INTRODUCTION

Quantum dots (QDs) are semiconductor nano-particles, which have many unique properties and show interesting phenomena, such as size dependent emission wavelength, narrow emission peak and broad excitation range[1-2]. Since they were first fabricated in the 80’s, QDs have been studied for almost three decades. However, a lot of physics behind those properties still remain unknown. Fluorescent resonant energy transfer (FRET) is a very common separation dependent phenomenon among many dyes. The unique properties of QDs make them ideal FRET pair. By measuring the separation dependent FRET level between QDs, we can discover the physics behind this phenomenon: whether the interaction is dipole-dipole or dipole-quadrupole dependence, which is an interest physics topic to be explored. If the separation dependence interaction is well controlled, it is highly possible to make QDs as a “calibrated” nano-scale ruler for potential biology application. In our experiment, we use a near-field scanning microscope (NSOM) to measure the FRET level between two different size CdSe/ZnS QDs. The idea for using NSOM is to bring small QDs very close to large QDs and use a 488nm argon laser to excite both the large QDs and small QDs. After collecting the combination of the two spectra from the two QDs, we analyze the spectra and measure the FRET interaction.

1.1 Quantum Dots

Quantum dots are semiconductor nano-crystals in which excitons are confined in all three spatial dimensions. The confinement can be realized by fabricating the semiconductor in very small size, typically several hundred to thousands of atoms per particle[1-2]. Due to quantum confinement effects, QDs act like artificial atoms, showing controllable discrete energy levels. QDs were first fabricated in the 80’s by Louis E. Brus[3] and the unique properties of these special nano-structures attracted interest from many fields[4-6].
QDs can be optically excited. When absorbing photons, electrons in QDs gain energy leading to the creation of excitons. An exciton is a bound state of an electron and a quasi particle called a hole. After relaxation from the excitonic excited state to its lower energy state, the electron and hole recombine (exciton recombination), emitting a photon. The overall process of optical excitation, relaxation of excited state, recombination of electron and hole and fluorescent emission is called photoluminescence (PL). The number of photons emitted can be measured as a function of energy, which gives the PL spectrum. Different from many organic dyes, QDs can be excited by many light sources within a large wavelength range, since QDs have continuous and broad absorption spectra. Furthermore, many kinds of QDs can be excited by the same light source and their emission can be easily separated. The emission light spectra of QDs are narrower and more symmetric than conventional organic dyes[7-9], making the sensitivity of detection higher than for organic dyes. Because of this property, QDs are attractive fluorophores for biological imaging (biological tagging)[4-6].

Figure 1.1. Size dependent Photoluminescence (PL) spectra of CdSe QDs (from Benoit Dubertret and Hideki Ooba)
The energy gap of excitons in QDs is strongly size dependent. This size dependent phenomenon is due to the effect of confinement: The smaller QDs have stronger confinement making the energy gap larger. Similarly, a larger size gives a smaller energy gap[2]. Hence, QDs with different emission colors can be made from the same material by changing their size[10]. Hence, shown in Figure 1.1, for larger (small) size the emission is more towards the red (blue). Colloidal QDs, which are synthesized by relatively inexpensive wet chemistry methods, make it possible to have the desired particle size which makes it easy to find QDs with the energy spectrum we need and high quantum yield (number of photons absorbed over the number of photons emitted), even at room temperature[2]. Currently, CdSe or CdTe QDs with a ZnS shell (see Figure 1.2) are commonly used and studied. The Core/Shell structure diminishes chemical damage to the fluorescence core. ZnS is optically transparent to the emission range, therefore there are no photon losses associated to the shell with visible light emission[10].

![Figure 1.2. Core/Shell structure of CdSe/ZnS Quantum Dots (QDs)](image URL)
1. 2 Förster Resonant Energy Transfer (FRET)

When two molecules, nano-crystals or fluorophores are very close together and one of them gets excited, the energy may transfer to another one by means of dipole-dipole interaction. This phenomenon is termed Förster Resonant Energy Transfer (FRET)[11]. The particle which releases the energy in the FRET pair is called the donor while the other one is called the acceptor. FRET only happens when the emission energy of the donor matches the excitation energy level of the acceptor, i.e. the donor and the acceptor need to be resonant. The phenomenon is named after the German scientist Theodor Förster who proposed a theory describing long range molecular interaction by resonance energy transfer. The transfer rate $k$ is[11-13].

$$k = \frac{1}{\tau_D} \left(\frac{R_0}{r}\right)^6,$$

$$R_0^6 = 8.758 \times 10^{-5} \frac{\kappa^2 \phi_D}{n^4},$$

where $\tau_D$ is the donor PL lifetime in the absence of an acceptor, $r$ is the donor-acceptor distance and $R_0$ is Förster radius, typically within 100nm. $\kappa^2$ is the dipole orientation factor (the dipole of donor relative to that of the acceptor), $\phi_D$ is the quantum yield of the donor in the absence of accepter. $n$ is the reflective index of the medium and $J$ is an overlap integral of the donor's emission spectrum with acceptor's absorption spectrum indicating how well the donor resonate with acceptor. Because of its strong distance dependence, FRET has been used as a nano-scale ruler to determine molecular distances in structure biology and biochemistry[14].

Since FRET represents a transfer of energy, it can be detected by measuring the quenching of the emission of the donor or the enhancement of the emission of the acceptor. In our case, we measure the ratio of the area of PL spectra of small dots
(donors) versus the area of PL spectra of large dots (acceptors) to determine the FRET level. A smaller ratio indicates a larger FRET level and vice versa.

1.3 NSOM and Separation Dependence of Resonant Energy Transfer between CdSe/ZnS Nanocrystals

Conventional far-field optical approaches are always limited by diffraction, allowing a resolution of several hundred nanometers (Abbe’s diffraction limits). To overcome diffraction limitations, researchers developed many techniques including NSOM. E.H Synge was the first person to give the concept of the near-field technique in 1928[15]. A. O’Keefe also developed similar theories in 1956 without knowledge of Synge’s earlier work. It was Ash and Nichols who, in 1972, first broke Abbe’s diffraction limit using 3cm radiation[16]. They were able to reach a resolution of $\lambda_0/60$ in their work. In 1980’s Pohl et al. [17] and Lewis et al.[18] both developed techniques with a sub-wavelength metal coated optical aperture at the tip of a sharp pointed probe, and a feedback mechanism to maintain a constant distance of a few nanometers between the sample and the probe. A 25nm (about $\lambda_0/20$) resolution was achieved in the work by Pohl et al. The main reason behind using a NSOM system was to illuminate an area much smaller than what can be achieved in far field microscopy. Second, the NSOM system can be used to move one group of QDs with respect to another.

