Chapter 2

Experimental Techniques
In previous chapter basics of semiconductor nanocrystals (NCs), and synthesis methods are outlined. Colloidal synthesis route is the most widely used for synthesis of NCs. The route is favored because of the ease of synthesis, and the control over shape, size, and morphology of the NCs. This versatile route allows the synthesis of doped and compositionally tunable NCs. The synthesis details of NCs studied in this thesis are presented in this chapter. The various experimental techniques used to characterize the NCs are also briefly presented in this chapter.

The NCs are synthesized by high temperature wet chemical method. High temperature method is favored over the low temperature, because of the quality in terms of crystallinity and absence of the defects in NCs. The high crystallinity, and absence of defects is reflected in photoluminescence (PL) spectrum of the particles as highly luminescent band edge emission. One of the advantages of this high temperature method is the use of organic solvents which many times can be used as capping agents to passivate the dangling bonds at the surface of the NCs. Moreover, high temperature method provides control on size and size distribution of NCs, which is very difficult to obtain by low temperature method. The size of the NCs can be tuned by varying the growth time, precursor concentration, and/or the reaction temperature. Even though the size distribution of NCs prepared by high temperature method is good, it can further be improved by "size selective precipitation". In size selective precipitation method, solvent-non solvent pair is used to narrow down the size distribution via centrifugation. Particles are centrifuged for fixed time so that only larger particles will be settled down. The supernatant contains relatively smaller particles; centrifugation of this supernatant further selects the larger particles from the supernatant. In this way different sized particles were obtained from the same batch. Obviously the difference in size of the particles (collected in separate centrifugation) is very small and can practically be ignored. However, the size distribution among these is very narrow (<5%). Size distribution of particles can also be controlled by choosing proper injection temperature. One can achieve a decent size distribution by injecting the precursor rapidly so that almost all the nucleation takes place at constant temperature. Owing to these advantages, high temperature wet chemical method is employed for synthesis of NCs used in this study.
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2.1 Preparation techniques

NCs studied in the present work are synthesized by chemical route. High temperature chemical method provide control over size, shape, and morphology.\textsuperscript{1} the typical reaction taking place is depicted in Fig. 2.1. The underlying details are presented below.

2.1.1 Preparation of undoped ZnSe, ZnSe\textsubscript{1-y}Te\textsubscript{y}, and Mn doped ZnSe\textsubscript{1-y}Te\textsubscript{y} NCs

**Synthesis of undoped ZnSe:** ZnSe NCs were synthesized by modifying the method reported by Hines \textit{et al.}\textsuperscript{2} The size of NCs in this method can be controlled by varying injection temperature.\textsuperscript{2,3} At lower injection temperatures ZnSe with smaller sizes is formed.\textsuperscript{2,3} In a typical reaction, 10 g octadecylamine (ODA) was degassed and heated to 260 - 300 °C. Diethyl zinc (0.8 mM), 1 M selenium (Se) and 2 mL trioctylphosphine (TOP) complex was prepared in glove box in nitrogen atmosphere and swiftly injected in hot ODA solution. The reaction was allowed to proceed for 5 min. Reaction solution was cooled to 60-70 °C. To isolate nanocrystals, the reaction solution was redispersed in 20 mL butanol and precipitated with 20 mL methanol. After centrifugation, the settled NCs were retained and the supernatant was discarded. The dispersion, precipitation, and centrifugation process was repeated 3-4 times to remove un-reacted chemicals and excess capping agent. The collected NCs can be dispersed in variety of organic solvents like butanol, hexane, chloroform etc.

