction from immersion oil of the objective lens to the nanofluidic channels. *d.*, *e.*, and *f.* y-z scan images of nanochannels with an increasing order of CNDs' concentration flowing through nanochannels. *g.* High concentration of CND. All the horizontal scale bars denotes 2 μm . The vertical scale bar denotes photon counts as 0.0 (lowest) to 1.0 (highest).

without being adsorbed. We chose carbon-nanodots (CND) as the first probe of interest. They are <2 nm in size [19] and negatively charged (see appendix C). A confocal scanned image was recorded along the x - y plane when CNDs were flowing inside nanochannel. In the schematic top-view of the nanochannels in figure 3.5a – dark lines are the nanochannels. They were filled with CNDs (figure 3.5b). The pixel size (p_a) of the image is 320 nm with dwell time (δt) of 5 ms. To record this focused z plane in figure 3.5b, we first performed a y - z scanned image, and went to the position where the count-rates were maximum. The scheme of y - z piezo-scan is shown in figure 3.5c. This enables us to record the cross-sectional image of periodically arranged nanochannels. When single CNDs are flowing in unidirectionally, jittery (or fluctuating) fluorescent signals were recorded as observed in the periodic PSFs – figure 3.5d, e, and f. Figure 3.5d, e, and f are presented here in ascending order of concentrations of aqueous CND solutions. In figure 3.5d to g the volume percentages of CNDs in water relative to its stock solution are 1%, 2%, 5%, and 50%, respectively. Here, p_a is 100 nm and δt is 2 ms. The applied electric field to induce electro-osmotic flow during the measurement was 15 V/mm. As the concentration of CNDs increased the fluctuations of the photon signal decreased due to increasing rate of CNDs arrival in the focal volume (figure 3.5d - g).

3.5 Electro-osmotic flow of single molecules

3.5.1 2fFCS

Time correlated analysis of photon counts (2fFCS) was carried out to investigate flow velocities of single molecules. These measurements were carried out at the positions of y - z scanned PSFs where the photon counts were maximum – considering that is the position of the nanochannel where single molecules were flowing. Prior to performing every 2fFCS measurement a y - z scan was taken as shown in figure 3.6. To substantiate that our method is not restricted to a particular fluorescent probe, we performed 2fFCS of Atto 488 dye (see appendix D for a control measurement of diffusion) and 48 base-pair of DNA labelled with single Alexa-647 fluorophore (purchased from IBA GmbH, Goettingen, Germany). The electrolytic aqueous buffer solution used for the electro-osmotic flow is detailed in method section (page number 69). We measured the flow velocities of single DNA molecules inside 1D nanochannel within a range of applied electric field from 27 to 300 V/mm (see appendix F). This measurements were performed inside a nanochannel with d_{nc} of 30 nm. The correlated photon counts provide two auto-correlations for two foci, and one forward and one backward cross-correlation between them [24, 25]. A difference should be observed in two cross-correlations due to a unidirectional flow profile as shown earlier by Arbour and Enderlein [25]. The correlated data points from 2fFCS is fitted with the Fokker-Planck equation [38] considering the 1D electro-osmotic flow.

An exemplary correlated photon counts of 1D flow measurement performed at 220 V/mm is shown in figure 3.6a. Here, light-blue and red fitted curves are two cross-correlations, and other two

Figure 3.6: 1D Flow of 48bp DNA. **a.** 2fFCS measured correlation plots of photon counts from two APDs. Here, applied electric field was of 220 V/mm. The data was fitted with 1D Fokker-Planck equation. From the fitting, we found diffusion coefficient of $1.51 \times 10^{-7} \text{ cm}^2/\text{s}$. **b.** Plot of velocity versus applied voltage along the nanochannel. The data was fitted with a linear fit. **c.** The burst size distribution of single molecule transits was fitted with two Poissonian distributions. The total time trace was binned with 5 ms. The inset showing total time trace — the dashed bordered grey region is shown in **d.** **d.** A part of the time trace plots of photon bursts from single DNA molecules transiting through focal volume.

curves are autocorrelations. The velocity and diffusion coefficient from the curve fitting are $1.51 \times 10^{-7} \text{ cm}^2/\text{s}$ and -206.93 μm/s , respectively. The negative value of the flow velocity infers the direction of the flow, which can be altered by changing the applied polarity of the electric field. The linear relationship of electro-osmotic flows at different applied electric fields are plotted in figure 3.6b. The linear fit has an r^2 value of 0.992.

3.5.2 Photon burst size distribution

To confirm whether the photon used in 2fFCS were from aggregates of single molecules, photon burst sizes of molecular transits were analysed [39]. Burst size distribution (BSD) of the complete time trace for a flow measurement is shown in figure 3.6c. The complete time trace used in the BSD analysis is shown in the inset. We fit the BSD with Poisson distributions. The fitting used more than one Poissonian functions to fit the total BSD histogram. The first peak is due to the single molecule bursts, and the second is due to multiple-molecule-events as observed by Enderlein et al. [39]. Multiple-molecule-events occur when the concentration of flowing molecule is slightly high or in other words, two or more molecules transit through focus in close succession that the detectors cannot resolve temporally between two or more molecules. Enderlein et al. named this as molecular shot noise [39]. Figure 3.6d shows a magnified region of the inset 5 ms binned time-trace of figure 3.6c where 0 to 600 ms is highlighted with grey inside a dashed box. We clearly observe single molecule bursts in figure 3.6d.

3.6 Summary

In summary, this chapter presented an efficient approach of nanofabricating nanochannels and their usage in single molecule nanofluidics to suppress the thermal fluctuations. The fabrication process demonstrated here is simple and high-throughput for large scale production of nanofluidic chips. The process is not material restricted process, unlike the oxidation and bonding based techniques. Single fluorophore molecules of 0.5 - 1 nm, CNDs of 1-2 nm, and DNA of 11 nm were studied inside nanochannels. 1D flows of them inside nanochannel were achieved using electro-osmosis. Wide-field and confocal microscopy were used for qualitative analysis of the 1D flow. Using 2fFCS method the 1D flows of the mentioned species were quantitatively analysed. A broad range of velocities were observed by varying the applied electric field along the nanochannels. The 1D flow observed in 2fFCS data analysis was from transits of single molecules, to confirm that we performed BSD analysis. All the experiments were performed inside nanochannels of 30 nm × 35 nm in cross-section. This simple and high-throughput approach of fabricating nanochannels paves the way towards detecting early onset of any disease at single molecule level. In future, trapping nanoscale objects less than 2 nm for large residence time should be also feasible using these nanochannels. Biomolecular interactions such as dynamics of DNA, protein aggregation, and structural biology of molecules in physiological condition can be also studied at single molecule level using the SAEBD based nanofluidic devices.

Methods

Nanofabrication: A rigorous cleaning of the surface of interest is required to eliminate unwanted organic or dust-particle contaminations for nanofabrication. The silicon wafers were ultrasonicated with acetone prior to piranha cleaning at 120° C for 15 min. The piranha cleaned wafers were then rinsed with de-ionised water. The wafers were heated at 180° C for 10 min to make sure all the water molecules were evaporated. The cleaning protocol was carried out inside a class 100 cleanroom. Prior to spin coating the wafers with e-beam resist, an upright optical microscope with 100× objective lens was used to investigate the surface of the silicon. Fused silica wafers were cleaned with RCA1 reagents instead of piranha. The pristine wafers were spin-coated with the e-beam resist PMMA-A2 (polymethylene methacrylate from MicroChem Corp., Newton, United States) at 2000 rpm for 60 s. The spin-coated wafers were then baked at 180° C for 90 s. A uniform 100 nm thick PMMA film was produced on the wafers. EBL was carried out on the PMMA coated wafers using fixed-e-beam-moving-stage condition [40] The first experiment where silicon wafers was used – nanochannel dimensions were 2 mm long and 30 - 100 nm wide. The final fused silica based nanofluidic device contained 100 – 150 µm long nanochannels. To avoid overlapping periodic PSFs, the distance between every two nanochannels was 2 – 3 µm. The EBL exposed PMMA was monomerised and removed by immersing the wafers into MIBK:IPA (methyl isobutyl ketone dissolved in isopropanol purchased from MicroChem Corp., Newton, United States) developer at -20° C for 30 s. After 30 s, the wafers were immersed into isopropanol to stop over-development and nitrogen blow dried. Nanometre scale openings were formed revealing the silicon or silica where PMMA was removed with developer. The remaining undeveloped PMMA regions acted as a mask where no etching was performed for RIE. The reactive plasma was created at high vacuum. Detail parameteres of the plasma are detailed in appendix A. This process-flow is schematically described in figure 3.1a. After the RIE treatment, the left-over PMMA (mask-region) was removed with acetone, piranha, and oxygen RIE.

SAEBD: SAEBD was performed using a Leybold Univex 350 e-beam evaporation system purchased from Oerlikon Leybold Vacuum GmbH, Cologne, Germany. The vacuum pressure inside the chamber while evaporation was 1×10^{-6} mbar. The dicing system used to cross-section the silicon wafers was Disco Dad 320 purchased from Disco Corporation, Tokyo, Japan. The diamond blade used in the system was ZH05-SD2000-N1-70 (Disco Corporation, Tokyo, Japan).

FIB: FIB used here is Nova NanoLab 600 DualBeam purchased from FEI Company, Hillsboro, USA. Specifications of the system is detailed in appendix E. The protection layer of platinum was deposited in two separate growth rates. At the interface of Ti-2, we used 1 pA current for the deposition to achieve a smooth cross-sections. The top most surface (Pt-4) was grown at fast deposition rate because this surface is the first interacting surface with FIB dissipating majority of the damaging ionic bombardments. To avoid any damage at the region of interest, ion beam was

created with 1 pA current at system vacuum of 1.5×10^{-6} mbar.

2fFCS setup: We used the commercial setup of Microtime 200 (PicoQuant, Berlin, Germany) for fluorescence confocal scan imaging and performing 2fFCS. The details of the setup is included in the appendix F.

Buffer: Electro-osmotic flow was performed using 300 pM of 48 base-paired DNA tagged with Alexa647 was diluted with Tris Buffer and 30% Glycerol where the pH was 8.5.

Bibliography

- [1] Jan CT Eijkel, and Albert Van Den Berg. *Nanofluidics: what is it and what can we expect from it?* Microfluidics and Nanofluidics 1, no. 3 (2005): 249-267. [80](#)
- [2] Sumita Pennathur, and Juan G. Santiago. *Electrokinetic transport in nanochannels. 1. Theory.* Analytical Chemistry 77, no. 21 (2005): 6772-6781. [80](#)
- [3] Miao Wang, Nan Jing, Chin B. Su, Jun Kameoka, Chao-Kai Chou, Mien-Chie Hung, and Kuang-An Chang. *Electrospinning of silica nanochannels for single molecule detection.* Applied physics letters 88, no. 3 (2006): 033106. [80](#)
- [4] Prakash Goswami, and Suman Chakraborty. *Energy transfer through streaming effects in time-periodic pressure-driven nanochannel flows with interfacial slip.* Langmuir 26, no. 1 (2009): 581-590. [80](#)
- [5] Fredrik Persson, and Jonas O Tegenfeldt, *DNA in Nanochannels—Directly Visualizing Genomic Information.* Chemical Society Reviews 39 (2010): 985-999. [80](#)
- [6] Seung Kyu Min, Woo Youn Kim, Yeonchoo Cho, and Kwang S. Kim. *Fast DNA sequencing with a graphene-based nanochannel device.* Nature Nanotechnology 6, no. 3 (2011): 162-165. [80](#)
- [7] Bala Murali Venkatesan and Rashid Bashir. *Nanopore sensors for nucleic acid analysis.* Nature Nanotechnology 6, no. 10 (2011): 615-624. [80](#)
- [8] Chao Wang, Robert L. Bruce, Elizabeth A. Duch, Jyotica V. Patel, Joshua T. Smith, Yann Astier, Benjamin H. Wunsch et al. *Hydrodynamics of diamond-shaped gradient nanopillar arrays for effective DNA translocation into nanochannels.* ACS nano 9, no. 2 (2015): 1206-1218. [80](#)
- [9] Anirban Mitra, Bradley Deutsch, Filipp Ignatovich, Carrie Dykes, and Lukas Novotny. *Nano-optofluidic detection of single viruses and nanoparticles.* ACS nano 4, no. 3 (2010): 1305-1312. [80](#)

- [10] Mark N. Hamblin, Jie Xuan, Daniel Maynes, H. Dennis Tolley, David M. Belnap, Adam T. Woolley, Milton L. Lee, and Aaron R. Hawkins. *Selective trapping and concentration of nanoparticles and viruses in dual-height nanofluidic channels*. Lab on a Chip 10, no. 2 (2010): 173-178. [80](#)
- [11] Jean-Luc Fraikin,, Tambet Teesalu, Christopher M. McKenney, Erkki Ruoslahti, and Andrew N. Cleland. *A high-throughput label-free nanoparticle analyser*. Nature nanotechnology 6, no. 5 (2011): 308-313. [80](#)
- [12] Alfredo de la Escosura-Muniz, and Arben Merkoci. *A Nanochannel/Nanoparticle-Based Filtering and Sensing Platform for Direct Detection of a Cancer Biomarker in Blood*. Small 7, no. 5 (2011): 675-682. [80](#)
- [13] Adam E. Cohen *Trapping and manipulating single molecules in solution*. Diss. Stanford University, 2006.
- [14] Manoj Kumbakhar, Dirk Hähnel, Ingo Gregor, and Jörg Enderlein. *Feedback-controlled electro-kinetic traps for single-molecule spectroscopy*. Pramana 82, no. 1 (2014): 121-134.
- [15] Marco Braun, Andreas Paul Bregulla, Katrin Guenther, Michael Mertig, and Frank Cichos. *Single Molecules Trapped by Dynamic Inhomogeneous Temperature Fields*. Nano letters (2015).
- [16] Fredrik Persson, L H Thamdrup, M BL Mikkelsen, S E Jaarlgar, P Skafte-Pedersen, H Bruus, and A Kristensen, *Double thermal oxidation scheme for the fabrication of SiO₂ nanochannels*. Nanotechnology 18 (2007): 245301-245305. [80](#)
- [17] Daniel C S Bien, Hing Wah Lee and Rahimah Mohd Saman, *Fabrication of High aspect ratio silicon nanochannels arrays*. ECS Solid State Letters1 (2012): 45-47. [80](#)
- [18] So Hyun Kim, Yidan Cui, Min Jung Lee, Seong-Won Nam, Doori Oh, Seong Ho Kang, Youn Sang Kim and Sungsu Park, *Simple fabrication of hydrophilic nanochannels sing the chemical bonding between activatied ultrathin PDMS layer and cover glass by oxygen plasma*. Lab Chip 11 (2011): 348-353. [80](#)
- [19] Fredrik Westerlund, Fredrik Persson, Anders Kristensenb and Jonas O. Tegenfeldt, *Fluorescence enhancement of single DNA molecules confined in Si/SiO₂ nanochannels* Lab Chip 10 (2010): 2049. [80](#)
- [20] Pan Mao and Jongyoon Han, *Fabrication and characterization of 20 nm planar nanofluidic channels by glass-glass and glass-silicon bonding*. Lab Chip 5 (2005): 837-8. [80](#)