According to the Förster rate equation, FRET is a separation dependent phenomenon. My former colleague did a series of experiments to investigate the separation dependence of the interaction between two groups of dots[19]. He attached small QDs (in orange color, see Figure 1.3) to the surface and apex of an Al-coated probe of a NSOM setup, large QDs (in red color, see Figure 1.3) were deposited on the flat side of a solid immersion lens (SIL). Figure 1.3 shows the schematic of the NSOM probe with small QDs and the SIL with large QDs. By
using the NSOM system, the two groups of QDs were brought in close proximity (20-50nm). Then by changing the distance between two groups of dots and collecting their spectra, he obtained the FRET level as a function of separation (shown in Figure 1.4).

Figure 1.3. Schematic of NSOM probe and SIL (not to scale) with two resonant group of QDs deposited on probe and SIL. Red spheres representing large QDs and orange spheres representing small QDs. Gray hatched area is Al coating on the NSOM probe. The coated area prevents the excitation of dots which are not on the clear apex region.
Figure 1.4. a) PL signal of small and large QDs as a function of separation. Horizontal, lateral and vertical axes show wavelength, separation between two groups of QDS and PL signal respectively. b) Quenching of PL signal of small QDs. Horizontal and vertical axis shows distance between two groups of QDs and the PL signal. The small QDs PL signal has been normalized by large QDs PL signal [19].

The trend in Figure 1.4 shows that when two groups of dots approach each other, the FRET level becomes higher. The problem is that it is very difficult to have uniformly separated dots on the SIL when we deposit the large QDs on the SIL. The reason is that the dots will aggregate when the solvent of QDs evaporate. The interaction we observed is actually between a group of QDs and another group of QDs. Therefore, the trend of the FRET level versus the distance shown in Figure 1.4 is clear, but the data does not fit in to the model my former colleague proposed, which is only appropriate for single QD-single QD interaction. Hence, it would be ideal if we can identify two QDs’ interaction with each other to fit the data in the model. One solution is to use polymethylmethacrylate (PMMA) to embed large QDs on the cover glass using a spin coating method (see Figure 1.5). PMMA has the potential to separate QDs from each other, which make it possible for a NSOM raster scan to localize single QDs on the cover glass. After we locate a single QD on the cover
glass, we need to perform a fine scan (constant distance between the probe and sample) to make sure the small QD is right on top of the large QD. Since the excited excitonic energy level of large QD matches the ground excitonic energy level of small QD, the strongest quenching of the PL of the small QD should be observed when the two dots are closer, see Eq. (1.1). If we find the strongest quenching, we are sure that the two dots are well aligned. Ideally we can finally achieve the interaction between two single QDs. However, there are also some disadvantages if we use PMMA method. For example, since we are not able to coat PMMA on the SIL, the signal collection efficiency is reduced. Furthermore, the thickness of the PMMA is over 10 times larger than the QDs; there is a possibility that the QDs may not be on the surface of the PMMA film, since that we may not observe the interaction.

Figure 1.5. Schematic of NSOM probe and cover glass (not to scale) with two resonant group of QDs deposited on probe and cover glass.
2 EXPERIMENTAL SETUP

As I described in the previous chapter, we use a NSOM to measure the FRET level between two different size QD. The idea for using NSOM is to bring the small QDs very close to large QDs and to excite both of them. The combination of the two spectra allows us to measure the interaction. In order to achieve our goal, we need several components for our experimental setup: first of all, the setup is based on a NSOM, which allows us to bring the two dots close enough (nano-scale) to have FRET interaction. As the aperture of the NSOM probe is smaller than the wavelength, we can have a sub-wavelength image, which can help us locate single QDs; secondly, a stable feedback loop is needed to control the distance between the two dots. Ideally we want to measure two dots interaction. In order to do that, polymethylmethacrylate (PMMA) is used to embed large QDs on the cover glass with spin coating. With PMMA, we assumed that the large QDs are separated from each other. Raster scan of the QDs-PMMA thin film allows us to locate single large QD on the cover glass.

2.1 Main Design

A near-field scanning optical microscope (NSOM) which was designed and built by former lab members is used for this experiment to excite the CdSe/ZnS QDs[19]. NSOM is a technique which works near the surface of the object. Unlike far-field techniques, which allow light to diffract and propagate, NSOM uses an evanescent wave that exists only near the surface of the probe as the excitation light source. Since this evanescent wave decays exponentially along the distance from the boundary, the light source and sample should be in close proximity. The resolution is then limited by the aperture of the NSOM probe rather than by diffraction as in far-field techniques. NSOM uses a feedback loop to control the metal coated fiber tip to give
a nano-scale separation from the sample. The optical images are generated by raster scanning the sample by means of a piezo-stage.

Our NSOM system is able to move the small QDs (which are attached to the apex of the probe) laterally and vertically\[19\]. Lateral motion of the probe is necessary to raster scan to find large QDs-PMMA embedded in the thin film on the cover glass. Vertical motion is used to bring the small QDs close to the large QDs. Figure 2.1 shows a schematic diagram with the most important parts of the NSOM that we used.

Figure 2.1. Schematic of experimental setup including near-field scanning optical microscope (NSOM) and spectrometer.
The probe's vertical motion is controlled by a feedback loop system while its lateral motion is controlled by a Labview program in the computer. The signal from the feedback loop system and the computer are amplified and then sent to a 3-axis piezo stage. The NSOM probe is attached to a quartz tuning fork and then assembled on a tripod which sits on the piezo stage. There is a hole in the sample holder which allows the fiber tip go through the sample holder and approach the cover glass sample.

As shown in Figure 2.1, an Ar laser with the wavelength at 488nm has been used for optical excitation. For accurate monitoring of the laser intensity signal, a light chopper is used before the laser is coupled into a single mode optical fiber. The laser is then splitted and a silicon photodiode and a lock-in amplifier are used to detect the laser intensity to normalize the final spectra data. The rest of the laser light is sent to the NSOM probe. After excitation of the small QDs on the tip and large dots within the PMMA thin film on the cover glass, photons emitted from the QDs are collected by two objectives and sent to a grating spectrometer where the spectra information is collected by a Charged Coupled Device (CCD).

2.2 Near-field Scanning Optical Microscopy

2.2.1 Overview

In NSOM the lateral resolution is limited by the size of the probe (light source) as well as the probe-sample distance which can be made small by suitable feedback schemes. Al-coated pulled optical fibers have been used as probe in this system. After pulling the fiber using specific parameters[19], the fiber’s apex becomes smaller than the wavelength of light. Al coating controls the output and lets the light come out only from the probe’s apex. Resolution is determined by the size of the aperture of the fiber probe, in our case, 100-200nm.
2.2.2 Fiber Tips

In order to have an aperture which is smaller than the wavelength of light, a specific tip making process has been used. The idea of the method is to heat the glass fiber, pull the fiber and then coat the fiber with Cr and Al[19]. The quality of the fiber probe rules the quality of the final data. For example, the apex size determines the resolution; pinholes on the tip cause light loss and sometimes mis-focusing. My former colleague developed a fine protocol to produce the fiber used in my experiment[19].