**Synthesis of ZnSe\textsubscript{1-y}Te\textsubscript{y} NCs:** ZnSe\textsubscript{1-y}Te\textsubscript{y} NCs were synthesized by the method similar to that of undoped ZnSe NCs. However in this reaction along with Se small amount of tellurium (Te) was incorporated. In order to dissolve Te, TOP-Se-Te solution was slightly heated. The organometallic precursor containing diethyl zinc (0.8 mM) and a mixture of Se and Te powder (1M in total) in TOP was rapidly injected into the hot ODA at high temperature. The amount of Se and Te was varied to attain different Te doping levels. Particle size was varied by injecting precursor at different temperature (260 – 300 °C). The particles were isolated as discussed above and a fine powder of ZnSe\textsubscript{1-y}Te\textsubscript{y} NCs was obtained. About 5% Te can be incorporated by this method. When reaction was carried for higher amount of Te NCs were observed to be of very poor quality showing no optical absorption or photoluminescence.
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Synthesis of Mn doped ZnSe and ZnSe\textsubscript{1-x}Te\textsubscript{x} NCs: Mn was incorporated in ZnSe and ZnSe\textsubscript{1-x}Te\textsubscript{x} NCs using the same protocol, reported by Norris \textit{et al.}\textsuperscript{5} The precursor, dimethylmanganese (MnMe\textsubscript{2}) was prepared by using 1.5 mL of 0.2 M manganese chloride slurry in tetrahydrofuran (THF) with 0.2 mL of 3 M methylmagnesium chloride in THF. MnMe\textsubscript{2} solution was diluted with 5.4 mL of toluene. Part of the MnMe\textsubscript{2} solution was added to 2 mL of TOP, 1 mL of 1 M Se and Te in TOP, and 0.18 mL of 1 M diethyl zinc. The organometallic precursor was rapidly injected into a vigorously stirred reaction vessel containing degassed ODA at 280 °C under dry nitrogen. The particles were isolated and a fine powder of NCs was obtained. Mn concentration was varied in the reaction solution by varying the amount of MnMe\textsubscript{2} in the organometallic precursor. Zn\textsubscript{1-x}SeMn\textsubscript{x} NCs were also synthesized using the same method. Mn precursor concentration in the reaction solutions was varied from 2.5 to 10.0 %.

2.1.2 Synthesis of compositionally tuned Zn\textsubscript{1-x}Cd\textsubscript{x}Se NCs

Synthesis of graded core/shell NCs: Zn\textsubscript{1-x}Cd\textsubscript{x}Se NCs were synthesized chemically by modifying the method used for formation of cobalt doped ZnSe NCs.\textsuperscript{6} In a typical synthesis zinc acetate dihydrate (Zn(Ac)\textsubscript{2}.2H\textsubscript{2}O) and cadmium acetate dihydrate [Cd(Ac)\textsubscript{2}.2H\textsubscript{2}O,] in total 0.4 mM, oleic acid (OA, 1.5 mL), octadecene (ODE, 16 g), and hexadecylamine (HDA, 1.6 mM) were loaded in three neck flask and purged at 120 °C for 1 hour. The solution was heated to 300 °C. A solution of Se (2.4 mM), TOP (2 mL), and ODE (1.5 mL) was prepared in nitrogen atmosphere in glove box and rapidly injected in reaction solution at 300 °C. The NCs were annealed at 290 °C for 10 min. The reaction solution is cooled to 80 °C. In order to precipitate nanocrystals, reaction solution was dispersed in toluene, and precipitated using ethanol. NCs were separated by centrifugation. On centrifugation two separate layers of NCs solution and unreacted supernatant are observed. The unreacted supernatant was discarded and NC solution was collected by decantation. The procedure of dispersion, precipitation, centrifugation, and decantation was repeated several times. Initial oily sample turned powdery after several washing steps. Pure ZnSe is also synthesized using the same method by not using Cd(Ac)\textsubscript{2}.2H\textsubscript{2}O in the reaction solution. The typical chemical reaction taking place in this synthesis is depicted in Fig. 2.1. Amount of oleic acid controls the size in this reaction. More amount of oleic
acid yield larger NCs. Shape, size, and optical properties of NCs were observed to be unaltered even without use of HDA.

FIG. 2.1 Typical chemical reaction for synthesis of ZnSe and compositionally tuned semiconductor NCs in non coordinating solvent using Zn and Cd carboxylate and TOP as precursors [adapted from P. Reiss, New J. Chem. 31, 1843 (2007)].

**Synthesis of alloy Zn$_{1-x}$Cd$_x$Se NCs:** To form an alloy NCs, graded core-shell NCs were annealed for longer time duration. Annealing for longer time duration facilitate diffusion of Zn from shell to the core of NCs forming a uniform alloy. The NCs are characterized by various techniques. The experimental techniques are outlined below.