- [21] Siddharth Ghosh, Anna M. Chizhik, Narain Karedla, Mariia O. Dekaliuk, Ingo Gregor, Henning Schuhmann, Michael Seibt et al. *Photoluminescence of Carbon Nanodots: Dipole Emission Centers and Electron-Phonon Coupling*. Nano letters 14, no. 10 (2014): 5656-5661. [80](#), [88](#), [116](#), [117](#), [118](#), [121](#), [123](#), [144](#), [145](#)
- [22] Michael Brinkmeier, Klaus Dörre, Jens Stephan, and Manfred Eigen. *Two-beam cross-correlation: a method to characterize transport phenomena in micrometre-sized structures*. Analytical chemistry 71, no. 3 (1999): 609-616. [80](#)
- [23] Thomas Dertinger, Victor Pacheco, Iris von der Hocht, Rudolf Hartmann, Ingo Gregor, and Jörg Enderlein. *Two-focus fluorescence correlation spectroscopy: A new tool for accurate and absolute diffusion measurements*. ChemPhysChem 8, no. 3 (2007): 433-443. [80](#), [84](#)
- [24] Thomas Dertinger, Anastasia Loman, Benjamin Ewers, Claus B. Moeller, Benedikt Kraemer, and Jörg Enderlein. *The optics and performance of dual-focus fluorescence correlation spectroscopy*. Optics express 16, no. 19 (2008): 14353-14368. [80](#), [88](#)
- [25] Tyler J. Arbour, and Jörg Enderlein. *Application of dual-focus fluorescence correlation spectroscopy to microfluidic flow-velocity measurement*. Lab on a Chip 10, no. 10 (2010): 1286-1292. [29](#), [80](#), [88](#)
- [26] J. J. Merkel, T. Sontheimer, B. Rech and C. Becker. *Directional growth and crystallization of silicon thin films prepared by electron-beam evaporation on oblique and textured surfaces*. Journal of Crystal Growth 367 (2013): 126-130. [80](#)
- [27] Stefan Egger, Adelina Ilie, Shinichi Machida, and Tomonobu Nakayama. *Integration of individual nanoscale structures into devices using dynamic nanostenciling*. Nano letters 7, no. 11 (2007): 3399-3404. [80](#)
- [28] Mattox, Donald M. *Handbook of physical vapor deposition (PVD) processing*. William Andrew, 2010. [80](#)
- [29] Siddharth Ghosh, Manoj Kumbhakar, Mitja Platen, Ingo Gregor, and Jörg Enderlein. *Single-molecule fluorescence inside solid-state nanochannels*. In SPIE BiOS, pp. 895008-895008. International Society for Optics and Photonics, 2014. [81](#), [84](#)
- [30] Xiaohua Huang, Manuel J. Gordon, and Richard N. Zare. *Current-monitoring method for measuring the electroosmotic flow rate in capillary zone electrophoresis*. Analytical Chemistry 60, no. 17 (1988): 1837-1838. [83](#)
- [31] Neelesh A. Patankar, and Howard H. Hu. *Numerical simulation of electroosmotic flow*. Analytical Chemistry 70, no. 9 (1998): 1870-1881. [83](#)

- [32] Norman J. Dovichi, and Jianzhong Zhang. *How capillary electrophoresis sequenced the human genome*. Angewandte Chemie International Edition 39, no. 24 (2000): 4463-4468. 83
- [33] Suman Chakraborty, and Amit Kumar Srivastava. *Generalized model for time periodic electroosmotic flows with overlapping electrical double layers*. Langmuir 23, no. 24 (2007): 12421-12428. 83
- [34] **Video file 1** Shared through GWDG Powerfolder cloud service
URL:<https://powerfolder.gwdg.de/getlink/fiQ4s5es9aeixBYUnK994DQh/110g0%2C05sec4Best.gif> 84, 87
- [35] **Video file 2** Shared through GWDG Powerfolder cloud service
URL:<https://powerfolder.gwdg.de/getlink/fi8SWHh4rEsuTsv0GMJnagyn/100g0%2C1sec.gif> 84, 87
- [36] Kosmulski, Marek. *Chemical properties of material surfaces*. Vol. 102. CRC press, 2001. 86
- [37] Manish Keswani, Srini Raghavan, Pierre Deymier, and Steven Verhaverbeke. *Megasonic cleaning of wafers in electrolyte solutions: Possible role of electro-acoustic and cavitation effects*. Microelectronic Engineering 86, no. 2 (2009): 132-139. 86
- [38] Hannes Risken, *Fokker-Planck Equation*. Springer Berlin Heidelberg, 1984. 88
- [39] Jörg Enderlein, David L. Robbins, W. Patrick Ambrose, and Richard A. Keller. *Molecular shot noise, burst size distribution, and single-molecule detection in fluid flow: effects of multiple occupancy*. The Journal Of Physical Chemistry A 102, no. 30 (1998): 6089-6094. 90
- [40] M. Kirchner, and M. Kahl. *Raith-Electron Beam Lithography for Research*. Acta Physica Polonica A 116, no. s (2009): s198-s200. 91
- [41] Michael Wahl, Hans-Juergen Rahn, Tino Roehlicke, Gerald Kell, Daniel Nettels, Frank Hillger, Ben Schuler, and Rainer Erdmann. *Scalable time-correlated photon counting system with multiple independent input channels*. Review of Scientific Instruments 79, no. 12 (2008): 123113. 141

Chapter 4

Carbon nanodots - single dipole emitters

In this chapter, I will introduce a class of carbon-based nanostructures with single-photon emission behaviour. Although conventional quantum dots have high photostability and quantum yield, frequently their usage is restricted due to high toxicity. This motivated us to study the newly discovered carbon nanodots towards their fluorescence associated properties to enable their application in biophysical single-molecule research. Here, I have investigated the origin of their photoluminescence by developing a single-nanoparticle fluorescence correlated electron microscopy technique. The size distribution of the studied nanoparticles is found to be from 2 nm to 20 nm. Using the defocused optical imaging and a stepwise photobleaching they were found to be single-photon emitters. The optical absorption and emission of them evidenced the presence of single electric dipoles in each single nanoparticles. The photoluminescence spectra of them showed a strong electron-phonon coupling proving their emission from crystalline nanostruc-

tures. One of the predictions from this investigation was the carbon nanodots are fluorescent due to localised defects in forms of functional groups.

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4.1 Introduction

Single photon emitters are useful for detection of biomolecules, super-resolution microscopy, and quantum optical information processing. In this chapter, we demonstrate properties of a novel fluorescent probe – carbon nanodots (CNDs) which were used in single molecule nanofluidics (chapter 3) as well as in super-resolution microscopy [1]. Among other carbon-based nanomaterials (such as nanodiamonds, fullerenes, graphene, carbon nanotubes [2]) CNDs have unique photophysical behaviour. CNDs were first found while purifying single-walled carbon nanotubes by Scrivens and co-workers in 2004 [3]. Since then CNDs are a material of scientific interest due to their bright photoluminescence (PL), high aqueous solubility, easy functionalisation, low toxicity, high photostability, and chemical inertness [4, 5, 6, 7]. Recent demonstrations of one-step synthesis of CNDs [8, 9, 10] introduce their use in biological imaging [11], drug delivery [12], biomolecule sensing [13], photovoltaic devices [14], lasing [15], or catalysis [16]. CNDs are already used in flexible optoelectronic devices, solid-state lighting, color displays, and luminescent solar concentrators [17].

The scientific question which motivated us to study CND was – what is the photophysical behaviour of individual CNDs? At single CND level the fundamental photophysical and spectroscopic properties are not clear. Ensemble optical spectroscopic studies showed that PL spectrum of CNDs is dependent on excitation wavelength with suggestions of multi-chromophoric sites [5]. The possible origins of the multiple emissive centres in CNDs can be from single CNDs or from aggregates of many CNDs which remained unanswered. The emission properties of CNDs are

shown to be dependent on surface passivation and pH [19]. PL studies of single CND revealed moderate blinking characteristics [20] to complete dark states [21]. Combining all the reports the PL origin in CNDs still remain inconclusive.

In this chapter, we demonstrate some of the fundamental PL behaviours of individual CNDs by correlating with its structural properties. We combined single-nanoparticle optical spectroscopic methods with high-resolution transmission electron microscopy (HRTEM) to correlate optical and structural properties of same CNDs. The CNDs presented here are single dipole emitter with a strong electron phonon coupling behaviour in their emission spectra. We performed defocused optical imaging and azimuthally polarised scanning confocal fluorescence microscopy to confirm the presence single transition dipoles.

4.2 PL correlated HRTEM

Structural information at atomic resolution is unavailable in any standalone fluorescence microscopy. For fundamental understanding of light-matter interaction in nanostructures as well as to synthesise fluorescent probes for super-resolution microscopy structural information of single dipole emitters are necessary to be known. A scanning confocal fluorescence microscopy (SCFM) technique is correlated with an HRTEM. Here, spatial correlation of photon count map and electron density map reveals underlying structures of single dipole emitters at atomic resolution. The motivation behind this technique was to investigate the atomic structure of hetero-dispersed carbon nanodots (CND) to understand their behaviour as single photon sources. Here, position markers of standard TEM grids were utilised to locate CNDs of interest under SCFM and HRTEM. To avoid electron beam induced radiation damage, SCFM was performed prior to studying the CNDs under HRTEM. The photon emitting regions obtained from SCFM were overlapped and correlated with electron density map of HRTEM. The correlative investigation en-

Figure 4.1: Process steps of SCFM correlated HRTEM (a) 10 μ l of CND solution dispersed on TEM grid. (b) Scanning of a confocal laser beam on CND coated TEM grids. (c) PL scan image of CNDs with spatial coordinates. (d) HRTEM at the SCFM obtained spatial coordinates. (e) HRTEM images of single dipole emitters.

ables us to extract the atomic parameters of CNDs.

4.2.1 Process steps

In figure 4.1 the process steps of the SCFM correlated HRTEM is schematically described. Here, a drop of CND solutions are dispersed on standard TEM grids (figure 4.1a). The black lines represent copper support of the suspended amorphous carbon thin-film (gray regions). The droplet was dried overnight in a clean-room environment. The grids were placed on a silica coverslip and spatially scanned with a focused laser beam of SCFM [4]. The relative scanning was performed using an xy piezoelectric stage (figure 4.1(b)). The photoluminescence map of the grid was recorded using single photon avalanche photodiodes. The spatial coordinates of the scanned grid was noted - marked in figure 4.1(b) with a red box. Figure 4.1(c) shows a single excitation dipole patterns of the CNDs (the dipole pattern are simulated here). The xy coordinates of the dipole was recorded using a piezoelectric scanner. The same grid was imaged under HRTEM as shown in figure 4.1(d). The transmitted electron density map of the grid (figure 4.1(e) - here simulated HRTEM image of CNDs) was spatially correlated with SCFM image (figure 4.1(c)). The electron density map and photoluminescent map overlap with excellent agreement. The technique reveals the underlying nanostructure of single dipole patterns. In other words, the technique enable us to identify the florescence active CNDs.

4.2.2 Optically active CNDs

The particles were dispersed on the surface of a standard carbon/copper TEM grid (figure 4.2a). For the PL measurements, the grid was placed on the surface of a clean glass cover slide to avoid contact of the grid with the immersion oil of a high numerical aperture objective lens. An exemplary PL image obtained by confocal scanning of one of the grid sections is shown in Figure 4.2b. Then, HRTEM measurements were performed in areas where the PL image exhibited bright spots, corresponding to individual luminescent CNDs.

Figure 4.2 shows HRTEM images of the observed nanostructures. They can be subdivided into the two types of particles. The first type of particles, shown in figure 4.2(c)-(f), possesses a lattice spacing of around 0.35 nm to 0.36 nm, which agrees well with the $\langle 002 \rangle$ plane of graphitic carbon [7]. These will be referred as type I CND. The observed nanostructures have different diameters varying from near 20 nm to 7 nm, different aspect ratios from near 1.5 (figure 4.2(c), (d)) to 1 (figure 4.2(e), (f)), and characteristic onion-like shape structure [22]. Their localisation in the PL image (figure 4.2(b)) corresponds to the bright spots in area (1), (2), and (3), respectively. The second type of CNDs, observed in the HRTEM images is characterised by fully crystalline particles, with 2 nm to 5 nm in diameter (figure 4.2(g),(h)). The lattice spacing of nearly 0.24 nm

Figure 4.2: (a) Scanning electron microscopy image of a the carbon/copper grid, used for PL-correlated TEM imaging. (b) PL confocal scanning image recorded in one of the grid sections. (c)-(h) HRTEM images of individual CNDs possessing various crystal structures and shapes. Localisation of the CNDs on the PL image: (c) and (d) two bright spots in area 1; (e) and (f) bright spots in areas 2 and 3, respectively; (g) and (h) two bright spots in area 4. The white bars at the right bottom corners of (c)-(h) are 5 nm scale bars.

Figure 4.3: (a) and (d): TEM images of single CNDs crystal structure, shown in figures 1(c) and (g) of the article, respectively. (b) and (e): Fourier transform of the areas shown in figure (a) and (d), respectively. (c) and (f): cross sections through the images (a) and (d), respectively, along the white lines. FFT and cross sections through TEM images show the lattice constant values of 0.35 and 0.25 nm for type I and type II CNDs, respectively.

corresponds to the $\langle 100 \rangle$ crystal plane of graphite [7]. These will be referred as type II CNDs. All the observed CNDs are composed of multiple layers of graphene, as determined by recording electron diffraction patterns. Similar carbonic nanostructures have been observed in other studies using alternative top-down or bottom-up synthetic methods (see [6, 7] and citations therein).

The lattice constants of single CNDs were determined with the electron diffractograms and verified with the atomic plane profile. Figure 4.3 shows FFT and atomic plane profile of individual CNDs. Figure 4.3(a) exhibits a part of the CND TEM image, shown in figure 4.2 (c) (type I CND). The diffractogram (figure 4.3(b)) and atomic plane profile (figure 4.3(c)) agree with the lattice constant of 0.35 nm at all the positions within the particle. For the structures, shown in figure 4.2(d), (e) and (f), the lattice constant values varied from 0.35 nm to 0.36 nm. Figure 4.3(d) corresponds to the particle, shown in figure 4.2(g). The FFT and the atomic plane profile of the particle are shown in figure 4.3(e) and (f), respectively. For type II CNDs the lattice constant was found to be 0.24 nm - 0.25 nm.

The chemical analysis of CNDs was carried out with energy dispersive X-ray spectroscopy (EDX). Figure 4.4(a) and (b) show the EDX spectra within the whole measurable energy range and the area of interest for better signal to noise ratio, respectively. Besides signal from copper, which arises

Figure 4.4: Energy dispersive x-ray spectroscopy of amorphous shells of single CNDs.

from the TEM grid, EDX shows the presence of carbon and oxygen atoms. Note that the presence of hydrogen atoms cannot be resolved with the EDX spectroscopy. CNDs are mainly composed of carbon atoms with some oxygen atoms. Presence of oxygen suggests -COOH and -OH groups at the edges, which have been frequently observed in previous studies [23].