A P-2000 micropipette puller from Sutter Company was used to taper the fiber. This system includes a $CO_2$ laser and a two step pulling process using a constant weak pulling force (gravity) and a strong pulling force (solenoid). These two pulling steps have a well known effect on shortening the taper length and decreasing the probe size. The parameters such as heat, delay, pull and velocity of pulling control the length of the fiber taper parts, its aperture size and shape of the probe. The heat parameter is used to control the heating of the fiber. If the heating is too high or too low, then the fiber melts too fast or does not melt well, which does not give a conical tip. The delay parameter controls the delay between the heating and pulling. The velocity parameter is the pulling speed. If the speed is too fast, the fiber breaks or the apex of the tip is too large. If the speed is too slow, the fiber apex is too thin and will easily break during the experiment. The pull parameter is the pulling strength during the pulling process. Only if all the parameters are in the right range are we able to get the tips we want.

The heating-pulling process is as follows: strip the plastic cladding from the glass fiber and stabilize the optical fiber in the puller. Make sure the stripped part is in the center of the puller and the fiber is stable and tight. The $CO_2$ laser (heat parameter) starts heating up the fiber, which softens under the weak pull. When the fiber is
moving at velocity parameter, after the delay parameter the strong pull starts. The magnitude of the strong pull is defined by the pull parameter, and it could start either before or after the laser heat terminates (delay parameter). Figure 2.2.a shows the apex of the pulled fiber (image collected by my former colleague) using the parameters and description explained above[19]. SEM images give us an idea that what is the dimension of the aperture of the probe. From the SEM images we estimated that the diameter of the probe aperture is ~100nm.

![SEM images of fiber probes](image)

**Figure 2.2.** a) SEM of 5-10nm Au coated fiber probe, magnification=35,000X. The arrow shows the flat apex. b) SEM of 15nm Cr plus 150nm Al coated fiber probe, magnification=150,000X[19]

After pulling the fibers, we use Cr and Al to coat them. Soon after the pulling process, we take the pulled fibers to a thermal evaporator, where the coating takes place. The faster this is done the less dust attached to the probe. We coat 10 fibers at a time. The fibers are clamped in a fiber holder and then placed in the thermal evaporator in a specific angle range. This angle needs to be adjusted correctly in order to coat the fiber completely but not to cover the apex of the fiber probe. The fibers are rotating while the evaporation process is ongoing to uniformize their coating.
Before the Al deposition, around 10nm thickness Cr layer is deposited onto the fiber probe to increase the adhesion of Al to the surface of the fiber probe. Compared to Cr, the deposition rate of Al is a much more important parameter. Fast deposition causes an uncontrollable coating while slow deposition rate causes more pinholes and optical losses on the fiber probe. As it was explained above, the fibers are mounted on a rotation system which is placed directly above the evaporation source. 20-40 rpm is an acceptable rotation rate. Figure 2.2.b shows the scanning electron microscopy image of 10nm Cr/150nm Al coated fiber probe.

2.3 Feedback Loop System

FRET happens at a nano-scale separation of two groups of molecules. Therefore, the probe-sample distance plays an important role in our experiment. This distance is crucial as we measure the energy transfer between two dots as a function of probe-sample separation. In order to achieve a small and stable separation, a feedback mechanism was utilized. Before discussing the feedback control, we need to know what will reversibly happen if two objects are brought close together. In other words, we need a ruler of the separation. When a vibrating object with a constant excitation force gets close to another object, the amplitude of the vibrating object will decrease due to the shear-force between the two objects, which is an external force acting tangent to the material. This shear force only happens when the two objects are close together, which also satisfies the near-field requirement. This amplitude change is a “ruler” for the separation measurement and control. In our case, if the probe has a vibration with a constant excitation force, we can control the separation between the probe and cover glass.

To use this property as a parameter for controlling the probe-sample distance a tuning fork has been used to oscillate the probe and this vibration is monitored using a lock-in amplifier technique. The coated fiber probe is glued to the tuning fork with a
nominal natural resonance frequency at 32768 Hz. The probe/tuning fork resonance amplitude is used as an input for a feedback loop system to control the probe-sample separation. The feedback loop circuit (PI) which is the main part of feedback loop system, is an integrator system (I) which integrates the difference between two inputs to the circuit in the integration branch and adds it to the proportional branch (P) signal. Appendix A shows the detail of feedback loop circuit (PI). One input of the circuit is a setpoint for desired amplitude of the probe/tuning fork which is a function of probe-sample separation. Another input is the current amplitude of the probe/tuning fork. Mechanical and piezo stages are used to bring the probe/tuning fork close to the cover glass surface.

The system has to be calibrated to keep the probe-sample distance in a safe range (setpoint has to be not less than 90 percent of the probe/tuning fork amplitude at large separation) and parameters have to be optimized for better stability. If the NSOM probe is initially too far from the cover glass (out of range of piezo stage displacement which is ~20μm), engagement of the probe and cover glass does not happen. In this case, the NSOM probe has be moved mechanically close enough to the cover glass to allow for the final engagement to happen. Figure 2.3 shows the schematic of the feedback loop system.

2.3.1 Tuning Fork

The tuning fork provides the NSOM probe a vibration with constant frequency and constant force. The amplitude of oscillation decreases when the probe approaches the cover glass within a nanometer range because of the shear force between the two objects. By using this property we are able to control the probe to cover glass distance.
Figure 2.3. Schematic of feedback loop system. Direction of probe to cover glass approach and direction of probe/tuning fork oscillation are identified by black arrows.

Quartz tuning forks can be considered as a series R-C-L (resistance, capacitance and inductance) in parallel with another capacitor, see Figure 2.4. By fitting the theoretical value of the frequency dependence of the tuning fork's amplitude to the measured one, the used model can be checked and information about the tuning fork can be obtained[19].

The driving voltage on the tuning fork is considered as $V = V_0 e^{i\omega t}$. Voltages for RCL and C branches can be written as:
Figure 2.4. Schematic of RLC circuit representing tuning fork

\[ V = I_2 R + L \frac{dI_2}{dt} + \frac{Q_2}{c_2} \]  
\[ (2.1) \]

\[ V = \frac{Q_1}{c_1} \]  
\[ (2.2) \]

Charge in the RLC branch is \( Q_2 = Q_0 e^{i(\omega t + \varphi)} \). Considering \( I_2 = \frac{dQ_2}{dt} \) an expression for \( Q_0 \) can be derived: \( Q_0 = \frac{V_0}{(iR\omega - L\omega^2 + \frac{1}{c_2})} e^{i\varphi} \) and \( I_2 \) can be derived as:

\[ I_2 = \frac{i\omega V_0 e^{i\omega t}}{(iR\omega - L\omega^2 + \frac{1}{c_2})} \]

Knowing that \( I_1 = \frac{dQ_1}{dt} \), \( I_1 \) can be derived as \( I_1 = iC_1 \omega V_0 e^{i\omega t} \), the total current \( I_{T_F} \) is the sum of these two currents:

\[ I_{T_F} = \frac{V_0 R \omega^2 / L^2}{R^2 \omega^2 + (\omega_0^2 - \omega^2)^2} + i[C_1 V_0 + \frac{V_0 \omega (\omega_0^2 - \omega^2)}{L \left( \frac{R^2 \omega^2}{L^2} + (\omega_0^2 - \omega^2)^2 \right)}] \]

The real part is what it is measured in phase (X channel) on a standard lock-in amplifier:
\[ I_{TF} = \frac{V_0 R \omega^2 / L^2}{\frac{R^2 \omega^2}{L^2} + (\omega_0^2 - \omega^2)^2} \]

where \( \omega_0 = \sqrt{\frac{1}{L C_2}} \) is the resonance frequency of the tuning fork and \( C_1 \) is 12.5 pF as provided by the manufacturer. A measured response and fitting for the tuning fork is shown in Figure 2.5. By fitting the data we get the resonance of the tuning fork which is 32809Hz.