### 2.2 Characterization Techniques Used

#### 2.2.1 X-ray diffraction

X-ray diffraction (XRD) is most widely used technique to determine the structure and phase of the solid. It can be used to characterize thin films, bulk materials and NCs. The working principle of this technique is the diffraction of electromagnetic radiation by crystalline solid. Diffraction is possible only when the wavelength of incident electromagnetic waves is comparable to the interplaner spacing i.e. $d \sim \lambda$. Here, $d$ is the interplaner spacing and $\lambda$ is wavelength of incident radiation. Wavelength of x-rays is typically few Å. Constructive interference occurs for which Bragg equation $2dsin\theta = n\lambda$ is satisfied.
Strain present in the lattice is reflected as change in the lattice spacing in the XRD pattern. Using XRD one can determine defects produced due to strain in the sample. For crystals with long range periodicity the XRD peaks are intense and sharp. Lack of extended periodicity in NCs broadens the XRD peak. As a result, broadening in peak is used to determine the size of NCs, using Scherrer formula.\(^7\)\(^-\)\(^9\) The Scherrer formula is
\[
\text{average crystallite size} = \frac{\lambda}{\beta \cos \theta_b}
\]
Here A is a constant that depends on shape and morphology of NCs. For spherical particle A=0.9, \(\beta\) is full width at half maximum of particular XRD peak, and \(\theta_b\) is angle of the peak in consideration. The average crystallite size can be estimated by XRD.

XRD can also used to study semiconductor alloy formation. In case of alloy, change in lattice constant is governed by Vegard’s rule. Vegard’s rule states that lattice constant of alloy vary linearly with composition. If \(A_{1-x}B_x\) is alloy then lattice constant \(A_{1-x}B_x\) can be calculated as

\[
A_{1-x}B_x = (1-x)A + xB
\]

where \(A\) is lattice constant of alloy \(A_{1-x}B_x\), \(B\) is lattice constant of B, \(A\) lattice constant of A. Using equation (1) and XRD one can confirm alloy formation and estimate composition.

FIG. 2.2 Schematic diagram of x-ray diffractometer.
Figure 2.2 shows the typical block diagram of XRD machine. X-ray diffractometer comprises of (i) source, (ii) sample holder and (iii) detector. The source, sample, and detectors are placed in such a way that the sample is at the center of the circle and detector on the peripheral of the circle.

Main advantage of XRD is the ease with which the structure can be determined, the ability to find strain present in the sample, and determination of alloy composition. The main disadvantage of XRD is that, the size determined is an average size of crystallite. The details of shape and size distribution in the sample cannot be determined by XRD.

In the present work, XRD is used to determine the phase and alloy formation. All the measurement were performed on Bruker D8 advanced powder diffractometer, using Cu K$_\alpha$ ($\lambda=1.5402$ Å) as an incident radiation.

2.2.2 Transmission electron microscopy (TEM), selected area electron diffraction (SAED) and energy dispersive analysis of x-ray (EDAX)

TEM is the most widely used to find out shape, size, and morphology of NCs. Direct visual image of nanocrystals with atomic precision is obtained by TEM. The working principle of optical microscope and TEM is same. Only difference between TEM and optical microscopy is use of electrons instead of photons. Ideally, the resolution of optical microscope is diffraction limited (i.e. $\sim \lambda/2$). Wave associated with energetic electron is much smaller than the wavelength of light. So, one can attain atomic scale resolution with electron microscope. Practically, much higher resolution (even better than 0.2 nm) can be achieved in this technique. Moreover, electron diffraction from a selected area can be obtained. In TEM crystallographic defects can also be revealed by varying the orientation of the crystal with respect to the incident beam. Disadvantages of TEM are, expensive instrumentation, time consuming sample preparation, and sensitivity of some materials to electron beam radiation which results in loss of crystallinity. Samples need to be stable in vacuum.

The quantitative measurement of shape, size and size distribution of nanocrystals is routinely performed by TEM. Moreover, simultaneous determination of the structure with the higher sensitivity by SAED is feasible. Better imaging resolution of TEM can be exploited to determine the structural changes like strain and stress produced by core/shell sample along with the distribution of composition in
single NC etc. This information is crucial to explain the observed optical behavior and energy level variation. Using energy dispersive analysis of x-rays (EDAX) one can determine the elemental composition and its relative concentration in the sample. EDAX in conjunction with TEM and x-ray photoelectron spectroscopy (XPS) gives the exact composition of NCs.

TEM is used mostly to determine the size, shape and size distribution of NCs in the present work. TEM measurements on NCs studied in present endeavor were performed by Tecnie G² 20 ultra twin microscope operating at 200 kV. Diluted solution of sample was coated on copper grid. The solution was allowed to evaporate before recording the TEM. The ultimate resolution available with this microscope is 1.4 Å.