4.3 Transition dipole moment

One of the key parameters of any single quantum emitter is its transition dipole moment (TDM). It has been shown that depending on the origin of PL in semiconductor nanocrystals [24, 25, 26, 27] and dielectric nanoparticles [28], the dimensionality of emission and excitation TDMs changes. For instance, both the excitation and emission TDMs of spherical silicon nanocrystals coated with a SiO₂ amorphous shell are distributed in one- or three- dimensions, depending on whether their PL originates from surface defects or from a quantum-confined electron-hole pair, respectively [27].

A straightforward way for measuring the orientation and dimensionality of the excitation TDM is scanning an emitter through the focal region of an azimuthally polarised laser beam (APLB) [26, 29]. Figure 4.5(a) shows excitation patterns of individual CNDs dispersed on the surface of a glass cover slide. All of the recorded patterns consist of two nearby bright spots of elliptical shape, which corresponds to a fixed linear excitation TDM. The projection of the TDM on the substrate surface is oriented along the dark gap in the middle of the double-lobe pattern, as shown in the inset of figure 4.5(a). Out-of-plane orientations of the dipole moment lead to a weaker intensity of the double-lobe pattern, while dipole moments oriented exactly perpendicular to the surface cannot be observed at all.

Figure 4.5: (a) Single CNDs excited with an azimuthally polarised laser beam. Inset shows a theoretically calculated excitation pattern for identical experimental conditions assuming a linear horizontal dipole. The orientation of the dipole is indicated by the double arrow. (b)-(g) Consecutive images of the same sample area recorded by using an azimuthally polarised laser beam (scanning direction up-down). Each double-lobe pattern corresponds to the same CND. The images show PL intermittency (d) and single-step photobleaching (f) of the particle. Scanning direction is top-down. (h)-(q) Defocused images of single CNDs: experimental data (h)-(l) and fitted patterns (m)-(q), respectively. All the patterns correspond to the emission of a single fixed dipole.

Figure 4.5(b)-(g) shows a series of successive single CND excitation patterns. Acquisition time for one image was 6 seconds. A small asymmetry of the double-lobe pattern is caused by a slight

distortion of the excitation laser beam. Fixed orientation of the pattern indicates that the TDM did not undergo reorientation during the acquisition time. Since excitation patterns are obtained by point scanning, a dark line in figure 4.5(d) indicates PL intermittency. A single step transition from an on-state to background in PL intensity as shown in figure 4.5(f) and (e) corresponds to single step bleaching of CND PL. This is yet another proof of the single quantum emitter nature of CND PL.

Whereas imaging with an APLB provides information about the excitation TDM, the orientation and dimensionality of the emission TDM can be determined using defocused imaging [30, 31]. By moving the objective lens towards the sample and imaging the PL of single CNDs with a CCD camera, one observes a defocused image of a single CND's PL, which provides information about the angular distribution of the emission of the imaged particle. Figure 4.5(h)-(l) shows five exemplary PL patterns of individual CNDs recorded with defocused imaging. The defocusing values (displacement of objective towards sample) were 280 nm for figure 4.5(h) and 300 nm for (i)-(l). Experimental images can be very well fitted with a model which assumes that the emission originates from a single fixed dipole, as shown in figure 4.5(m)-(q) [31].

We measured PL lifetime values of 474 individual CNDs dispersed on the surface of a glass cover slide. The relatively broad distribution covers a range from 0.8 ns to 6 ns with a maximum close to 3 ns.

4.4 Single CND PL spectroscopy

Using a home-built confocal microscope, we measured PL spectra of single CNDs dispersed on the surface of a glass cover slide. Figure 4.6(a) shows spectra of different CNDs (spectra (2)-(5)) as well as an ensemble spectrum, recorded from particles suspended in aqueous solution (spectrum 1). The inset shows a photograph of a vial with CNDs in water illuminated by ambient light (left) and upon excitation with a 488 excitation laser (right). The right picture was taken through a 500 nm long pass filter to block the scattered laser radiation. The bulk emission covers a broad spectral range centred at 550 nm (near 2.27 eV).

Individual CND spectra are much narrower than the bulk emission spectrum, and show random spectral shifts from 500 to 650 nm (near 2.5 eV to 1.9 eV) in agreement with the width of the ensemble PL spectrum. All single particle spectra are broadened towards the low-energy side, exhibiting there a second less-intense band. To calculate the energy splitting of the two bands, we fitted the single CNDs spectra by two Gaussian functions. The fit results are plotted in figure 4.6(a) (red curves). Solid circles in figure 4.6(b) show the separation between the two spectral lines vs. the maximum of the high-energy line. The splitting varies from 70 to 150 meV and exhibits no clear dependence on the PL energy maximum.

Figure 4.6: (a) Bulk PL spectrum of CNDs in water (spectrum 1) and exemplary PL spectra of single CNDs dispersed on the surface of a glass cover slide (spectra 2-5). Before plotting, the experimental data has been subjected to averaging over five data points. Inset shows two photographs of a cuvette containing CNDs in an aqueous solution under ambient light excitation (left) and upon excitation with a 488 nm laser (right). The left photograph is taken through a 500 nm long-pass filter to block scattered laser light. (b) Splitting between zero-phonon and phonon-assisted bands for all PL spectra measured in this study as a function of zero-phonon line energy (solid circles). Dashed and dotted lines represent the result of the phonon energy calculations for the crystal structures I and II, respectively.

4.4.1 Electron-phonon coupling

From the knowledge of the crystal structures of single CNDs, obtained by a Fourier transform of the HRTEM data, we calculated all possible optical phonon modes using a density-function tight-binding method [32]. Within the experimentally observed 70 meV to 150 meV energy range, two values for optical phonon modes were found: 98 and 112 meV, corresponding to crystal structures I and II, respectively. The obtained values are shown in figure 4.6(b) by dashed and dotted lines, respectively. It should be emphasised that the other theoretically obtained phonon energy values sufficiently differ and show no overlap with the experimental data. The moderate dispersion of the measured values can be attributed to the high curvature of the crystal structure, especially in the case of onion-shaped particles. Hence, we assign the lower energy satellite peak to the excitation of optical phonons, coupled to the radiative recombination of the charge carriers. Interactions between electrons and lattice vibrations result in a bound state, which leads to a decrease of energy as compared to a non-interacting system. This manifests itself in the lowering of the energy of emitted photons [33]. As the CNDs were not embedded into a polymer matrix during PL measurements, the emission energy as well as the phonon energy are solely related to the structure of the particles and are not affected by any local chemical environment. A strong coupling between electronic transitions and phonons due to a relaxation mechanism involving charges has been also observed in other types of nanostructures, for example, SiO₂ nanoparticles [28], Si [34], or CdSe [35] nanocrystals.

Recent studies have shown that the shape of the bulk PL spectrum from CNDs is strongly dependent on the excitation wavelength [5]. To find out whether tuning of the excitation wavelength changes the shape of a single particle PL spectrum, we measured single CNDs emission spectra using different excitation wavelengths. Figure 4.7 shows the normalized PL spectra recorded from five individual CNDs using 467 nm (red curve) and 488 nm (black curve) excitation laser

Figure 4.7: Normalised PL spectra of single CNDs dispersed on the surface of a glass cover slide excited with 467 nm (red curve) and 488 nm (black curve) laser beam. Before plotting, the experimental data has been subjected to averaging over five data points.

beams. For all the measured CNDs, the shape of the emission spectra did not exhibit any noticeable change. Table 4.1 shows the values of the main emission band maxima, which were obtained as a result of fitting of the spectra with two Gaussian functions. The difference between the maxima of the spectra recorded from the same CND at different excitation wavelengths does not exceed 1%, which can be attributed to the error of fitting. This finding suggests that the wavelength-dependent modification of the ensemble PL spectrum of CNDs is caused by excitation of different subsets of CNDs due to sample heterogeneity.

Table 4.1: Values of the main emission band maxima of the spectra, shown in figure 4.1 of the article. The values were obtained as a result of fitting of the spectra with two Gaussian functions.

λ_{exc} (nm)	1 (eV)	2 (eV)	3 (eV)	4 (eV)	5 (eV)
467	2.37	2.31	2.27	2.24	2.21
488	2.37	2.31	2.27	2.23	2.21

The wavelength-dependent modulation of the CNDs' ensemble PL, along with the large difference between the absorption and excitation spectra makes it difficult to use standard methods for measuring the quantum yield (QY) of CNDs. However, our recently developed nanocavity-based method of QY determination measures only the cavity-modulated radiative decay rate of an emitter [36], which makes it also applicable for such complex systems as CNDs [37, 38]. Placing CNDs between the metal mirrors of a nanocavity changes their emission behavior due to a cavity-modified electromagnetic field mode density [39, 40]. Since the cavity changes only the radiative rate of the embedded emitters, measuring the modulation of the PL lifetime as a function of the cavity length allows for determining an absolute value of an emitters QY [36]. Moreover, as the rotational diffusion of the emission dipoles modifies the coupling of the emitters to the cavity modes [41], measuring the cavity-induced lifetime modification allows us also to estimate the rotational diffusion time of the CNDs. Beside HRTEM, rotational diffusion time value gives the size of the CNDs via the Stokes-Einstein-Debye relation [42]. The measured rotational diffusion time of 16 ns corresponds to spherical particles of nearly 5 nm diameter, which is in good agreement with the average CND size obtained from the HRTEM studies.

The observed properties of CND PL, i.e. fixed linear emission and excitation TDMs, broad distribution of PL lifetimes, and random shifts of the emission spectra, resemble the PL properties of SiO₂ nanoparticles, where the emission originates from defects in the SiO₂ structure [28, 41]. The fact that the PL of CNDs with different crystal structures does not show pronounced differences is a strong indication that the emission originates from a charge recombination on defect centers on the surface of a CND, rather than originating from its core. The homogenous dispersion of

PL spectral emission maxima and lifetime values suggests that all emission originates from the same type of defects, while the dispersion is induced by random fluctuations in the local structural environment.

The spectral dispersion of a single CNDs' PL and the linearity of their emission and excitation TDMs show that multiple emission centres, observed in previous studies (see [5] and citations therein), result from different particles, while each CND contains only one single optically active emission center. It should be emphasised that the relatively high structural heterogeneity of the studied CNDs does not lead to a similarly heterogeneous behaviour in their PL.

4.5 Summary

In summary, we have presented a comprehensive PL, HRTEM, and AFM study of single CNDs, which allowed us to relate their fundamental optical and structural properties. We showed that the PL of CNDs originates from a charge recombination on surface emission centres, involving a strong coupling between the electronic transition and collective vibrational modes of the lattice structure. Whereas the PL properties of individual CNDs resemble those of typical dye molecules, the temporal and spectral dispersion of their PL reflects the complex nature of the local chemical environment around each PL center. Finally, we showed that CNDs, which can be synthesised without the need for sophisticated and expensive equipment, possess a high PL QY and photostability, which makes them ideal candidates for luminescent centres in bio-imaging.

Methods

Synthesis of CNDs: The CND synthesis started by mixing, in flat-bottomed flask, 4 g sucrose with 0.5 g PEG-150, which was then diluted in 26 ml distilled water. Subsequently, the solution was treated in a microwave oven (600 W, 4 min) to form a black precipitate. The final product was collected by adding distilled water to the flask which was then centrifuged at 5000 rpm for 5 min. The solid precipitate was re-dispersed into water. Thereafter, the supernatant was selected for further work. To obtain a maximally homogeneous fraction of particles, a size separation by column chromatography using Sephadex-200 was performed.

PL measurements: Photoluminescence measurements were performed with a home-built confocal microscope equipped with an objective lens of high numerical aperture (Apo N, 60×/1.49 NA oil immersion, Olympus). A pulsed white light laser system (Fianium SC400-4-20, pulse width 50 ps, repetition rate 20 MHz) with a tunable filter (AOTFnc- 400.650-TN) served as the excitation source at 488 nm. The light was reflected by a non-polarising beam-splitter towards the objective, and backscattered excitation light was blocked with a long pass filter (Semrock EdgeBasic BLP01-

488R). Collected photoluminescence was focused onto the active area of an avalanche photo diode (PicoQuant τ -SPAD). Data acquisition was accomplished with a multichannel picosecond event timer (PicoQuant HydraHarp 400). PL spectra of single CNDs were recorded using a spectrograph (Andor SR 303i) and a CCD camera (Andor iXon DU897 BV).

HRTEM measurements: For the HRTEM measurements we used FEI Titan 80-300 ETEM G2 aberration corrected microscope. The accelerating voltage was 80 kV.

Phonon modes: The details of the calculation is shown in the theory chapter (section Phonon modes).

Full list of the calculated optical phonon energies Phonon energy (meV):

Type I CND: 3, 20, 98, 218.

Type II CND: 112, 113, 388.

Bibliography

- [1] Anna M. Chizhik, Simon Stein, Mariia O. Dekaliuk, Christopher Battle, Weixing Li, Anja Huss, Mitja Platen et al. *Super-Resolution Optical Fluctuation Bio-Imaging with Dual-Color Carbon Nanodots*. Nano letters 16, (2016): 237–242. [98](#)
- [2] Deep Jariwala, Vinod K. Sangwan, Lincoln J. Lauhon, Tobin J. Marks, and Mark C. Hersam. *Carbon nanomaterials for electronics, optoelectronics, photovoltaics, and sensing*. Chemical Society Reviews 42, no. 7 (2013): 2824-2860. [98](#)
- [3] Xiaoyou Xu, Robert Ray, Yunlong Gu, Harry J. Ploehn, Latha Gearheart, Kyle Raker, and Walter A. Scrivens. *Electrophoretic analysis and purification of fluorescent single-walled carbon nanotube fragments*. Journal of the American Chemical Society 126, no. 40 (2004): 12736-12737. [98](#)
- [4] Jariwala, Deep, Vinod K. Sangwan, Lincoln J. Lauhon, Tobin J. Marks, and Mark C. Hersam. *Carbon nanomaterials for electronics, optoelectronics, photovoltaics, and sensing*. Chemical Society Reviews 42, no. 7 (2013): 2824-2860. [98](#)
- [5] Demchenko, Alexander P., and Mariia O. Dekaliuk. *Novel fluorescent carbonic nanomaterials for sensing and imaging*. Methods and Applications in Fluorescence 1, no. 4 (2013): 042001. [98](#), [107](#), [109](#)
- [6] Haitao Li, Zhenhui Kang, Yang Liu, and Shuit-Tong Lee. *Carbon nanodots: synthesis, properties and applications*. Journal of materials chemistry 22, no. 46 (2012): 24230-24253. [98](#), [102](#)