![Figure 2.5. Data and fitting of the real part of the current on a tuning fork as a function of frequency. The resonance frequency of the tuning fork is 32809Hz.](image)

After gluing the fiber probe, the resonance frequency of the combination (tuning fork plus fiber probe) does shift from its original value, the full width at half maximum (FWHM) of the resonance amplitude also becomes larger and the amplitude at resonance decreases. The reason for those is that gluing the fiber provides an extra force to the tuning fork. The glued fiber damps the tuning fork. The system is a driven damping oscillator and the damping factor is directly associated with the
FWHM of the resonate peak. After the probe gluing, the spring constant and the mass of the tuning fork/fiber probe both increase. However the spring constant increases more, which causes an increase of the resonant frequency. Figure 2.6 shows the shift of the resonant frequency (from 32809 Hz to 33662 Hz), broadening of the width (from 4.1Hz to 38.4Hz) and decreasing of resonance amplitude (from 38nA to 4.29nA). The driven force of both tuning fork individually and tuning fork with probe attached to it is the same.

![Figure 2.6.](image)

**2.3.2 Quality of Engagement**

The quality of the engagement between the probe and the cover glass was checked by using a square wave as set point (with maximum and minimum voltage within the engaging range voltage) and monitoring the probe's amplitude as it follows the set point.
Figure 2.7. Response of the NSOM probe to a square wave set point. Vertical axis shows the amplitude of set-point (in red) as square wave and the NSOM probe amplitude (in black). Horizontal axis is time.

Figure 2.7 shows the response of the NSOM probe to the change of the set point. By knowing how fast the probe can respond, we can set the integration time when we scan the sample (in this case, the fastest we can achieve is ~150ms per point). This response time varies when we change some of the parameters of the PI circuit. As shown in Appendix A, the capacitor in the integrator branch is the main factor which determines the response time. By changing this capacitor, the response time varies from 70ms to 200ms. Larger response time gives a more stable engagement of the system, but slower scanning speed. Also, the selection of capacitor, resistors and variable resistors in PI circuit affect the stability of the engagement. Even after optimization, when the tuning fork/fiber reaches the set point value, it shows noise. Since the amplitude of the tuning fork/fiber corresponded to the distance between the probe and cover glass, the amplitude fluctuation is considered as the noise of the engagement. By using the calibration (shown later in this chapter, Figure 2.9), we calculate that the fluctuation is ~3.2nm. Also we monitor the fluctuation of the
output voltage which is sent to the piezo stage to drive the z-direction movement. We record the voltage and calculate the standard derivation which corresponds to ~3nm displacement of the piezo stage.

In order to check the stability of the scanning system, we deposited 2.9 µm micro beads to the cover glass as the sample and checked the topography information. The image is taken by a raster scan with 90×50 pixels. The data acquisition time for each point is 300ms, in which the probe can fully relax from the movement (according the response time of the probe). The step sizes in both X and Y directions is 100nm.

![Topography image of the micro beads](image)

Figure 2.8. Topography image of the micro beads. The right up corner shows the 2-D image and the left down corner shows the surface plot.

As shown in Figure 2.8, the gray value shows the topography information of the sample. The noise for the topography reading is around 5nm which also indicates the limitation for the distance measurement between our NSOM probe and the cover glass sample. The 2-D plot (shown in the top right corner in Figure 2.8) gives us information of the lateral calibration of the piezo-stage. The points a, b and c are at the centers of the 3 spheres we image. The distance between those centers should be the diameter of the spheres, which are 2.9µm. Since the distance measurement from the
original calibration does not match the beads diameters, we can tell that the lateral calibration of the piezo stage is wrong. Therefore, I modified the original calibration from my former colleague, using the micro-beads images.

2.3.3 Calibration of the distance between probe and cover glass

As it was explained before, when the probe moves towards the cover glass at constant excitation, the amplitude of the probe/tuning fork decreases. When the NSOM probe engages to the cover glass, the separation between the probe and cover glass should be a function of the amplitude set point of the probe/tuning fork. The set point should not be higher than the original amplitude of the probe/tuning fork so that the engagement happens. The range in between the original amplitude and 90% of original amplitude is where our measurements take place. Hence, the decrease of the amplitude of the probe/tuning fork can be used to measure the distance between the probe and cover glass.

In order to measure the distance, we engage the probe to the cover glass in the near-field and bring the probe towards or away from the cover glass by changing the set-point in the feedback-loop system. Differences between the set-point and the amplitude of the probe/tuning fork force the feedback loop system to change the voltage sent to the piezo-stage. The voltage sent to the stage determines the displacement of the piezo-stage. The piezo-stage response to the voltage, calibrated by my former colleague, is that 290V provide 35 μm displacement. This calibration is consistent with the beads image (see Figure 2.8). Hence, we have stage displacements corresponding to different set-points. Considering that we reach a set-point, at which the probe and the cover glass are almost in contact (zero separation), then we pull back the probe by changing the set-point. The extra voltage applied to the stage is proportional to the distance between the probe and cover glass. So we can calibrate the distance by increasing the set-point after the contact point and
have the separation information as a function of the set-point. Figure 2.9 shows the amplitude of probe/tuning fork as a function of the separation between NSOM probe and sample surface. The x-axis shows the distance between the cover glass and NSOM probe while y-axis shows the probe/tuning fork amplitude. We usually use 90% of the original amplitude as the engagement set-point, where the separation is ~20nm[19]. The tip will break then because the QDs have ~10nm diameter.

Figure 2.9. Amplitude of NSOM probe as function of separation between NSOM probe and cover glass. Y-axis shows the resonance amplitude of the NSOM probe (measured by lock-in amplifier connected to the tuning fork and this value is the same as the setpoint). X-axis shows the separation between NSOM probe and cover glass.