2.2.3 Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

Any element absorbs or emits its characteristic line corresponding to the atomic energy level difference which essentially varies with the atomic number value. Thereby, measuring the absorption and emission wavelength from atoms in the material is helpful to determine elements in the material. This concept is the heart of ICP-AES technique. The material whose composition is to be determined, is atomized and the atoms are excited by plasma. The intensity of emitted light is directly proportional to the amount of atoms present in the sample. The emitted light intensity as a function of wavelength is collected. For quantitative analysis, solution of known concentration of element under consideration is prepared. The emitted light intensity from sample is compared with intensity of reference signal and concentration of the element is calculated.

This technique is very powerful and can detect even trace amount of impurities (few microgram per kg) present in the sample. In the present work, determination of the exact composition of sample and presence of impurity is carried out by ICP-AES. All the measurements in the present work are performed on Spectra Arcos spectrometer. This spectrometer (750 mm focal length) is capable of collecting emission in the region 130 to 770 nm using 32 linear CCD detectors with a resolution of 8.5 pm for 130 to 340 nm and 15 pm for higher wavelengths.
2.2.4 Optical Absorption

Band gap of semiconductor depends not only on size of NCs,\textsuperscript{16-17} but also on the composition.\textsuperscript{18-20} In order to understand the physical properties of semiconductor, band gap determination is mandatory. When photon is incident on semiconductor, photon with energy below band gap will be transmitted where as photons with energy more than or equal to band gap will be absorbed. On-set of the absorption is the band edge of the material. This technique is extended for NCs. Here, the energy levels are discrete hence rather than a continuously increasing absorption above band edge, distinct peaks are observed. The intensity of the peaks depends on the absorption cross section, concentration of the material, and path length of light in the material. The relation between these parameters is given by Beer-Lamberts law.

\[ A = abc \]

Where \( A \) is the absorbance, \( a \) is absorption cross section, \( b \) is path length in the material, and \( c \) is number of absorbing species.

In the present work, optical absorption is used to probe variation of band gap with composition and size. Optical absorption measurements were carried out on Perkin Elmer Lambda 950 spectrophotometer. This spectrometer is fitted with double holographic grating (1440 lines/mm blazed at 240 nm for UV visible region, and 360 lines/mm blazed at 1100 nm for NIR region). This spectrometer is able to record the spectrum in a wavelength range from 175 to 3300 nm. The slits for UV –VIS region were maintained at 0.5 nm for all measurements presented in the thesis.

2.2.5 Photoluminescence and photoluminescence excitation (PLE) spectroscopy

When semiconductor is excited with photon energy greater than the band gap energy, exciton is created. Radiative recombination of the exciton gives out the energy in the form of light. This process of exciting a sample using light and detection of emitted photon is called as PL. Due to defects or impurities present in the sample, the emitted photon can have energy much lower than the band gap energy. This technique is thus useful to detect defects present in the material and is extensively used to study Mn doped ZnSe,\textsuperscript{21-23} ZnS,\textsuperscript{24-33} and CdS\textsuperscript{34-37} NCs.

The PL spectrometer comprises of source, excitation monochromator, emission monochromator, detector and focusing optics as shown in Fig. 2.3. For PLE
white light source is required. Xenon or mercury lamps are usually used as a source. Desired wavelength from white light source is selected by excitation monochromator. Focal length, grating, exit and entrance slit width determines the resolution of the monochromator. Higher groove density grating and long focal length monochromator is used when good resolution is needed. However higher density grooves and long focal length monochromator spreads the light resulting in lower throughput of the spectrometer. For a given monochromator with fixed grooves density, resolution can be controlled by entrance and exit slits. Smaller the slit width higher will be the resolution and lesser is the light intensity. One needs to optimize the resolution and light throughput to get a good signal to noise ratio. Optics focuses excitation light on the sample and emitted light at the exit slit of emission monochromator as shown in Fig. 2.3. Emission monochromator separate the emitted light in its component. Photomultiplier tube (PMT) or charge coupled device (CCD) are generally used as a detector. However for recording PLE spectrum PMT is used. With CCD a range of wavelength is scanned in a single shot, hence it is not possible to record PLE using CCD. The scan speed of spectrometer can be improved significantly with CCD, resulting in faster acquisition. PMT scans the entire wavelength region point by point making the data acquisition time consuming. It is easier to select component of light with PMT. This is a reason why PMT is used when PLE is to be recorded. Emission monochromator and detector together gives intensity profile as a function of energy of emitted light. For recording PL spectrum, emission wavelength is selected by fixing excitation monochromator and emission monochromator is scanned. In other words intensity of emitted light as a function of emission wavelength is recorded in PL. For PLE spectroscopy, emission monochromator is fixed and excitation monochromator is scanned. PLE spectroscopy records intensity of selected emission wavelength as a function of excitation energy. To reduce thermal noise and improve signal to noise ratio PL and PLE measurements are performed at low temperature. Liquid helium filled or liquid He closed cycle cryostats are used to reduce the temperature of the sample. Sample is mounted on the cold head of the refrigerator. The measurements reported in this thesis is performed on liquid He closed cycle cryostat.
FIG. 2.3 Schematic of PL spectrometer, the sample is placed in the sample holder which can be attached to a cryostat refrigerator to study PL and PLE at low temperature.