- [7] Sheila N. Baker and Gary A. Baker. *Luminescent carbon nanodots: emergent nanolights*. *Angewandte Chemie International Edition* 49, no. 38 (2010): 6726-6744. [98](#), [100](#), [102](#)
- [8] Jie Jiang, Yi He, Shiyuan Li, and Hua Cui. *Amino acids as the source for producing carbon nanodots: microwave assisted one-step synthesis, intrinsic photoluminescence property and intense chemiluminescence enhancement*. *Chemical communications* 48, no. 77 (2012): 9634-9636. [98](#)
- [9] Changjun Liu, Peng Zhang, Feng Tian, Wenchen Li, Fan Li, and Wenguang Liu. *One-step synthesis of surface passivated carbon nanodots by microwave assisted pyrolysis for enhanced multicolor photoluminescence and bioimaging*. *J. Mater. Chem.* 21, no. 35 (2011): 13163-13167. [98](#)
- [10] Pin-Che Hsu and Huan-Tsung Chang. *Synthesis of high-quality carbon nanodots from hydrophilic compounds: role of functional groups*. *Chemical communications* 48, no. 33 (2012): 3984-3986. [98](#)
- [11] Songnan Qu, Xiaoyun Wang, Qipeng Lu, Xingyuan Liu, and Lijun Wang. *A Biocompatible Fluorescent Ink Based on Water-Soluble Luminescent Carbon Nanodots*. *Angewandte Chemie* 124, no. 49 (2012): 12381-12384. [98](#)
- [12] Jing Tang, Biao Kong, Hao Wu, Ming Xu, Yongcheng Wang, Yanli Wang, Dongyuan Zhao, and Gengfeng Zheng. *Carbon nanodots featuring efficient FRET for real-time monitoring of drug delivery and two-photon imaging*. *Advanced Materials* 25, no. 45 (2013): 6569-6574. [98](#)
- [13] Changmin Yu, Xizhen Li, Fang Zeng, Fangyuan Zheng, and Shuizhu Wu. *Carbon-dot-based ratiometric fluorescent sensor for detecting hydrogen sulfide in aqueous media and inside live cells*. *Chemical Communications* 49, no. 4 (2013): 403-405. [98](#)
- [14] Xin Wang, Li Cao, Fushen Lu, Mohammed J. Meziani, Heting Li, Gang Qi, Bing Zhou, Barbara A. Harruff, Fabien Kermarrec, and Ya-Ping Sun. *Photoinduced electron transfers with carbon dots*. *Chemical Communications* 25 (2009): 3774-3776. [98](#)
- [15] W. F. Zhang, H. Zhu, S. F. Yu, and H. Y. Yang. *Observation of lasing emission from carbon nanodots in organic solvents*. *Advanced Materials* 24, no. 17 (2012): 2263-2267. [98](#)
- [16] Haitao Li, Xiaodie He, Zhenhui Kang, Hui Huang, Yang Liu, Jinglin Liu, Suoyuan Lian, Chi Him A. Tsang, Xiaobao Yang, and Shuit-Tong Lee. *Water-soluble fluorescent carbon quantum dots and photocatalyst design*. *Angewandte Chemie International Edition* 49, no. 26 (2010): 4430-4434. [98](#)

- [17] Yu Wang, Sergii Kalytchuk, Yu Zhang, Hengchong Shi, Stephen V. Kershaw, and Andrey L. Rogach. *Thickness-dependent full-color emission tunability in a flexible carbon dot ionogel*. The journal of physical chemistry letters 5, no. 8 (2014): 1412-1420. [98](#)
- [18] Marta J. Krysmann, Antonios Kelarakis, Panagiotis Dallas, and Emmanuel P. Giannelis. *Formation mechanism of carbogenic nanoparticles with dual photoluminescence emission*. Journal of the American Chemical Society 134, no. 2 (2011): 747-750. [99](#)
- [19] Ya-Ping Sun, Bing Zhou, Yi Lin, Wei Wang, KA Shiral Fernando, Pankaj Pathak, Mohammed Jaouad Meziani et al. *Quantum-sized carbon dots for bright and colorful photoluminescence*. Journal of the American Chemical Society 128, no. 24 (2006): 7756-7757. [99](#)
- [20] Somes K. Das, Yiyang Liu, Sinhea Yeom, Doo Young Kim, and Christopher I. Richards. *Single-particle fluorescence intensity fluctuations of carbon nanodots*. Nano letters 14, no. 2 (2014): 620-625. [99](#)
- [21] Ya-Ping Sun, Bing Zhou, Yi Lin, Wei Wang, KA Shiral Fernando, Pankaj Pathak, Mohammed Jaouad Meziani et al. *Quantum-sized carbon dots for bright and colorful photoluminescence*. Journal of the American Chemical Society 128, no. 24 (2006): 7756-7757. [99](#)
- [22] David Pech, Magali Brunet, Hugo Durou, Peihua Huang, Vadym Mochalin, Yury Gogotsi, Pierre-Louis Taberna, and Patrice Simon. *Ultrahigh-power micrometre-sized supercapacitors based on onion-like carbon*. Nature nanotechnology 5, no. 9 (2010): 651-654. [100](#)
- [23] Lei Wang, Shou-Jun Zhu, Hai-Yu Wang, Song-Nan Qu, Yong-Lai Zhang, Jun-Hu Zhang, Qi-Dai Chen et al. *Common origin of green luminescence in carbon nanodots and graphene quantum dots*. ACS nano 8, no. 3 (2014): 2541-2547. [103](#)
- [24] S. A. Empedocles, R. Neuhauser, and M. G. Bawendi. *Three-dimensional orientation measurements of symmetric single chromophores using polarization microscopy*. Nature 399, no. 6732 (1999): 126-130. [103](#)
- [25] Lei Wang, Shou-Jun Zhu, Hai-Yu Wang, Song-Nan Qu, Yong-Lai Zhang, Jun-Hu Zhang, Qi-Dai Chen et al. *Common origin of green luminescence in carbon nanodots and graphene quantum dots*. ACS nano 8, no. 3 (2014): 2541-2547. [103](#)
- [26] Alexey I. Chizhik, Anna M. Chizhik, Dmitry Khoptyar, Sebastian Baer, and Alfred J. Meixner. *Excitation isotropy of single CdSe/ZnS nanocrystals*. Nano letters 11, no. 3 (2011): 1131-1135. [103](#)
- [27] Torsten Schmidt, Alexey I. Chizhik, Anna M. Chizhik, Karsten Potrick, Alfred J. Meixner, and Friedrich Huisken. *Radiative exciton recombination and defect luminescence observed in single silicon nanocrystals*. Physical Review B 86, no. 12 (2012): 125302. [103](#)

- [28] Anna M. Chizhik, Alexey I. Chizhik, Raphael Gutbrod, Alfred Johann Meixner, Torsten Schmidt, Jana Sommerfeld, and Friedrich Huisken. *Imaging and Spectroscopy of Defect Luminescence and Electron-Phonon Coupling in Single SiO₂ Nanoparticles*. Nano letters 9, no. 9 (2009): 3239-3244. [103](#), [107](#), [108](#)
- [29] Alexey I. Chizhik, Anna M. Chizhik, Anja Huss, Regina Jäger, and Alfred J. Meixner. Nanoscale Probing of Dielectric Interfaces with Single-Molecule Excitation Patterns and Radially Polarized Illumination. The Journal of Physical Chemistry Letters 2, no. 17 (2011): 2152-2157. [103](#)
- [30] Jan Jasny, and Jerzy Sepiol. *Single molecules observed by immersion mirror objective. A novel method of finding the orientation of a radiating dipole*. Chemical physics letters 273, no. 5 (1997): 439-443. [105](#)
- [31] Digambara Patra, Ingo Gregor, and Jörg Enderlein. *Image analysis of defocused single-molecule images for three-dimensional molecule orientation studies*. The Journal of Physical Chemistry A 108, no. 33 (2004): 6836-6841.
- [32] Marcus Elstner, Dirk Porezag, G. Jungnickel, J. Elsner, M. Haugk, Th Frauenheim, Sandor Suhai, and Gotthard Seifert. *Self-consistent-charge density-functional tight-binding method for simulations of complex materials properties*. Physical Review B 58, no. 11 (1998): 7260. [105](#)
[107](#)
- [33] Alexey Chizhik, Frank Schleifenbaum, Raphael Gutbrod, Anna Chizhik, Dmitry Khoptyar, Alfred J. Meixner, and Jörg Enderlein. *Tuning the fluorescence emission spectra of a single molecule with a variable optical subwavelength metal microcavity*. Physical review letters 102, no. 7 (2009): 073002. [107](#)
- [34] D. Kovalev, H. Heckler, M. Ben-Chorin, G. Polisski, M. Schwartzkopff, and F. Koch. *Break-down of the k-conservation rule in Si nanocrystals*. Physical review letters 81, no. 13 (1998): 2803. [107](#)
- [35] Stephen A. Empedocles, Robert Neuhauser, Kentaro Shimizu, and Mouni G. Bawendi. *Photoluminescence from single semiconductor nanostructures*. Advanced Materials 11, no. 15 (1999): 1243-1256. [107](#)
- [36] Alexey I. Chizhik, Ingo Gregor, Benedikt Ernst, and Jörg Enderlein. *Nanocavity-Based Determination of Absolute Values of Photoluminescence Quantum Yields*. ChemPhysChem 14, no. 3 (2013): 505-513. [108](#)
- [37] Alexey I. Chizhik, Ingo Gregor, and Jörg Enderlein. *Quantum yield measurement in a multicolor chromophore solution using a nanocavity*. Nano letters 13, no. 3 (2013): 1348-1351. [108](#)

- [38] Narain Karedla, Jörg Enderlein, Ingo Gregor, and Alexey I. Chizhik. *Absolute photoluminescence quantum yield measurement in a complex nanoscopic system with multiple overlapping states*. The journal of physical chemistry letters 5, no. 7 (2014): 1198-1202. [108](#)
- [39] Ph Goy, J. M. Raimond, M. Gross, and S. Haroche. *Observation of cavity-enhanced single-atom spontaneous emission*. Physical review letters 50, no. 24 (1983): 1903. [108](#)
- [40] A. I. Chizhik, Anna M. Chizhik, Dmitry Khoptyar, Sebastian Bär, Alfred J. Meixner, and Jörg Enderlein. *Probing the radiative transition of single molecules with a tunable microresonator*. Nano letters 11, no. 4 (2011): 1700-1703. [108](#)
- [41] Alexey I. Chizhik, Ingo Gregor, Frank Schleifenbaum, Claus B. Müller, Christian Röling, Alfred J. Meixner, and Jörg Enderlein. *Electrodynamic coupling of electric dipole emitters to a fluctuating mode density within a nanocavity*. Physical review letters 108, no. 16 (2012): 163002. [108](#)
- [42] P. Debye. *Polar Molecules; Chemical Catalog Company: New York, 1929*. There is no corresponding record for this reference. [108](#)

Chapter 5

Light-matter interaction in carbon nanodots

Luminescent carbon nanodots (CND) are a recent addition to the family of carbon nanostructures. Interestingly, a large group of CNDs are fluorescent in the visible spectrum and possess single dipole emitters with potential applications in super-resolution microscopy, quantum information science, and optoelectronics. There is a large diversity of CND's size as well as a strong variability of edge topology and functionalisation in real samples. This hampers a direct comparison of experimental and theoretical findings that is necessary to understand the unusual photophysics of these systems. Here, we derive atomistic models of finite sized ($<2.5 \text{ nm}$) CNDs from high resolution transmission electron microscopy (HRTEM) which are studied using approximate time-dependent density functional theory. The atomistic models are found to be primarily two-dimensional (2D) and can hence be categorised as graphene quantum dots (GQD). The GQD model structures that are presented here show excitation energies in the visible spectrum matching previous single GQD level photoluminescence studies. We also present the effect of edge hydroxyl and carboxyl functional groups on the absorption spectrum. Overall, the study reveals the atomistic origin of CNDs photoluminescence in the visible range.

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5.1 Introduction

Toxic and environment unfriendly nanomaterials are a matter of current public concern [21, 32, 29, 3, 14]. At the moment, there are several nanomaterials that pose an alarming threat to the environment [32]. Cadmium, selenium, and lead based nanomaterials are some of them with exemplary quantum optical behaviour at low dimensions [2]. However, the level of toxicity raises questions towards their usage in opto-electronic and biological applications [1, 20], and hence, there is a transcending need of non-toxic quantum materials [11, 28]. This leads to focus towards carbon based nontoxic quantum-materials given their numerous advantageous physical properties at the nanoscale [6, 18, 33, 19, 17, 47, 36]. One of the recent developments in this direction are carbon nanodots (CND), among them two-dimensional, monolayered CND termed graphene quantum dots (GQD) which have shown strong impact immediately after their discovery [47]. GQDs are found to be fluorescent in the visible range [19, 5, 12, 24, 13]. They feature promising biophysical applications from structural biology to active transport using superresolution microscopy [22]. Recent studies show that they are excellent nontoxic and superresolution probes for animal cells [10, 38], unlike conventional quantum dots [21, 30, 25].

There are several ensemble spectroscopic studies of GQD [5, 24, 13]. Only a few reported at the single nanodot level [12, 19]. Among them, our previous investigation as presented in Chapter 4 showed presence of single-dipole excitation and emission in single GQDs [19] with excitation energies at 2.54 eV and 2.65 eV. From a correlative study of HRTEM and scanning confocal fluorescence microscopy we had estimated the atomic structure of the optically active CNDs. Later, Chizhik et al. used the same species of CNDs for super-resolution imaging and reported other excitation energies at 3.02 eV and 3.64 eV [10]. Another study by Das et al. showed single photon emission when excited at 1.93 eV, 2.21 eV, and 2.54 eV [12]. Several groups have speculated that certain functional groups are responsible for GQD’s to be fluorescent in the visible range. The other potential reason could be emission from the carbon core governed by quantum confinement. Unfortunately, there is hardly any control study of GQDs ranging from 2 to 10 nm to investigate this effect. To use them as an ideal superresolution microscopy probe, GQD should be small enough to omit probe induced spatial artefacts [7, 40]. In order to use them as inner cellular fluorescent probes, they need to sieve through or permeable to the restricted size of the membrane pores. One such pore is the nuclear pore complex with an opening diameter of 2.6 nm [31]. Therefore, it seems worthwhile to study the physical behaviour of GQDs of less than 2.5 nm

in size.

In this work, we present atomistic models of such GQDs and their corresponding excitation energies, which are in excellent agreement with our previous single GQD level experimental quantum optical measurements [19] and other studies [12, 10]. Here, we calculate the electronic and optical properties of GQDs using the time-dependent density functional based tight binding method (TD-DFTB) [35], which is a numerically efficient and parameter free approximation to time-dependent density functional theory (for a review see [34]). TD-DFTB has proven to be an accurate method to unfold the excited state behaviour of different finite nanostructures [8, 45], and the ground state DFTB method has been successfully applied to a variety of graphene nanoflakes before [36, 42, 46]. The GQDs studied here are single-layer, two-dimensional carbon sheets, in other words, few nanometers wide and long graphene. Therefore, the edge termination plays a crucial role in their optical properties [39, 43, 23, 26, 44]. The edges of the model GQDs shown here are dominated by armchair type termination [23, 26, 44]. HRTEM simulations of these models match well with our experimentally found GQDs. We also study the effect of edge modifications by hydroxyl (OH) and carboxyl (COOH) functional groups since previously energy dispersive X-ray spectroscopy showed presence of only oxygen besides carbon [19]. Depending on the number of functional groups the excited states of GQD are strongly modified. While some of the experimentally found excitation energies are not present in pure GQD, we find that OH and COOH groups are not mandatory for the GQDs to be optically active in the visible range.