2.4 Poly(methyl methacrylate) (PMMA) and QDs Sample Preparation

PMMA is a polymer which is wildly used in industry. It has very good visible light transparency. In our case, we dilute the large QDs in toluene and then mix with 4% PMMA in anisole (details of the QDs concentration are discussed in Chapter 3) and spin coat the mixture to the cover glass making a thin film. The large polymer molecules prevent the QDs from aggregation, which allow us to locate single dots in
the sample. However, we cannot be sure that there are large QDs staying at the top of the thin film which can interact with the small one on the probe (deeper dots do not interact because of short Förster radius). Therefore we need to make the PMMA film as thin as possible. The thickness of the PMMA thin film can be measured by Atomic Force Microscopy (AFM). After the spin coating, the sample is scratched in order to provide an edge between the sample and the cover glass. Then we image using an AFM the edge between the PMMA film and cover glass where the PMMA is taken away.

Figure 2.10. a) AFM image of the topography of the PMMA thin film. b) Line cut along dotted line in a).

Figure 2.10 shows the AFM image of the PMMA thin film spin coated at 1000rpm. The thickness of the PMMA film for this specific rotation speed is about 480nm. The way that we calculate the thickness is to treat the glass surface on the right as a background and subtract the background. The remaining part should be the height of the PMMA sample. We spin coated a series of PMMA thin film at different rotation speeds (3-4 samples in each speed). As shown in Figure 2.11, the thickness
of the thinnest film with PMMA we can make is ~150nm. However, if we mix the 4%PMMA solution (in Anisole) with toluene (1:1 mix), which is the solvent for QDs, the thin film can be coated at a thickness of 100nm with the rotation speed set at 10000rpm. The error bars come from measurements from multiple samples.

Figure 2.11. Thickness of 4% PMMA on anisole thin film as function of the spinning rate. The thickness does not decrease if the speed is above 6000rpm.

Small CdSe/ZnS with 6nm diameter were placed on NSOM probe by keeping the probe in the suspension of dots for the desired length of time. Typically, a few seconds are enough for the deposition of the small dots on the probe.

2.5 CdSe/ZnS Quantum Dots for Resonant Energy Transfer

Single band effective mass approximation has been used to study the excitonic energy level in QDs. The Coulomb interaction between electron and hole has been taken into account as a perturbation[20-21]. As calculated by my former colleague[19], the energy levels of the large QDs are shown in Table 2.1. The PL
spectra (Figure. 2.14, described later in this chapter) of the large QDs shows that the
ground state energy level of the large QDs correspond to 623nm wavelength. The
ground energy state of the small QDs which corresponds to 580nm wavelength
matches the 3rd excitation energy state of the large QDs.

Table 2.1
Table of calculated energy levels for the large QDs

<table>
<thead>
<tr>
<th>Energy State</th>
<th>Total Energy (J)</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground State</td>
<td>$3.13 \times 10^{-19}$</td>
<td>631</td>
</tr>
<tr>
<td>1st Excitated State</td>
<td>$3.23 \times 10^{-19}$</td>
<td>612</td>
</tr>
<tr>
<td>2nd Excitated State</td>
<td>$3.38 \times 10^{-19}$</td>
<td>584</td>
</tr>
<tr>
<td>3rd Excited State</td>
<td>$3.48 \times 10^{-19}$</td>
<td>567</td>
</tr>
</tbody>
</table>

When the QDs are excited, excitons are formed. Those QDs, which are also
charge distributions, generate an external electrical potential. The electrical potential
energy, using a multipolar expansion, can be written as:

$$E = q\varphi(0) - \hat{p} \cdot \vec{E} - \frac{1}{6} \sum_{l,j} Q_{l,j} \frac{\partial E_j}{\partial x_l} + \cdots$$  \hspace{1cm} (2.3)

where $q$ is a point charge, $p$ is the dipole term and $Q$ is the quadrupole term. $\varphi$ is
the potential due to the charge ($\propto \frac{1}{r}$), dipole ($\propto \frac{1}{r^2}$) and quadrupole ($\propto \frac{1}{r^3}$). $\vec{E}$ is the
electric due to the charge ($\propto \frac{1}{r^2}$), dipole ($\propto \frac{1}{r^3}$) and quadrupole ($\propto \frac{1}{r^4}$). $\frac{\partial E_j}{\partial x_l}$ is the
electric field gradient due to the charge ($\propto \frac{1}{r^3}$), dipole ($\propto \frac{1}{r^4}$) and quadrupole ($\propto \frac{1}{r^5}$).

Each term above have component contributed by point charge, dipole and quadrupole.
In our case, charge distribution of QDs, there is no net charge. Therefore, the point
charge components in each term shown in equation 2.3 are zero (entire first term
$q\varphi(0)$ is zero, charge contribution to term $\vec{p}.\vec{E}$ is zero and charge contribution to
term $\frac{1}{\varepsilon}\sum_{i,j} Q_{ij} \frac{\partial E_j}{\partial x_i}$ is zero, etc.). The first term we expect to see is the dipole-dipole
interaction energy (dipole contribution of term $\vec{p}.\vec{E}$), which is the dipolar electric field
of excitons in the small QDs interacting with dipole of excitons in the large QDs.

In our case the ground state of the small QDs is resonant with the third excited
state of the large QDs. The interactions between electron in small dot with electron
in large dot, electron in small dot with hole in large dot, hole in small dot with electron
in large dot, and hole in small dot with hole in large dot need to be taken into account.

![Diagram](image)

Figure 2.12. Initial and final states of combination of small and large QDs.
At initial state, the small QD exciton is in its ground state and there is no
exciton in the large QD. In final state, there is not an exciton in the small
QD and large QD exciton is in its third excited state.

The initial state and the final state after the energy transfer are shown in Figure
2.12. Initially, the small QD exciton is in its ground state and there is no exciton in the
large QD. In the final state, there is no exciton in the small QD and large QD exciton
is present in its third excited state. The transition rate is \( W = \frac{2\pi}{\hbar} |< V_{12}>|^2 \rho \)
where \(< V_{12}>\) is the electrostatic potential energy between the excitons in small and large QDs, and \( \rho \) is the density of the states (Fermi’s golden rule). The transition probability is \( P = W \tau \) where \( \tau \) is the PL lifetime. Using equation 2.4 and introducing the initial and final state of combination of the small and large QDs, we can calculate the transition rate \( W \). Then the transition probability \( P \) can also be calculated by knowing the PL lifetimes is a few nanoseconds. The theoretical transition probability calculated by former colleague is \( \approx 3 \times 10^{-47} m^6/r^6 \)[19].

### 2.6 Spectrometer Setup

Light from small and large QDs is or has been analyzed using a Horiba Jobin-Yvon Triaxs-550 spectrometer. In this spectrometer blazed gratings are used to shift the intensities out of the zeroth order into the first order spectra. Later, the diffracted light is collected in a CCD and provides an intensity profile as a function of energy (wavelength). The CCD has 85 percentage quantum efficiency at 650nm. The dark signal of this CCD is \(< 3 \ e/\text{pixel/hour} \ @ \ 133K \). The spectrometer includes two gratings which were used, one with 300 grooves per millimeter yielding 0.16nm spectral resolution and another on with 1200 grooves per millimeter yielding 0.05nm spectral resolution.