PL spectroscopy is used to probe the defects present, if any. Not only radiative but also presence of non-radiative defects can be probed by measuring the quantum yield of the material. PLE technique is used to probe the energy transfer pathways to the emission bands. Information of higher lying energy state of NCs can be fetched by PLE spectroscopy. In other words PLE is complementary technique to optical absorption. In PLE experiment, emission monochromator is placed at a wavelength of interest and excitation wavelength is varied. Using PLE, one can probe the higher lying energy levels contributing to emission from levels of interest. Changing the size, cause variation in the band gap and hence the emission from band edge. In PLE a small portion of wavelength is selected. This wavelength band arises from small portion of particle having almost same size. Changing the detection wavelength (emission wavelength) one can select different portions of particle sizes. In this way PLE can be used to probe size selective energy levels. One major problem with PLE is the background correction. Background arises because the intensity of lamp is not uniform and it has a characteristic peaks of elements used (Xe, Hg). The variation in excitation profile is reflected in emission intensity. To get an actual data one needs to correct the background. Background correction is usually performed with dye with saturated concentration such that the emission intensity is independent of excitation wavelength and only depends on the excitation light intensity. PLE spectrum of
saturated dye is the lamp profile or background, and is used to correct the sample data. Various dyes are available for data correction in different spectral region. In the Present work, data correction was performed by using rhodamine B (RhB). The concentration of 3 g/L is used. This concentrated solution absorbs virtually all radiation from 220 to 600 nm. Hence, quantum yield and emission maxima are independent of excitation wavelength.

PLE is employed to determine the energy level structure of NCs as a function of composition. Using this information energy level evolution in semiconductor alloy NCs is determined. PLE is also used to determine the energy transfer pathways to defect or trap levels. Using PLE, change in the path of energy transfer by isoelectronic center formation is probed. PL and PLE measurements on doped NCs are performed at room temperature and at 77 K with Perkin Elmer LS55 spectrometer. LS55 spectrometer uses xenon flash lamp pulsed at 50 Hz, with pulse FWHM < 10 µs and power equivalent to 20 kW at continuous operation. The spectrometer has Monk-Gillieson type monochromators, covering the wavelength range for excitation 200-800 nm, and for emission 200-900 nm. The slit width was fixed to 5 nm for all the measurements so that a resolution <1 nm is obtained. The emitted light is detected by a PMT. PL and PLE of graded core/shell and alloy NCs at room temperature and 10 K are recorded with assembled PL setup. It is equipped with Jobin-Yvon 450 W Xe lamp as source, TRIAX 180 monochromator as an excitation monochromator, iHR 320 as emission monochromator, a photomultiplier tube, and Janis CCS 150 optical cryostat. All the measurements were performed with resolution 0.5 nm. Peak positions in PLE are extracted by fitting the corrected spectrum using multiple Gaussian peaks. Fitted spectrum with chi square close to one and residue with no derivative component were accepted as a final fitted spectrum.

2.2.6 X-ray photoelectron spectroscopy (XPS)

XPS can be used to determine the composition, ionization state, and position of element in the sample. Photoelectrons generated by x-rays are energy analyzed. The binding energy of photoelectrons is deduced from the kinetic energy of the ejected electron

\[ KE = h\nu - BE \]
where, KE is kinetic energy of photoelectron, BE is binding energy of photoelectron and \( h\nu \) is incident photon energy. Binding energy of photoelectron crucially depends on the environment of the atom. In short, measuring the binding energy gives information about chemical composition and ionization state of the constituents.