5.2 Model structure and structural optimisation

Figure 1(a) shows the proposed atomistic model for the type II CNDs or GQDs previously characterized by means of photoluminescence (PL) correlated HRTEM measurements [19]. Previously, the edge information of the 2 nm sized GQD was difficult to extract from the HRTEM images due to the amorphous carbon background of the TEM grid. We therefore experimented with different edge types by keeping the diameter of the GQD within 2 nm. For structures with extended zigzag edges (see appendix G - Figure G1) we often found triplet ground states in agreement with earlier theoretical work [39]. Depending on synthesis conditions, zigzag termination can dominate and lead to characteristic PL features related to the triplet groundstate [37]. A quantitative TEM simulation [27] of the proposed armchair model however agrees well when overlayed on experimentally measured HRTEM images. Figure 1(c), (d), and (f) show the simulated HRTEM phase, amplitude, and diffractogram images of GQD, where the lattice spacing (2.5 Å) matches with the experimental HRTEM lattice spacing [19]. The total number of carbon atoms in the model is 92 and the dangling bonds are passivated with 26 hydrogen atoms. We refer to this model as 2D-CND. The passivation with hydrogen is crucial to avoid any trapped states at the dangling bonds. It also simplifies the theoretical description by facilitating the convergence to electronic self-consistency.

The width and the length of the 2D-CND are 1.674 nm and 1.794 nm, respectively. The C-C bond lengths vary from 1.42 to 1.43 Å, and the lattice spacing ‘a’ is 2.48 Å as shown in the appendix G (Figure G3). All these parameters match well with the earlier experimental measurement [19].

In order to estimate the dependence of the optical spectrum on the precise edge topology we also investigated GQD with rough borders. Graphene nanoflakes of this kind have been recently analyzed by Sattler and coworkers [44]. Figure 1(b) shows such a type of GQD. We address this structure as Prot-2D-CND due to the newly introduced six carbon atoms to 2D-CND (Figure 1(a)) in the form of two protrusions (marked in green). The width of the Prot-2D-CND is the same as 2D-CND, while the length amounts to 2.208 nm. Bond lengths and lattice spacing are similar to the earlier model although the structure is slightly non-planar at the protrusions. Like the 2D-CND model this structure features a singlet groundstate (appendix G table G1).

5.2.1 Optical spectra

The calculated absorption spectra of 2D-CND and Prot-2D-CND are shown in Figure 1(f) and show significant differences. We are interested in the visible region of the spectrum, specifically, in the green and blue region because former experimental measurement of single CNDs showed excitation energies at 2.54 eV, 2.65 eV, and 3.0 eV [19, 10]. So, we have magnified the 2.4 eV to 3.1 eV region in Figure 1(g). The calculated TD-DFTB (singlet-singlet) excitation energies (ω_I) found for model 2D-CND are 2.47 eV (501.69 nm), 2.61 eV (475.03 nm), and 3.00 eV (413.28 nm). The last two states match well with our previous reports. In case of Prot-2D-CND, the strong peak of 2D-CND at 2.47 eV disappears, instead, two adjacent peaks appear at 2.43 eV (510.22 nm) and 2.51 eV (493.96 nm). Additionally, other peaks at 2.61 eV and 3.00 eV also disappear. Two new peaks appear within the 2.61 eV to 3.00 eV range – at 2.72 eV (455.82 nm) and 2.89 eV (429.01 nm). Outside the window, the lowest ω_I shifts towards higher energy compared to 2D-CND.

5.2.2 Molecular orbitals

For deeper understanding, we performed a detailed molecular orbital (MO) analysis. In time-dependent density functional theory, excited states may be represented in an approximate configuration interaction expansion which allows to identify the dominant single-particle transitions. In Figure 2(a), we show the corresponding occupied (hole) and virtual (electron) orbitals for the aforementioned excited states ω_I . Here, E_n refers to the Kohn-Sham energy of the MOs. The hole orbitals for 2D-CND are delocalised over the entire structure, while the electron orbital E_{204} (+2.05 eV) participating in the excited states at 2.61 eV and 2.47 eV shows no localization in the centre of the GQD. In the case of Prot-2D-CND, both electron and hole orbitals are delocalised over the

Figure 5.1: DFTB optimized model structures of GQD. (a) CND: Pure GQD with armchair edge termination. Terminating carbon atoms with dangling bonds are passivated with hydrogen atoms (red). The total number of carbon atoms (blue) here is 94. The relaxed structure remains planar as visible from two side views ($[1\bar{1}00]$ and $[1120]$ planes). (b) Prot.CND: GQD with two protrusions (green). The structure is slightly displaced out of $[1120]$ plane as shown in the longitudinal side view. (c) TD-DFTB calculated absorption spectra of CND (red) and Prot.CND (blue dashed). The highlighted gray region from 2.4 eV to 3.1 eV is further magnified in (d), here, the black lines correspond to absorption maxima for CND and Prot.CND. The spectra are broadened with Lorentzians (0.04 eV). (e) Phase, (f) amplitude, and (g) diffraction pattern of HRTEM simulation of GQD

Figure 5.2: Molecular orbitals (MOs) and density of states (DOS) for CND and Prot.CND. (a) Relevant hole and electron orbitals for the corresponding excitation energies of interest (ω_I) from Fig 1(d). (b) Total DOS and partial DOS (PDOS) for different shells (s,p) as well as PDOS for edge atoms only. (c) Same for Prot.CND. Orbital energies given in (a) are marked with black dashed lines. The Fermi level (E_F) was set to 0 eV in all plots and DOS curves have been broadened with Gaussian broadening factor 0.06 eV.

entire structure, except at E_{207} (-1.47 eV) and E_{214} (+1.56 eV) where the protruded regions do not show any participation. The protrusions play a major role in the modification of the absorption spectra.

5.2.3 Density of states

We further quantify the density of states (DOS). In Figure 2b and 2c, we plot the DOS of 2D-CND and Prot-2D-CND in an extended energy range as well as from -2.5 eV to +2.5 eV to make contact with the aforementioned E_n levels (Figure 2b). A clear bandgap is observed. The total DOS is primarily composed of the p-shell of carbon atoms with very little contribution of the

s-shell, evidencing the π -bonding network of the graphene flake. The local DOS obtained by considering only edge atoms reveals that the armchair edge significantly contributes to the total DOS. The clean bandgap observed is due to this armchair termination. Due to the small size of the GQD it is possible to identify the molecular orbitals contributing to the mentioned excited states as isolated resonances in the DOS. As already expected from the optical spectra, the DOS for Prot-2D-CND (Figure 2c) is different from 2D-CND. Here as well, we have magnified the -2.5 eV to +2.5 eV energy region and the Fermi energy (E_F) is set to 0 eV. The first noticeable difference is the bandgap, which is 1.92 times (almost twice) larger than in the 2D-CND case. We attribute this to the out-of-plane deformation of the 2D-CND backbone due to the protrusions which destabilizes and effectively shrinks the π -bonding network. The reduced band gap supports the aforementioned observation of the lowest ω_l moving towards higher energy. We marked the corresponding peaks of the hole (E_{207} , E_{208} , E_{209} , and E_{210}) and electron orbitals (E_{212} , E_{214} , E_{215} , and E_{217}). Like for 2D-CND, the p-shell contributes primarily to the total DOS.

5.3 Effect of functionalisation on optical spectra

Our previous experimental study found presence of oxygen in GQDs [19]. Therefore, we study the effect of two possible oxygen containing functional groups - OH and COOH on the optical spectrum. Our former report also suggests that the majority of the CNDs were structurally planar or 2D. This is due to the orientation of every single excitation and emission dipoles, which were always found perpendicular to the optical axis [19, 10]. If the CNDs had atoms in all three spatial dimensions, repeated findings of planar dipole orientation would have been less probable, contrary to the experimental results. So, it is likely that the CND remains fully planar after functionalization which justifies referring them as GQD.

5.3.1 Hydroxyl groups

Structural planarity

The number of OH groups introduced in model 2D-CND are one to four (1OH, 2OH, 3OH, and 4OH), six (6OH), eight (8OH), and 26 (full-OH). We refer to 2D-CND with 26 OH groups as ‘full-OH’, since it replaces all the passivating hydrogen atoms with OH groups. Until the introduction of 6OH the 2D-CND remains planar in the xy plane (Figure 3(a)) after structural relaxation, as observable from the yz side-view. Here, the xy plane is the top-view from the [0001] direction. 2D-CNDs with 8OH and full-OH show large out of the xy plane distortions.

Figure 5.3: Hydroxyl functionalisation. *a.* Effect of OH functionalisation on excitation behaviour of GQD. In contrast to excitation spectrum of pure GQD, calculated spectra of increasing number of OH (n OH) functional groups are presented. Red-shifts of 2.35 eV and 3 eV are observed. They are marked with red dotted lines. Several new excitation peaks are observed near 2.5 eV as a result of OH functionalisation. The planarity of the primary backbone of GQD is slightly distorted when three OH groups are introduced (3OH). Large out of plane strain is observed at 8OH and complete functionalisations. *b.* HOMOs and LUMOs near 2.54 eV based on number of OHs.

Optical spectra

In Figure 3(a), we also show the absorption spectra of 2D-CND with the above mentioned numbers of OH groups at the edge in comparison to pure 2D-CND. At first, we show the spectra until 4 eV for the overview. However, our region of interest is from 2.4 eV to 3.1 eV highlighted with grey. A magnified view of this grey region is shown in the same figure. The peak at 2.35 eV (527.59 nm) redshifts by 3.95% from no OH to full-OH. In appendix G (Figure G2) we show that the character of the participating MO for this excitation does not significantly change upon oxidation. Beside this redshift, the peak almost disappears in case of full-OH (Figure S2). The transition at 2.47 eV (501.96 nm) shows little dependence on functionalization, except in the case of 3OH, 8OH, and full-OH where the peak disappears completely. In addition, a new peak appears at 2.55 eV (486.21 nm) for 3OH and 2.54 eV (488.12 nm) for 4OH and full-OH. They are not shifted from the 2.47 eV state. The MOs involved in 2.47 eV transition in 2D-CND (E_{197} to E_{204} figure 2(a)) does match with MOs for 2.55, 2.53, and 2.54 eV for 3OH, 4OH, and full-OH, respectively (figure 3(b)). These peaks appear due to the presence of 3OH, 4OH, and full-OH functionalizations which ω_f s match very well with our previous report of 488 nm excitation [19]. The next excitation of pristine CND is at 2.61 eV (475.03 nm). A very small shift is observed for this peak from CND to 8OH (except at full-OH where it disappears completely). In 3OH and 4OH this peak shifts to 2.65 eV and 2.66 eV, respectively. Close in energy are two excitations at 2.71 eV (457.50 nm) and 2.77 eV (447.59) in 8OH and full-OH, respectively. The last absorption peak in the visible spectrum of CND is at 3.009 eV (412.04 nm). Here, a redshift of 3.62% is observed going from CND to 6OH (Figure G3 in the appendix G shows that the MO character is indeed conserved). At 8OH and full-OH this peak disappears completely. Comparing all the OH functionalisations, 4OH matches well with our previous report with ω_f s at 2.54 eV, 2.66 eV, and 3.00 eV [19, 10]. From the theoretical simulations it seems unlikely that the measured sample contained fully oxidized GQD. Due to the non-planarity of the structure the excitation dipole will also be out of the xy plane. Additionally, the oscillator strength reduces noticeably in full-OH and many prominent peaks disappear. In contrast, a GQD structure with three to four OH groups is consistent with the experimental data. Both the structures are planar and show ω_f matching with our previous report. Only a small out of xy plane displacement of 3OH is present.

5.3.2 Carboxyl groups

Structural planarity

Next, we study the influence of carboxylation on the optical properties. The number of COOH groups attached to the model CND are one to four (named as 1COOH, 2COOH, 3COOH, and 4COOH), six (6COOH), and eight (8COOH). We show the optimised structures in Figure 4 from

three different views (xy , yz , xz). 2D planarity of the original CND model is not broken until 6COOH. Similar to 8OH, a moderate distortion is observed for 8COOH. It is not possible to replace all the 26 hydrogen passivated sites with carboxyl groups due to steric hindrance at the GQD edges. GQD with 26 COOH groups never converged to a relaxed and optimal structure. While strongly carboxylated GQD have been speculated in the literature [41], our calculations do not support the stability of such structures.

Optical spectra

The calculated absorption spectra for the carboxylated 2D-CND are shown in Figure 4(a) up to 4 eV for a broad overview. The highlighted region of interest is further magnified. In contrast to the hydroxylated 2D-CND, the peak at 2.35 eV is not shifted noticeably while the next peak at 2.47 eV is redshifted by 2.44% (c.f. appendix G - Figure G5). Increasing the number of COOH groups affects the peak at 2.61 eV strongly. It gradually disappears from 1COOH to 8COOH. Additionally, new absorption bands appear at 2.54 eV (2COOH), 2.53 eV (3COOH), 2.52 eV (4COOH), and 2.51 eV (6COOH), close to the experimental excitation energy at 2.54 eV. We plot the respective MOs for these ω_{fs} in Figure 4(b). Except for 2COOH, the excited states near 2.54 eV derive from similar MOs in 3COOH, 4COOH, and 6COOH. The peak at 3.00 eV shows a total of 4.48% redshift from CND to 8COOH (appendix G - Figure G6). Another peak outside the visible region at 3.21 eV shows a noticeable redshift of 4.3% (appendix G - Figure G7). This redshift introduces a new band almost within the visible range. The behaviour of COOH groups on 2D-CND is significantly different from OH groups. In several instances, the ω_{fs} follows different trend with OH functionalisations. In 2COOH, 3COOH, 4COOH, and 6COOH ω_{fs} are found at or near 2.54 eV and match the experimental excitation energy. However, accompanying experimental excitation energies are not found simultaneously.

5.4 Summary

In summary, we have shown several theoretical models of 2D-CND. We have modelled the single nanodot level experimental study of GQD using TD-DFTB. The quantum mechanical reasoning behind the visible range photoluminescence of them are answered. The restricted size gives rise to exemplary quantum confined visible range photoluminescence of GQDs. At nanometer length scale, the armchair type borders significantly contributes to the DOS and a clean band gap of the proposed 2D-CND model. We also conclude that GQDs are not necessarily be functionalised to show excitation energies in the visible spectrum. The visible region of excitations are important for bio-imaging to avoid unwanted damage with high energy photons. The GQD by itself has excitation energies in visible wavelength which agrees well to our former report and other ex-

Figure 5.4: Carboxyl functionalisation. *a.* Effect of COOH functionalisations on excitation behaviour of GQD. In contrast to excitation spectrum of pure GQD, calculated excitation spectra of increasing number of COOH (n COOH) functional groups are presented. Red-shifts of 2.10 eV, 2.47 eV, 3 eV, and 3.21 eV are observed and marked with red dotted lines. Several new excitation peaks are observed near 2.5 eV as a result of COOH functionalisation, like OH functionalisation. The planarity of the primary backbone of GQD is slightly distorted when three COOH groups are introduced (3COOH). Large out of plane strain is observed at 8COOH functionalisation. *b.* HOMOs and LUMOs near 2.54 eV based on number of COOHs.

perimental studies. These excitation energies can be tuned by introducing certain number of OH or COOH groups. Comparing with experimental evidence, we infer that GQDs in our previous studies contain four hydroxyl groups because the 2D-CND-4OH shows all the accompanying excitation energies (2.53 eV, 2.66 eV, 2.91 eV, and 3.00 eV) and a matched atomistic structures. The excitation energies reported here will be useful to nanofabricate GQD based super-resolution fluorescent probes, single-photon quantum detectors, and solar cells. We envision the study will be used to efficiently synthesize non-toxic GQDs considering the structure, edge termination, and functional groups.