### 2.7 Photoluminescence Measurement

In PL measurements, parameters such as slit size and exposure time control the quality of the final result. Photons are collected into the spectrometer through the entrance slit. After collection of the photons and diffraction at the grating, photons reach the CCD as a function of energy (wavelength). Pixels on CCD absorb the photons and generate electrons. These electrons are collected and added together and the final result is an intensity as function of energy (wavelength) graph.
In order to get good quality results, we did many adjustments. Figure 2.13 shows the side view of parts used in our experiment (drawing by former colleague). After attaching and depositing the large and small NQDs to the probe and cover glass, we mount the x,y transitional stage (the combination of part 1, 2, 3 and 4) with the objective (part 5) on top of it. We use z translactor (part 2) to focus the objective on the probe using Ar laser light as excitation. As the probe and sample are in close proximity, the objective should also focus on the light emitted by QDs both on the tip of the probe and in the PMMA sample. X and Y translators (part 1) are used to center the signal on the objective. This alignment does center the laser spot on the mirror which is held on top of the objective by the mirror mount. Then the tilt and rock adjusting screws on the mirror holder (part 3) are used to deflect the light toward the entrance slit of the spectrometer. The light is collimated, and then focused into the spectrometer by another objective.

At this point, we make sure that the entrance slit is wide open (2mm) and align the laser light to the entrance of the spectrometer. We put a neutral density (ND) filter in front of the entrance slit in order not to saturate the CCD and then monitor the laser light intensity by the CCD. We focus the objectives (shown in Figure 2.11 part 4, right above the sample) by focusing the laser light. Then we reduce the slit size and maximize the signal by tilt and rock of the mirror (shown in Figure 2.13 part 3) to make sure the light goes through the center of the slit. We keep reducing the slit size and maximizing the signal until the slit size is 0.2mm. If the slit is further reduced, light collection from the probe is also attenuated. When the collection optimization is done, the QDs PL signal both from probe and PMMA samples can be observed (Figure 2.14). These spectra are taken in 30s exposure time at 0.2mm entrance slit of spectrometer in near field condition. Usually we use a notch filter to block the laser light. All the spectra we collect are based on these parameters. The fitting of the PL
spectra shows the peak wavelength of the large QDs is ~623 nm and the small QDs is ~579 nm. As explained before, these QDs with 2 different sizes form a resonant pair.

Figure 2.13. Side view of parts in the cryostat and out of the cryostat. The parts outside the cryostat have been designed to be used with objective for collecting the light and detecting it toward the spectrometer: 1-X-Y axis stage, 2-Z axis stage, 3-Mirror mount and mirror, 4-Objective tube, 5-Bracket for objective
Figure 2.14. Sample PL spectra of QDs both from the probe and the cover slide. The green line shows the multi-peak fitting by Gaussian functions. These spectra are taken in 30s exposure time at 0.2mm entrance slit of spectrometer with notch filter applied in near field condition.
3 RESULTS AND DISCUSSION

The measurement process contains two parts: Scan the sample to locate a single dot on the cover glass and then bring the tip and cover glass together to monitor the FRET level separation dependence of between two kinds of QDs. As we discussed before, the FRET level is obtained based on the PL signal of two groups of QDs collected by the spectrometer. As shown in Figure 3.1, Area 1 is the intensity of the small QDs signal and area 2 is the intensity of the large QDs signal. The FRET level is calculated as area 1/area 2, which is the small QDs signal over the large QDs signal. A lower value indicates a larger FRET interaction.

Figure 3.1 Schematic shows FRET level measurement process. Area 1 is the intensity of the small QDs signal and area 2 is the intensity of the large QDs signal. The FRET level is calculated as area 1/area 2, which is the small QDs signal over the large QDs signal. Lower values indicate larger FRET interaction.

3.1 Single Quantum Dot Localization by Coarse Scan

After optimizing the PL signal of the QDs, we engage the probe with the cover glass and record a spectrum in a proper exposure time. Since the probe is mounted on a piezo stage, we can scan a small area of the QDs-PMMA sample and get the spectra point by point and extract an image after analyzing the spectra. The signals are
based on the area under the spectra of the two peaks. The key part of this step is to have the right concentration of the QDs on the PMMA sample. If the QDs concentration on the sample is too high, a uniform signal as a function of position is seen. If the QDs concentration is too low, it is very hard to find any signal during the scan. By scanning QDs-PMMA samples with different QDs concentrations, we can figure out a concentration range in which we are able to easily locate a single QD. The concentration of QDs provided by Nanomaterials & Nanofabrication Laboratories (NN lab) is $\sim 10^{17} \frac{\text{dots}}{\text{ml}}$. We diluted the sample 160 times (5μl QDs solution provide by NN lab: 395μl toluene: 400μl 4%PMMA solution in Anisole). The QDs concentration in PMMA is $\sim 10^{15} \frac{\text{dots}}{\text{ml}}$. Figure 3.2 shows an image of an area of interest. Image A is the signal of the QDs in the PMMA (large ones) and image B is the signal of the QDs on the probe (small ones), obtained simultaneously. Image C is the signal of QDs from the PMMA normalized by the signal of QDs from the probe. The lower row in Figure 3.2 shows the pixel value of each image. The reason for the normalization is to minimize the effect of the laser fluctuation.

By analyzing the pixel value of the image C we can see the signal of each pixel shows discrete phenomenon. The value of pixel (0, 600), 2.31, is almost half of the value of pixel (0, 400) and (600, 400). 2.31 is also one third of the value of pixel (400, 0) and one fourth of the value of pixel (0, 200). We make an assumption that pixel (0, 600) is the single dot signal, pixel (0, 400) is the signal from 2 QDs and so on. We plot the number of pixels as a function of number of QDs we estimate and the pixel value as a function of number of QDs (shown in Figure 3.3). The linear correlation (R-square is 0.99719, P-value < 0.00001) between the pixel value and integer number of QDs in Figure 3.3.b indicates that the smallest non-zero pixel value should be the single dot signal, in this case, which is pixel (0, 600), (400, 800) and (400, 1200). We also extract the information that there are ~3 QDs in the field of excitation. We also plot the pixel value as a function of number of QDs using the un-normalized large
QDs signal (shown in Figure 3.3.c), which is not as good as the normalized one (R square is 0.98402). Also, the error bar for un-normalized signal is larger, indicating that the fitting is not accurate. Since we have multiple pixels corresponding to a same number of QDs, we can obtain an error bar for each data point by using those pixel values. It is still very hard to find a good image in which there is one dot in the center and no signal around. However, by analyzing the signal of each pixel we can still identify a single QD on the cover glass. Figure 3.4 shows the experimental data of the spectra of QDs which shows one large QD on the cover glass. The ratio of the area under the small QDs over the area under the large QDs is ~1.4. The separation between the probe and cover glass is ~50nm.