In the present work, x-ray photoelectron spectroscopic analysis is carried out on VG Micro-Tech ESCA 3000 instrument at a pressure, below \( 10^{-9} \) mbar. The sample is coated on silicon wafer. Charging effect is corrected by using graphite powder. C 1s is used as an internal reference. All parameters except peak position, and width used for fitting were kept constant. Fitting components with chi square close to one and residue with no derivative signal is accepted as final fitted spectra. In the present work, XPS is used to explore the composition profile in nanocrystals.

### 2.2.7 Electron paramagnetic resonance (EPR)

In EPR the spin degeneracy of a paramagnetic ion is lifted by application of magnetic field, and the transition between these degenerate energy levels can be used to get information of charge state and environment of the paramagnetic ion. Lifting degeneracy of single electron is explained in Fig. 2.4 (a). The transition between these states can be probed by using a microwave radiation. The energy levels of paramagnetic ion can be further splitted by the nuclear magnetic moment (I) of the paramagnetic ion. This splitting by nuclear magnetic moment is called as hyperfine splitting. Every paramagnetic ion will show its characteristic EPR spectra depending on \( S_z \) and nuclear magnetic moment of the ion. In EPR measurements, excitation microwave source is kept constant and the applied magnetic field is varied. In order to improve the signal to noise ratio the signal is recorded in derivative form. The selection rules for the transition are \( \Delta S_Z = 1 \) and \( \Delta I_Z = 0 \). In general for paramagnetic ions \( 2I + 1 \) transitions are observed. For example Mn\(^{2+}\) has a nuclear magnetic moment 5/2 hence in total 6 transition lines will be observed in the EPR spectrum of the Mn\(^{2+}\) ion as shown in Fig. 2.4.

EPR can be effectively used for probing the location of paramagnetic ion in the lattice. Crystal in which the paramagnetic ion is incorporated, influences ‘g’ (Lande’s g factor) value. Hence using this technique one can determine whether the paramagnetic ion is substitutionally doped or not.\(^{22}\) EPR is also exploited to determine the position of paramagnetic ion in the sample.\(^{23}\) In order to determine the position of
the paramagnetic ion, change in symmetry around paramagnetic ion at surface and near surface is examined.

**FIG. 2.4** (a) Splitting of spin degenerate energy level in single electron leading to two distinct energy levels. (b) Hyperfine splitting of splitted energy level in Mn$^{2+}$ ion due to nuclear spin magnetic moment $I_z$.

In the present work, EPR measurements were carried out on Bruker EMX spectrometer operating at 9.1 GHz (X band) at liquid nitrogen temperature (77 K). EPR technique is employed to confirm substitutional doping of paramagnetic ions, and estimation of the position impurity in NCs.
2.2.8 Time resolved photoluminescence (TRPL)

The exciton decay dynamics can be understood from TRPL measurements. In TRPL measurements an excitation pulse is incident on the sample and time needed to decay the emission intensity is evaluated. Emission process is random and decay time is an average relaxation time. PL rate depends on radiative rate (excited state to ground state), non-radiative rate (excited state to non-radiative trap state) and energy transfer rate (excited state to another excited state).

\[ \frac{1}{\Gamma} = \frac{1}{\Gamma_{\text{rad}}} + \frac{1}{\Gamma_{\text{non-rad}}} + \frac{1}{\Gamma_{\text{ET}}} \]

where, \( \frac{1}{\Gamma_{\text{rad}}} \), \( \frac{1}{\Gamma_{\text{non-rad}}} \) and \( \frac{1}{\Gamma_{\text{ET}}} \) are decay rate of sample, radiative decay rate, non-radiative decay rate and energy transfer rate respectively. Determining the decay rate gives the information of radiative, non-radiative, and energy transfer rate from the state under consideration.