Methods

All calculations have been performed with a development version of the DFTB+ code[4] and the mio-0-1 set of Slater-Koster files[15]. Geometries have been relaxed in the electronic ground state using a conjugate gradient optimizer with a force tolerance of $1\text{e-}4\text{ eV/\AA}$. For the partially functionalized dots only selected high symmetry structures have been considered. Electronic excited states have been computed using the frequency domain (Casida) formalism in TD-DFTB.[34] The obtained stick spectrum was Lorentzian broadened by 0.01 eV and DOS was Gaussian broadened by 0.06 eV. DFTB molecular orbitals were rendered with the DFTB package *waveplot* at an isovalue of ± 0.009 . The HRTEM simulation to extract the atomistic model from experimental HRTEM was performed using QSTEM code [27].

Bibliography

- [1] U.S. Environmental Protection Agency. Deposition of air pollutants to the great waters. pages EPA-453/R-93-055, 1994. First Report to Congress. 116
- [2] A Paul Alivisatos. Semiconductor clusters, nanocrystals, and quantum dots. *Science*, 271(5251):933, 1996. 116
- [3] Alaaeddin Alsbaiee, Brian J Smith, Leilei Xiao, Yuhang Ling, Damian E Helbling, and William R Dichtel. Rapid removal of organic micropollutants from water by a porous β -cyclodextrin polymer. *Nature*, 2015. 116
- [4] B. Aradi, B. Hourahine, and T. Frauenheim. Dftb+, a sparse matrix-based implementation of the dftb method. *J. Phys. Chem. A*, 111(26):5678–5684, 2007. 126
- [5] Sheila N Baker and Gary A Baker. Luminescent carbon nanodots: emergent nanolights. *Angewandte Chemie International Edition*, 49(38):6726–6744, 2010. 116

- [6] Verónica Barone, Oded Hod, and Gustavo E Scuseria. Electronic structure and stability of semiconducting graphene nanoribbons. *Nano letters*, 6(12):2748–2754, 2006. [116](#)
- [7] Eric Betzig. Nobel lecture: Single molecules, cells, and super-resolution optics. *Reviews of Modern Physics*, 87(4):1153, 2015. [116](#)
- [8] Juncao Bian, Jianfu Li, Sergii Kalytchuk, Yu Wang, Qian Li, Tsz Chun Lau, Thomas A Niehaus, Andrey L Rogach, and Rui-Qin Zhang. Efficient emission facilitated by multiple energy level transitions in uniform graphitic carbon nitride films deposited by thermal vapor condensation. *ChemPhysChem*, 16(5):954–959, 2015. [117](#)
- [9] M. E. Casida. *Recent Advances in Density Functional Methods, Part I*, chapter Time-dependent Density Functional Response Theory for Molecules, pages 155–192. World Scientific, Singapore, 1995. [146](#)
- [10] Anna M Chizhik, Simon Stein, Mariia O Dekaliuk, Christopher Battle, Weixing Li, Anja Huss, Mitja Platen, Iwan AT Schaap, Ingo Gregor, Alexander P Demchenko, Christoph F Schmidt, Jörg Enderlein, and Alexey I. Chizhik. Super-resolution optical fluctuation bio-imaging with dual-color carbon nanodots. *Nano letters*, 16:237–242, 2015. [116](#), [117](#), [118](#), [121](#), [123](#)
- [11] Eric Cross. Materials science: Lead-free at last. *Nature*, 432(7013):24–25, 2004. [116](#)
- [12] Somes K Das, Yiyang Liu, Sinhea Yeom, Doo Young Kim, and Christopher I Richards. Single-particle fluorescence intensity fluctuations of carbon nanodots. *Nano letters*, 14(2):620–625, 2014. [116](#), [117](#)
- [13] Mariia O Dekaliuk, Oleg Viagin, Yuriy V Malyukin, and Alexander P Demchenko. Fluorescent carbon nanomaterials: quantum dots or nanoclusters? *Physical Chemistry Chemical Physics*, 16(30):16075–16084, 2014. [116](#)
- [14] Curtis Deutsch, Aaron Ferrel, Brad Seibel, Hans-Otto Pörtner, and Raymond B Huey. Climate change tightens a metabolic constraint on marine habitats. *Science*, 348(6239):1132–1135, 2015. [116](#)
- [15] M. Elstner, D. Porezag, G. Jungnickel, J. Elsner, M. Haugk, T. Frauenheim, S. Suhai, and G. Seifert. Self-consistent-charge density-functional tight-binding method for simulations of complex materials properties. *Phys. Rev. B*, 58(11):7260–7268, 1998. [126](#)
- [16] Shuai Feng, Quan-Song Li, Ping-Ping Sun, Thomas A Niehaus, and Ze-Sheng Li. Dynamic characteristics of aggregation effects of organic dyes in dye-sensitized solar cells. *ACS applied materials & interfaces*, 7(40):22504–22514, 2015.

- [17] Andrea C Ferrari, Francesco Bonaccorso, Vladimir Fal'Ko, Konstantin S Novoselov, Stephan Roche, Peter Bøggild, Stefano Borini, Frank HL Koppens, Vincenzo Palermo, Nicola Pugno, Jose A. Garrido, Roman Sordan, Alberto Bianco, Laura Ballerini, Maurizio Prato, Elefterios Lidorikis, Jani Kivioja, Claudio Marinelli, Tapani Ryhänen, Alberto Morpurgo, Jonathan N. Coleman, Valeria Nicolosi, Luigi Colombo, Albert Fert, Mar Garcia-Hernandez, Adrian Bachtold, Gregory F. Schneider, Francisco Guinea, Cees Dekker, Matteo Barbone, Zhipei Sun, Costas Galiotis, Alexander N. Grigorenko, Gerasimos Konstantatos, Andras Kis, Mikhail Katsnelson, Lieven Vandersypen, Annick Loiseau, Vittorio Morandi, Daniel Neumaier, Emanuele Treossi, Vittorio Pellegrini, Marco Polini, Alessandro Tredicucci, Gareth M. Williams, Byung H. Hong, Jong-Hyun Ahn, Jong Min Kim, Herbert Zirath, Bart J. van Wees, Herre van der Zant, Luigi Occhipinti, Andrea Di Matteo, Ian A. Kinloch, Thomas Seyller, Etienne Quesnel, Xinliang Feng, Ken Teo, Nalin Rupesinghe, Pertti Hakonen, Simon R. T. Neil, Quentin Tannock, Tomas Löfwander, and Jari Kinaret. Science and technology roadmap for graphene, related two-dimensional crystals, and hybrid systems. *Nanoscale*, 7(11):4598–4810, 2015. [116](#)
- [18] Andre K Geim and Konstantin S Novoselov. The rise of graphene. *Nature materials*, 6(3):183–191, 2007. [116](#)
- [19] Siddharth Ghosh, Anna M Chizhik, Narain Karedla, Mariia O Dekaliuk, Ingo Gregor, Henning Schuhmann, Michael Seibt, Kai Bodensiek, Iwan AT Schaap, Olaf Schulz, Alexander P Demchenko, Jörg Enderlein, and Alexey I. Chizhik. Photoluminescence of carbon nanodots: Dipole emission centers and electron–phonon coupling. *Nano letters*, 14(10):5656–5661, 2014. [80](#), [88](#), [116](#), [117](#), [118](#), [121](#), [123](#), [144](#), [145](#)
- [20] Steven J Hamilton. Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment*, 326(1):1–31, 2004. [116](#)
- [21] Ron Hardman. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environmental health perspectives*, pages 165–172, 2006. [116](#)
- [22] Bo Huang, Mark Bates, and Xiaowei Zhuang. Super resolution fluorescence microscopy. *Annual review of biochemistry*, 78:993, 2009. [116](#)
- [23] Xiaoting Jia, Mario Hofmann, Vincent Meunier, Bobby G Sumpter, Jessica Campos-Delgado, José Manuel Romo-Herrera, Hyungbin Son, Ya-Ping Hsieh, Alfonso Reina, Jing Kong, Mauricio Terrones, and Mildred S. Dresselhaus. Controlled formation of sharp zigzag and armchair edges in graphitic nanoribbons. *Science*, 323(5922):1701–1705, 2009. [117](#)
- [24] Syamantak Khan, Abhishek Gupta, Navneet C Verma, and Chayan K Nandi. Time-resolved emission reveals ensemble of emissive states as the origin of multicolor fluorescence in carbon dots. *Nano letters*, 15(12):8300–8305, 2015. [116](#)

- [25] Christian Kirchner, Tim Liedl, Stefan Kudera, Teresa Pellegrino, Almudena Muñoz Javier, Hermann E Gaub, Sonja Stölzle, N Fertig, and Wolfgang J Parak. Cytotoxicity of colloidal cdse and cdse/zns nanoparticles. *Nano letters*, 5(2):331–338, 2005. [116](#)
- [26] Yousuke Kobayashi, Ken-ichi Fukui, Toshiaki Enoki, Koichi Kusakabe, and Yutaka Kaburagi. Observation of zigzag and armchair edges of graphite using scanning tunneling microscopy and spectroscopy. *Physical Review B*, 71(19):193406, 2005. [117](#)
- [27] Christoph Tobias Koch. *Determination of core structure periodicity and point defect density along dislocations*. 2002. [117](#), [126](#)
- [28] Liang Li, T Jean Daou, Isabelle Texier, Tran Thi Kim Chi, Nguyen Quang Liem, and Peter Reiss. Highly luminescent CuInS₂/ZnS core/shell nanocrystals: cadmium-free quantum dots for in vivo imaging. *Chemistry of Materials*, 21(12):2422–2429, 2009. [116](#)
- [29] Mohamed E Mahmoud, Azza EH Abdou, and Somia B Ahmed. Conversion of waste styro-foam into engineered adsorbents for efficient removal of cadmium, lead and mercury from water. *ACS Sustainable Chemistry & Engineering*, 2015. [116](#)
- [30] X Michalet, FF Pinaud, LA Bentolila, JM Tsay, SJL Doose, JJ Li, G Sundaresan, AM Wu, SS Gambhir, and S Weiss. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science*, 307(5709):538–544, 2005. [116](#)
- [31] Dagmar Mohr, Steffen Frey, Torsten Fischer, Thomas Güttler, and Dirk Görlich. Characterisation of the passive permeability barrier of nuclear pore complexes. *The EMBO journal*, 28(17):2541–2553, 2009. [116](#)
- [32] Andre Nel, Tian Xia, Lutz Mädler, and Ning Li. Toxic potential of materials at the nanolevel. *Science*, 311(5761):622–627, 2006. [116](#)
- [33] AH Castro Neto, F Guinea, NMR Peres, Kostya S Novoselov, and Andre K Geim. The electronic properties of graphene. *Reviews of modern physics*, 81(1):109, 2009. [116](#)
- [34] Thomas A Niehaus. Approximate time-dependent density functional theory. *Journal of Molecular Structure: THEOCHEM*, 914(1):38–49, 2009. [117](#), [126](#)
- [35] Thomas A Niehaus, S Suhai, F Della Sala, P Lugli, M Elstner, G Seifert, and Th Frauenheim. Tight-binding approach to time-dependent density-functional response theory. *Physical Review B*, 63(8):085108, 2001. [117](#)
- [36] Carlos-Andres Palma, Manohar Awasthi, Yenny Hernandez, Xinliang Feng, Klaus Müllen, Thomas A Niehaus, and Johannes V Barth. Sub-nanometer width armchair graphene nanoribbon energy gap atlas. *The Journal of Physical Chemistry Letters*, 6(16):3228–3235, 2015. [116](#), [117](#)

- [37] Dengyu Pan, Jingchun Zhang, Zhen Li, and Minghong Wu. Hydrothermal route for cutting graphene sheets into blue-luminescent graphene quantum dots. *Advanced Materials*, 22(6):734–738, 2010. [117](#)
- [38] Lulu Pan, Shan Sun, Aidi Zhang, Kai Jiang, Ling Zhang, Chaoqing Dong, Qing Huang, Aiguo Wu, and Hengwei Lin. Truly fluorescent excitation-dependent carbon dots and their applications in multicolor cellular imaging and multidimensional sensing. *Advanced Materials*, 27(47):7782–7787, 2015. [116](#)
- [39] Ljubisa R Radovic and Bradley Bockrath. On the chemical nature of graphene edges: origin of stability and potential for magnetism in carbon materials. *Journal of the American Chemical Society*, 127(16):5917–5927, 2005. [117](#)
- [40] Jonas Ries, Charlotte Kaplan, Evgenia Platonova, Hadi Eghlidi, and Helge Ewers. A simple, versatile method for gfp-based super-resolution microscopy via nanobodies. *Nature methods*, 9(6):582–584, 2012. [116](#)
- [41] Christoph G Salzmann, Valeria Nicolosi, and Malcolm LH Green. Edge-carboxylated graphene nanoflakes from nitric acid oxidised arc-discharge material. *Journal of Materials Chemistry*, 20(2):314–319, 2010. [124](#)
- [42] Hongqing Shi, Robert J Rees, Manolo C Per, and Amanda S Barnard. Impact of distributions and mixtures on the charge transfer properties of graphene nanoflakes. *Nanoscale*, 7(5):1864–1871, 2015. [117](#)
- [43] Young-Woo Son, Marvin L Cohen, and Steven G Louie. Energy gaps in graphene nanoribbons. *Physical review letters*, 97(21):216803, 2006. [117](#)
- [44] Natalie Wohner, Pui K Lam, and Klaus Sattler. Systematic energetics study of graphene nanoflakes: From armchair and zigzag to rough edges with pronounced protrusions and overcrowded bays. *Carbon*, 82:523–537, 2015. [117](#), [118](#)
- [45] Qi Wu, Xiao-Hui Wang, Thomas A Niehaus, and Rui-Qin Zhang. Boundary and symmetry determined exciton distribution in two dimensional silicon nanosheets. *The Journal of Physical Chemistry C*, 118(35):20070–20076, 2014. [117](#)
- [46] Sharma SRKC Yamijala, Madhuri Mukhopadhyay, and Swapan K Pati. Linear and nonlinear optical properties of graphene quantum dots: A computational study. *The Journal of Physical Chemistry C*, 119(21):12079–12087, 2015. [117](#)
- [47] Shoujun Zhu, Yubin Song, Xiaohuan Zhao, Jieren Shao, Junhu Zhang, and Bai Yang. The photoluminescence mechanism in carbon dots (graphene quantum dots, carbon nanodots, and polymer dots): current state and future perspective. *Nano Research*, 8(2):355–381, 2015. [116](#)

Chapter 6

Conclusion

This thesis gives an insight of nanoscale photonics with respect to single molecule nanofluidics and light-matter interaction in nanostructures. In near future, nanofluidics will play an important role in healthcare research and industry. Considering this fact, I presented the fundamentals of nanofluidics as well as single molecule nanofluidic experiments, where single fluorophore molecules, carbon nanodots (CND), and small DNA molecules were studied. With that, the strategies of realising nanofluidic devices are also shown by nanofabrication techniques. I show that shadow-angle-electron-beam-deposition is an efficient approach to preparing nanofluidic devices in a high-throughput manner. I also performed two-focus fluorescence correlation (2fFCS) spectroscopy using these nanofluidic devices. It is notable that there was no earlier report with successful 2fFCS measurement inside nanofluidic channels for single molecule detection. Overall, the study presented an efficient nanofluidic device fabrication technique as well as an efficient method of handling single molecules in liquid.

Another part of the thesis presents photophysics of newly discovered non-toxic CNDs as single-photon emitters. CNDs show a promising future in biomolecular detection. They are also a potential class of nanomaterials suitable for quantum optical and optoelectronic research and industry. Until now, there was no detail single molecule level study of carbon nanodots revealing the fundamental reasons behind their strong photoluminescence. Here, a correlative study of electron microscopy and confocal scanning fluorescence microscopy together with single-nanoparticle photoluminescence spectroscopy revealed an understanding of their fluorescence behaviour. We found two kinds of CNDs – multiwalled short structures with graphitic stacking and graphene quantum dots. The atomic structures of the CNDs showed good agreement between theoretically predicted phonon modes and electron-phonon coupling observed in the photoluminescence spectroscopy. The study revealed CNDs are single-photon emitter using defocused optical imaging and stepwise bleaching.

Finally, a time-dependent approximate density functional theoretical study revealed the molecular

insight of the CNDs' visible range photoluminescence. From this study, I confirmed that one of the CNDs studied experimentally is graphene-based quantum dots. Here, an HRTEM simulation was performed to extract the model structure of CNDs from the previous experimental study. Size at the nanoscale is an important factor to be considered since below 10 nm quantum confinement significantly affects the photoluminescence. Hence, I also investigated the size-dependent excitation energies of CNDs where structures of CNDs from 92 to 118 carbon atoms were used. A significant change in excitation energies was observed when the number of atoms was increased. I found that CNDs are not necessarily be functionalised or should have crystal defects to be photoluminescent in the visible range. However, the effect of chemical defects on the optical properties of CNDs was also investigated.

In future, I expect that the presented research work in this thesis should have a potential impact in:

- I. Single-molecule sensitive biomedical diagnostics using nanofluidics,
- II. Nanoelectronic research by using nanofluidic platform as an in-situ tool of wide range of dynamic studies,
- III. Quantum optical research by using CNDs as the source and detector of single photons,
- IV. Label-free nanofluidic detection of biomolecules by utilising the study of excited state properties of CNDs to fabricate on-chip graphene-based detectors which can sense single molecules by modifying the detectors' excited state with weak-coupling.

Appendix A

Reactive ion etching

Following are the material specific recipes used for reactive ion etching in an Oxford PlasmaLab – RIE and PECVD cluster (Oxford Instruments plc, Abington, UK):

Silicon: 40 sccm of CHF_3 and 10 sccm of SF_6 gases are used at a chamber pressure of $\sim 1 \times 10^{-6}$ mbar and 100 W power. Such combination produces an etch rate of 40 nm/min for [100] silicon. The average surface roughness was found to be of 2 Å to 3 Å.

Silica: 12 sccm of CHF_3 and 38 sccm of Ar gases are used at a chamber pressure of $\sim 1 \times 10^{-6}$ mbar and 100 W power. Such combination produces an etch rate of 25 nm/min to 30 nm/min for fused silica coverslips with an average surface roughness of 6 Å.

Gold: 30 sccm of Ar gas was used at $\sim 1 \times 10^{-6}$ mbar. At 50 W power, it produces 30 nm/min etch rate for gold (e-beam evaporated thin-films). The same recipe also etches PMMA where the etch rate is approximately same as gold. In case, gold is patterned with e-beam lithographed PMMA larger thickness of PMMA is spin coated than the intended depth of etching in gold.

Appendix B

Coupling nanochannels with outside world

Figure B.1: Device 1: 25 mm $\times$ 25 mm fused-silica coverslip with milli-reservoirs (mR) for the fluidic inlet and outlet. The mRs are connected with the nanochannels through micro-reservoirs as shown in Figure B.2.

Figure B.2: Device 2: (a) In this nanofluidic device, two mRs are clearly visible. (b) The mRs are joined with micro-reservoirs (μ R). (c) In between two μ Rs, the nanochannels are fabricated.

Figure B.3: Nanofluidic device with electrical inputs (platinum electrodes) for electro-osmotic flow; nanochannels are aligned with the optical path (as the objective lens is visible below the device) for 2fFCS measurement.

Appendix C

Carbon nanodots cation- π interaction

Carbon nanodots (CND) are found to be negatively charged. At high pH values above the isoelectric point of silica, when the surface of silica possesses high negative charges, CND repel to this surface. Considering the graphene nanoflake configuration of CND, the strong cation- π interaction due to benzene rings the adsorption-free behaviour of carbon nanodots to fused silica surface is well justified.

*Figure C.1: A drop of highly concentrated carbon nanodots solution was placed on silica cover slip. Using a scanning confocal fluorescence microscope **a.** y-z scan and **b.** x-y scan are performed. In the y-z scan, high photon counts from the aqueous solution of CND was recorded. When x-y scan was performed at the interface of silica and CND solution, few CNDs were observed to adsorb on the silica surface. On the contrary, at high concentration, on the silica surface one should expect large adsorption of fluorescent molecules as frequently observed if similar experiments are performed with organic fluorophore molecules.*

Appendix D

Electro-osmotic flow vs. applied electric field

Figure D.1: 1D diffusion of Atto 488

Figure D.2: 1D electro-osmotic flow of 48bp DNA at different electric field. The applied electric potential per mm were **a.** 27 V, **b.** 47 V, **c.** 90 V, **d.** 120 V, **e.** 175 V, **f.** 220 V, and **g.** 300 V. **h.** Distribution of velocity with applied voltage along the nanochannel.

Appendix E

FIB setup

The FEI Nova NanoLab 600 FIB is a high-performance dual beam system containing a Ga⁺ focused ion beam (FIB) together with a high resolution (1.5nm) scanning electron microscope (SEM). The main purpose of the Nova is site-specific specimen preparation and nano patterning. The system is equipped with a Pt deposition source and a gas injection system for enhanced etching (Iodine) and an Omniprobe for micro manipulation of the specimen and in-situ lift-out of experiments for example TEM lamellae. The piezo-driven 5-axis stage is fully computerized with reliable repeatability and sub μm step size. Up to 6 SEM stubs can be mounted at a time. The software package includes "Run Script" which allows automated imaging and processing tasks such as Auto TEM and an in-house built Slice and View script for 3-D reconstruction from serial sectioning (FIB-tomography) of the sample. Various sample holders and techniques are available to perform top-down and plane-view of lamellae.

Appendix F

2fFCS setup

A modified Olympus IX-71 microscope (Olympus Deutschland, Hamburg, Germany) with an accessible standard side port on the right side was used. The samples were scanned pixel by pixel using a three-axis piezo stage (P-562.3CD, Physik Instrumente, Germany) that was driven with a digital piezo controller (E-710 Physik Instrumente, Germany). A pulsed diode laser (λ 640nm, LDH-D-C-640, PicoQuant) with a pulse width of 100 ps full-width at half-maximum was operated at a pulsing frequency of 80MHz by using a multichannel picosecond diode laser driver (PDL 828 Sepia II PicoQuant). A clean-up filter (Z640/10X, Chroma Technology) was used to block unwanted wavelengths from the laser. The laser beam was then coupled to a polarization-maintaining single-mode optical fiber (PMC-400-4.2-NA010-3-APC- 250 V, Schaefer and Kirchhoff, Germany). The fiber output was collimated to a beam of 9mm diameter by coupling it to an infinity-corrected 4X objective (UPLSAPO4X, Olympus). The excitation beam was then reflected by using a dichroic mirror (FITC/CY5 (51008bs), Chroma Technology) to guide it into the side port of the microscope. The excitation power used was around $10 - 25 \text{ kWcm}^2$. A high numerical aperture objective was used to excite the samples (UAPON 100XOTIRF, 1.49 N.A., Olympus) and to collect the fluorescence emission. The sample is moved with respect to the excitation focus with the help of a piezo stage (P-562.3CD, Physik Instrumente (PI) GmbH & Co. KG) driven by a three-axes piezo controller (E.710.3CD, Physik Instrumente (PI) GmbH & Co. KG). The emission light was then passed through the dichroic mirror and focused onto a $50\mu\text{m}$ pinhole for confocal imaging. After the pinhole it was refocused by using a pair of achromatic lens doublets onto the active area ($\approx 75\mu\text{m}$) of a single-photon counting module (SPCM AQR-13, Perki-Elmer, ($\approx 70\%$ quantum yield of detection at $\lambda = 670\text{nm}$). A band-pass filter (BrightLine HC 692/40, Semrock) was used before the detector to block the back-scattered laser light and a major part of gold photoluminescence. The dark count rate of the detector was less than 150 counts per second. The transistor-transistor-logic (TTL) pulses from the SPCM were recorded with a 2ps time resolution by using a multichannel picosecond event timer and time correlated single-photon

counting (TCSPC) module (HydraHarp 400, PicoQuant) in the time-tagged time-resolved (TTTR) acquisition mode [41]. The periodic sync signal for the time gates was obtained from the Sepia II driver and the markers from the piezo controller representing the start and end of a scan line were recorded as 'virtual photons'. The collected photon data was processed using the commercial software SymPhoTime v.5.2 (PicoQuant) to obtain intensity and lifetime images.

Appendix G

2D carbon nanodots

1. Atomistic models of 2D-CND and Prot-2D-CND

Figure G.1: Armchair and zigzag edges, lattice constant, and relevant bond lengths of 2D-CND and Prot-2D-CND.

In Figure G1, the lattice constant ‘a’ for the 2D-CND model is 2.48 $\text{\AA}$. At the armchair edges the C-C bonds vary from 1.41 $\text{\AA}$ to 1.42 $\text{\AA}$ and at the zigzag edges C-C bonds vary from 1.37 $\text{\AA}$ to

1.43 Å. In the core the C-C bonds vary from 1.42 Å to 1.43 Å. The C-H bonds are 1.09 Å.

In Figure G1, the lattice constant 'a' of Prot-2D-CND is 2.485 Å. The C-C bonds vary from 1.38 Å to 1.45 Å at the armchair edge. In the core the C-C bonds vary from 1.42 Å to 1.44 Å. Near the edges C-C bonds vary from 1.42 Å to 1.46 Å. The C-H bonds are 1.09 Å.

2. HRTEM of GQD

The type II CNDs reported in chapter 4 are GQDs. In Figure G2(a), an experimental HRTEM image of GQD is Wiener-filtered to reduce the background noise of the substrate. An electron intensity profile obtained from Figure G2(a) is also shown in Figure G2(b). Here, the lattice distance matches to 'a'-parameter as shown in Figure G1.

Figure G.2: a. A Wiener-filtered HRTEM image of a GQD. b. Electron intensity profile integrated over the rectangular area shown in 'a'.

3. Singlet and triplet energies

4. HRTEM simulation

Since QSTEM is a simulation using periodic boundary conditions, a large vacuum space was used surrounding all the structures to avoid artefacts due to a reflected wavefunction. Following are experimental parameters used in the simulation:

Table G.1: Lowest singlet (S_0) and triplet (T_1) DFTB total energies of 2D-CND and Prot-2D-CND. The triplet energies were obtained by a spin-unrestricted DFTB calculation.

Model	Triplet (eV)	Singlet (eV)
2D-CND	-4607.1128	-4607.6549
Prot-2D-CND	-4910.2528	-4911.2857

Table G.2: List of HRTEM simulation parameters

Parameters	Values
Energy of electron beam	80 kV
Energy spread of source	0.6 eV
Convergence angle	15 mm
Temperature	300 K
TDS	on
Defocusing distance	0 nm
Focal spread	3 nm
Cs value	0.004 mm
Beam tilt	0°

Here, TDS = on refers to the fact that no Debye-Waller factor was used in the calculation. However, the atoms are allowed to vibrate using a position offset u (3D Gaussian distribution) so that the B factor is $8\pi^2\langle u^2 \rangle$, where $\langle u^2 \rangle$ is the mean squared displacement.

5. Size dependency of optical spectra

In order to determine the optimal model structure that fits to earlier experimental reports, we also investigated other GQD models with different sizes. These structures are $C_{100}H_{26}$ (1.925 nm $\times$ 1.789 nm – C_{100} -2D-CND), $C_{110}H_{28}$ (1.676 nm $\times$ 2.219 nm – C_{110} -2D-CND), and $C_{118}H_{28}$ (1.927 nm $\times$ 2.217 nm – C_{118} -2D-CND). Simulated HRTEM images of these additional models are shown in Figure G2. They are not similar to previously found experimental HRTEM images [19]. We compare their absorption spectra with 2D-CND ($C_{92}H_{26}$) and Prot-2D-CND ($C_{98}H_{28}$) in Figure G3. For C_{100} -2D-CND and C_{110} -2D-CND there are a small number of excitations in the visible range (highlighted in grey – Figure G3). Increasing the number of carbon atoms by eight, going from C_{110} -2D-CND to C_{118} -2D-CND, strongly modifies the spectrum. Many excitations are found in the visible region and some of them match with 2D-CND, such as peaks at 2.10 eV, 2.61 eV, 3.00 eV, and outside the visible range at 3.44 eV. C_{118} -2D-CND could be a possible model

with optical behaviour similar to type II CNDs which are larger than 2 nm but smaller than 2.5 nm (considering our previously reported size distribution of type II CNDs as 2–5 nm) [19].

Figure G.3: HRTEM simulation of (a) Prot-2D-CND, (b) C_{100} -2D-CND, (c) C_{110} -2D-CND, and (d) C_{118} -2D-CND.

6. Excitation energies

Here, we list additional information from the TD-DFTB simulations for the excitation energies (ω_I) within the visible range. Data for the most relevant models from the chapter 5 are provided. In

Figure G.4: **Size dependency.** TD-DFTB calculated absorption spectra of 2D-CND, Prot-2D-CND, C_{100} -2D-CND, C_{110} -2D-CND, and C_{118} -2D-CND. All the curves are broadened with Lorentzians (0.1 eV)

the following tables, the term weight refers to the maximal coefficient in the CI singles expansion given by Casida [9] and ω_{KS} is the Kohn-Sham orbital energy difference for the corresponding single-particle transition.

Table G.3: Excitation energies ω_I , oscillator strengths, weight in CI expansion, and Kohn-Sham orbital energy differences of 2D-CNDs within visible range

ω_I [nm (eV)]	osc. str.	weight	ω_{KS} [eV]
502 (2.47)	0.31	0.91	2.42
475 (2.61)	0.08	0.69	2.42
413 (3.00)	0.10	0.79	2.95
386 (3.21)	0.38	0.69	3.16

Table G.4: Prot-2D-CND

ω_I [nm (eV)]	osc. str.	weight	ω_{KS} [eV]
510 (2.43)	0.08	0.77	2.42
492 (2.52)	0.02	0.61	2.47
456 (2.72)	0.31	0.87	2.58
429 (2.89)	0.30	0.66	2.80

Table G.5: 2D-CND-4OH

ω_I [nm (eV)]	osc. str.	weight	ω_{KS} [eV]
489 (2.53)	0.10	0.71	2.56
466 (2.66)	0.05	0.94	2.62
426 (2.91)	0.25	0.99	2.83
405 (3.00)	0.09	0.99	2.94

7. Detailed investigation of redshifts induced by functionalisation

The MOs associated with the redshifts of ω_I in 2D-CND due to introduction of OH and COOH groups are investigated here.

7.1 OH associated redshift:

Figure G5 shows the electron and hole orbitals associated with the redshift of the 2D-CND excited state at 2.354 eV. The structure of the MOs remains the same until 2D-CND-8OH, which confirms that the peaks for the different functionalisations from 2.354 eV to 2.261 eV actually correspond

Table G.6: 2D-CND-2COOH

ω_I [nm (eV)]	osc. str.	weight	ω_{KS} [eV]
605 (2.05)	0.34	0.84	1.796
528 (2.35)	0.41	0.97	2.171
508 (2.44)	0.27	0.99	2.358
488 (2.54)	0.15	0.62	2.558
393 (3.15)	0.25	0.87	3.132
392 (3.16)	0.24	0.82	3.142

to the same quantum state. However, when all the hydrogen atoms are replaced by OH groups in 2D-CND-full-OH, the redshift is discontinued. The MOs related to the 2.232 eV transition have no similarity with the native MOs for 2D-CND. Until 2D-CND-8OH, the electron orbitals are slightly modified by the OH functional groups but the hole orbitals remain unchanged.

Figure G6 shows the same information for the 2D-CND excited state at 3.009 eV. The structure of the relevant MOs remains the same until 2D-CND-6OH, accompanied by a shift of ω_I from 3.009 eV to 2.900 eV. Here, electron and hole orbitals are affected by the the OH functional groups. The redshift is discontinued from 2D-CND-8OH. Similar MO-structures of 2D-CND are not found for 8OH and full-OH. In other words, more than six hydroxyl groups diminishes the native excitation band near 3.00 eV.

7.2 COOH associated redshift:

Figure G7 shows the electron and hole orbitals associated with the redshift of the 2D-CND state at 2.100 eV. The structure of the MOs remains the same from 2D-CND to 2D-CND-8COOH with an accompanying shift from 2.100 eV to 1.997 eV.

Figure G8 shows the electron and hole orbitals associated with the redshift of the 2D-CND state at 2.477 eV. The structure of MOs remains the same from 2D-CND to 2D-CND-8COOH with an accompanying shift of ω_I from 2.477 eV to 2.418 eV. The hole orbitals are delocalised over the carbon network in all cases (exceptions are 6COOH and 8COOH where two COOH groups contribute). However, the electron orbitals are always affected by the COOH functional groups.

Figure G9 shows the electron and hole orbitals associated with the redshift of the 2D-CND state at 3.009 eV. The structure of MOs remain similar from 2D-CND to 2D-CND-8COOH with an accompanying shift of ω_I from 2.477 eV to 2.418 eV. Here, electron and hole orbitals are both affected by COOH functional groups.

Figure G.5: Upper panel: Redshift of the 2D-CND excited state at 2.354 eV due to OH functionalisation. Lower panel: Shown are the relevant electron and hole orbitals for the states in functionalised 2D-CND that are energetically close to the peak in native 2D-CND. Black tick marks indicate MOs similar to the respective 2D-CND MO, red crosses otherwise.

In Figure G10 we finally show the consistent redshift for the excitation at 3.21 eV in the absorption spectrum.

Figure G.6: Same as Fig. G5, but for the 2D-CND excited state at 3.009 eV. The cross-marks for 8OH and full-OH denote no similar MOs are found related to transitions near 3.009 eV.

Figure G.7: Red shift of 2D-CND excited state at 2.100 eV due to COOH functionalisation. See Fig. G5 for details.

Figure G.8: Red shift of 2D-CND excited state at 2.477 eV due to COOH functionalisation. See Fig. G4 for details.

Figure G.9: Red shift of 2D-CND excited state at 3.009 eV due to COOH. See Fig G5 for details.

Figure G.10: Redshifted transition energies from 3.210 eV due to COOH functionalisation.

About the author

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Academic Preparations

☐ **Doctor of Philosophy in Physics** Oct 12-Aug 16

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☐ **Master of Philosophy in Mechanical Engineering** Nov 10-Sep 12

School of Mechanical Engineering, University of Birmingham, Birmingham.

Thesis title: *Investigation of techniques for the surface roughness characterisation of bovine articular cartilage.*

Review remarks: Excellent.

☐ **Junior Research Fellow in Mechanical Engineering** Jul 09-Oct 10

Centre for Nanoscience and Engineering, Indian Institute of Science, Bangalore.

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Visvesvaraya Technological University, Karnataka.

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Academic Accolades

☐ 2013 Excellence stipend: Full doctoral Scholarship from the International Max Planck Research School for Physics of Biological and Complex Systems (Source: Ministry of Science and Culture (Nds. Ministerium für Wissenschaft und Kultur)).

☐ 2012 Thomas Andrew Common Grants from the Institution of Mechanical Engineers (IMechE).

☐ 2008 Best technical paper while pursuing undergraduate in the ‘International Conference on Total Engineering, Analysis and Manufacturing Technologies’.

Other Research Works

☐ **Indian Institute of Science, Bangalore** 2008 and 2009

Project Trainee (Summer and Winter Intern), Department of Mechanical Engineering, Research. '*MEMS based device for human antigen detection*'

☐ **Indian Institute of Science, Bangalore** 2008 and 2009

Project Trainee (Summer and Winter Intern), Department of Instrumentation and Applied Physics.

Research: '*Surface modification of PDMS using atmospheric glow discharge plasma*'.

☐ **Centre for Food Technological Research Institute, Mysore** 2007

Summer Internship, Department of Fermentation Technology and Bioengineering.

Research: '*Immunoassays and Quantum Dots labelling*'

☐ Subjective Strengths

Quantum mechanics, electrodynamics, nano-optics and plasmonics, density functional theory, nanoelectronics, immunology, theoretical biophysics, active transport, fluid dynamics, non-linear dynamics.

☐ Theoretical/Computational Skills

Quantum mechanical calculations (Tight binding and density functional theory), HRTEM simulations, electrodynamics, finite element analysis. Packages: Python, Fortran, C/C++, Matlab/Octave, LAMMPS, Comsol/Ansys, QSTEM, Gatan microscopy, MeX Alicona, CleWin, L-Edit, Raith NanoSuite.

☐ Experimental Skills

Micro and nanofabrication (single and multi-photon lithography, focused ion beam, e-beam lithography), electron microscopy, atomic force microscopy, e-beam and physical vapour deposition, reactive ion etching, nanostructure synthesis, heterostructure quantum well fabrication, time-correlated single photon counting, optical imaging and spectroscopy, correlative microscopy.

Unrestricted Research Interests

Nanophotonics, quantum well, 2D materials, defect in nanocrystal, quantum optics, single-molecule fluorescence, electron-phonon interactions, fast non-periodic time-dependent first principle calculations, charge transport at low dimension, nanofabrication/lithography developments, nanomechanics of solid-state and soft materials, nanofluidics, and active transport.

Teaching

☐ Optics in Microscale to Nanoscale: From Rainbow to Plasmonics (3×), Göttingen

☐ Introduction to Biophysics (1×), Göttingen

☐ Quantum Mechanics – I (1×), Göttingen

☐ Crash course on Time Dependent Density Functional Theory (1×), Göttingen

Publications

18. **S. Ghosh**, M. Ghosh, A. Kukliansky, A. Natan, G. M. Rao, ‘Experimental and theoretical point defects induced Raman spectra of ZnO nanostructure’, in preparation.
17. **S. Ghosh**, N. Karedla, M. Platen, I. Gregor, ‘High-throughput nanofluidic device for one-dimensional confined detection of single molecules and nanodots’, *arXiv*, 1702.03992, 2017 (DOI).
16. **S. Ghosh**, M. Awasthi, M. Ghosh, M. Seibt, T. Niehaus, ‘Graphene quantum dots with visible light absorption of the carbon core: Insights from single-particle spectroscopy and first principles based theory’, *2D Materials* 3, 041008, 2016 (DOI).
15. M. Ghosh, **S. Ghosh**, M. Seibt, I. Schaap, C. F. Schmidt, G. M. Rao, ‘Deoxidation inhibiting encapsulation of ZnO nanostructures for fluidic and biological applications’, *Applied Surface Science*, 390, 924-928, 2016 (DOI).
14. M. Ghosh*, **S. Ghosh***, H. Attariani, K. Momeni, M. Seibt, G. M. Rao, ‘Atomic defects influenced mechanics of II-VI nanocrystals’, *Nano Letters* 16, 5969-5974, 2016 (DOI). [*equally contributed]
13. **S. Ghosh**, ‘Atomic structure of single dipole emitters: Scanning confocal fluorescence correlated HRTEM’, *Imaging & Microscopy*, 3, 2016 [Invited article - (pdf)].
12. **S. Ghosh**, M. Ghosh, M. Seibt, G.M. Rao, ‘Detection of quantum well induced single degenerate-transition-dipoles in ZnO nanorods’. *Nanoscale*, 8, 2632-2638, 2016, (DOI).
11. M. Ghosh, **S. Ghosh**, M. Seibt, K.Y. Rao, P. Peretzki, G.M. Rao ‘Ferroelectric origin in one-dimensional undoped ZnO towards high electromechanical response’, *Cryst. Eng. Comm.*, 18, 622-630, 2016, (DOI).
10. **S. Ghosh**, G.K. Ananthasuresh, ‘Single-photon-multi-layer-interference lithography for high-aspect-ratio and three-dimensional SU-8 micro-/nanostructures’ *Scientific Reports*, 6, 18428, 2016, (DOI).
9. **S. Ghosh**, A.M. Chizhik, N. Karedla, M.O. Dekaliuk, I. Gregor, H. Schuhmann, M. Seibt, K. Bodensiek, I.A.T Schaap, O. Schulz, A.P. Demchenko, J. Enderlein, A.I. Chizhik., ‘Photoluminescence of Carbon Nanodots: Dipole Emission Centers and Electron-Phonon Coupling’. *Nano Letters* 14 (10), 5656-5661, 2014, (DOI).
8. **S. Ghosh**, M. Kumbhakar, M. Platen, I. Gregor, J. Enderlein ‘Single-molecule fluorescence inside solid-state nanochannels’ *SPIE BiOS*, 895008-895008-7, 2014, (DOI).

7. **S. Ghosh**, J. Bowen, D.E.D. Shepherd ‘Investigation of techniques for the measurement of articular cartilage surface roughness’ *Micron*, 44, 179-184, 2012, (DOI).
6. **S. Ghosh**, G.K. Ananthasuresh ‘A note on high aspect-ratio SU-8 micromechanical structures using mask-less direct laser writing’ *ASME* 2012 International Manufacturing Science and Engineering Conference + 40th North American Manufacturing Research Conference (NAMRC40), 2012, (DOI).
5. **S. Ghosh**. ‘Investigation of techniques for the surface roughness characterisation of bovine articular cartilage’ M.Phil. thesis, *University of Birmingham*, 2012, (DOI) Excellent comment by the reviewers.
4. **S. Ghosh**, D.E.D Shepherd, K. Jiang ‘Preliminary Investigation of Surface Characteristics of Articular Cartilage with 3-D Tomography’ *IEEE* Nanoscience, Technology and Societal Implications, 2011, (DOI).
3. V. Anand*, **S. Ghosh***, M. Ghosh*, G.M. Rao, R. Railkar, R.R. Dighe, ‘Surface modification of PDMS using atmospheric glow discharge polymerization of tetrafluoroethane for immobilization of biomolecules’, *Applied Surface Science* 257 (20), 8378-8384, 2011, (DOI).
* equal contributions.
2. **S. Ghosh**, G.K. Ananthasuresh, ‘High aspect ratio SU-8 microstructures using Laser writing’, Fourth International *ISSS* Conference on Microsystems, Smart Materials and Structure, Publisher: *Institute of Smart Structures and Systems*, 2010.
1. M. Ghosh, **S. Ghosh**, ‘MEMS based device for Human antigen detection’, *International Conference on Total Engineering, Analysis and Manufacturing Technologies*, 2008.

Academic Activities

◁ *Selected Scientific meetings and talks:*

5. ‘**Microscopy Conference 2015**’. Abstract accepted - oral presentation - ‘Fluorescence confocal scanning correlated high resolution transmission electron microscopy’ (Göttingen).
4. ‘**Nanoscale Assemblies of Semiconductor Nanocrystals, Metal Nanoparticles and Single Molecules: Theory, Experiment and Application**’ *NANOSA 2015*, Abstract and application accepted for participation (MPI-PKS, Dresden).
3. Invited talk (Nanocrystals - Single dipole emitters and Nanochannel - 1D photonic spectroscopy) at the Centre for Nano Science and Engineering, Indian Institute of Science, Bangalore, 20th November 2014.
3. ‘**High performance models for charge transport in large scale materials system 2014**’. Abstract accepted - poster presentation - Nanocrystals - Single dipole emitters: Electron-phonon interaction

in crystalline nanostructures' 2014, (BCCMS, Bremen).

2. '20th International Workshop on Single Molecule Spectroscopy and Ultrasensitive Analysis in the Life Sciences', Abstract accepted - poster presentation 2014, 'Single molecule spectroscopy inside 1D nanochannels' (Pico Quant, Berlin).

1. 'Photonics West 2014'. Paper accepted - oral presentation - 'Single-molecule fluorescence inside solid-state nanochannels' (San Francisco)

◁ **Conference Organised**

2. Third Infinity 2015 - A conference on complex systems, Göttingen, Germany ([website](#)).

1. Third Infinity 2013 - A conference on complex systems, Göttingen, Germany ([website](#)).

◁ **Student Representative**

Göttingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences (GGNB) 2012-2013 elected as a student representative of 10 graduate schools ([GGNB](#)).

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Favourite books

Recent: *Thing Explainer* and *What if?* by Randall Munroe.

All time: *The Feynman's Lecture on Physics (Vol. I, II, and III)* and *Quantum Electrodynamics* by R. P. Feynman

Surely You're Joking, Mr. Feynman! by Ralph Leighton and R. P. Feynman

Feynman's Rainbow by Leonard Mlodinow,

The Theoretical Minimum book series by Leonard Susskind,

For the love of physics by Walter Lewin,

One Two Three... Infinity by George Gamow,

Hyperspace and *Physics of the impossible* by Michio Kaku.

Brian Greene's digital resources at <http://www.worldscienceu.com/>,

Sherlock Holmes by Arthur Conan Doyle,

Feluda and *Shonku* series by Satyajit Ray,

Rabindra Rachanabali (total body of Tagore's works) by Rabindranath Tagore.