CHAPTER 3

THE TECHNOLOGIES OF NANODEVICE FABRICATION PROCESS

3.1 DNA HYBRIDIZATION SYSTEM FOR NANODEVICE FABRICATION

As we have discussed in the chapter 1, the forces that direct and contribute to the assembly of nanoparticles (NPs) are similar to those involving in the interaction between molecules, such as hydrogen bonds, Coulombic force, and Van der Waal Force. In this work, we develop two significant fabrication systems, ***DNA hybridization system*** and ***Coulombic force system*****,** which are able to efficiently control the assembly processes and the nanostructures of NPs on substrate. DNA hybridization system utilizes the nature of DNA primers that the two well-designed complementary oligonucleotides will hybridize with each other automatically under proper environment and will denatured when the temperature rises---the self-assembly process. Coulombic force system directly takes advantage of the positive or negative charges on the NPs or the substrate. For instance, when the Au NPs are negative-charged, they will repulse each other to prevent self-aggregation and will be attracted by the positive-charged substrate or another type of NPs, like CdSe NPs, which are positive-charged. Coulombic force system will be discussed in detail in later paragraphs, and, here we will focus on DNA hybridization system. The overall fabrication process of the photo-sensing nanodevice composed of Au and CdSe NPs on silicon chip by DNA hybridization system is shown in Fig. 3.1. The passivation window, 86 μm square, was opened up on silicon chip, resulting in the electrodes structures where the two closely

separated Al metal lines served as electrodes and the oxide region are the place for nanodevice construction. Then, the surface of oxide surface that naturally has –OH groups was chemically modified with 10% N-[3-(trimethoxysilyl)propyl]-ethylene diamine (TMSPED)/methanol (or 3-aminopropyltriethoxysilane (APTES)/ethanol) solution in order to label amino ($-NH_2$) groups on oxide surface for further reaction with CdSe NPs. The CdSe NPs, less than 10 nm in diameter, were converted form oil-soluble to water-soluble by modifying the surfaces of NPs with carboxylate ($-COO^-$) group. The chip was immersed in CdSe NPs suspension, where the negative charges on CdSe NPs surface repulse each other to prevent self-aggregation. Then, Cyanamide (HN=C=NH) was added in suspension to activate carboxylate ($-COO^-$) groups and not protonated amino ($-NH_2$) groups on oxide surface attacked the carboxylate ($-COO^-$) groups, to form covalent bond between CdSe NPs and substrate. In the same way, the Primer 3 with amino group on 5' end was added to form covalent bond with CdSe NPs. The Au NPs, 20~40 nm in diameter, are labeled with Primer 1 that are complementary to Primer 3 by utilizing Au-S bond. The chip was immersed in the Au NPs suspension and the Primer 1 on Au NPs are hybridized with Primer 3 on CdSe NPs, performing the self-assembly process. The CdSe NPs are labeled with Primer 3 by using the Cyanamide method as described above. Again, the chip was immersed in CdSe NPs suspension labeled with Primer 3 and the CdSe NPs will be assembled onto the Au NPs by self-assembly process. Finally, the photo-sensing nanodevice structure was formed on silicon chip.

3.1.1 The Synthesis of Au Nanoparticles and Modification with DNA Primers

Before we start to construct the specific nanostructure composed of Au and CdSe NPs by utilizing DNA self-assembly property, the conjugation of the NPs and DNA primers is one of

the primary and critical technologies for the further experiment. As long as the NPs are firmly labeled with the DNA primers, instead of random and unpredictable organization, they can be directed and assembled into a well-designed nanostructure we desired. The method to make DNA primers attached to the Au NPs surface is simply through the formation of Au-S bond, which in literature is commonly adapted in many experiments involving DNA and Au NPs. To get a more efficient and complete attachment, the DNA primers must be carefully and thoroughly modified with thiol reagent and the surface of the Au NPs must keep as "fresh" as possible, which means the absence of any chemical substance capping on the Au NPs. In addition to these, it also needs very careful manipulation and verification during the process. We will discuss all of them in detail in the following paragraph.

1) ***The two thiol (-SH) modified and complementary DNA primers:*** The two thiol modified DNA primers we used here were purchased from *Alpha DNA, Inc., Canada.* The sequences of the two primers were designed, by simulation of software *Primer 5*, to have 15 bps in length and complementary to each other. Furthermore, by meticulous estimation, we can efficiently eliminate the unexpected inter- or intra-oligonucleotides interactions, such as hairpin loop formation of one single primer and primer-dimmer structure between two primers of the same sequence. The reason why we choose 15 bps for the length of each primer is primarily because we want to make the distance between the two NPs that are ready to be assembled by primers hybridization as short as possible. And we found that 15 bps is the optimized and effective length for two primers to recognize and hybridize each other easily. The distance made by the primers between NPs is estimated to be 0.34 nm * 15 + 5 nm ~ 10 nm, considered that the two complementary primers are hybridized to form double helix structure, B form, and the rest part of primer, like alkyl chain, is assumed to be less than 5 nm. The denature temperature of cross dimmer between two complementary primers is expected to be 60^{o}C. The sequence of the two DNA primers is shown bellow:

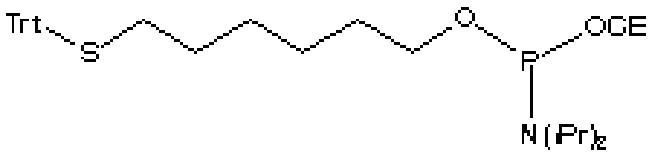
Primer1: $SH(CH_2)_6$*-CGGCT GGGGA TAATG (5' to 3')*

Primer2: *$SH(CH_2)_6$-CATTA TCCCC AGCCG (5' to 3')*

Primer1: $SH(CH_2)_6$-CGGCT GGGGA TAATG

Primer2: GCCGA CCCCT ATTAC-$(CH_2)_6$ SH

Besides the sequences play an important role in DNA primers recognition and hybridization, the thiol modification is also one of the very critical factors for primers to be firmly attached to the surface of Au NPs or Au plate through Au-S bond formation. The modification reagent, 5' Thio C6 Modifier (Trityl-6-Thiohexyl Amidite) is shown bellow:



After modification, the reduced thiol group (SH-) at the 5' end of each primer is highly reactive, therefore it tends to be quickly oxidized and "lost" because it enters various unfavorable chemical reactions against the Au-S bond formation. The most common figures are the primer-primer dimmer or primer-modification reagent dimmer formation, i.e.

Oligonucleotide#1-S-S-Oligonucleotide#2

or

Oligonucleotide#1-S-S-reagent

The first one is inevitable unless we treat the primers with dithiothreitol (DTT), a kind of reductant to reduce and eliminate the primer-primer dimmer structure prior to the usage of primers, which however will instead induce another complicated problem of removing the DTT by ethanol precipitation after reduction. As a result, for the sake of simplicity of experiment design, we finally choose to use the primers without DTT and we must use our primers as soon as possible once we dissolved our oligonucleotides. The oligonucleotides were originally shipped lyophilized (dried under vacuum) and under Argon. If not all amount of primers is used, the remaining quantity should be divided into convenient aliquots, lyophilized again, and stored frozen at –80°C for up to several weeks. In addition, before the

usage of primers, it is also necessary to check if there are still active, un-oxidized, thiol groups at the 5' end of primer by 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) reagents, which will be introduced in detail in the following. The second one can be eliminated thoroughly since there is no free thiol contamination. The chemical synthesis of DNA goes from 3' to 5'. At the last cycle of the synthesis, the primer is in the solid phase (attached to the synthesis column), while the thiol reagent is in the liquid phase that passes through the column. Only the thiol that is coupled to the primer is retained on the column, any "free" thiol reagent is removed by the extensive washes at the end of the cycle, plus the washing step at the end of the synthesis.

Diminishing the possibility of all unfavorable oxidation reactions involving thiol group to minimum, the Au NPs can be labeled with DNA efficiently.

2) ***The Au NPs (by physical method) with diameter 20 nm~40 nm:*** The Au NPs we used here were purchased from *Global Nano Tech. Inc., Taipei.* The NPs were synthesized by novel physical method instead of conventional chemical method, citrate reduction of $HAuCl_4$. The size of NPs, analyzed by TEM, is ranging from 20 nm to 40 nm in diameter, as shown in Fig. 3.2. The concentration of the original Au NPs suspension we obtained was 3518 ppm, that is 17.86 mM. The Au NPs suspending in water are characterized by the minor peak at 530 nm of UV-visible spectrum, as shown in Fig. 3.3.

3) ***Verification of the active thiol group of two DNA primers:*** As we mentioned above, the thiol groups at the 5'end of primers we used in this experiment are so reactive that they tend to become dimmer structures with each other before the Au-S bond is form. Therefore, every time before the usage of primers, it is necessary to check if there are still plenty of active thiol groups of primers. The most common and effective method we adapted here is using the DTNB as test reagent. The mechanism is illustrated in Fig. 3.4. The original DTNB solution at pH 8 is transparent and achromatic. Then, the primers with thiol groups are added into the solution and the disulfide bonds of DTNBs are attacked (reduced) by the

thiol groups of primers. If there are plentiful active and free thiol groups in the solution, the color of solution will become yellow conspicuously and the UV-visible absorbance spectrum will have an apparent increment at 400 nm due to the present of reduced TNBs in the solution. The detailed process of this experiment is shown in the following paragraph.

All the water we used here was purified through deionization and distillation to prevent any unfavorable redox reaction. An aqueous phosphate buffer (50 mM Na_2HPO_4, pH 8, 100 mL) was prepared to dissolve both the DTNB reagent and the primers. The DTNB reagent we used here was 5 mM and solved in phosphate buffer. The two primers solutions, Primer1 sol. (1.46 mM) and Primer2 sol. (1.96 mM), were (estimated from OD-260 of UV-visible absorbance spectrum of each solution) solved in the phosphate buffer. Then, we made three samples, shown as bellow, to observe and identify the difference in UV-visible absorbance spectrum at 400 nm and the changing color of solution among them after standing for 10 minutes.

"DTNB + primer1" = 100 μL DTNB reagent + 400 μL phosphate buffer + 20 μL Primer1 sol.

"DTNB + primer2" = 100 μL DTNB reagent + 400 μL phosphate buffer + 20 μL Primer2 sol.

"DTNB only" = 100 μL DTNB reagent + 420 μL phosphate buffer

The UV-visible spectrums of the three samples are shown in Fig. 3.5, where the concentration of the samples were diluted to 1 / 5 of its original value by adding 400uL of the phosphate buffer (50 mM Na_2HPO_4, pH 8, 100 mL) to 100 μL of the sample solution. As we have seen in the spectra, there is an increment at OD-400 in the spectra of "DTNB + primer1" and "DTNB + primer2" compared to "DTNB only", due to the presence of TNBs in solution. Theoretically, one active and free thiol group of a primer will produce one TNB molecule. Therefore, by estimating the concentration of TNBs, we can calculate the concentration of Primer1 sol. and Primer2 sol. that have active and free thiol groups at 5' end.

The calculation process is shown bellow:

"DTNB + primer1":

(a) OD-400 increment due to *Primer1*

= 0.17 - 0.11 = $(1.63 * 10^4) * 1 * (C / 5)$

=>C (The concentration of active thiol group) = 21 μM

(b) The total *Primer1* concentration of "DTNB + primer1" = 1.46 / 26 = 56.2 μM

(c) The ratio of the number of *Primer1* that has active thiol end to the total number of *Primer1* = 21 / 56.2 = 37.4%

"DTNB + primer2":

(a) OD-400 increment due to *Primer2*

= 0.20 - 0.11 = $(1.63 * 10^4) * 1 * (C / 5)$

=>C (The concentration of active thiol group) = 28 μM

(b) The total *Primer2* concentration of "DTNB + primer2" = 1.96 / 26 = 75.4 μM

(c) The ratio of the number of *Primer2* that has active thiol end to the total number of *Primer2* = 28 / 75.4 = 37.1%

, where $1.63 * 10^4$ is the Molar Absorptivity of TNBs.

The color of the samples with primers, "DTNB + primer1" and "DTNB + primer2", were changing form achromatic to yellow, while the color of the sample without primers, "DTNB only", remained achromatic. The color of three samples after standing for 10 minutes is shown in Fig. 3.6.

4) *The conjugation of DNA primers with Au NPs (by physical method):* After the verification of active thiol groups at 5' end of the primers, we start to make the conjugation of the primers with Au NPs by forming Au-S bond. Again, all the water we used here was

purified through deionization and distillation to prevent any unfavorable redox reaction. An aqueous phosphate buffer (10 mM Na_2HPO_4, 100 mM NaCl, pH 7, 100 mL) was prepared. Before mixing the Au NPs suspension and the primer solution, we should "wash" the Au NPs suspension by replacing the solvent of the original suspension with the phosphate buffer we had prepared, in order to prevent any contamination that might affect the Au-S bond formation. The "wash" process is illustrated in Fig. 3.7. After the process, we obtained the purified Au NPs suspension, removing 99.99% of its original water solvent. However, during the purification, we might lose 30%~40% Au NPs, approximately. Therefore, the resulting concentration of purified Au NPs suspension would be 2287 ppm, which is 11.61 mM. The two primers solutions, Primer1 sol. (57.23 μM) and Primer2 sol. (62.30 μM), were (estimated from OD-260 of UV-visible absorbance spectrum of each solution) both solved in the phosphate buffer. When everything mentioned above was ready, we start the reaction between the Au NPs and primers. The same as we did in DTNB experiment, we made three comparing samples to distinguish the difference in UV-visible absorbance spectrum of the samples with or without primers. The detailed process is illustrated in Fig. 3.8. This experiment is designed and controlled simply by the fact that the Au NPs, which are much bigger and heavier than the DNA molecules, can be separated from the solvent and precipitate on the bottom of tube during centrifugation (14k rpm), while the primers remains completely resolved in the solvent and cannot be separated by centrifugation. If a certain portion of primers is labeled onto the surface of Au NPs, those primers coupling to Au NPs will be brought to the bottom of tube by centrifugation and the free primers will remain staying in the supernatant of original NPs suspension. Therefore, by taking UV-visible absorbance spectrum of the supernatant, we will observe a decrement at OD-260. The UV-visible absorbance spectrum of each sample, Sam1,2,3, Sam1s,2s, Sam1DNA, and Sam2DNA are shown in Fig. 3.9, where Sam1s and Sam2s are compared with Sam1DNA, and Sam2DNA to examine and note the decrement at OD-260 and all the concentration of the

samples were diluted to 30 / 430 of its original value by adding 400 μL of the phosphate buffer to 30 μL of the sample solution. In Fig. 3.9, we can obviously identify the decrement at OD-260 after centrifugation because the DNA primers attached to the Au NPs are brought to the bottom of tube, resulting in the lower concentration of primers in the supernatant. The decrement at OD-260 of Sam1s and Sam2s compared to Sam1DNA and Sam2DNA is calculated bellow:

OD-260(Sam1DNA) - OD260(Sam1s) = 0.25 - 0.21 = 0.04

OD-260(Sam2DNA) - OD260(Sam2s) = 0.26 - 0.22 = 0.04

The OD-530 of UV-visible absorbance spectrum after centrifugation (OD-530 of Sam1s and Sam2s) is almost zero, which means that the centrifugation process has successfully brought almost all of the Au NPs to the bottom of tube.

After carefully measuring the UV-visible absorbance spectrum, we begin to mix the two Au NPs that have already been individually labeled with complementary DNA primers. Consequently, if we mix the two Au NPs samples, we are able to observe the changing color and eventually the precipitation of Au NPs due to DNA primers hybridization and self-assembly. However, before mixing, it is very important to remove the excess DNA primers of both samples because the excess primers will hybridize with the primers that are attached to the Au NPs, disabling the normal hybridization between Au NPs. The "washing process" is shown in Fig. 3.10. After the process, we can mix the two final samples to observe the changing color and precipitation. As we can see in Fig. 3.11, there is an obvious precipitation in the mixture due to self-assembly process between promer1 and 2, and the color of suspension changes from ***purplish red*** to ***light blue***.

3.1.2 The Synthesis of CdSe Nanoparticles and Modification with DNA Primers

The method to make DNA primers attached to the CdSe NPs surface is more complicated compared to the method of Au NPs, including converting the oil-soluble CdSe NPs to water-soluble, activating the carboxylate ($-COO^-$) group by Cyanamide and the nucleophile attack reaction by the primary amino ($-NH_2$) group of DNA primers. The detailed process is discussed in the following paragraph.

1) ***The two amine ($-NH_2$) modified and complementary DNA primers:*** The two amine modified DNA primers we used here were purchased from *Alpha DNA, Inc., Canada.* The sequences of the two DNA primers were designed to be the same with those used for Au NPs except the 5' modification. The reason for this design is simple that in DNA hybridization system, as shown in Fig. 3.1, all the DNA primers used for self-assembly process have only two complementary sequences. As a result, Au and CdSe NPs are able to stack on the substrate sequentially to form the photo-sensing nanodevice. The only difference between the primer sequences for Au and CdSe NPs is that we make thiol modification at 5' end for conjugation to Au NPs, and amine modification at 5' end for conjugation to CdSe NPs. The denature temperature of cross dimmer between two complementary primers is expected to be 60^oC. The sequences of the four DNA primers are shown bellow:

Primer1: $SH(CH_2)_6$-CGGCT GGGGA TAATG (5' to 3')

Primer2: $SH(CH_2)_6$-CATTA TCCCC AGCCG (5' to 3')

Primer3: $NH_2(CH_2)_6$-CATTA TCCCC AGCCG (5' to 3')

Primer4: $NH_2(CH_2)_6$-CGGCT GGGGA TAATG (5' to 3')

Primer4: $NH_2(CH_2)_6$-CGGCT GGGGA TAATG

Primer3: GCCGA CCCCT ATTAC-$(CH_2)_6$ NH_2

Unlike thiol (-SH) groups, the amine (-NH2) groups at the 5' end of DNA primers 3 and

4 are relatively more stable because they will not form primer-primer dimmer structure. Again, if not all amount of primers is used, the remaining quantity should be divided into convenient aliquots, lyophilized again, and stored frozen at –80^{o}C for up to several weeks.

2) ***The water-soluble (carboxylate (-COO^{-}) modified) CdSe NPs with diameter less than 10 nm:*** The original fluorescent oil-soluble CdSe NPs are obtained from *Prof. Teng-Ming Chen's lab, National Chiao Tung University, Taiwan.* The detailed synthesis process and characteristics of oil-soluble CdSe NPs with diameter less than 10 nm is reported in literature [10]. In this part, we will focus on the technology of conversion to water-soluble NPs. As we discussed in Chapter 2, it is usually to cap the CdSe NPs core with ZnS shell in order to enhance the fluorescent property of CdSe NPs, resulting in CdSe/ZnS (core/shell) structures. In the experiments involving probing specific sequence of DNA or structure of proteins, the strength of fluorescence is very important for the precise detection. However, in this work, we focus on the electrical property of CdSe NPs instead of fluorescent property because we want to develop a photo sensor, not a fluorescent device. Theoretically, the ZnS (band gap of bulk = 3.68 eV at 300K) shell is a high band gap semiconductor material compared to the CdSe (band gap of bulk = 1.7 eV at 300K) core, resulting in blockade of the electrons shuttling across the shell---the quantum confinement effect. Therefore, we choose CdSe NPs instead of CdSe/ZnS NPs in this work. The structures of CdSe NP are illustrated in Fig. 3.12(a).

In the conversion process to water-soluble CdSe NPs, we use 11-mercaptoundecanoic acid (MUA) (HS-$(CH_2)_{10}$-$CH(COOH)_2$) as the new ligands to partially replace the original trioctylphosphine oxide (TOPO) coated on the CdSe NPs by forming S-Se bond on the surface. Theoretically, the covalent S-Se bond is relatively more stable than the ionic bond of TOPO to CdSe NPs. The structure of CdSe NP after MUA modification is illustrated in Fig. 3.12(b).

Typically, the TOPO-coated CdSe NPs are stored in powders and protected from illumination. After the oil-soluble CdSe NPs of the specific size (typically less than 10 nm) and MUA are ready, we are able to start the conversion process. First, we thoroughly dissolved 20 mg CdSe NPs powders in 5 mL methanol under room temperature and added this CdSe NPs suspension to MUA/methanol solution, which was prepared by adding 200 mg MUA into 10 mL methanol and stirring until clear under room temperature. Then, the solution was added 2 CC. base (tetramethyl ammonium hydroxide) to make pH>10, and was brought to reflux for 3 hr under 70°C and prevented form illumination. After reflux for 3 hr, the solution was left without reflux in room temperature to cool down for 30 min. Then, 10 mL chloroform ($CHCl_3$) and 10 mL PBS buffer (5 mM Na_2HPO_4, pH 7.5) were added into the solution, followed by stirring softly for 3 min to make complete mixture. After standing for 20 min, the solution became two layers, the upper PBS buffer solution of strong color corresponding to that of original CdSe powders and the lower chloroform solution of relatively light color. The successfully MUA-modified CdSe NPs became water-soluble and would stay in the PBS buffer layer. However, the remaining TOPO-modified CdSe NPs were still oil-soluble and would stay in the chloroform layer. Because methanol is soluble with both PBS buffer and chloroform and MUA was soluble in methanol, it is possible that both layers contained the unused MUA. Finally, we collected the upper layer of water-soluble MUA-modified CdSe NPs suspension and washed the NPs with methanol by centrifuge method to remove unused MUA. After wash, the precipitation of MUA-modified CdSe NPs was re-suspended in PBS buffer (5 mM Na_2HPO_4, pH 7.5) [11]. The overall detailed process of conversion is illustrated in Fig. 3.13.