To detect the TRPL, time correlated single photon counting (TCSPC) method is used. This system measures the time elapsed between excitation and detection of emitted photon. Histogram of these events is plotted. The resultant plot is exponential decay plot. The decay curve could be single exponential or multi-exponential. Few studies suggest that the multi-exponential decay can arise from change in the charge state of surrounding medium of matrix\(^{42,43}\) or fluctuation in non-radiative decay paths.\(^{44}\) However, the origin of multi-exponential decay is not well understood. A reference signal generally called as instrument response function (IRF) is collected and subtracted from the data. The data is then fitted with different exponential fitting functions and relaxation time is extracted. The fitting parameters are so chosen that the chi square value is close to one and no derivative feature in the residual signal is observable. Figure 2.5 is a schematic of the TCSPC system. The laser line is divided in to two signals one reference and other excitation. The experiments start with a pulse. The reference pulse starts the electronic counter. The electronic counter consists of constant function discriminator (CFD) and time to amplitude converter (TAC). CFD accurately measures the arrival of signal and TAC generates a ramp pulse which increases linearly as time elapsed. Single photon detector detects emitted photon. Signal of single photon detector is fed to CFD which sends a signal to stop the voltage ramp. TAC now contain a voltage proportional to time elapsed between excitation and emission. Electronic circuit converts this voltage to a numerical value. Histogram is generated by repeating this procedure several times with a pulsed light...
source. When the photon is detected, the corresponding time is stored by adding 1 in the memory assigned for the time. After many photons in the memory histogram of detection time builds up. This is illustrated in Fig. 2.6.

**Fig. 2.5** Schematic diagram of TCSPC system

**Fig. 2.6** Detection and generation of histogram in TCSPC different memory channels are used for different period and as peaks arrives in the particular period value of memory is increased by one [adapted from http://www.becker-hickl.de/pdf/tcspc11.pdf]
Life time of NCs was recorded on Edinburgh Instruments, FLS 920 system. Sample is excited with nanosecond flash lamp operating with 6.8 kV voltage and 40 kHz pulse frequency with 0.1 ns time resolution. IRF signal is recorded by scattering the light and recording the signal with same experimental parameter.

2.2.9 Single particle imaging and spectroscopy

2.2.9.1 Instrumentation: All the methods described above provide information of ensemble. However individual NCs behave differently.\textsuperscript{45,46} In order to study the behavior of single NCs single particle imaging and spectroscopy is employed in wide optical field configuration. Figure 2.7 shows diagram of typical wide field optical microscope. The working principle of this technique is epi-fluorescence. In epi-fluorescence technique, excitation and emission light is passed through the same objective lens. Emitted light is fed to a spectrometer or grating to resolve the components. There are three basic components of the fluorescence microscope; (a) excitation (b) illumination and collection optics, and (c) detection. With wide field microscope it is possible to excite large area and collect the signal from several particles in a single run. The instrumental details are explained below.

(a) **Excitation**: The excitation light is provided by a continuous wave air cooled argon ion laser system. (Mellos Griot, Model no 35-MAP-321-100). The typical power of 488 / 514 nm line of Ar\textsuperscript{+} ion is 60 mW. All single particle experiments were carried out on 488 nm. Combination of neutral density filters (not shown in figure) were used to adjust the power. The neutral density filters are placed in front of beam expander. Usually a power between ~0.1 – 10 mW is used for imaging and spectroscopy. The light from laser is expanded using a beam expander. The beam expander and lens is used to focus the laser beam at back focal plane of the objective lens. Focusing at the back focal plane is important because it allows using the microscope in wide field mode. Area of illumination can be controlled by the divergence of the beam by beam expander. The dichroic mirror is chosen in such a way that it reflects the excitation beam and transmit the emitted light.

(b) **Illumination and collection optics**: Most important part of the illumination optics is the objective lens. Nikon oil immersion high numerical aperture (NA =1.49, 60X) objective lens is used. The excitation beam is focused at the back focal plane of this objective. High NA allows collection of light emitted from the sample with better
signal to noise ratio. The arrangement is called wide field because it illuminates large area of the sample and collect signal from the complete area. Figure 2.7 explains how focusing the excitation beam at the back focal plane allows to illuminate large area. The collected signal passes through a dichroic mirror which cutoffs most of the excitation signal. This light then passes through laser edge filter and notch filter which eliminate the residual excitation light and the signal so obtained consist of pure emission signal from the sample. A commercial inverted microscope (Nikon TE 2000) is used to perform these experiments. In order to perform the spectroscopic measurements the detector is placed away from the microscope exit. The magnification and focus of the image is maintained by using two lenses called as relay lenses. Imaging is performed by feeding the light from NCs through the relay lenses to the CCD.