Figure 3.2. The upper row shows the 4x7 image of a) large QDs signal, b) small QDs signal and c) large QDs signal over small QDs signal. The lower row shows the pixel matrix corresponded with the images on the upper row.
Figure 3.3. a) number of pixels as a function of number of QDs we estimate. b) linear relationship between the pixel value and number of QDs. c) linear relationship between the pixel value and number of QDs with the un-normalized large QDs signal. The fitting parameters are also included.
As shown in Figure 3.2, the step size of the scan to locate the single QD is ~200nm. At this point, we know that there are small QDs on the probe and there are large QD within the PMMA on the cover glass in the field of excitation, however, we still cannot make sure that the FRET interaction happened between the two kinds of QDs, because the theoretical Foster radius calculated by my former colleague is only ~17nm. Therefore, the reasons why we cannot have the FRET interaction are very clear: first, the small QDs may not be right on top of the large QDs. If there is a very large horizontal separation between the two QDs (much larger than the Forster radius, there is no energy transfer between them. Secondly, since the PMMA thin film has a thickness around 100nm, the large QDs we locate may be buried inside the PMMA rather than at the surface of PMMA. If the large QD is much away from the PMMA surface, we can hardly get the interaction data.

3.2 Small QDs-Large QDs Alignment by Fine Scan

To overcome these problems and have the QDs aligned, a fine scan is needed. The idea is to engage the NSOM probe to the cover glass and scan a small area with a very small step size. When there is a large QD at the surface and aligned with the
small QD on the NSOM probe, the PL signal of the small QDs on the probe is quenched. Therefore, the quenching spot on the image will help us identify if the small and large QDs are well aligned. We need to increase the concentration which we used in coarse scan. The reason is that this dipole of the single dot on the cover glass may not be parallel to the one of the QD on the probe and the QD may be buried inside the PMMA thin film. Hence, it is very difficult to see the quenching if we have small amount of QDs in the field of view. Assuming that the QDs are uniformly distributed in PMMA, therefore there are QDs on the surface or inside the PMMA thin film. If we have more QDs in the field of excitation, we have higher possibility to obtain the QDs on the surface and match the dipole direction. Hence, we diluted the sample from NN lab for 40 times (20μl QDs solution provide by NN lab: 380μl toluene: 400μl 4%PMMA solution in Anisole) to increase the QDs concentration in PMMA.

Figure 3.5. a) 7×7 fine scan image of small QDs signal (normalized by laser intensity. b) intensity value corresponding to the image.
Figure 3.5 shows a 7×7 fine scan image of the small QDs signal normalized by laser intensity with the step size at 15nm. The separation between the probe and cover glass is ~18nm. In this image, we identify some of the spots which have ~11% signal quenching (assuming that the brightest spot is the small QDs signal without quenching). There is a possibility that this 11% signal quenching is due to the noise and error. Hence, I scanned a blank cover glass without PMMA and QDs. Figure 3.6 shows the 7×5 fine scan image of a small QDs signal normalized by laser intensity with 15nm step size. The difference between the smallest pixel value and the largest pixel value is ~5% of the largest pixel value, which is much smaller than the difference in Figure 3.5. Furthermore, the standard derivation of the pixel value in Figure 3.6 is ~1.4% of the average pixel value. The fitting error of the area under the small QDs spectra is ~1.2% which is comparable to the standard derivation of the pixel value in Figure 3.6. However, the standard deviation of the pixel value in Figure 3.5 is ~3% of the average pixel value. Due to the difference of the standard deviation and ratio of smallest and largest pixel value between Figure 3.5 and Figure 3.6, the fluctuation of the pixel value in Figure 3.5 is not caused by measurement errors. Therefore, the quenching of the small QDs signal shown in Figure 3.5 is very likely caused by the large QDs in the PMMA.
Figure 3.6. a) 5×7 fine scan image of small QDs signal (normalized by laser intensity) scanning a blank cover glass. b) pixel value correspond to the image

Large QDs signal is not used to normalize the small QDs signal since the large QDs signal does not correspond to the laser intensity very well, even when I scan a small area with a very small step size (shown in Figure 3.7, which also corresponding to Figure 3.5 since they are from same sets of spectra). When we move the probe, some of the large QDs get out of the field of excitation and some go into it. If the number of large QDs going into the field of excitation is different from the number getting out, it causes a signal loss or gain in the overall large QDs signal. If we plot the pixels value of large QDs as in Figure 3.8, we can tell that the pixel value represents three different populations of QDs (shown in different circles in Figure 3.8). The average pixel values of the three populations are 6.18, 6.73 and 7.21. The difference between the values should be a single large QD signal times a constant (the constant is probably one). From Figure 3.8.b), the intensity corresponding to single
QD signal is probably ~0.51. So the number of QDs in the field of excitation is ~13. However, without knowing the near-field intensity profile along z-direction, this number is an estimation based on the assumption that the excitation intensity profile is uniform in ~50nm-150nm range along the z direction. We will prove that the intensity profile is uniform in the 20nm-50nm range in z direction (in Chapter 3.3). We still need more proof of the uniform range of the laser intensity in the future.

Figure 3.8 c) is the histogram showing the distribution of the number of large QDs on the cover glass. Compared to the number of large QDs in the field of excitation analyzed in our coarse scan (~3-4), the concentration increase in the fine scan (~4 times) corresponds to the increase of the dilution very well.

Figure 3.7. a) 7×7 fine scan image of large QDs signal (normalized by laser intensity. b) Intensity corresponding to the image.
Figure 3.8. a) The histogram of pixels value can represent three different populations of large QDs on the PMMA. b) fitting for the average pixel value which correspond to the three different population of large QDs

In the quenching spots shown in Figure 3.5, we can make a statement that FRET interaction happens. In some pixels such as (0, 60), (45, 90), (60, 75) and (90, 45), we see the largest quenching of small QDs signal. The largest quenching indicates that the large QD are probably at the surface of the PMMA film and the small QDs are on top of large QDs. Assuming that the QDs are uniformly distributed in PMMA (according to Figure 3.8.c), the aperture size is ~100nm and the concentration of QDs is not too high (~10 in the field of excitation), each pixel size (15nm×15nm) should contain ~1 large QD. However, we still cannot tell that the largest quenching indicates that the small QDs interact with only one large QD. Figure 3.9 shows the situation which likely is happening: the signal we collect from each pixel is the total
signal from the small QDs on the probe containing the interaction information from every FRET pair. It is not necessary that the quenching is caused by only one large QD on the cover glass. As in Figure 3.9, the yellow spots represent the small QDs on the probe and red ones represent the large QDs on the cover glass.

Figure 3.9. Schematic shows the possible situation happened in fine scan.

3.3 Resonant Energy Transfer between CdSe/ZnS Nanocrystal Quantum Dots as a Function of Separation

Even thought we cannot confirm only one single QD on the cover glass interacting with one dot on the probe, as long as we find the largest quenching spot, we still want to use this information. Figure 3.10 shows the experimental data for the FRET as a function of separation. Figure 3.10.a shows the PL spectra of small QDs and large QDs as a function of separation. We fit the spectra with Gaussian functions and obtain the area under the PL spectra of small QDs and the large QDs. We then normalize the area under the PL spectra of the small QDs by the area of the large QDs. The results are shown in Figure 3.10.b, as the quenching of the PL signal of the small dots as function of separation from large dots. Figure 3.10.c shows the PL signal of the small QDs when approaching to a blank cover glass. This result is consistent
with our former member’s data. The quenching is stronger when the distance is shorter, which match our expectation. Data on a blank glass shown in Figure 3.10.c indicates that the quenching is not caused by the movement of the probe in the z direction, but by the interaction among dots. The signal from large QDs on the cover glass does not vary much, which means the light intensity profile does not change appreciably in ~20nm-50nm range in z-direction. In this figure, we see that the largest quenching is ~13% which is comparable to the quenching we see in Figure 3.5 (~11%). This is further evidence that the fine scan data shown in Figure 3.5 is reasonable. We also compare Figure 3.10 to Figure 3.4, which data is presumed to arise from a single large QD. The ratio between small QDs signal and large QDs signal shown in Figure 3.4 is ~0.2. Considering that the procedure to attach small QDs does not change, the number of small QDs on the probe in both experiments should be similar. So the ratio between the small QDs signal and large QDs signal also indicates how many QDs intensity are in the field of excitation. Figure 3.9 shows 7 times more large QDs in the field of excitation than Figure 3.4, which means there are ~7 QDs on the field of view. Compared to the estimation from Figure 3.8 which is ~13 QDs in the field of view, the value is almost half. This error is still tolerable since the amount of small QDs on the probe is not perfectly controlled.
Figure 3.10. a) PL spectra of small and large QDs as function of separation. Horizontal, lateral and vertical axes show wavelength, separation between the small QDs and large QDs. b) PL signal of small QDs normalized by the PL signal of large QDs. The horizontal axis shows the separation between small QDs and large QDs. The vertical axis shows the area of the PL spectra of the small QDs over the area of the PL spectra of the large QDs. c) small QDs signal when the probe approach to a blank cover glass.

The transition probability is the particle number transit from one energy state to another energy state in a unit time versus the particle number in the original energy state before transition. It can also be described as the probability of the energy in the
particle transferring to another energy state. We consider the farthest separation is ~53nm. We assume that there is no transition happening at this distance. As shown in Figure 3.10 b), the normalized small dots PL signal should be maximum at ~53nm. The difference between other separation data point and the maximum value should be proportional to the energy which transfers to the large dots. We then normalized that by the maximum value at the farthest separation. The outcome represents the number of the small QDs in which the energy transits to large dots versus the total number of the small QDs. It can be also interpreted as the probability of transition. The transition probability as a function of the separation with the theoretical line is shown in Figure 3.11. The red curve is the theoretical curve calculate by former colleague: \( P = \frac{k}{r^6} \), where \( P \) is the transition probability, \( k = 3 \times 10^{-47} m^6 \) and \( r \) is the separation between the two QDs and \( k \) is a constant. The red curve in Figure 3.11 shows the theoretical calculation of the transition probability curve.

![Figure 3.11. transition probability as a function of the separation. The solid line shows the theoretical calculation by the former colleague.](image.png)
As we see, the theoretical curve doesn’t fit the data point very well. Theoretically, what I want to see is the data point should be close to the red curve, at least shows the $\frac{1}{r^6}$ dependence with a different parameter. There are some factors need to be taken into the consideration. First of all, not all the small QDs on the probe participated in the FRET interaction with large QDs on the cover glass. The signal emitted by the potion of QDs participating in the interaction with the large QDs is $n(r)$. When $r$ is large and we consider that there is little interaction, then $n(r)$ become a constant $n_0$. $n_0$ is the signal of total QDs. The signal coming from the potion which doesn’t contribute to the interaction is $N$. Therefore, the transition probability should be represented as $1 - \frac{n(r)}{n_0}$. The “transition probability” we used in Figure 3.11 is actually $1 - \frac{N+n(r)}{N+n_0}$, which is $Pq$, where $P$ is transition probability and $q = \frac{n_0}{N+n_0}$, which is the fraction between QDs involved in FRET and the total QDs number.

Second, the situation schematically shown in Figure 3.10. needs to be considered. When we reach the highest quenching point, it does not necessarily indicate that all the small QDs on the probe are exactly aligned with the large QDs on the cover glass. In Figure 3.10, dots 1 and 3 are aligned but dots 2 4 are not well aligned, but also have FRET interaction. That’s why it is important to have single dot. Assuming that the FRET happens independently in every QDs pair (in case of Figure 3.10, the dot 2 does not interact noticeably with dot 3), the transition probability for each QDs pair is $\frac{k}{(r^2+x_n^2)^{\frac{3}{2}}}$, where $r$ is the distance between the probe and cover glass and $x_n$ is the horizontal separation for QDs pair $n$. After averaging we get the overall probability should be $\bar{P} = \frac{\sum_{k=1}^{K} k}{(r^2+x_n^2)^{\frac{3}{2}}}$, $K$ is the total number of QDs pair. Other thing needs to be considered is that the loss of FRET efficiency can be caused by the different polarization of small and large QDs. Furthermore, the small QDs distribution on the probe is not necessarily uniform or even may have aggregation. Therefore it is
possible that there are several small QDs interacting with a large QD on the cover glass, which makes it more complicated.

In this work, I still cannot identify single QD-single QD interaction. However, a systematic experimental method about how to indentify single QD on cover glass and how to align small large QDs, including the analysis methods are introduced. We can successfully reduce the number of large QDs which interact with small QDs. Many modifications are needed in the future. The first improvement should be decrease of the thickness of the PMMA thin film, to 10-20nm[22]. If we can obtain a thinner PMMA film, we can locate a large QD embedding on the surface of the PMMA by coarse scan. Then we can directly use a fine scan to align the single QD to the small QDs on the probe. By using a ∼10nm thickness PMMA we can probably achieve several small QDs interacting with one large QD. If we cannot make the PMMA film to the desired thickness, the intensity profile of the excitation light needs to be measured. Another potential improvement is the functionalization of the NSOM probe with signal small QD. Some paper introduce ways to functionalize AFM tips, including CdSe QDs[23]. This method makes it possible that only one QD on the tip of NSOM system by chemical way. Finally, the polarization of the QDs also needs to be taken into account.
Figure a. Specification of OPAs and resistors and capacitor of the feedback loop are shown in the diagram. Inputs (NSOM probe oscillation amplitude and set point) are at A and B. To optimize the stability of engaging of probe and cover glass, capacitor, resistor and variable resistors have been changed to find the best values. These values change as the engaging needs to be optimized. Capacitor and variable resistor on integrator and proportional branches can be changed to stabilize the engagement. This circuit is built by our former colleague[19].
REFERENCES