3) ***The conjugation of DNA primers with CdSe NPs:*** The conjugation between 5' amine modified DNA primers and MUA-modified CdSe NPs is achieved by the method of Jason R. Taylor et al. In this bioconjugation, the cross-linking reagent Cyanamide (NH=C=NH) is commonly used. This compound reacts with the carboxylate ($-COO^-$) groups on the surface

of CdSe NPs to yield an *O*-acyl-isourea active intermediate. Then, the amino (-NH_2) groups at 5' end of DNA primers attack these intermediates, to form a stable covalent bond between CdSe NPs and DNA primers. However, one significant drawback of this method is that the *O*-acyl-isourea active intermediate is also subject to hydrolysis in aqueous media, resulting in competition between H_2O and amine group of DNA primers. As a result, we added another reagent 1-Hydroxy-2,5-pyrrolidinedione (NHS) ($C_4H_5NO_3$) to form a more stable ester intermediate that is less susceptible to hydrolysis and more rapidly to react with amine group of DNA primers. The conceptual diagram of this conjugation process is illustrated in Fig. 3.14.

The detailed conjugation process is described as follow: First, the Primer3 sol. and Primer 4 sol. were both prepared in PBS buffer (5 mM Na_2HPO_4, pH 7.5). Then, we added 250 μL MUA-modified CdSe NPs we had prepared to 250 μL Primer3 sol. and 250 μL Primer4 sol., respectively. After standing for 15 min, 4 mg Cyanamide and 10 mg NHS were added and the mixture was vortexed slowly for 3 hr. The excess DNA primers, NHS, hydrolyzed Cyanamide were removed by using centrifugation method, removing the supernatant after spin. After wash, the precipitation of DNA modified CdSe NPs was re-suspended in PBS buffer (5 mM Na_2HPO_4, pH 7.5).

3.1.3 The Assembly of Au Nanoparticles on Gold Substrate by DNA Self-Assembly Process

After we have finished the synthesis of DNA primers modified Au NPs (from physical method) and CdSe NPs (surface MUA modified), we are able to assemble the NPs into a well-controlled array on the substrate by DNA self-assembly process. Eventually, we will construct the photo-sensing nanodevice on silicon oxide substrate following the process illustrated in Fig. 3.1. However, we first chose the gold substrate as a tester to make sure the

mechanism of DNA self-assembly process in nanostructure formation, because the gold substrate is naturally capable to connect with DNA primers by forming the Au-S bond.

The Au chip that provides an absolutely clean and flat gold substrate was purchased from BIACORE. The structure of the Au chip is a thin layer of gold (50 nm) on glass substrate. First, we made two batches of Au NPs, which were labeled with *Primer1* and *Primer2* respectively. The detailed process is described in Fig. 3.8 and Fig. 3.10. Then, the two Au chips were immersed in Primer1 sol. (171.6 μM) and Primer2 sol. (186.9 μM) (estimated from OD-260 of UV-visible absorbance spectrum of each solution) respectively and washed by phosphate buffer (10 mM Na_2HPO_4, 100 mM NaCl, pH 7) to remove excess DNA primers. The Au chip that was labeled with *Primer1* was again immersed into Au NPs suspension labeled with *Primer2*, and vice versa. Finally, by DNA self-assembly process, the Au NPs were assembled on the surface of Au chip, and the results of the assembly were verified by SEM. The detailed process is illustrated in Fig. 3.15. The SEM images (50k, 100k, 150k magnification) of the Au NPs modified Au chip substrate are shown in Fig. 3.16.

3.1.4 The Assembly of CdSe and Au Nanoparticles on Silicon Oxide Substrate by DNA Self-assembly Process

After the verification of the DNA self-assembly mechanism on gold substrate in 3.1.3, we started to construct the photo-sensing nanodevice on silicon oxide substrate following the steps shown in Fig. 3.1. First, the SiO_2 (30 nm in thickness)/Si wafer fragments (~5 mm^2 in area) were cleaned with strong N_2 flow for 3 min to remove unspecific particles attached on the surface. Then, we immersed the wafer fragments into 10% TMSPED (N-[3-(trimethoxysilyl)propyl]-ethylene diamine)/dry methanol (or 10% APTES (3-aminopropyltriethoxysilane)/ dry ethanol) solution for 20 min to modify the silicon oxide surface with amino ($-NH_2$) group [12]. It is very important to note that the TMSPED or the

APTES reagent is very sensitive to moisture since the electrons pairs of –OH groups will attack the partial positive Si of TMSPED or APTES molecules. Once the $–OCH_2CH_3$ or $–OCH_3$ are replaced by –OH, the molecules are no longer able to modify the silicon oxide, as illustrated in Fig. 3.17. After the modification, the excess reagent (TMSPED or APTES) was completely washed out by ethanol for several times to remove excess reagent. So far, we finished the step (b) in Fig. 3.1. To make the assembly of MUA (carboxylate)-modified CdSe NPs on the silicon oxide, we immersed the 10% TMSPED-modified wafer fragments into 100 μL CdSe NPs suspension we have made previously in Fig. 13.13. Then, we added 4 mg Cyanamide (NH=C=NH) powders into the solution to react with the carboxylate ($-COO^-$) groups on the surface of MUA-modified CdSe NPs to yield an *O*-acyl-isourea active intermediate. Then, the amino ($-NH_2$) groups of TMSPED or APTES on silicon oxide surface attacked this intermediate, to form a stable covalent bond between CdSe NPs and DNA primers. After standing for 3 hr, the wafer fragments were completely washed with DI water for several times to remove excess reagent. The SEM images (50k, 100k, 150k magnification) of CdSe NPs modified silicon oxide substrate are shown in Fig. 3.18.

The remaining steps shown in Fig.3.1 will be accomplished in the future by colleagues. The conjugation of 5' amine modified DNA primers to MUA-modified CdSe NPs (step (d) of Fig. 3.1.) is similar to the process described in detail in 3.1.2. After the conjugation, the following steps are nothing more than DNA self-assembly process. All the operations are in phosphate buffer (10 mM Na_2HPO_4, 100 mM NaCl, pH 7) that was used in 3.1.1.

3.2 COULOMBIC FORCE SYSTEM FOR NANODEVICE FABRICATION

As we have discussed in 3.1, in ***Coulombic force system,*** we directly take advantage of the positive or negative charges on the surfaces of nanoparticles (NPs) to induce repulsion or attraction forces between different NPs or between NPs and substrate. The repulsion force will prevent NPs from random aggregation before assembled on the substrate. In contrast, the attraction force will result in the assembly process of NPs on substrate. By well controlling the two forces, we are able to construct the structure of photo-sensing nanodevice on silicon oxide substrate efficiently. The overall fabrication process of the photo-sensing nanodevice composed of Au and CdSe NPs on silicon chip by DNA hybridization system is shown in Fig. 3.19.

General Methods. $HAuCl_4 \cdot 3H_2O$ was purchased from Aldrich chemical company. Citric acid Monohydrate was purchased from Fluka chemical company. Tyramine was purchased form Aldrich chemical company. Tetramethylammonium hydroxide 25 wt% in methanol was purchased from ACROS ORGANICS. N-[3-(trimethoxysilyl)propyl]-ethylene diamine (TMSPED) used to modify silicon oxide substrate was purchased from Aldrich. The 0.45 μm Nylon filter used to filter Au NPs after synthesis was purchased from NALGENE LABWARE. A DENVILLE 260D brushless microcentrifuge was used for centrifugation of CdSe NPs solution. The vacuum drier used to remove water or organic solvent was purchased form PANCHUM. Transmission electron microscopy (TEM) was performed with Philips TECNAI 20 transmission electron microscope. Scanning electron microscopy (SEM) was performed with Hitachi S-4700 high-resolution scanning microscope.

3.2.1 The Synthesis of Au Nanoparticles by Citrate Reduction Method

Approximately 15 nm diameter Au NPs were prepared by citrate reduction of $HAuCl_4$ as described in literature [8]. The pale yellow $HAuCl_4$ solution (1 mM, 500 mL) was prepared and brought to reflux while stirring for 20 min. Then, citric acid solution (38.8 mM, 50 mM) was injected quickly, resulting in solution color changing form pale yellow to deep red. After color changing, the solution was kept in reflux for additional 20 min and then standing in room temperature for another 30 min. Finally, the solution was filtered through 0.45 μm Nylon filter. The close photographs of the Au NPs solution is shown in Fig. 3.21(a). The TEM image of the approximately 15 nm diameter Au NPs is shown in Fig. 3.22(a) and the UV-visible absorbance spectrum of Au NPs solution is shown in Fig. 3.22(c).

3.2.2 The Synthesis of CdSe Nanoparticles and Modification with Amino ($-NH_3^+$) groups

The original approximately 5 nm diameter fluorescent oil-soluble (n-Hexadecylamine (HDA)-coated) CdSe NPs are obtained from *Prof. Teng-Ming Chen's lab, National Chiao Tung University, Taiwan* [10]. The HDA-coated CdSe NPs are very unstable with respect to photooxidation. Therefore, the NPs are stored in completely dried powders and dark environment.

To be able to connect with negative-charged (due to citric acid) Au NPs in aqueous solution, the original HDA-coated CdSe NPs must be converted to positive-charged CdSe NPs. In this work, we use Tyramine that has amino ($-NH_3^+$ after protonation) group at one end and –OH group at the other end as reagent to replace the HDA molecules adsorbed on the surface of CdSe NPs. Because the HDA-Cd interaction is weak, the HDA molecules can be displaced and form relatively more stable covalent bond (Tyramine-O-Cd) between Tyramine and Cd. First, 80 mg of HDA-coated CdSe NPs powders and 600 mg Tyramine powders were solved in 10 mL and 20 mL of dried methanol respectively. Generally,

Tyramine can be completely solved in methanol easily and HDA-coated CdSe NPs powders can suspend in methanol well after ultrasonic vibration. The two solutions were brought together, followed by adding 0.5 CC. 25wt% tetramethylammonium hydroxide/methanol which served as catalyst to displace HDA molecules. Then, the mixture was brought to reflux at 50°C for 12 hrs while stirring in dark environment. After reflux, the mixture was kept standing in room temperature for 30 min for cooling down. Then, 30 mL of chloroform and 10 mL of PBS buffer (5mM Na_2HPO_4, pH 7.0) were added into the mixture. After shaking the mixture softly and standing 10 min for separation between oil and water, the upper layer of Tyramine-modified CdSe NPs aqueous solution was collected and brought to centrifugation at 6 krpm to remove large aggregates. Because Tyramine was largely solved in methanol and methanol was solved in both PBS buffer and chloroform, there was part of excess Tyramine staying in the upper aqueous solution. Therefore, after centrifugation, the Tyramine-modified CdSe NPs solution was washed by 50 mL dichloromethane to remove excess Tyramine. Finally, the solution was dried in vacuum and resolved in DI water prior to use, which will completely remove organic solvents, including methanol, chloroform and dichloromethane [11]. The detailed modification process is shown in Fig. 3.20. The close photographs of the Tyramine-modified CdSe NPs solution is shown in Fig. 3.21(a). The UV-visible absorbance spectrum of original HDA-coated CdSe NPs in organic solvent is shown in Fig. 3.22(d). The TEM image of the approximately 5 nm diameter Tyramine-modified CdSe NPs is shown in Fig. 3.22(a). Fig. 3.21(b) shows the close photographs of the mixture of 100 μL Au NPs solution and 100 μL Tyramine-modified CdSe NPs solution just after mixing, the mixture after standing 6 hrs in room temperature, and the mixture after standing 5 days in room temperature. As we can see, the color of mixture just after mixing was like that of Au NPs solution. However, after 6 hrs, it became dark purplish red. After 5 days, there was obvious precipitate at the bottom and the supernatant became pale yellow, which means that part of CdSe NPs are assembled with Au NPs, forming large

aggregates and precipitate at bottom.

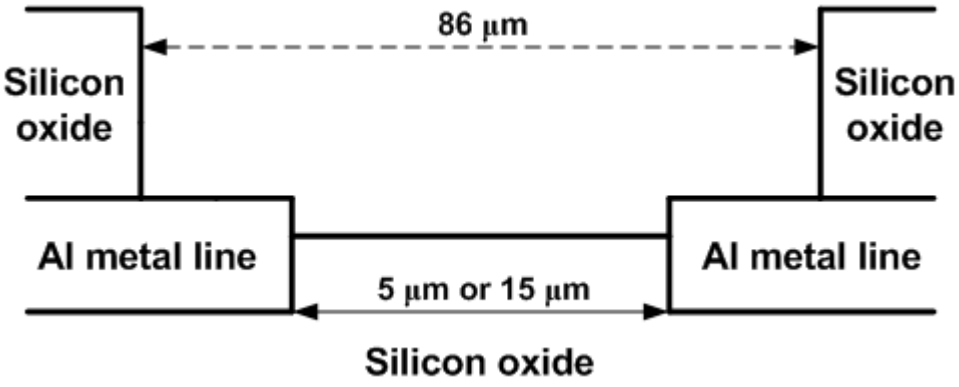
3.2.3 The Assembly of CdSe and Au Nanoparticles on Silicon Oxide Substrate Through Ionic Force Interaction

In this work, we use two kinds of silicon oxide substrate--- the SiO_2 (30 nm in thickness)/Si wafer fragments (~5 mm^2 in area) fabricated by National Nano Device Laboratories, Taiwan, and the silicon chip with electrodes structure fabricated by TSMC 0.35 μm 2P4M process. Generally, the process to construct the nanodevice structure of Au and CdSe NPs is the same for the two kinds of silicon oxide substrate.

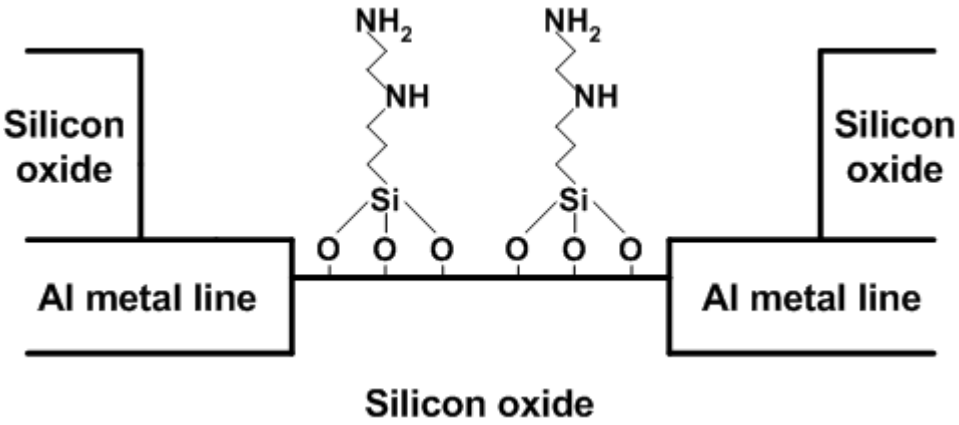
In order to modify the silicon oxide substrate with positive charges, we use N-[3-(trimethoxysilyl)propyl]-ethylene diamine (TMSPED) that has two amino groups on one alkyl chain as reagent to react with the –OH groups on silicon oxide surface. First, the silicon oxide substrate was cleaned by strong N_2 flow to remove nonspecific particles attached on the surface. Then, the silicon oxide was immersed in 10% TMSPED/methanol solution for 20 min [12]. Note that because TMSPED is very moisture sensitive, the methanol used here must be completely dried and all cleaned glasswares must also be dried in oven at 65°C for 30 min prior to use. After immersion, the silicon oxide substrate was cleaned by plenty of methanol to remove excess TMSPED staying on the surface, followed by dipping in 30 mM HCl for 3 seconds to protonate the amino group. Finally, the silicon oxide substrate was cleaned by using plenty of DI water for several times and dried in vacuum.

To construct photo-sensing nanodevice structure, the TMSPED-modified silicon oxide substrate was immersed in the Au NPs solution for 12 hr to make negative-charged ($-COO^-$) Au NPs assembled on the positive-charged ($-NH_3^+$) substrate by ionic interaction. After 12 hr immersion, the silicon oxide was cleaned by DI water for several times to remove free Au NPs and then dried in vacuum. Subsequently, the silicon oxide substrate was immersed in

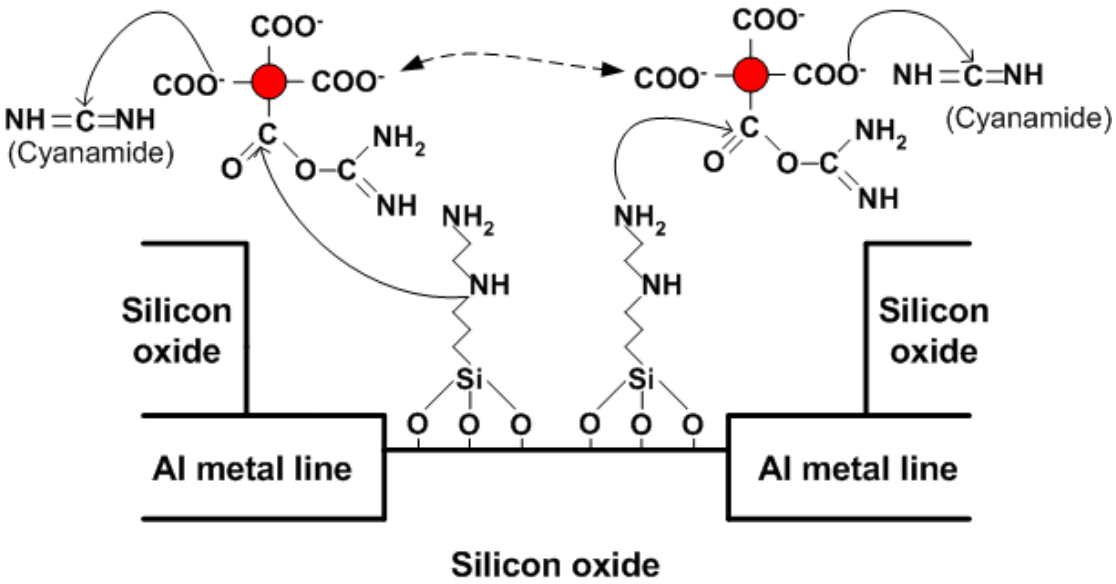
Tyramine-modified CdSe NPs solution for 12 hr to make positive-charged ($-NH_3^+$) CdSe NPs assembled to negative-charged ($-COO^-$) Au NPs, followed by DI water cleaning and vacuum dry as described above. Theoretically, we can repeat this process for several times to form layers of closely packed Au NPs and CdSe NPs structure. The close photographs of SiO_2/Si wafer fragments before and after repeated assembly process are shown in Fig. 3.23. The SEM images of the surface of SiO_2/Si wafer fragments after repeated assembly process are shown in Fig. 3.24 (50k magnification) and Fig. 3.25 (150k magnification). As we can see in the images, at first, the approximately 5 nm diameter CdSe NPs wrap the approximately 15 nm diameter Au NPs by ionic interaction, forming a larger core/shell (Au NPs/CdSe NPs) nanocrystal structure of 30 nm to 40 nm in diameter. Then, the Au NPs find and stick to CdSe NPs attached on former Au NPs surface, forming a closely packed layer of Au NPs. This closely packed Au NPs layer will result in obvious gold color on silicon oxide surface, which can be easily observed by naked eye. Again, the CdSe NPs wrap Au NPs. Finally, Au NPs form another closely packed layer. Theoretically, any two NPs of the same kind will not attach to each other because they are both positive or negative-charged.


86 μm
Silicon oxide
Silicon oxide
Al metal line
Al metal line
5 μm or 15 μm
Silicon oxide

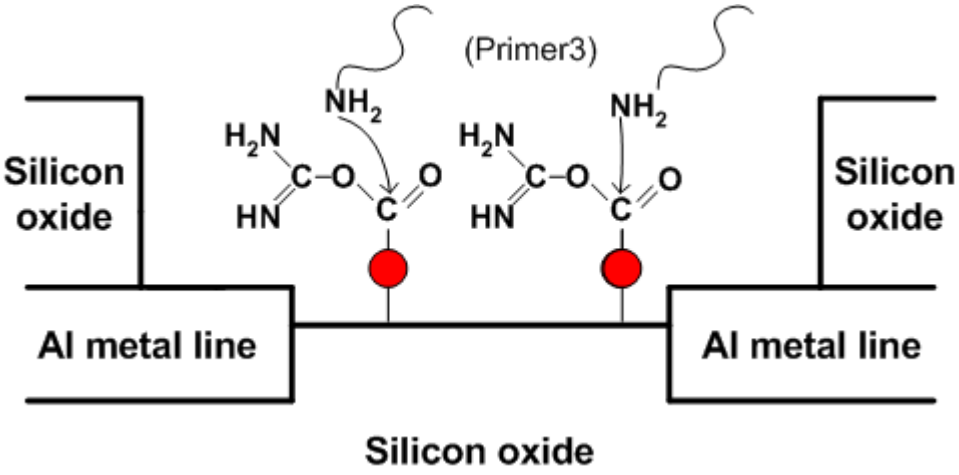
(a)


NH2
NH
Si
O O O
Silicon oxide
Silicon oxide
Al metal line
Al metal line
Silicon oxide

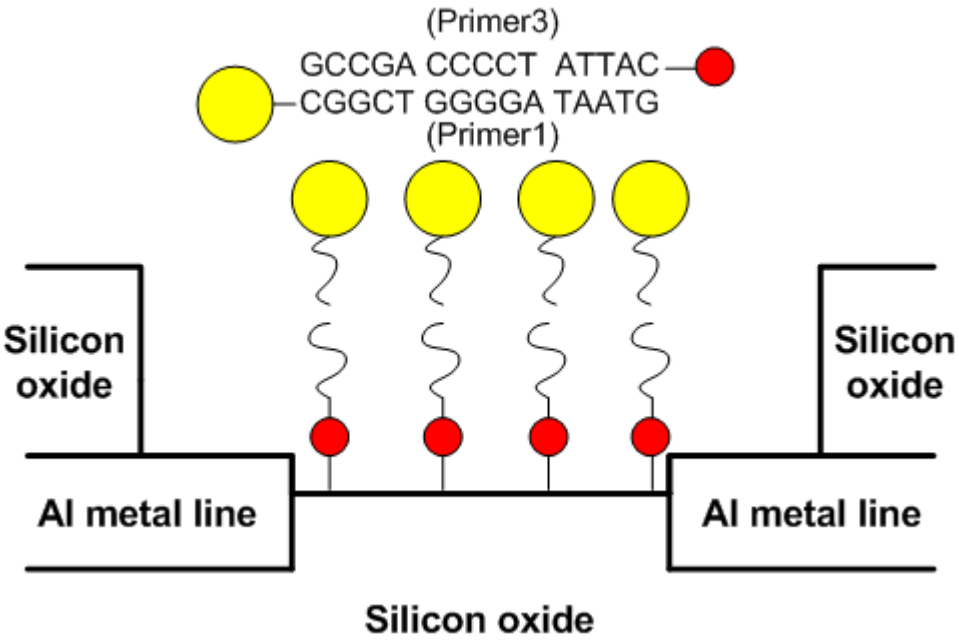
(b)


COO-
NH=C=NH
(Cyanamide)
NH2
NH
Si
Silicon oxide
Al metal line
Silicon oxide

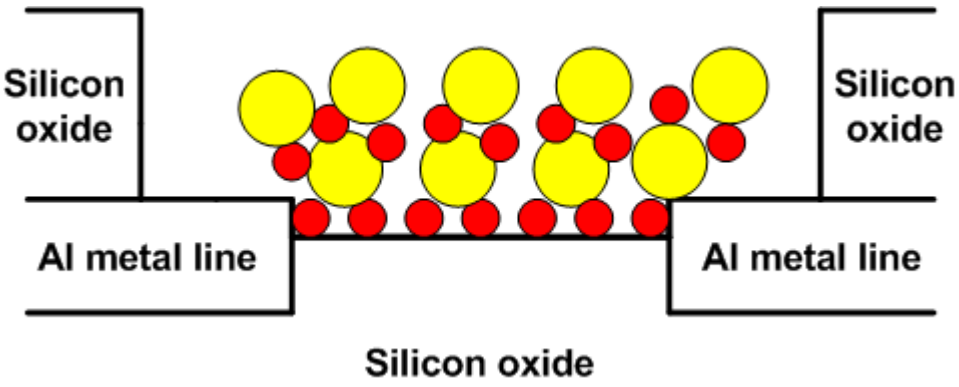
(c)


(Primer3)
NH2
H2N
C
O
HN
Silicon oxide
Al metal line
Silicon oxide

(d)


(Primer3)
GCCGA CCCCT ATTAC
CGGCT GGGGA TAATG
(Primer1)
Silicon oxide
Al metal line
Silicon oxide

(e)


Silicon oxide
Al metal line
Silicon oxide

(f)

Fig. 3.1. The overall construction process of photo-sensing nanodevice by DNA hybridization system on silicon chip substrate is shown above. (a) The cross-section figure of the surface of silicon chip designed for photo-sensing nanodevice construction, (b) The surface of silicon oxide region after TMSPED (or APTES) modification, (c) The assembly process of CdSe NPs on silicon oxide substrate, (d) The connection between DNA primer and CdSe NPs, (e) The assembly of Au NPs on CdSe NPs by self-assembly process and (f) The nanodevice structure after repeated assembly process.

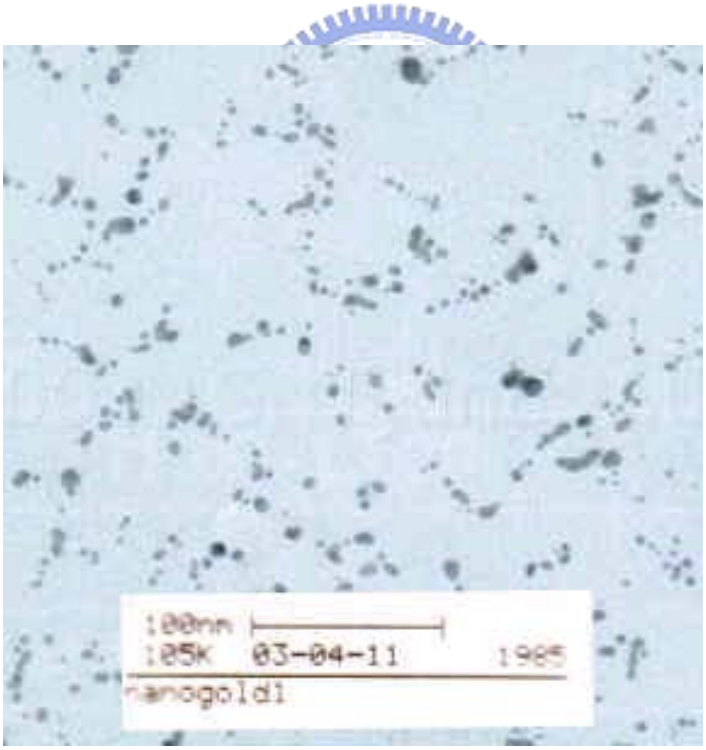


Fig. 3.2. The TEM analysis of Au NPs with diameter range from 20 nm~40 nm is shown above. (The photo is obtained from *Global Nano Tech. Inc., Taipei*)

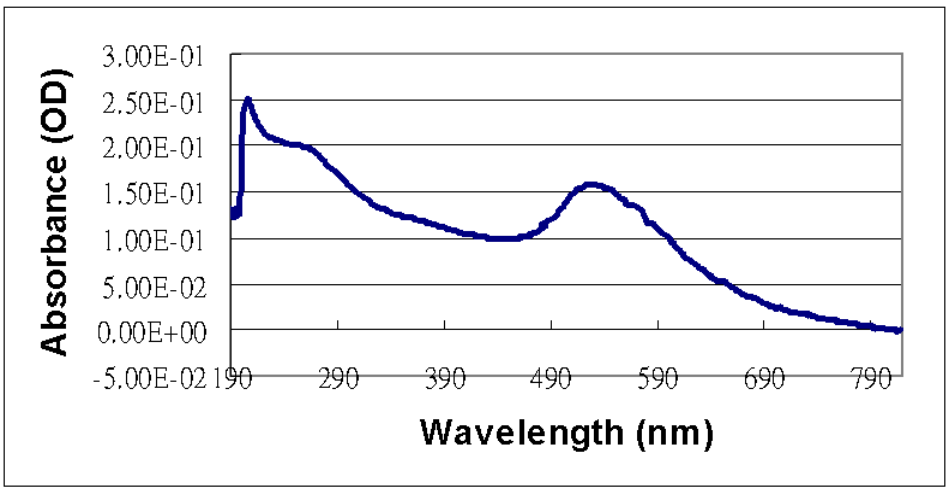


Fig. 3.3. The typical UV-visible spectrum of Au NPs suspension in water is shown above. There is a minor peak at 530 nm, which is usually used to determine the concentration of NPs. The concentration here is about 123 ppm (623 μM).

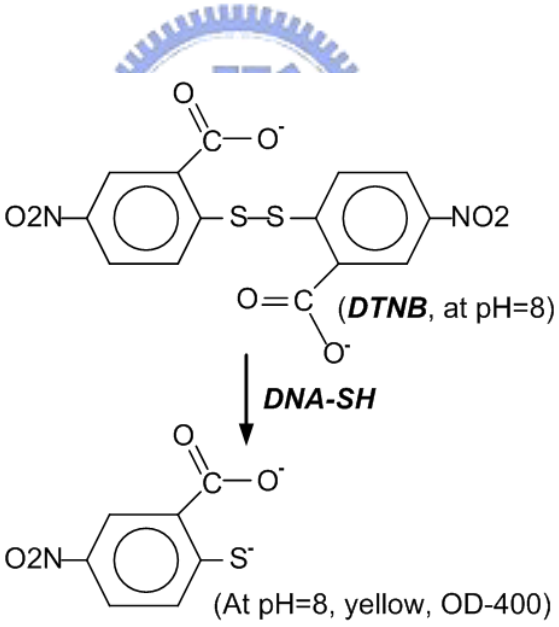
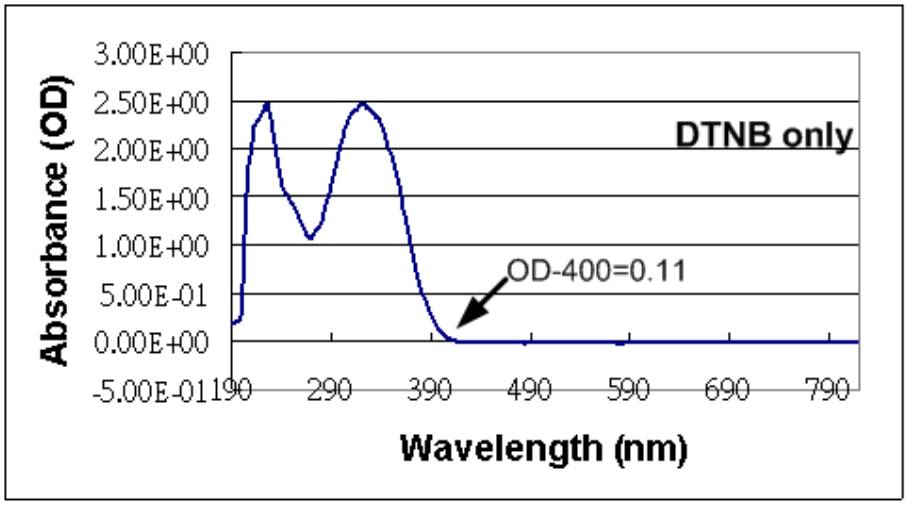
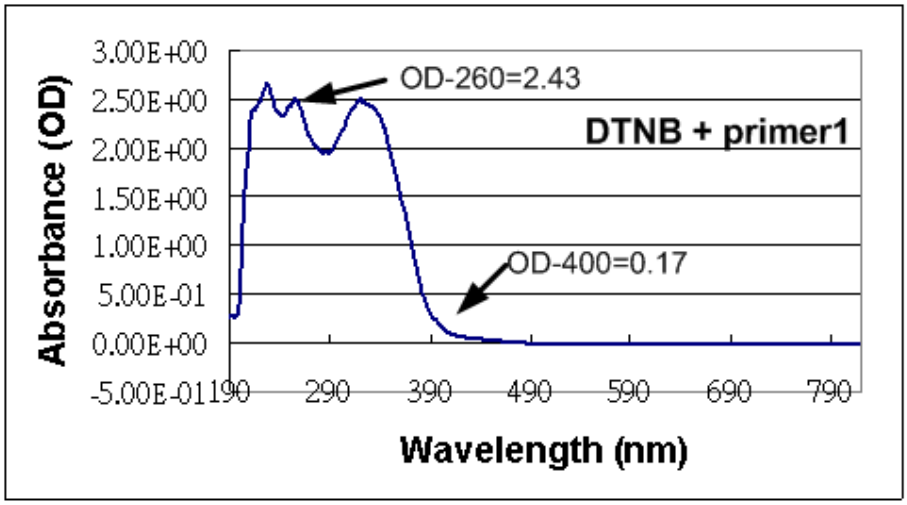


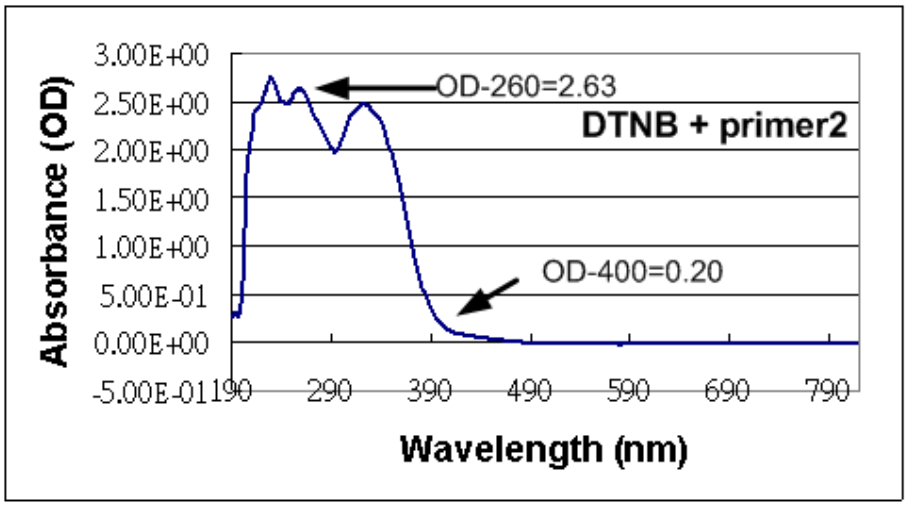
Fig. 3.4. The original DTNB solution at pH 8 is transparent and achromatic. Then, the primers with thiol groups are added into the solution. After the disulfide bond (-S-S-) is attacked by the thiol group of primer, the color of solution will become yellow and the UV-visible spectrum will have an increment at 400 nm due to TNBs presented in the solution.



(a)



(b)



(c)

Fig. 3.5. The UV-visible spectrums of the three samples are shown above. There is an obvious increment at 260 nm and 400 nm in the spectra due the addition of primers.

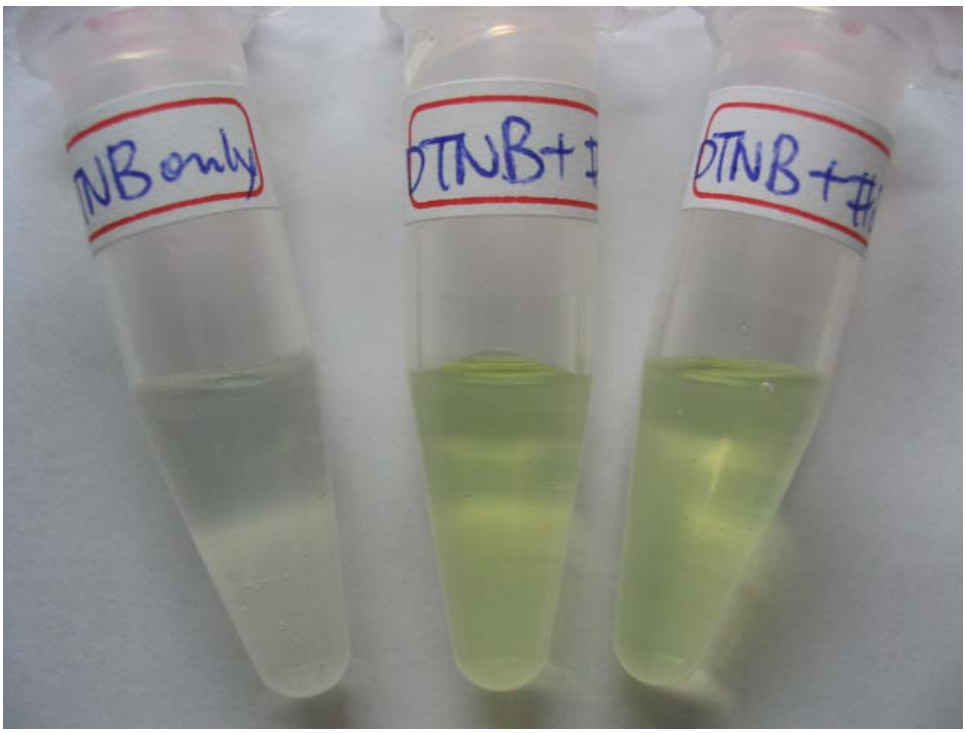


Fig. 3.6. After standing for 10 minutes, the color of three samples, "DTNB + primer1", "DTNB + primer2", and "DTNB only" (form right to left) are shown above.

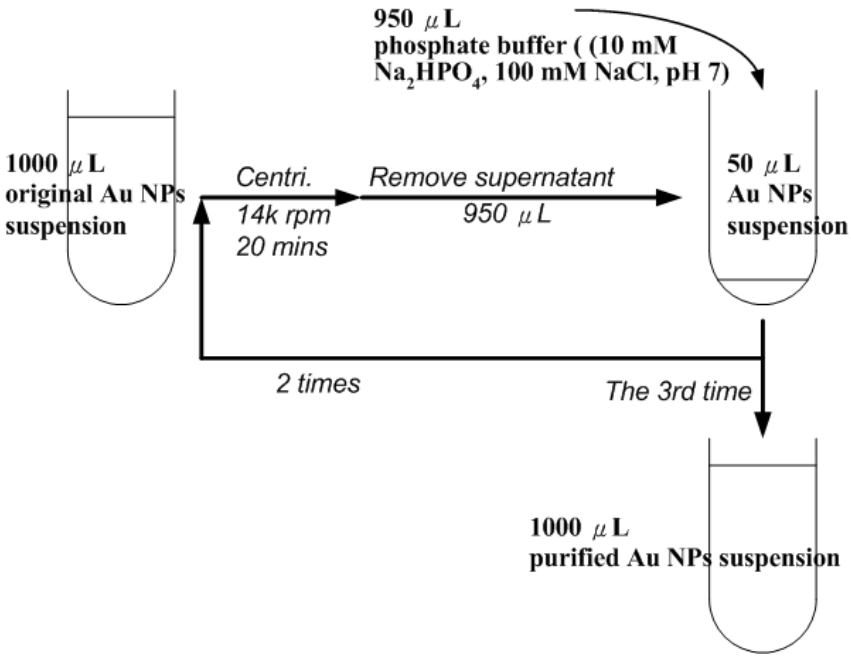


Fig. 3.7. The original Au NPs water suspension was "washed" by the method illustrated above.

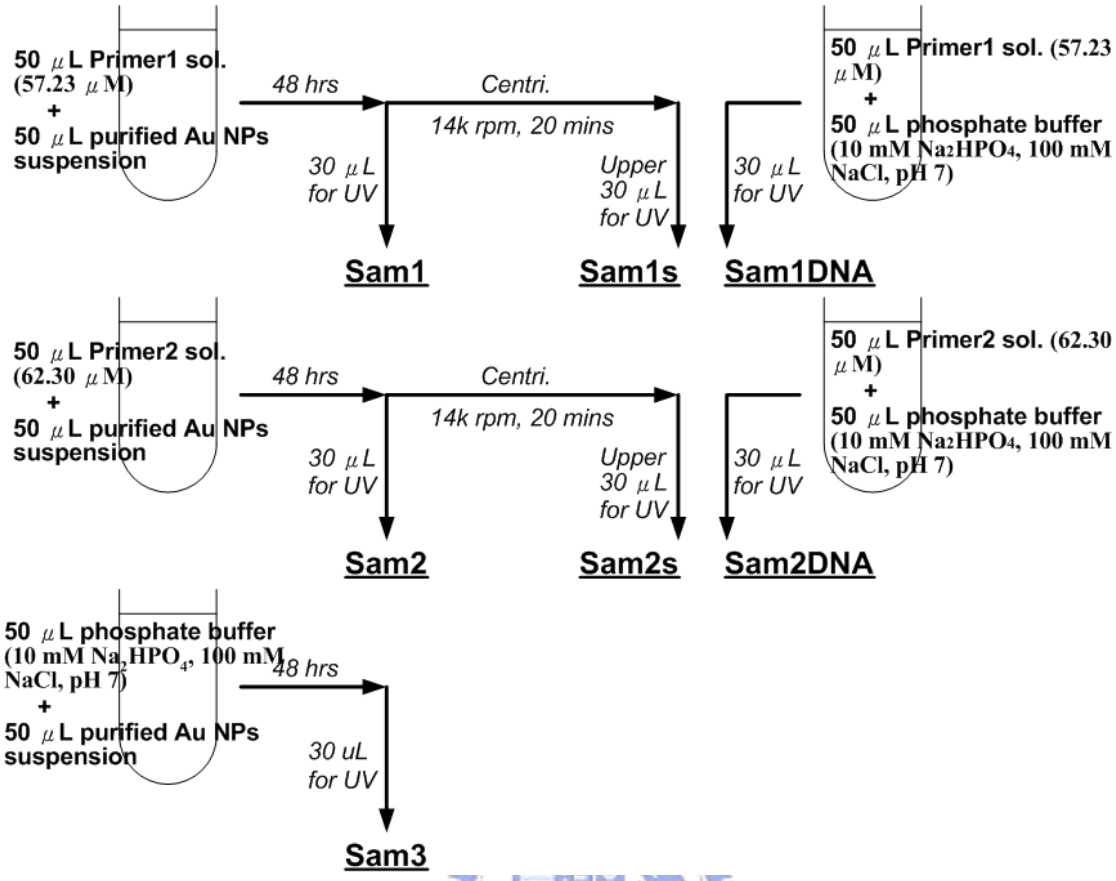
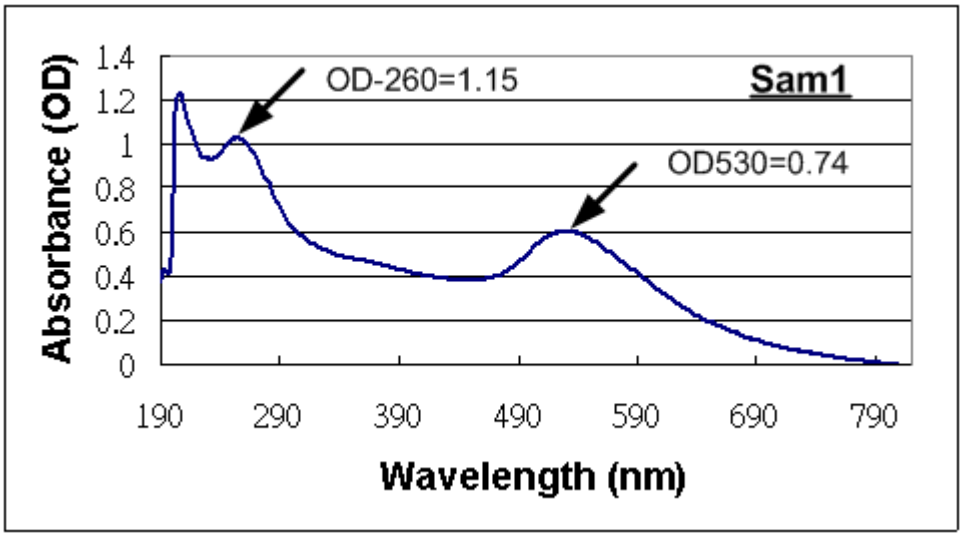
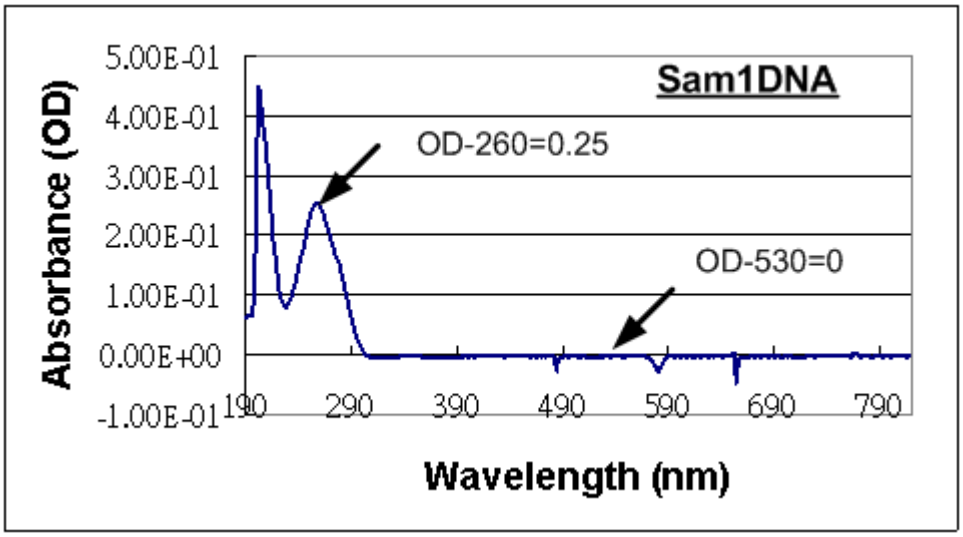


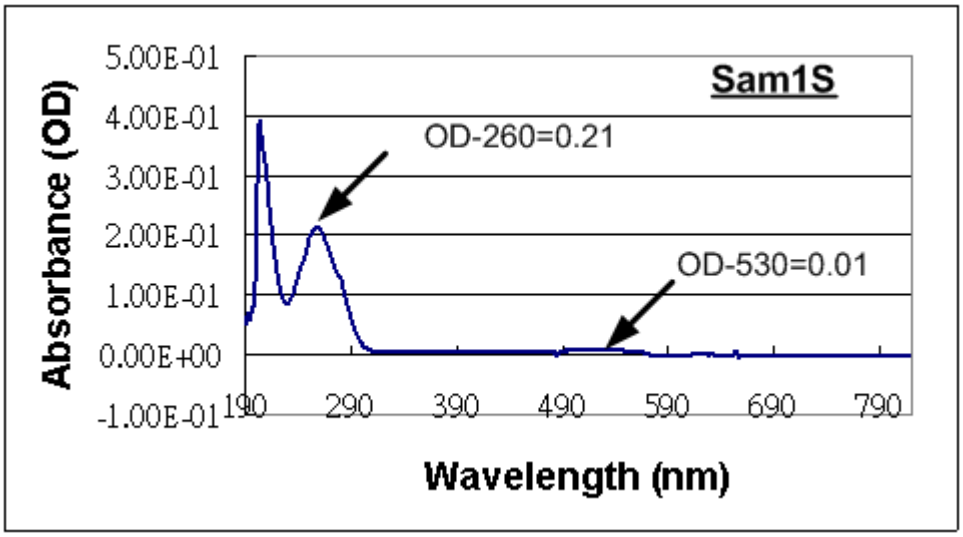
Fig. 3.8. The process of conjugating the Au NPs with two complementary primers is shown above. Sam1DNA, and Sam2DNA are compared with the experimental results, Sam1s and Sam2s.


Absorbance (OD)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD-260=1.15
Sam1
OD530=0.74
190
290
390
490
590
690
790
Wavelength (nm)

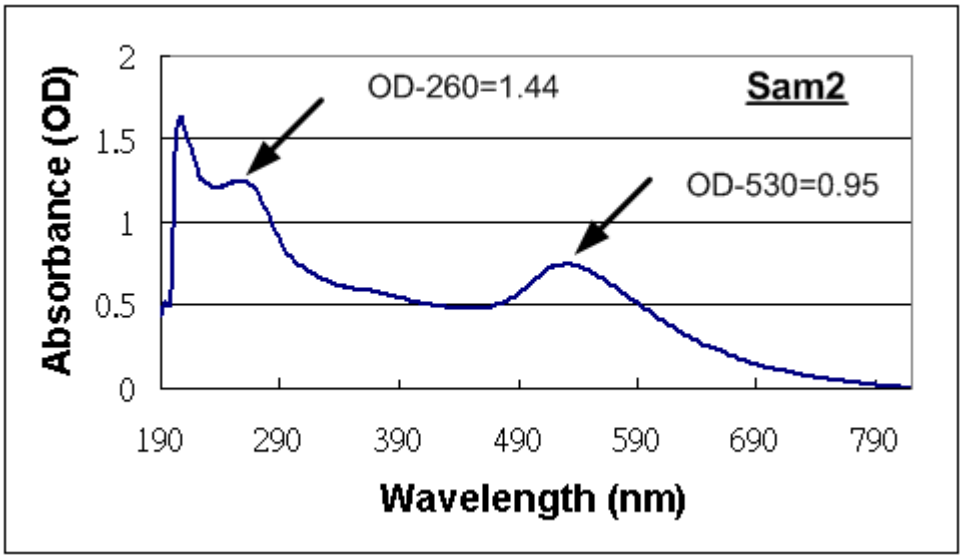
(a)


Absorbance (OD)
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
-1.00E-01
Sam1DNA
OD-260=0.25
OD-530=0
190
290
390
490
590
690
790
Wavelength (nm)

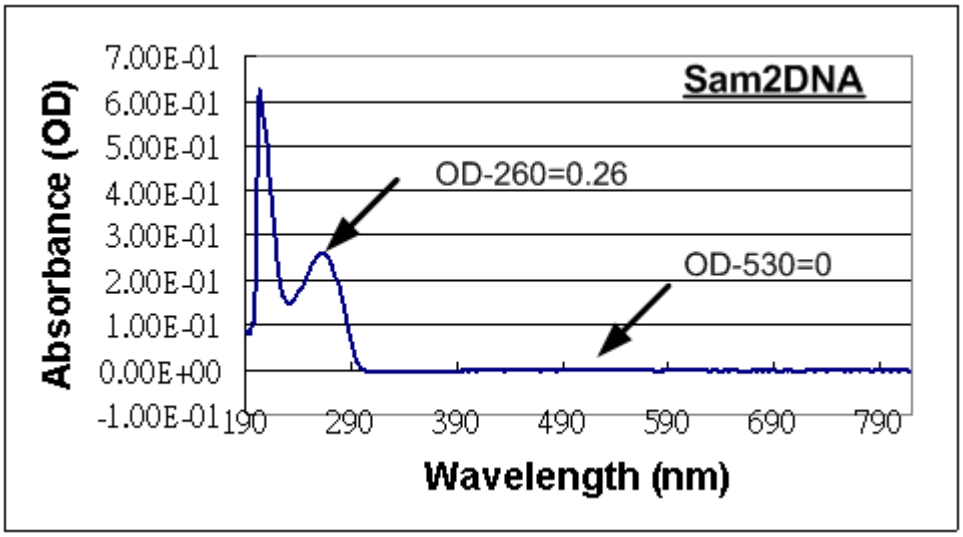
(b)


Absorbance (OD)
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
-1.00E-01
Sam1S
OD-260=0.21
OD-530=0.01
190
290
390
490
590
690
790
Wavelength (nm)

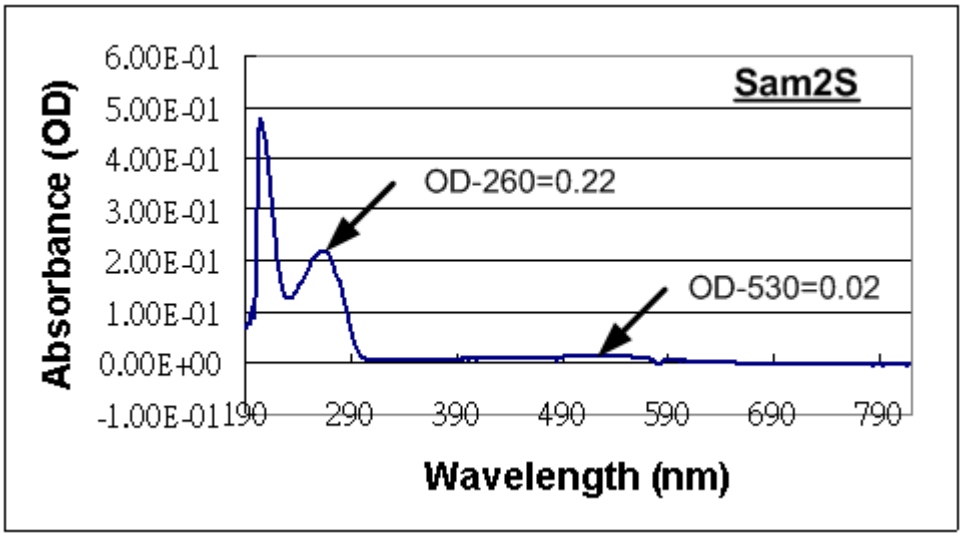
(c)


Absorbance (OD)
2
1.5
1
0.5
0
OD-260=1.44
Sam2
OD-530=0.95
190
290
390
490
590
690
790
Wavelength (nm)

(d)


Absorbance (OD)
7.00E-01
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
-1.00E-01
Sam2DNA
OD-260=0.26
OD-530=0
190
290
390
490
590
690
790
Wavelength (nm)

(e)


Absorbance (OD)
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
-1.00E-01
Sam2S
OD-260=0.22
OD-530=0.02
190
290
390
490
590
690
790
Wavelength (nm)

(f)

Fig. 3.9. The UV-visible absorbance spectrums of Sam1,2, Sam1s,2s, Sam1DNA, and Sam2DNA (a)~(f) are shown above. We can obviously identify the decrement at OD-260 after centrifugation because the DNA primers attached to the Au NPs are brought to the bottom of tube, resulting in the lower concentration of primers in the supernatant. While measuring the UV-visible absorbance spectrum, all the concentration of the samples were diluted to 30/430 of its original value by adding 400 μL of the phosphate buffer to 30 μL of the sample solution.

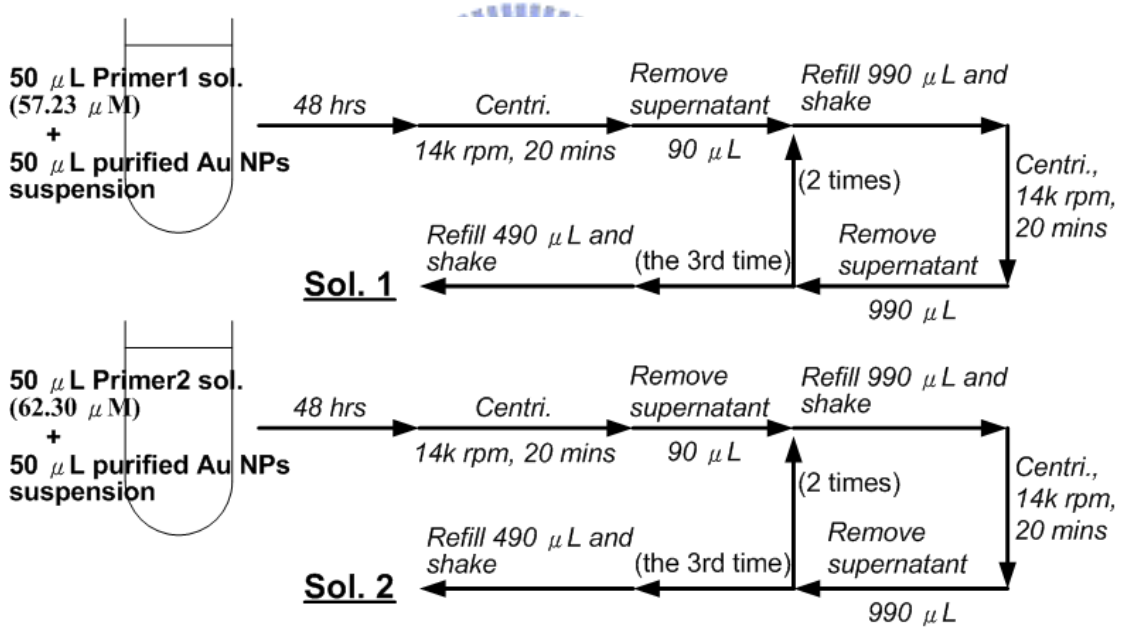


Fig. 3.10. The figure above shows the "washing process" to remove excess DNA primers of both samples before mixing them.

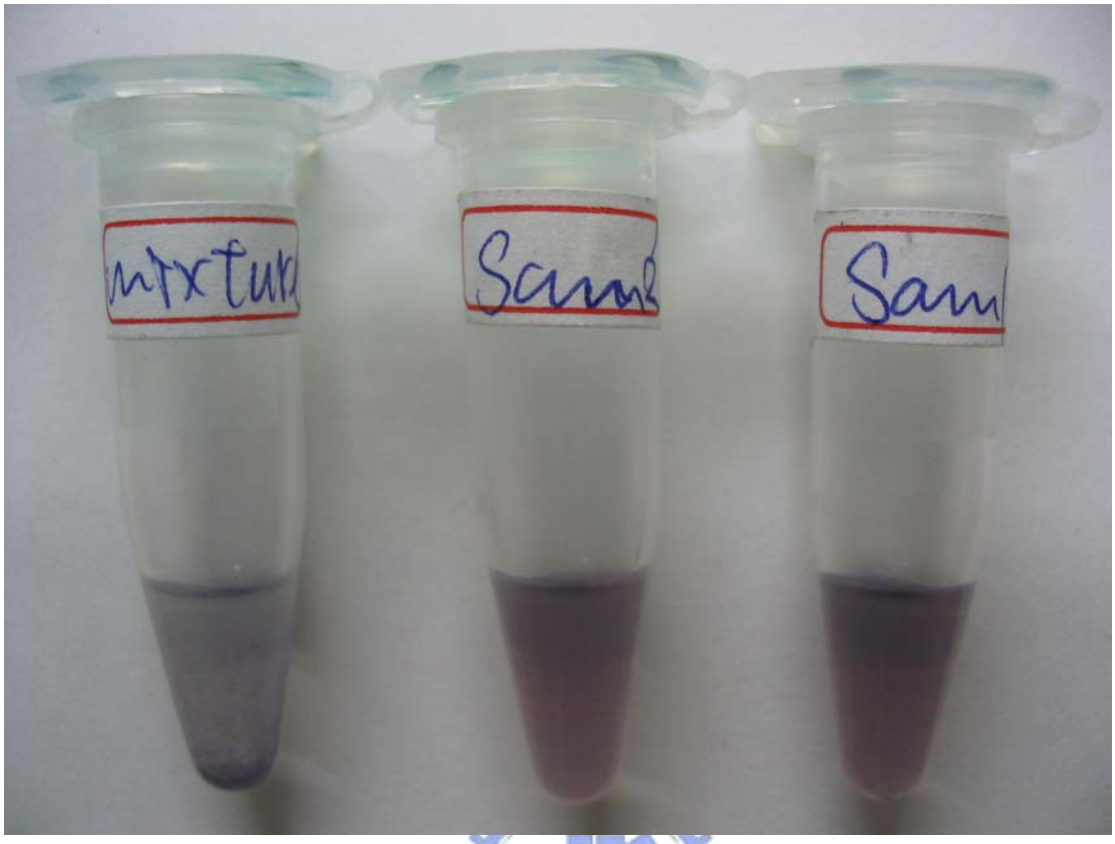
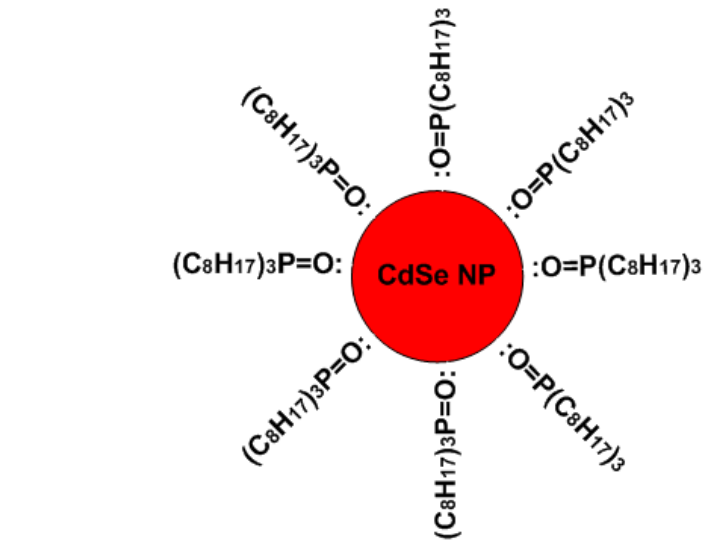
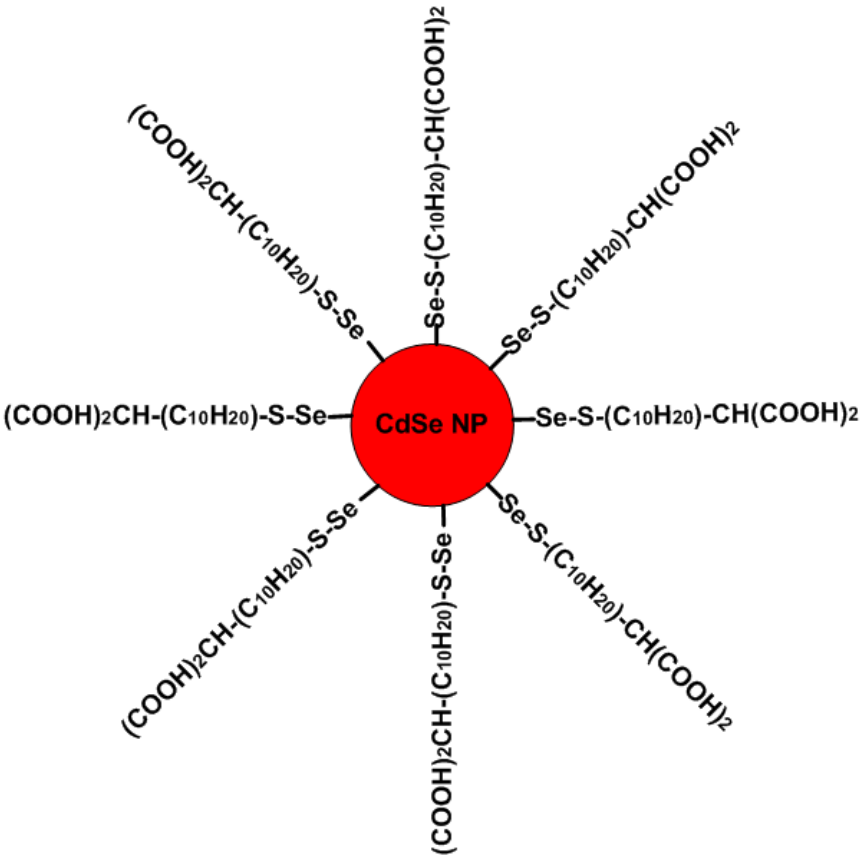


Fig. 3.11. The right and middle ones are the samples where Au NPs suspensions are labeled with DNA primer1 and 2 respectively, while the left one is the mixture of the right and middle. As we can see, there is obvious precipitation in the mixture due to self-assembly process between promer1 and 2, and the color of suspension changes from purplish red to light blue.



(a)



(b)

Fig. 3.12. The structure of TOPO-coated CdSe NP and MUA-modified CdSe NP. The covalent Se-S bond between MUA and CdSe NP is more stable than the ionic or Van der Waal bond between TOPO and Cd site on CdSe NPs.

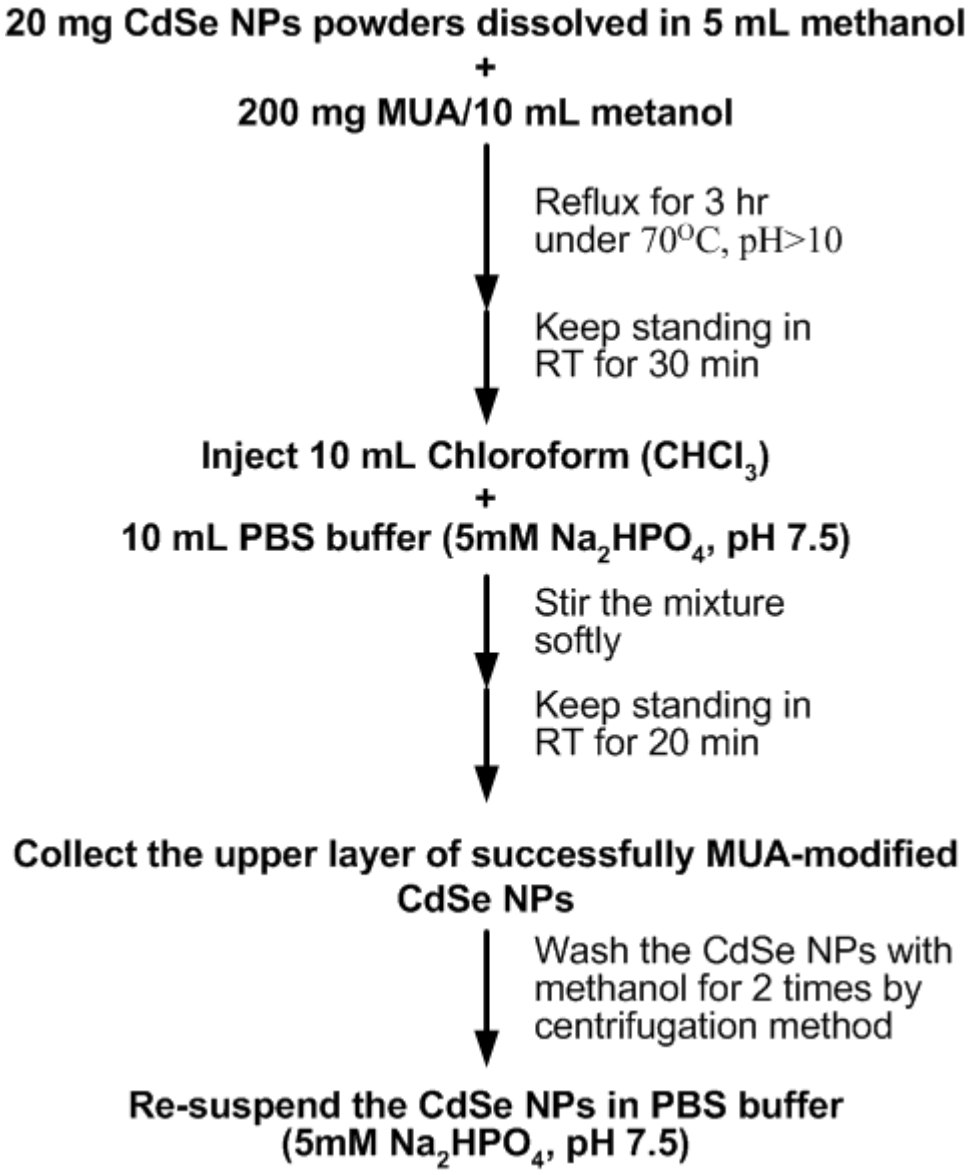


Fig. 3.13. The overall and detailed process of conversion of oil-soluble TOPO-coated CdSe NPs to water-soluble MUA-modified CdSe NPs is shown above.

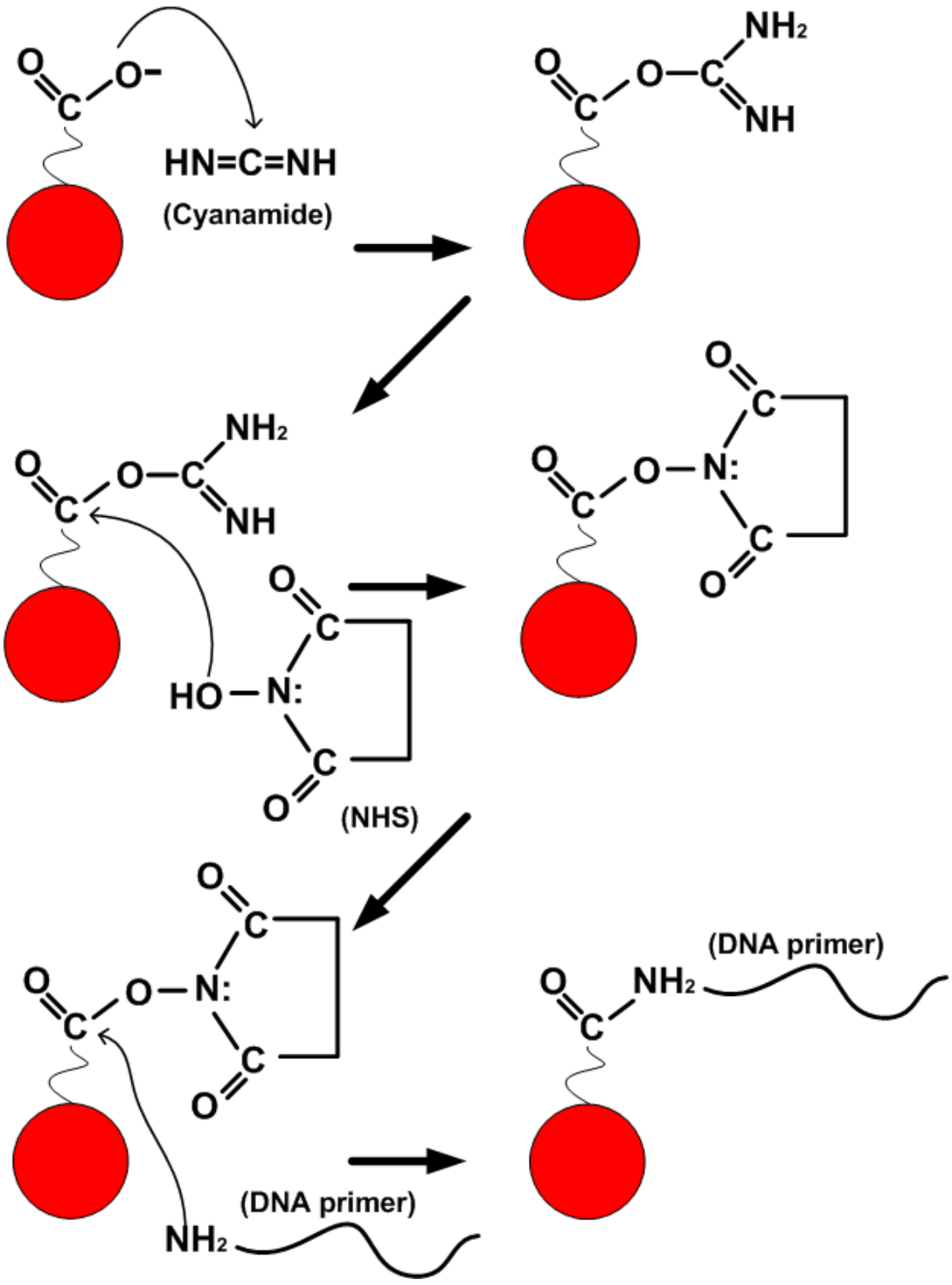


Fig. 3.14. The conceptual diagram of the conjugation of DNA primers with MUA-modified CdSe NPs is shown above.

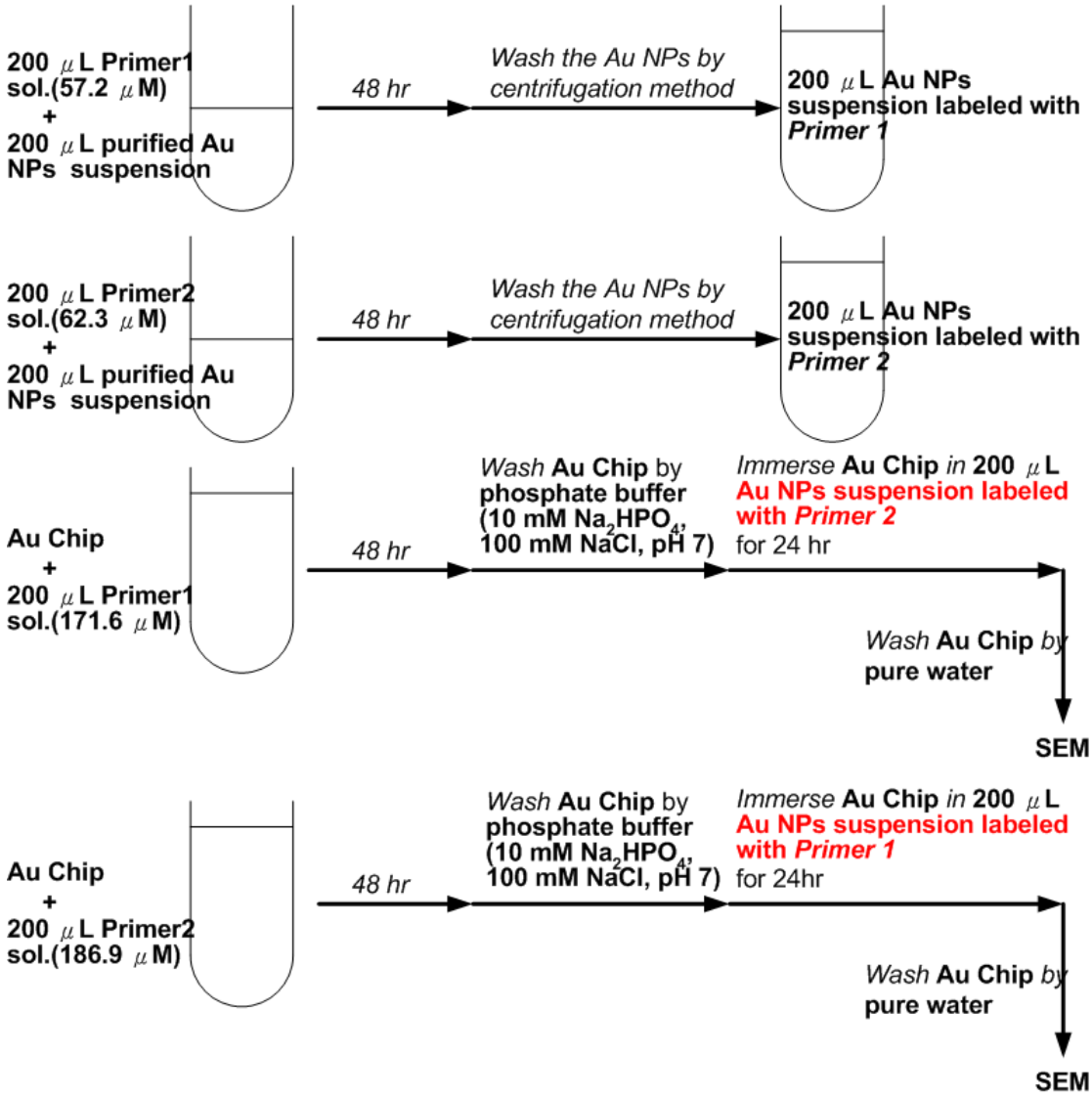
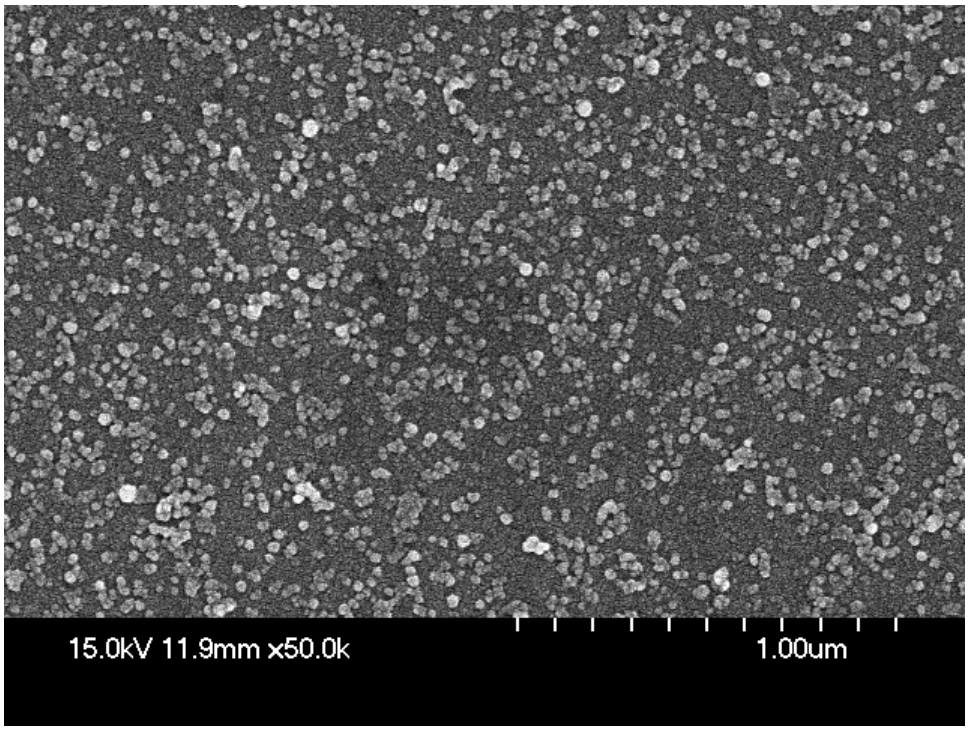
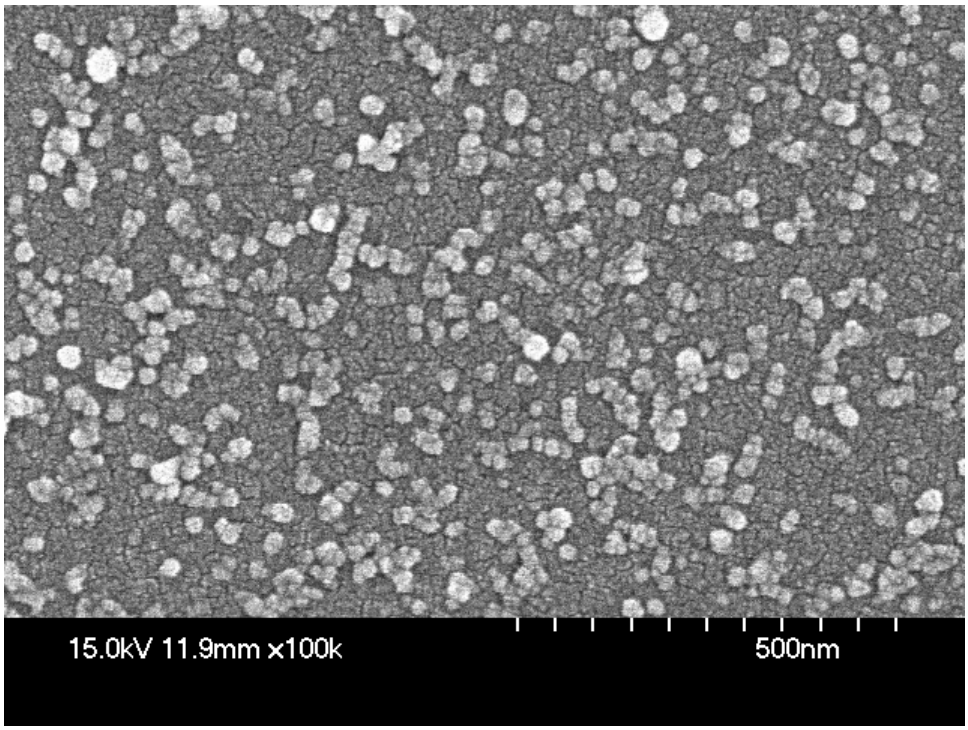


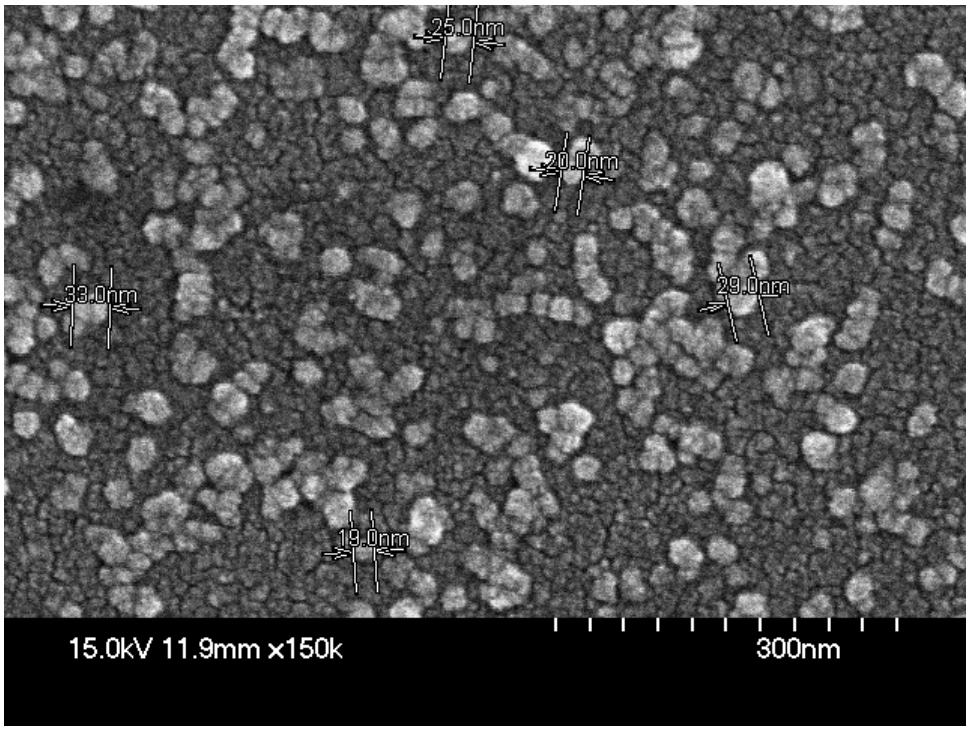
Fig. 3.15. The detailed assembly process of Au NPs on gold substrate is shown above. The purified Au NPs suspension was prepared by the method illustrated in Fig. 3.7.



(a) (50k magnification)

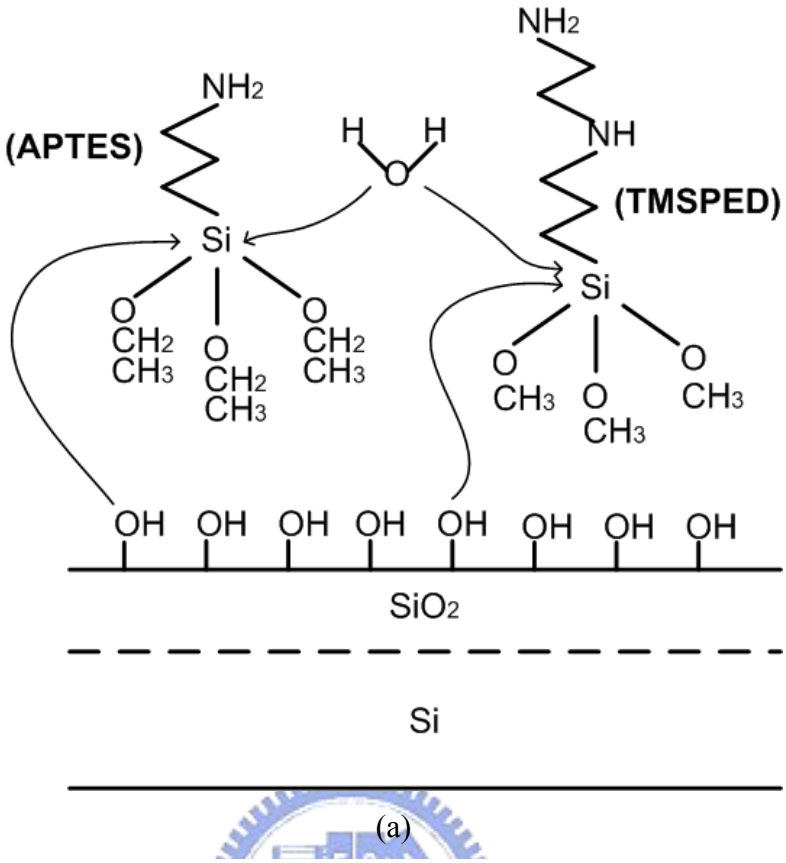


(b) (100k magnification)

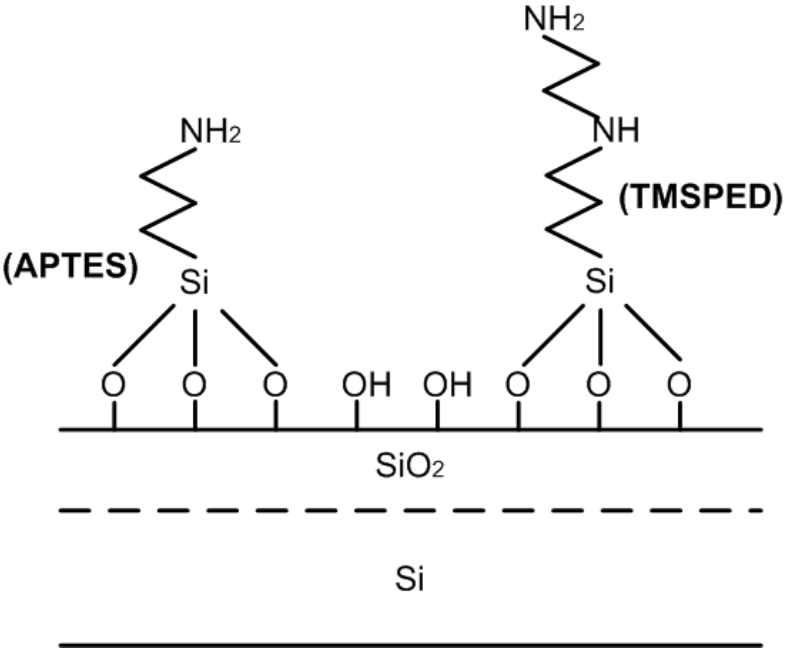


(c) (150k magnification)

Fig. 3.16. The SEM images of the Au NPs modified gold substrate by DNA self-assembly process with 50k (a), 100k (b) and 150k (c) magnification. Note that the diameter of the Au NPs (synthesis form physical method) is in the range of 20~40 nm. To increase the resolution of images, 3 nm thick Pt layer is placed on the surface to increase conductivity of samples.

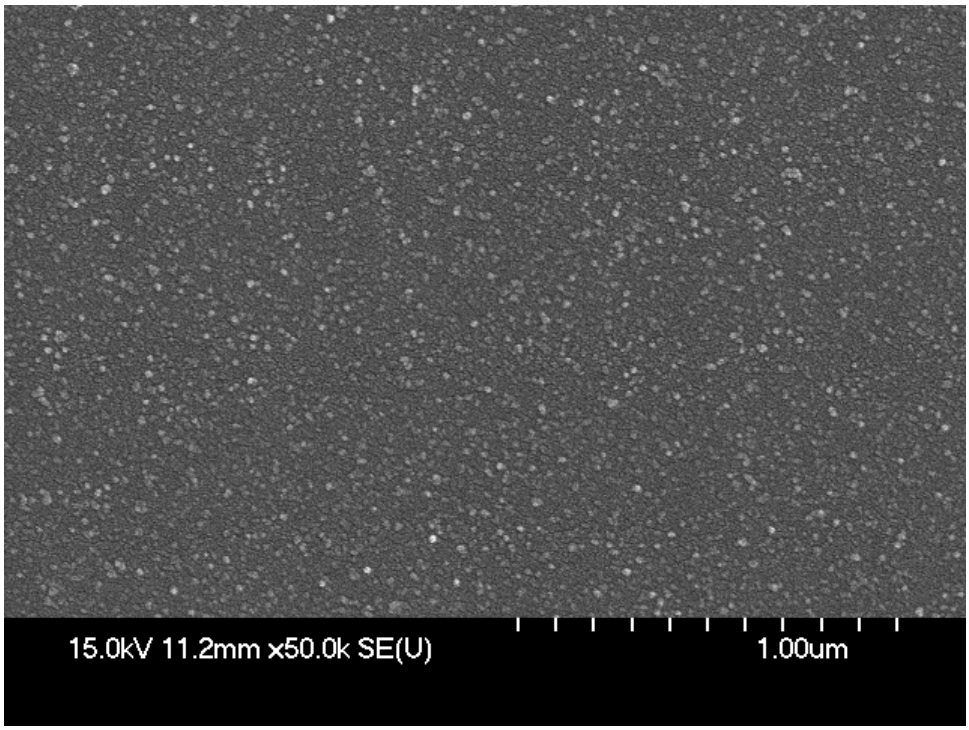


(a)

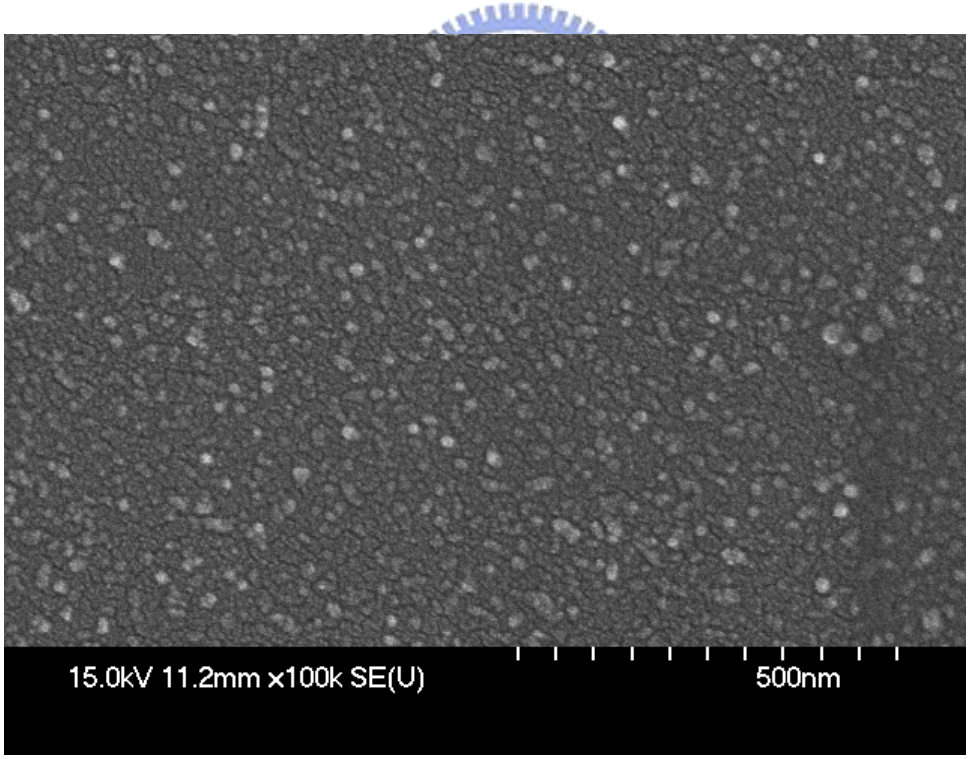


(b)

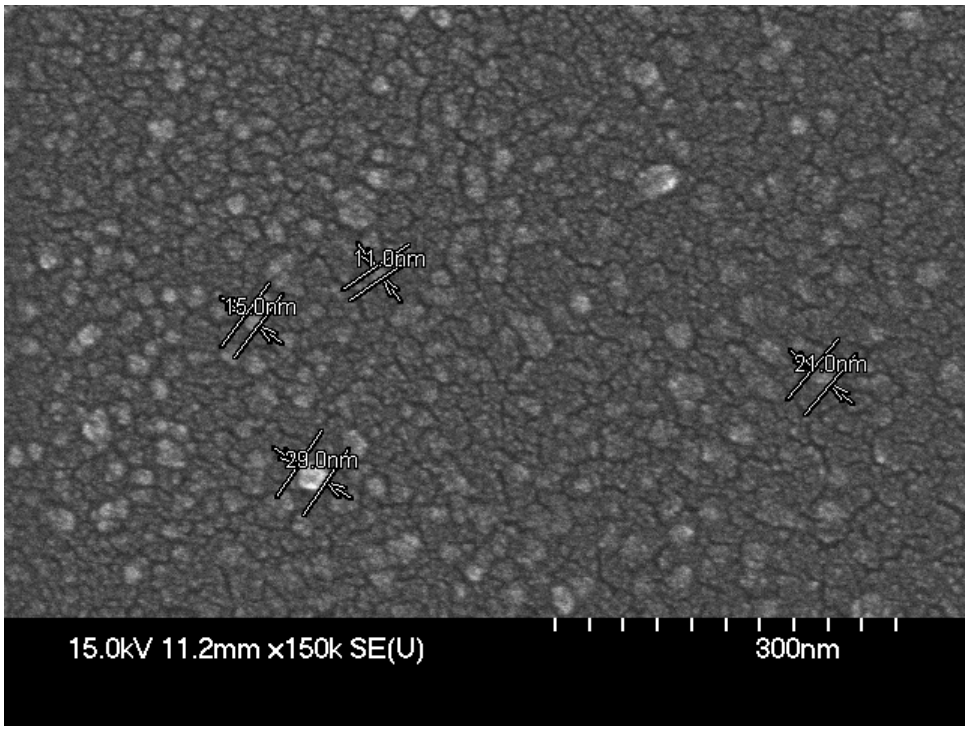
Fig. 3.17. The conceptual diagram of TMSPED and APTES modifying the silicon oxide surface is shown above. (a) Before modification, the H_2O molecules will attack the Si of TMSPED or APTES, competing with the –OH group on silicon oxide. (b) After modification, the TMSPED or APTES will form stable covalent bond with the silicon oxide.



(a) (50 k magnification)

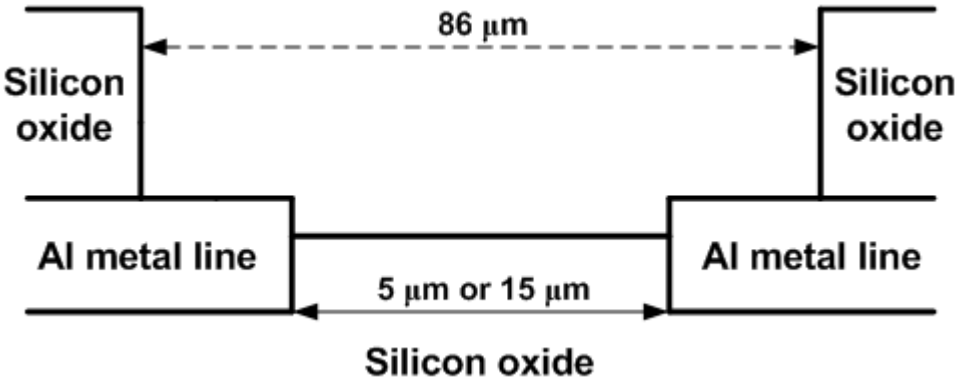


(b) (100 k magnification)

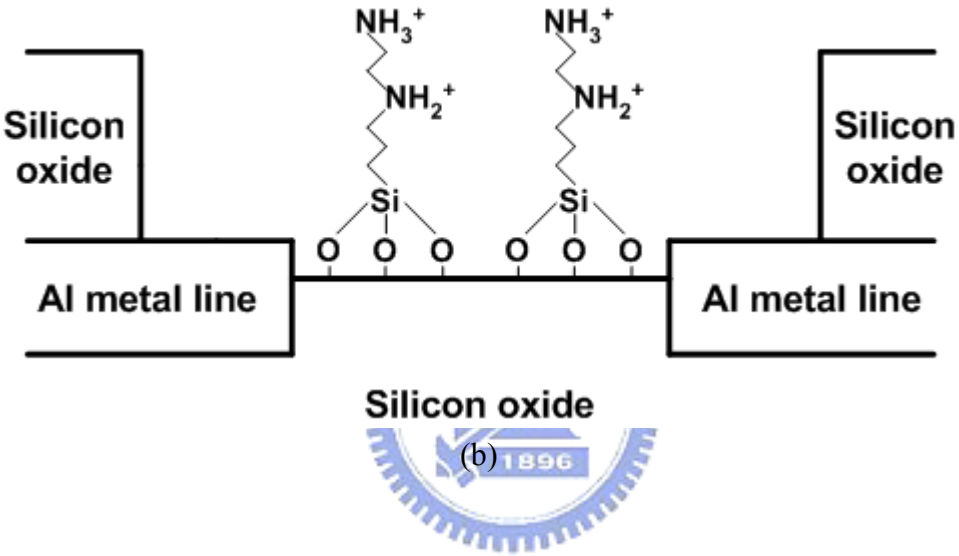


(c) (150 k magnification)

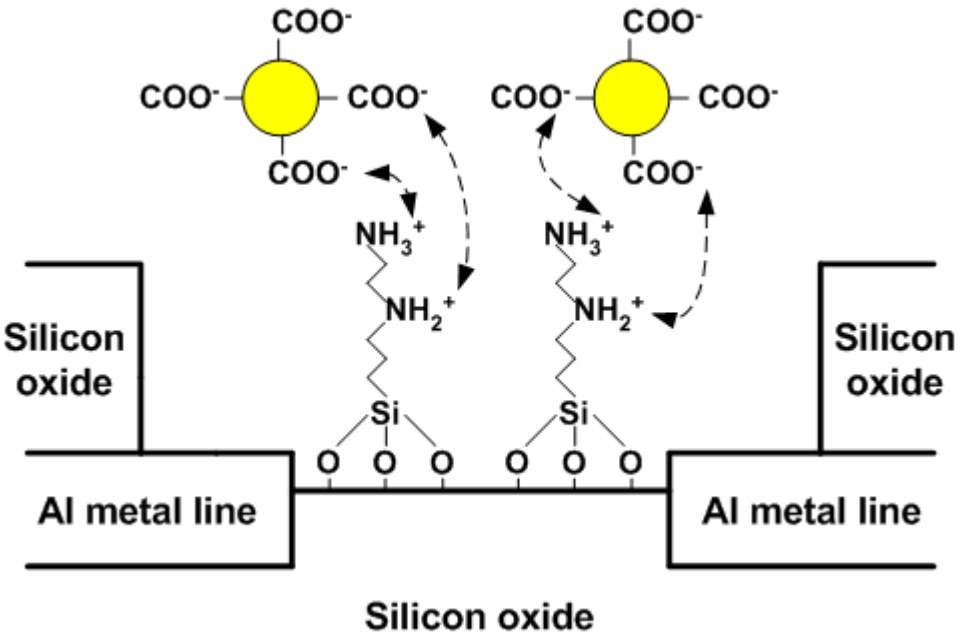
Fig. 3.18. The SEM images of CdSe NPs modified silicon oxide substrate with 50k (a), 100k (b), 150k (c) magnification. Note that the diameter of most CdSe NPs is less than 10 nm. To increase the resolution of images, 3 nm thick Pt layer is placed on the surface to increase conductivity of samples.


86 μm
Silicon oxide
Silicon oxide
Al metal line
Al metal line
5 μm or 15 μm
Silicon oxide

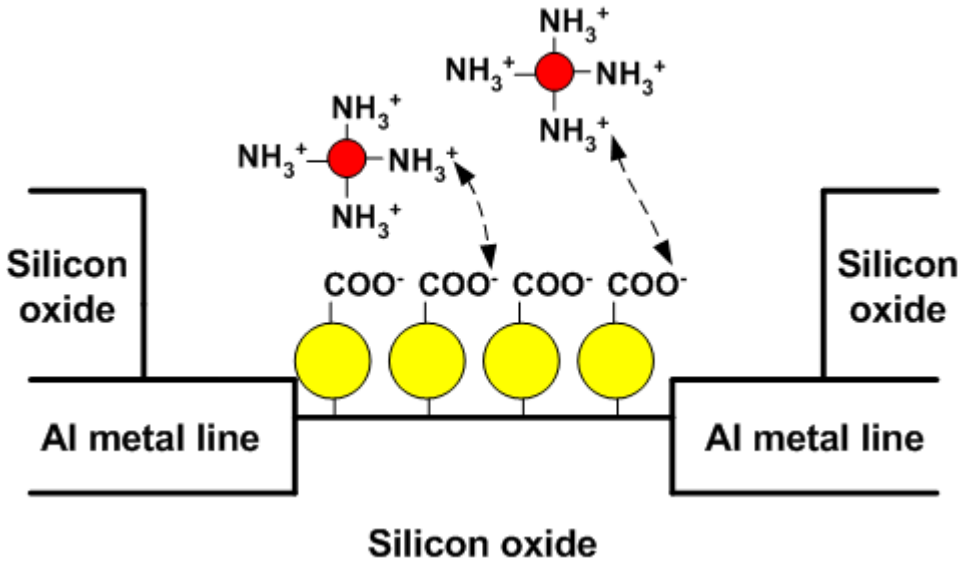
(a)


NH3+
NH2+
Si
O
Silicon oxide
Al metal line
Silicon oxide
Silicon oxide

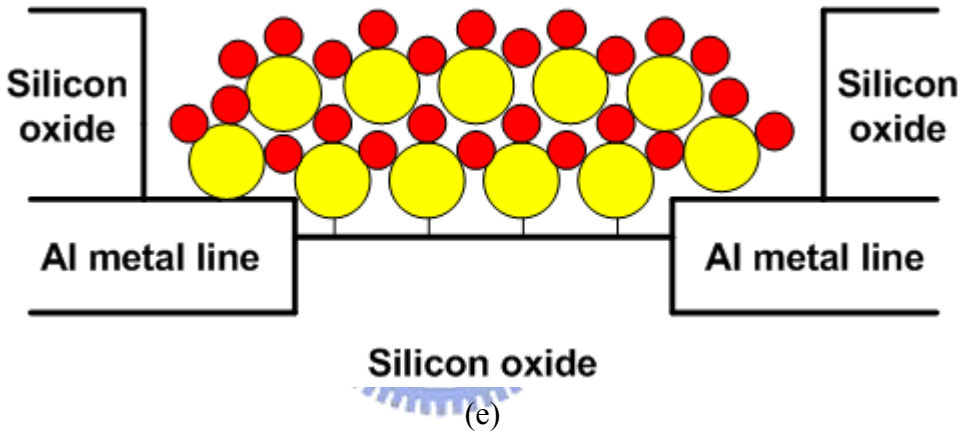
(b)


COO-
NH3+
NH2+
Si
O
Silicon oxide
Al metal line
Silicon oxide
Silicon oxide

(c)



(d)



(e)

Fig. 3.19. The overall construction process of photo-sensing nanodevice by Coulombic force system on silicon chip substrate is shown above. (a) The cross-section figure of the surface of silicon chip designed for photo-sensing nanodevice construction, (b) The modification of TMSPED (or APTES) on silicon oxide surface and the protonation of amino ($-NH_3^+$) groups, (c) The assembly of approximately 15 nm diameter Au NPs on silicon oxide substrate by ionic interaction, (d) The assembly of approximately 5 nm diameter Tyramine-modified CdSe NPs on silicon oxide substrate by ionic interaction, and (e) The formation of photo-sensing nanodevice structure after repeated assembly process.

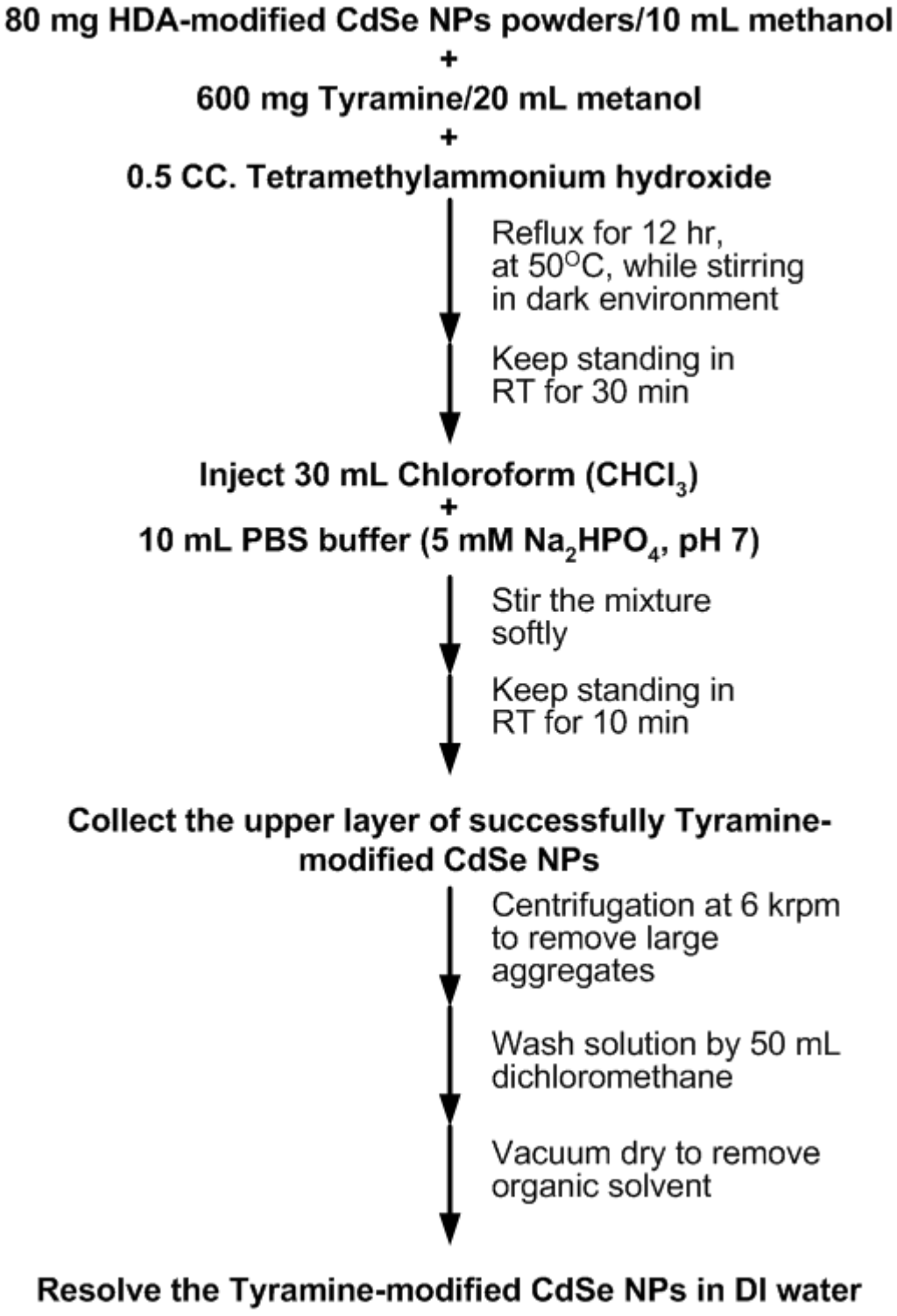
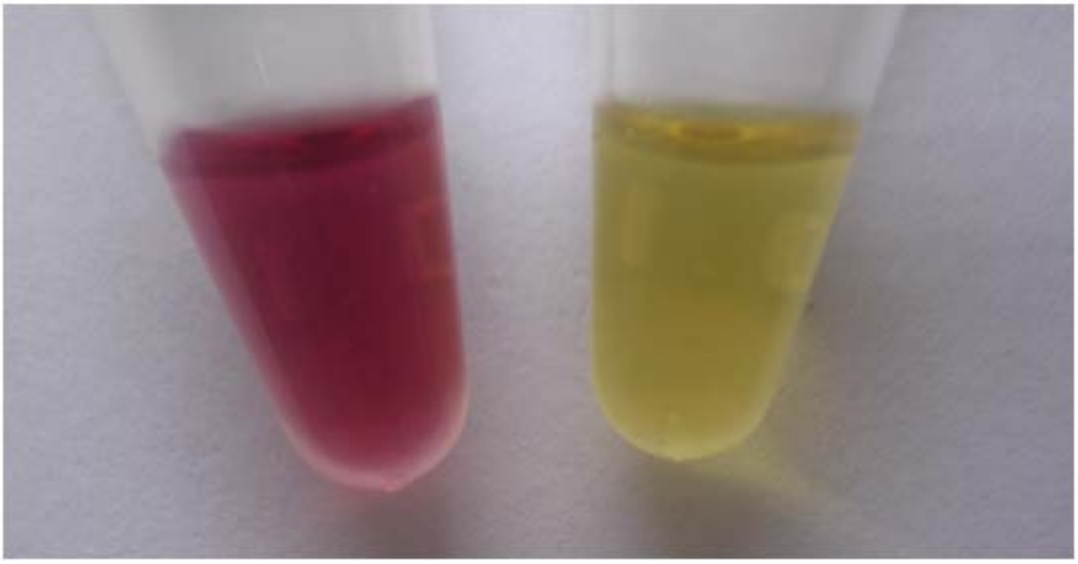
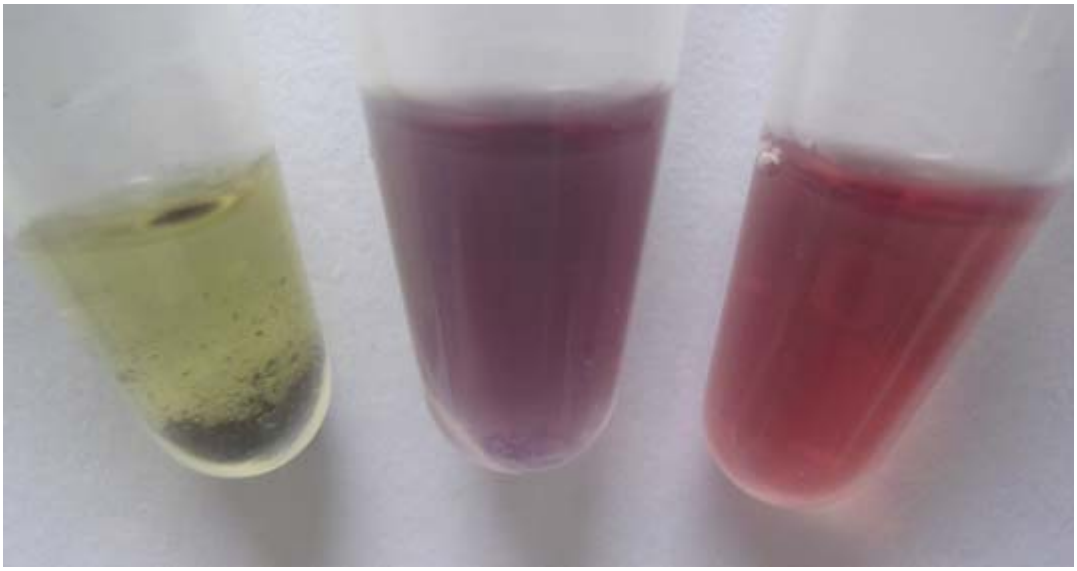


Fig. 3.20. The detailed Tyramine modification process of HDA-coated CdSe NPs is shown above.

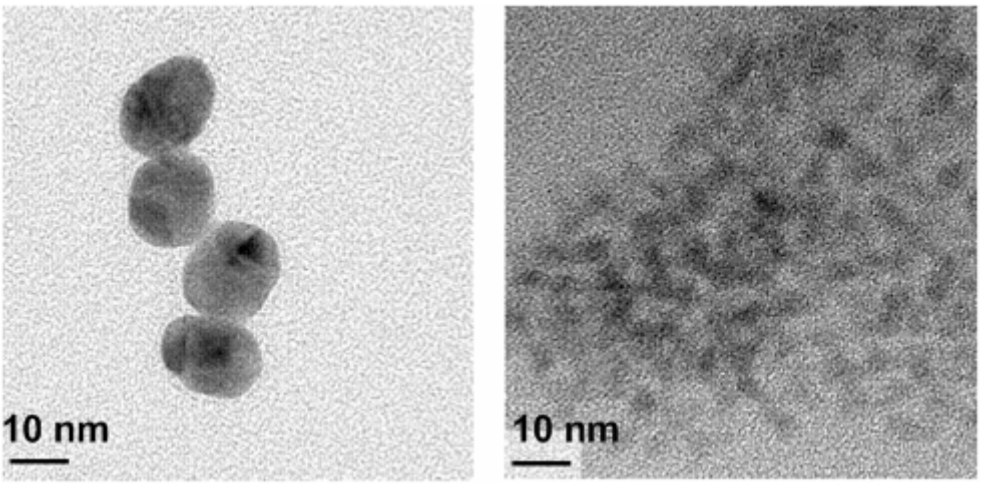


(a)

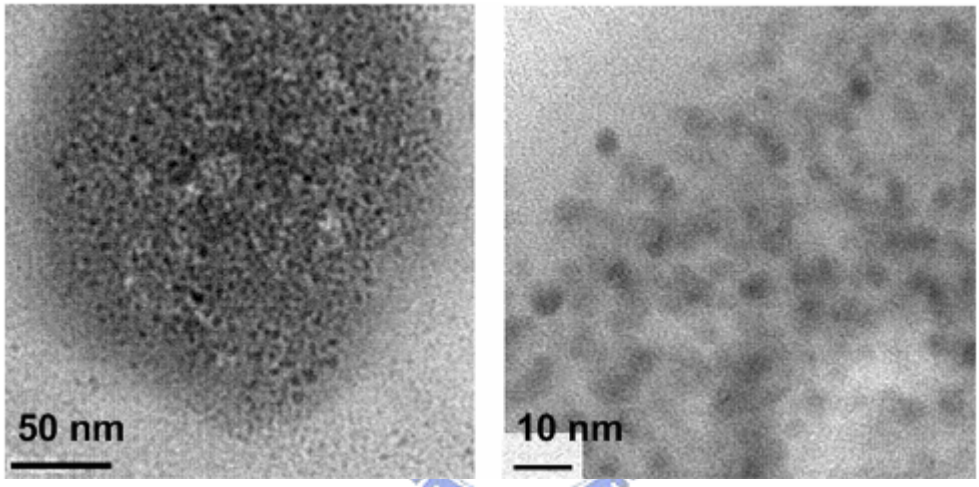


(b)

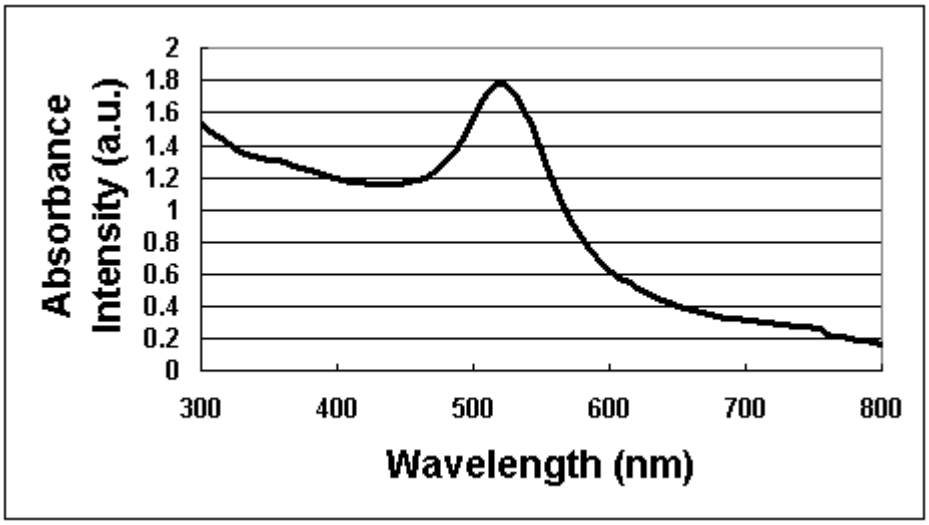
Fig. 3.21. (a) The close photographs of 100 μL of approximately 15 nm diameter Au NPs solution + 100 μL DI water (left) and 100 μL of approximately 5 nm diameter CdSe NPs solution + 100 μL DI water (right). The Au NPs solution was in deep red while the Tyramine-modified CdSe NPs solution was in yellow. (b) The close photographs of the mixture of 100 μL Au NPs solution and 100 μL Tyramine-modified CdSe NPs solution just after mixing (right), the mixture after standing 6 hrs (middle) in room temperature, and the mixture after standing 5 days in room temperature (left). As we can see, the color of mixture just after mixing was like that of Au NPs solution. However, after 6 hrs, it became dark purplish red. After 5 days, there was obvious precipitate at the bottom and the supernatant became pale yellow.


10 nm
10 nm

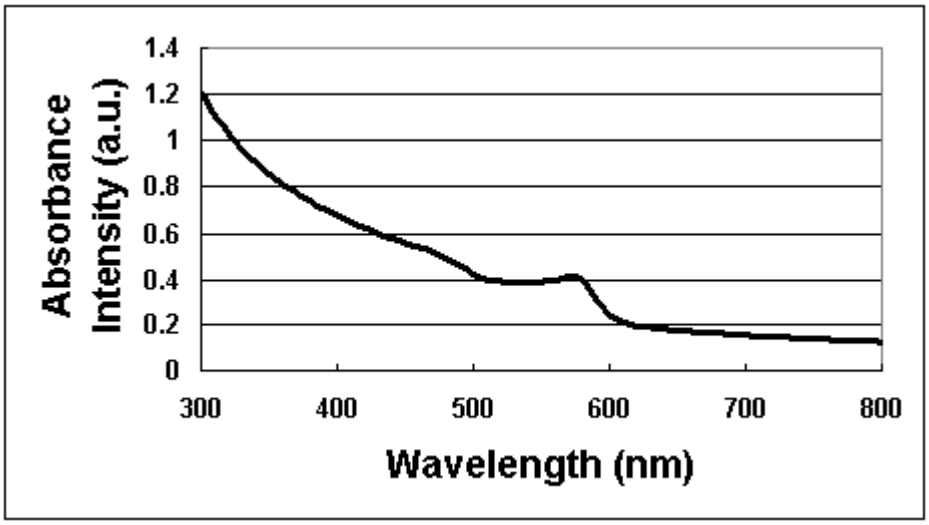
(a)


50 nm
10 nm

(b)


Absorbance Intensity (a.u.)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
300
400
500
600
700
800
Wavelength (nm)

(c)



(d)

Fig. 3.22. (a) The TEM images of approximately 15 nm diameter Au NPs (left) and approximately 5 nm diameter Tyramine-modified CdSe NPs. (b) The TEM images of the mixture of Au and Tyramine-modified CdSe NPs after standing 24 hrs (Fig. 3.20 (b)-middle) The right is larger magnification of part of the left. (c) The UV-visible spectrum of An NPs solution. (d) The UV-visible spectrum of original HDA-coated CdSe NPs solution in organic solvent.

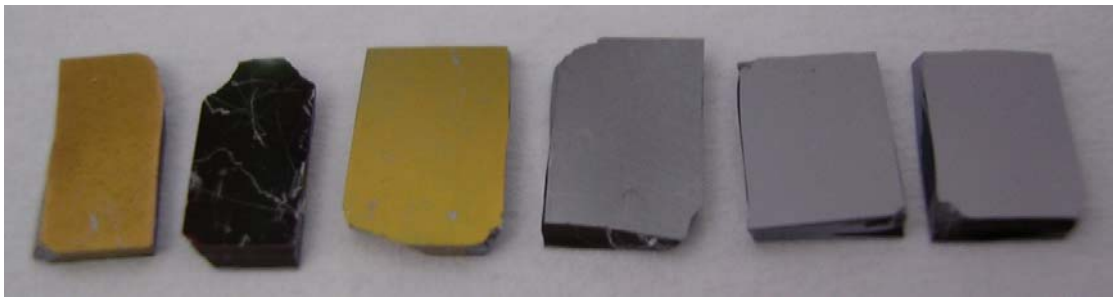
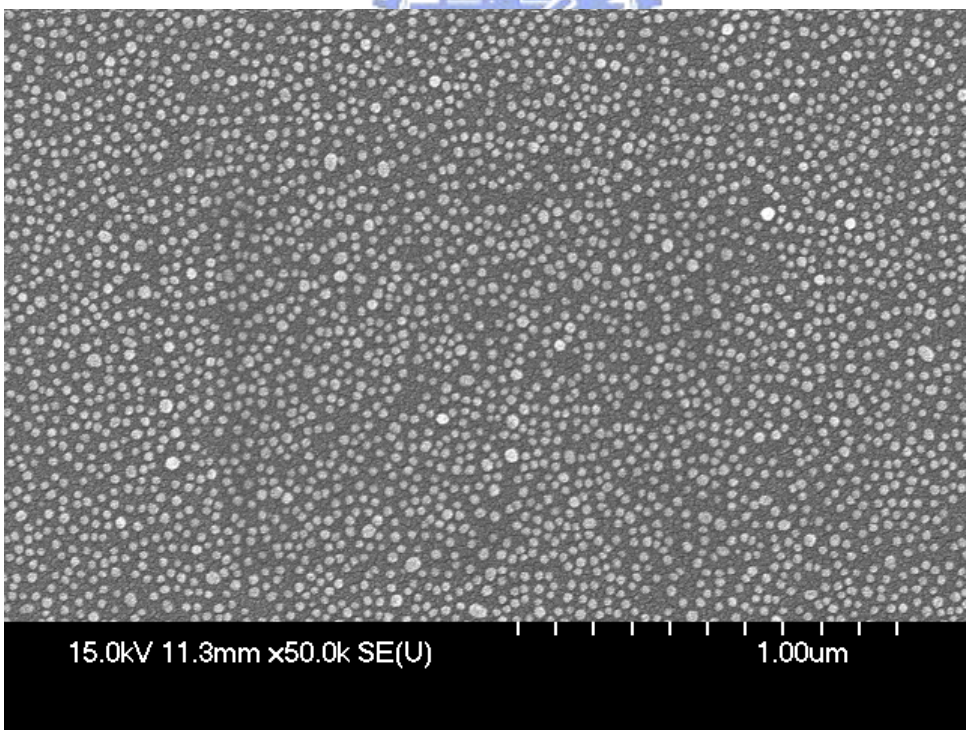
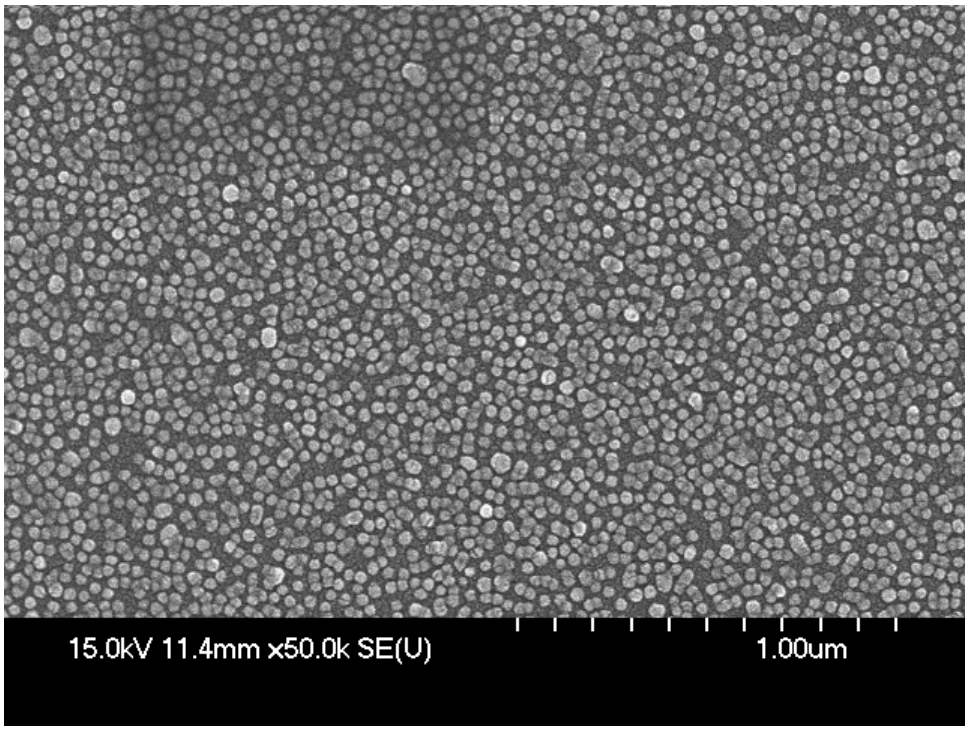


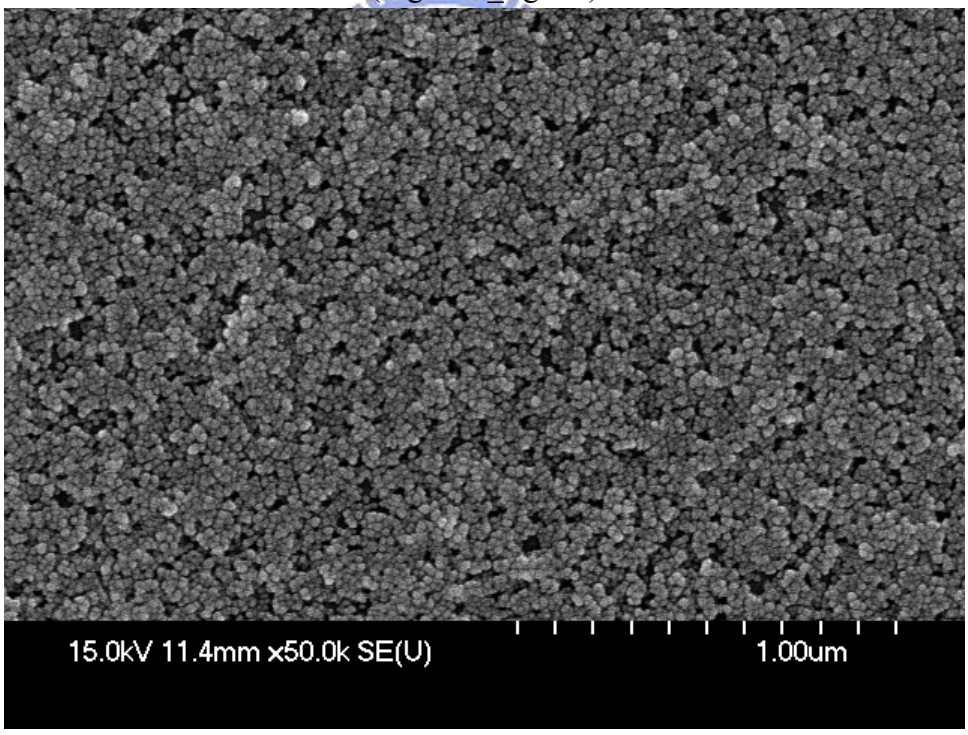
Fig.3.23. The close photographs of SiO_2/Si wafer fragments of different level assembly process. (right 1) blank SiO_2/Si wafer fragment. (right 2) Au NPs on SiO_2/Si wafer fragment. (right 3) CdSe NPs + Au NPs on SiO_2/Si wafer fragment. (right 4) Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment. (right 5) Cdse NPs + Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment. (right 6) Au NPs + CdSe NPs + Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment.



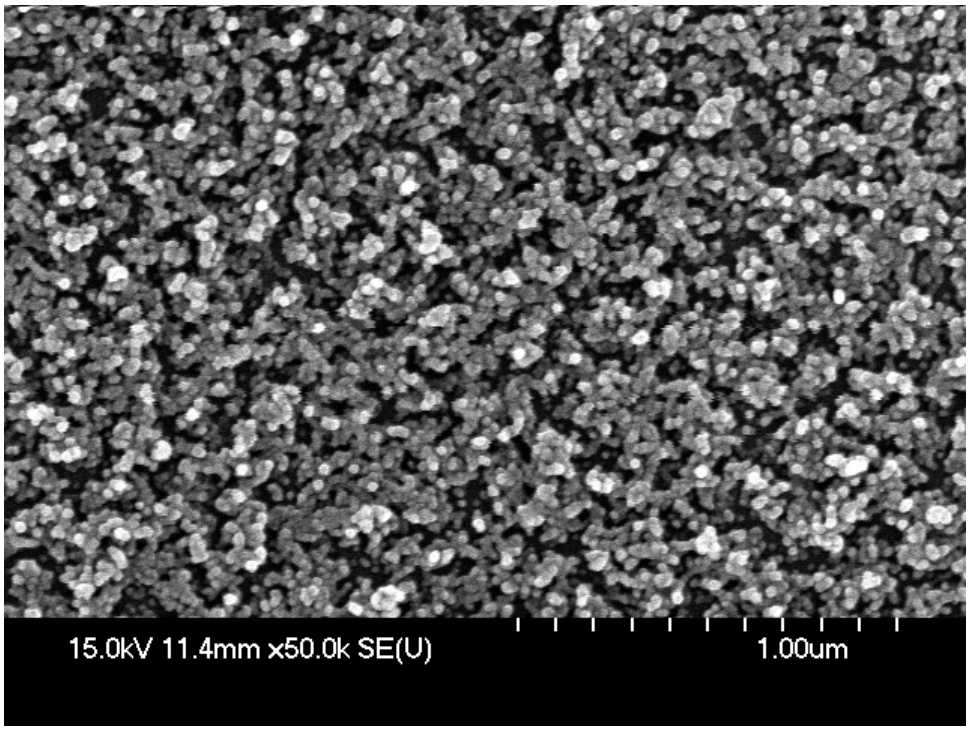
(a) Au NPs on SiO_2/Si wafer fragment
(Fig.3.22_right 2)



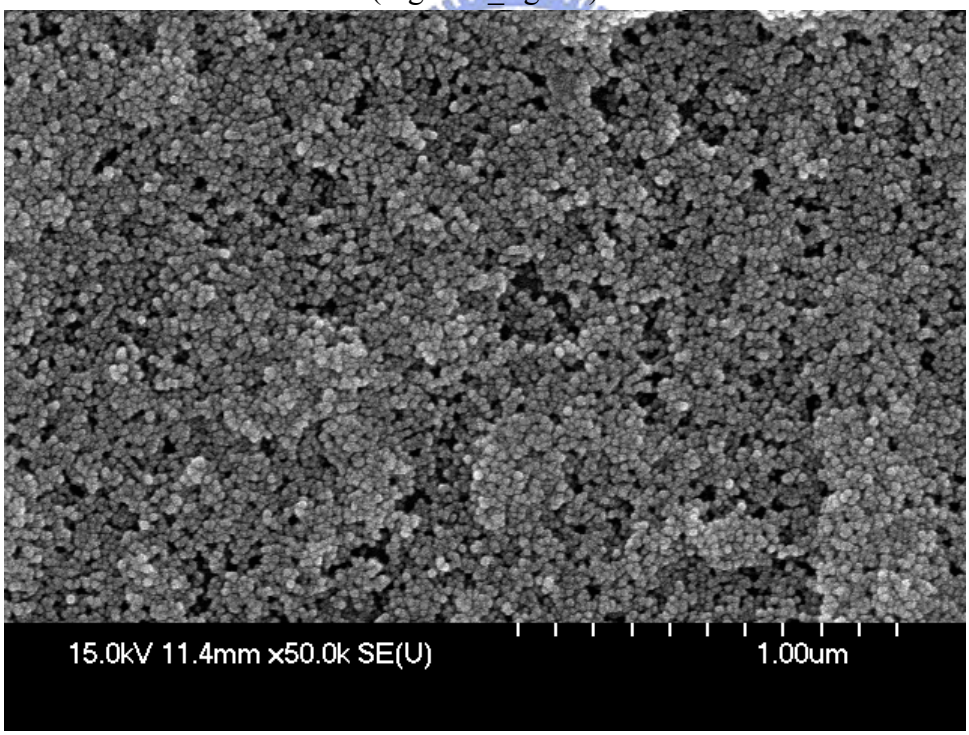
(b) CdSe NPs + Au NPs on SiO_2/Si wafer fragment
(Fig.3.22_right 3)



(c) Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment
(Fig.3.22_right 4)

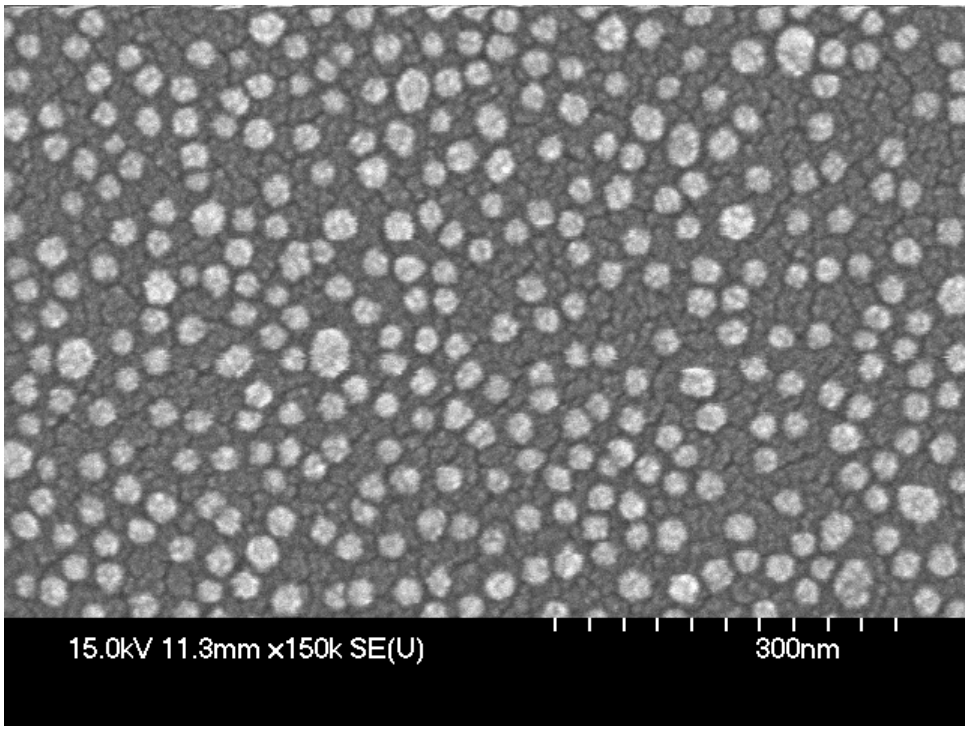


(d) CdSe NPs + Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment (Fig.3.22_right 5)



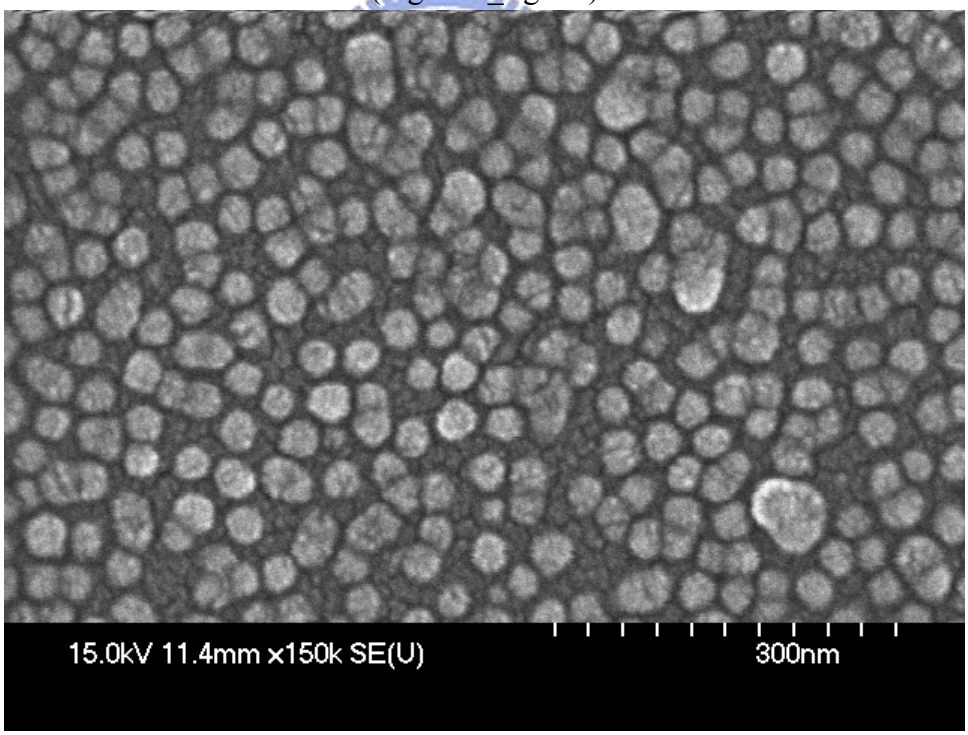
(e) Au NPs + CdSe NPs + Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment (Fig.3.22_right 6)

Fig. 3.24. The SEM images (50k magnification) of photo-sensing nanodevice structure of different level construction are shown above (a)~(e). Each SEM image has its corresponding close photographs in Fig. 3.22.



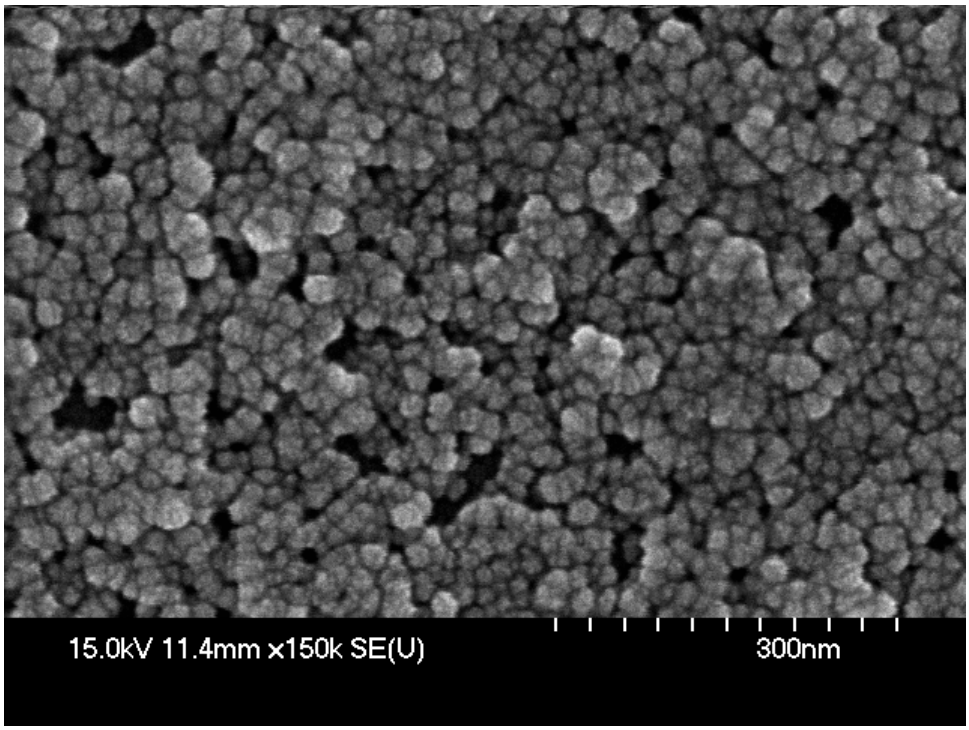
(a) Au NPs on SiO_2/Si wafer fragment

(Fig.3.22_right 2)



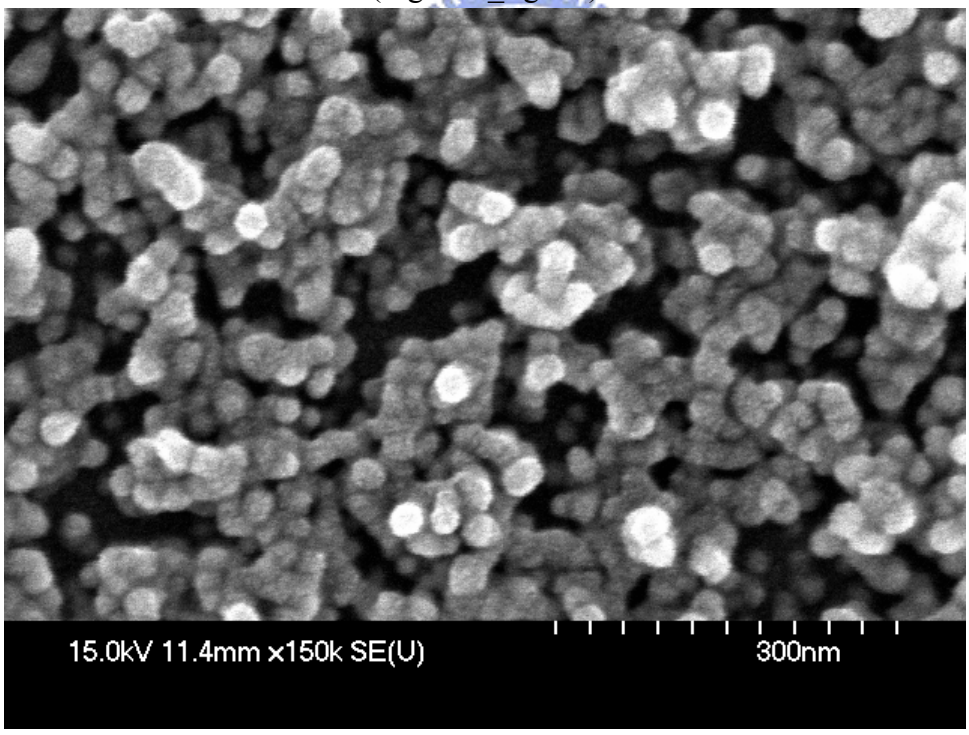
(b) CdSe NPs + Au NPs on SiO_2/Si wafer fragment

(Fig.3.22_right 3)



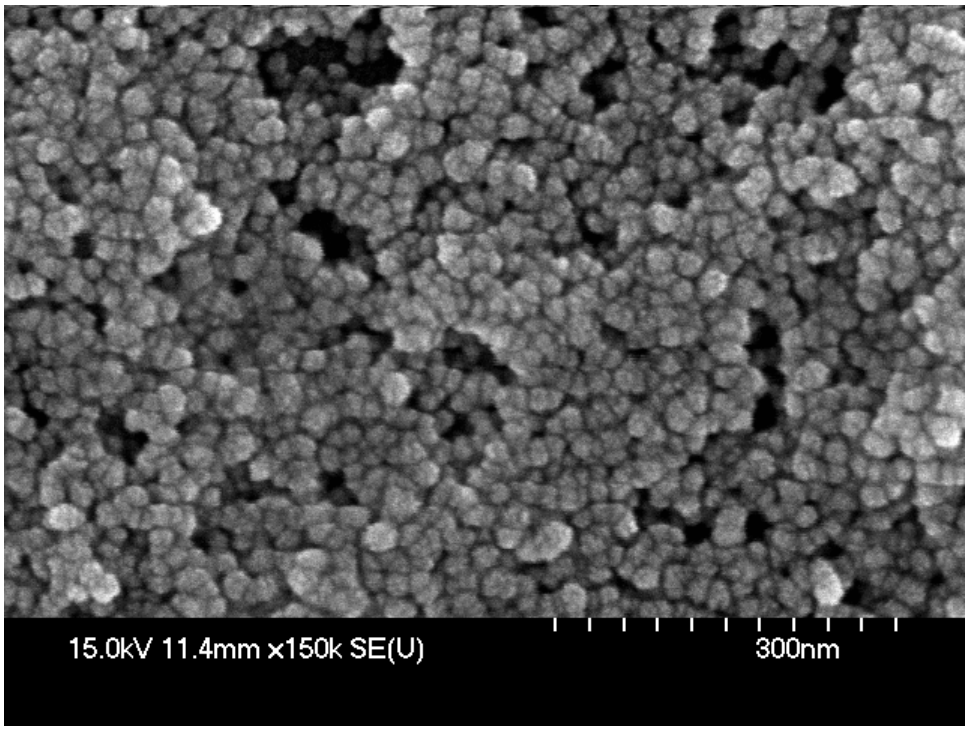
(c) Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment

(Fig.3.22_right 4)



(d) CdSe NPs + Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment

(Fig.3.22_right 5)



(e) Au NPs + CdSe NPs + Au NPs + CdSe NPs + Au NPs on SiO_2/Si wafer fragment (Fig.3.22_right 6)

Fig. 3.25. The SEM images (150k magnification) of photo-sensing nanodevice structure of different level construction are shown above (a)~(e). Each SEM image has its corresponding close photographs in Fig. 3.22.