FIG. 2.7 Wide field fluorescence optical microscope setup used for individual NCs imaging and spectroscopy. Focusing the excitation beam at the back focal plane excite large volume of area, where as luminescence from only particles in focus of objective is collected.
Figure 2.8 shows the image and the spectra recorded with sample. In order to make spectroscopic measurements a slit is placed between the first relay lens and microscope exit port. A grating (70 grooves/mm) is placed between the two relay lenses. Smallest possible slit width is selected to avoid interference from the neighboring NCs. The slit selects slice of the image, emission from the particle in the slice is then dispersed by the grating and dispersive image is produced. Smaller groove density grating allows sufficient light to be detected by the detector and imaging of zero order and first order diffraction from the grating. Zero order diffraction is nothing but the NC image. And the first order diffraction is the spectra or energy dispersive image of NCs. In this way the image of dot and its corresponding spectra can be recorded as shown in Fig. 2.8 (b).

(c) Detection: In order to record the blinking event a fast and efficient detector is needed. In the present work the measurements were performed by using a CCD camera. The light from the grating is fed to CCD camera through a second relay lens. Image and spectra are recorded with Peltier cooled (-25 °C) interline high efficiency (maximum efficiency 65% at 550 nm) CCD camera (DVC Co. 1412 monochromatic). The camera is electronically controlled by DVC view, a window based software program for real time viewing and image acquisition.

2.2.9.3 Sample preparation: A film of very dilute colloidal dispersion in polymethylmethacrylate (PMMA) was cast on a cleaned fused silica cover slide. The concentration of NCs is ~0.5 - 1 nM. The amount of PMMA is ~2 mg/mL. The spin casting is performed at ~2000 rpm using a spin coater. The thickness of the matrix is within 200 nm. For imaging the separation of the NCs in the matrix is ~1µm or more.

2.2.9.4 Data Analysis: The acquired images are analyzed using Image J (NIH) software. 

Image analysis: A film of images with acquisition time of 50-100 ms is recorded. All the images were analyzed after proper background fitting due to slight modulations in excitation field. These films were then analyzed for blinking behavior. The variation in intensity of individual dots is extracted from the image in binary data and is plotted in Origin 8. An intensity more than background intensity is used as reference OFF state. Using Origin 8 statistical analysis is performed to find out % ON and OFF time of the NCs. More than 150 dots were analyzed from each sample to generate reliable
statistical data. \%ON and \%OFF time data from individual NCs is statistically analyzed to get the ON and OFF statistics of the NCs. Figure 2.9 shows a typical blinking behavior of the NC.

**FIG. 2.8** (a) image of single NCs, the emission from these dots is very intense as seen by the intensity scale. (b) Spectra collected from individual NC. In a single collection, spectra from more than 1 dot are collected. The figure also shows the NCs and the corresponding spectra.

*Spectral Analysis:* CCD camera captures the spectrally diffuse image of NCs. This image is transformed into spectral data using Image J software. A strip from the center of NC (zero order diffraction) to the spectra is drawn (as shown in Fig. 2.10). The intensity profile as a function of pixel number is then extracted. The center of NC is numbered as pixel number 0.
FIG. 2.9 Blinking profile of single NC, the reference OFF state is indicated in the plot with a dashed line.

FIG. 2.10 Extraction of spectral data from spectrally resolved image. Data from the area enclosed will be transformed to intensity profile as a function of pixel number. Pixel number 0 is the pixel at the center of NC (start of the selection box).

Reference back ground of equal area is collected from above and below the NC emission. Actual emission from the NC is obtained by subtracting the background (bg1 and bg2) from the signal. The intensity profile so obtained is in pixel, in order to convert pixel to nm, wavelength calibration is performed by using three known laser lines, 488, 532, and 656 nm. The spectrally dispersed image is collected using the same slit width and position of grating and slit. The data in pixel form is extracted and the calibration curve is obtained by plotting laser wavelength versus pixel number of the laser signal as shown in Fig. 2.11 (a). The plot fitted with a linear scale fitting
equation is then used to convert pixel to nm. The spectra are further corrected for CCD response shown in Fig. 2.11 (b). More than 150 NCs per sample were analyzed in this way to generate statistically reliable spectral data. The data is analyzed for peak position, full width at half maxima of emission peak etc.

**FIG. 2.11** (a) wavelength calibration performed to convert pixel in to nm. (b) CCD response of the CCD used. The data is corrected for this CCD response.
References:


