Current and Future Directions

This group is focused on studying surface and interface science and developing novel characterization techniques using surface analysis. By tailoring the surface properties using organic self-assembled monolayers (SAMs) we are studying the interfacial interaction between materials and SAMs to develop a molecular transportation platform based on electrostatic interactions. Based on tools like SPM, XPS and SIMS that are generally used to characterize the monolayer, we are developing novel techniques for analyzing and imaging the volume distribution of organic materials using cluster ion beams as well. These topics are detailed below.

1) Surface and Interface Sciences

Nanoscale science and technology rely on control of phenomena occurring at the molecular and meso-structural level. Recently, nanoparticles and nanowires were applied to biosensing and biolabeling. For stability and biocompatibility, gold has long been used in biological studies. Gold nanoparticles are now being developed for labeling,[1-3] DNA/drug delivery,[4-9] and gene regulation.[10] Most of these methods rely on modifying the surface with a specific molecule to which the molecule of interest is covalently bonded. For example, gold nanoparticle-oligonucleotide complexes are often used due to their high stability in saline solutions.[11] These complexes are then used to bind complementary nucleic acids in a selective and cooperative manner.[12] One of the limitations of this approach is that once a molecule is bound to the carrier particle, the strong covalent bond prohibits release of the molecule into solution.

Recently, a technique applicable to research on low-temperature deposition of ceramic thin films has been developed, which is based on the use of organic self-assembled monolayers (SAMs)[13] to promote film deposition.[14] With different surface functional groups, different types of SAMs are found to either promote or hinder the deposition of different ceramic thin films based on electrostatic interactions.[13-18] Because bio-materials, such as DNA, peptides, or proteins also hydrolyze in aqueous solution in a similar manner to inorganic materials, the fundamental understanding gathered by studying the interaction between inorganic materials and SAMs could be applied to nano-biotechnology. In other words, bio-materials could be selectively adsorbed and desorbed by SAMs through electrostatic interactions.

For example, by tailoring the surface properties of organic SAMs such that the attraction interaction occurs, bio-materials can be adsorbed selectively on SAMs-bearing nano-particles. In addition, by changing the environmental pH across the isoelectric point (IEP) of either the adsorbent or the SAM, the electrostatic interaction will be inverted, and the molecule will be repelled and released into the environment. Our previous research reported the IEP of organic SAMs with different surface functional groups. For example, the IEP of sulfonate, carboxylate, amine, and alkylammonium groups on Si is pH <2, 3.0, 7.5, and >14, respectively.[13]

For biological applications, the available pH range is often limited and requires the carrier to have a specific IEP so that the surface potential inverts in the biological pH range. In other words, carriers with a specific IEP are required. For a given organic functional group, however, the surface properties are controlled mainly by the nature of the group. Therefore, it will be difficult to fine-tune the properties simply by using different functional groups. One of the possibilities to tailor the properties of a substrate is to prepare a SAM that has multiple functional groups. The resultant chemical composition of the surface can be determined with x-ray photoelectron spectroscopy (XPS) and secondary ion mass spectroscopy (SIMS).
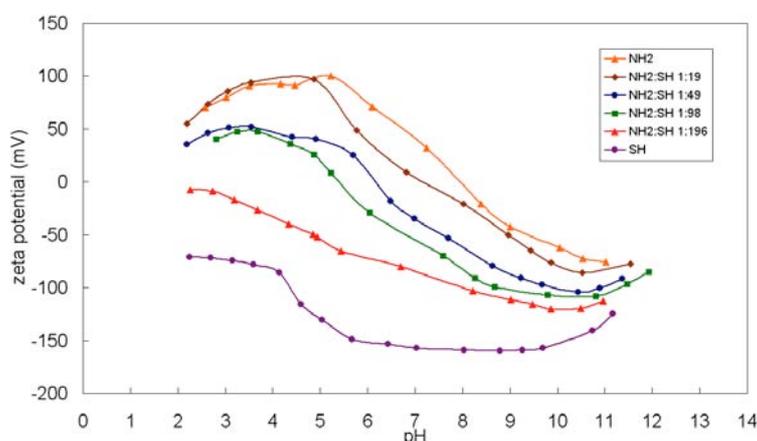
Figure 1. Zeta potential versus pH curves of SH-, NH_2-, and mixed-SAMs on silicon.

The interfacial charge density at solid-liquid interfaces is a controlling factor in a number of phenomena, such as adhesion, wetting, ion adsorption, and biocompatibility. The zeta potential, which is the potential at the shear plane between the compact layer and the diffuse layer, is an important indicator of the surface charge. This property is a useful parameter for determining the electrokinetic chemical properties of both pristine and modified surfaces. For particles smaller than tens of micrometers,

electrophoresis light scattering (ELS) or phase analysis light scattering (PALS) methods are used in this group. For larger particles or bulk substrates, streaming current is measured by pumping electrolytes through a micro-channel formed by the sample. Using carboxylic acid and amine bearing SAMs on a flat Au surface and a Au nanoparticle as an example, we have demonstrated that arbitrary IEP values between the extremes defined by exclusive use of an amine or a carboxylic acid could be achieved.[19,20] Preliminary work was done on a Si/SiO_2 surface with mixed amine and thiol functional groups as well (**Figure 1**). It seems that only a small amine content is required to affect the resulting zeta potential.
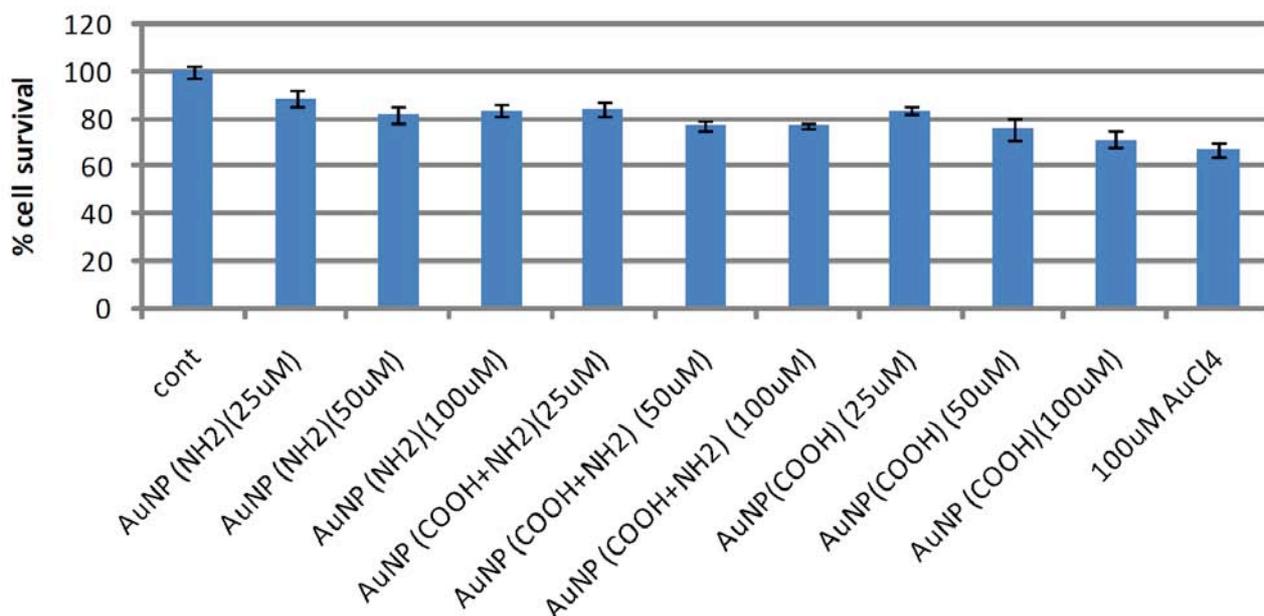

Figure 2. The cytotoxicity of SAM-modified Au nanoparticles to living cells as determined with MTT assay.

Aiming at biological applications, the cellular uptake and cytotoxicity of materials modified with SAMs with mixed functional groups needs to be investigated. A standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to measure the cell activity after culturing NIH/3T3 cells in enviornments containing Au nanoparticles modified with mixed $-NH_2$ and $-COOH$ functional groups for 24 h. Preliminary results (**Figure 2**) show that the cytotoxicity increased slightly with increasing $-COOH$ concentration on the surface presumably due to the stronger surface charge. Nevertheless, the nanoparticle is less toxic than the gold salt precursor at concentrations below 100 μM. The nanoparticle could be used to deliver payloads to the cell, and the properties of the surface charge could control its delivery efficiency. To examine this proposal, a fluorescent protein-expressing plasmid DNA will be used as the payload of modified nanoparticles. By examining the fluorescent properties of the resulting cells, the ability of carrying and protecting the

molecule in the solution and then regulating the payload release inside the cell will be studied.

Because the objective of this work is to develop a molecule transportation system by using SAMs with multiple functional groups, the quantification of the bio-adsorption is an important aspect. Direct measurement of the interaction between biological molecules and SAM-modified substrates will allow us to compare the effects of differently structured surfaces. By modifying the tip of an atomic force microscope (AFM) cantilever with a SAM that represents a SAM-modified nanoparticle (both have a radius curvature around 10 nm), the absolute interactions between the monolayer and bio-molecule can be measured in an aqueous environment. The resulting interaction force will allow the examination of the proposed electrostatic adsorption and desorption model. Preliminary work has investigated Au-coated AFM cantilevers that are subsequently modified by SAMs with multiple functional groups. The force curve between Si (with native oxide) and modified tips at different pH are shown in **Figure 3** as a model system.
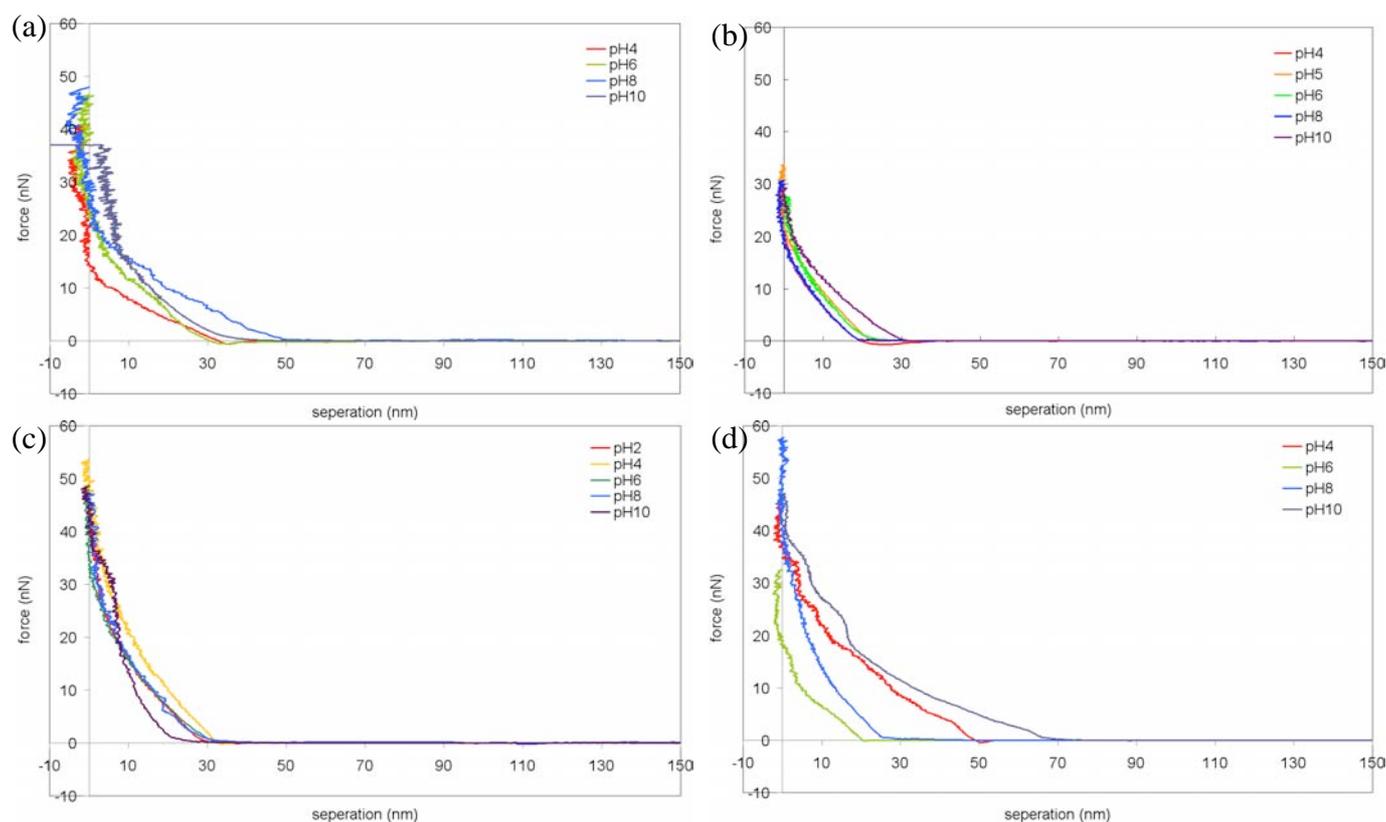
Figure 3. AFM force curve of SAMs with mixed functional groups of (a) 1:0, (b) 1:1, (c) 1:5, (d) 0:1 –COOH: –NH$_2$ and native oxide on Si.

The directly measured forced of the interaction was as expected. At pHs below and above the IEP of SiO$_2$ and the SAM, a repulsion force is observed. Conversely, for the pH between the IEP of SiO$_2$ and the SAM, an attractive interaction is observed. In other words, by tailoring the IEP using SAMs with mixed

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functional groups, molecules could be selectively immobilized, and a molecular transportation platform could be developed by considering the electrostatic interactions.

Due to its high weight sensitivity, a quartz crystal microbalance is often used to monitor the weight change of a substrate. **Figure 4** shows the frequency shift of Au during exposure to an amino-thiol solution. An unstable multilayer of the film quickly immobilized on the surface and was removed by an ethanol rinse. The net frequency shift indicated a stable monolayer was formed on the surface. Combined with energy dissipation, the change in the elastic property of the immobilized phase can be studied as well. Using this technique, the adsorption and desorption kinetics of biological molecules on SAMs will be studied and compared to the AFM force curve and the zeta-potential of the surface.
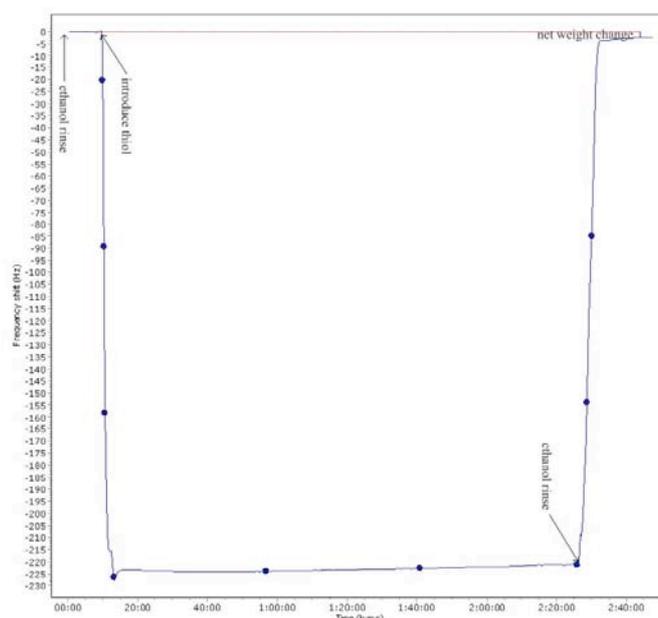
Figure 4. QCM frequency shift of Au after exposure to an amino-thiol solution followed by an ethanol rinse.

In addition to acting as an adaptor for immobilizing molecules, SAMs are also known to affect cell adhesion and spread. Such properties are crucial aspects of biomaterial research. For example, phosphonic acid-bearing SAMs have been used to modify Ti-Nb-based medical implants.[21] The extracellular matrix (ECM) affects cell growth and adhesion significantly through focal adhesions (FAs). By tailoring the surface properties with SAMs, FA proteins could be adsorbed selectively on the substrate and in turn affect cell adhesion. For example, by tailoring the surface properties of organic SAMs such that the attraction interaction takes place, FA protein can be adsorbed selectively, and cells could adhere to the substrate. On the other hand, by modifying the surface chemistry to repeal the FA protein, an

anti-adhesive surface can be prepared. Using QCM, the absorption kinetic of FA proteins on the SAM-modified surface can also be examined and compared to the results of bio-adhesion tests.

2) Analysis and Imaging of Soft Matter

Based on surface analysis techniques like XPS and SIMS that are regularly used to characterize the chemical composition of organic SAMs discussed in the previous section, we are extending these analytical methods to study complicated organic structures. The nanostructure of a material significantly affects the properties of the resultant opto-electronic devices. Unlike inorganic semiconductors, which are usually crystalline, organic materials are mostly amorphous. As a result, it has been difficult to analyze the nanostructure inside organic electronics using well-established analytical techniques. Furthermore, the segregated amorphous and oriented phases of polymers used in organic electronics is highly sensitive to the fabrication process.[22,23] Additional phase separation in polymer films is often found upon mixing with small molecules that have significantly different chemical structures. This phase separation is crucial to device efficiency.[24] Therefore, investigating and understanding the relationship between fabrication parameters, nanostructures in the polymer film and device performance is valuable to prepare highly efficient, long lifetime organic electronics.

Based on surface analysis techniques and ion-beam depth profiling we recently developed a series of novel analytical methods that allow for the analysis of the interior structure of organic materials. For example, the vertical nanostructure of organic opto-electronic devices has been studied with XPS[25-28] and ToF-SIMS.[27] Although cluster ion sputtering significantly altered the outer-most surface of inorganic materials,[29] it caused insignificant damage to the organic surface. This difference from the generally used atomic ion-beams is because of the shallower damage range[30] and enhanced sputtering rate[31] of the cluster ion. Using this novel analytical technique we reported the observation of electron-migration of small molecules inside organic light emitting diodes (OLEDs).[28]

It is proposed that using host materials with higher stereo hindrance could suppress the migration of electron-injecting materials and enhance the device lifetime (**Figure 5**). For example, by replacing the CBP host with mCP, which has higher dipole moment, the migration of electron-injecting TPBi molecules into mCP is suppressed because of the stronger van der Waals force between mCP molecules.

Furthermore, using SimCP2 as the host material, the migration of TPBi can also be suppressed because of the higher molecular weight and higher dipole moment of the new material. Finally, using a SimCP that has a higher density than SimCP2 resulted in more densely packed molecules, and the electron-migration can be further suppressed. By fabricating these OLED devices and analyzing the distribution of molecules at different operation times, the device lifetime will be examined.
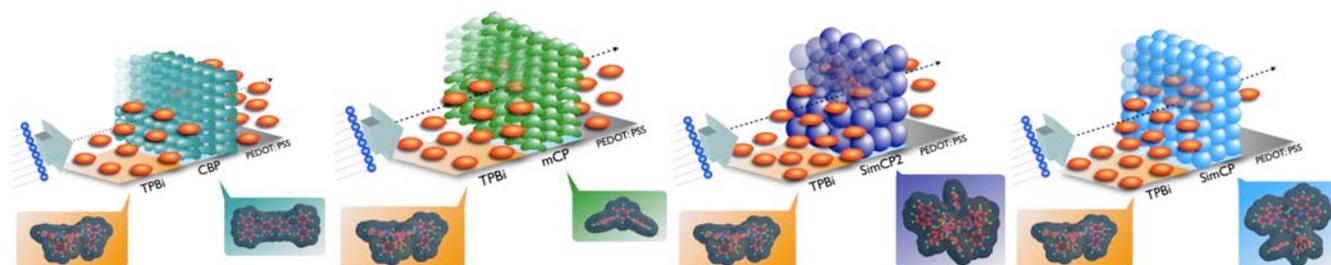
Figure 5. Schematic illustration of suppressing the electron migration of electron-injecting material using different host materials.

SIMS is known to has extremely high detection sensitivity for analyzing the surface.. In addition, SIMS provides complementary information to XPS. Taking the electron-injecting TPBi molecule (m.w. 654) as an example, **Figure 6** shows the ToF-SIMS spectra excited by 10 kV C_{60+} sputtering. It is clear that structural information of the TPBi molecule can be acquired while XPS can only reveal the C to N ratio of the molecule. Due to the unsteady sputtering rate and limited sputtering depth, however, ToF-SIMS with a pulsing C_{60}^{+} ion source has its limitations on sampling depth. Furthermore, it is difficult to integrate a post-ionization system with a ToF-SIMS as in secondary neutral mass spectrometry (SNMS). As a result, less than 5% of the particles ejected from the surface can be collected, and the signal intensity is controlled by the matrix effect. These issues limited the application of SIMS in quantification. Using a more economical approach, we are integrating a quadrupole mass analyzer onto the existing available C_{60}^{+} ion source. Operating in DC mode, co-sputtering can be applied, and post-ionization using electron and/or photon can be easily integrated. In other words, we will be developing a molecular SNMS (or secondary molecule mass spectrometry, SMMS) system for parallel detection of mixed organic/biological materials.
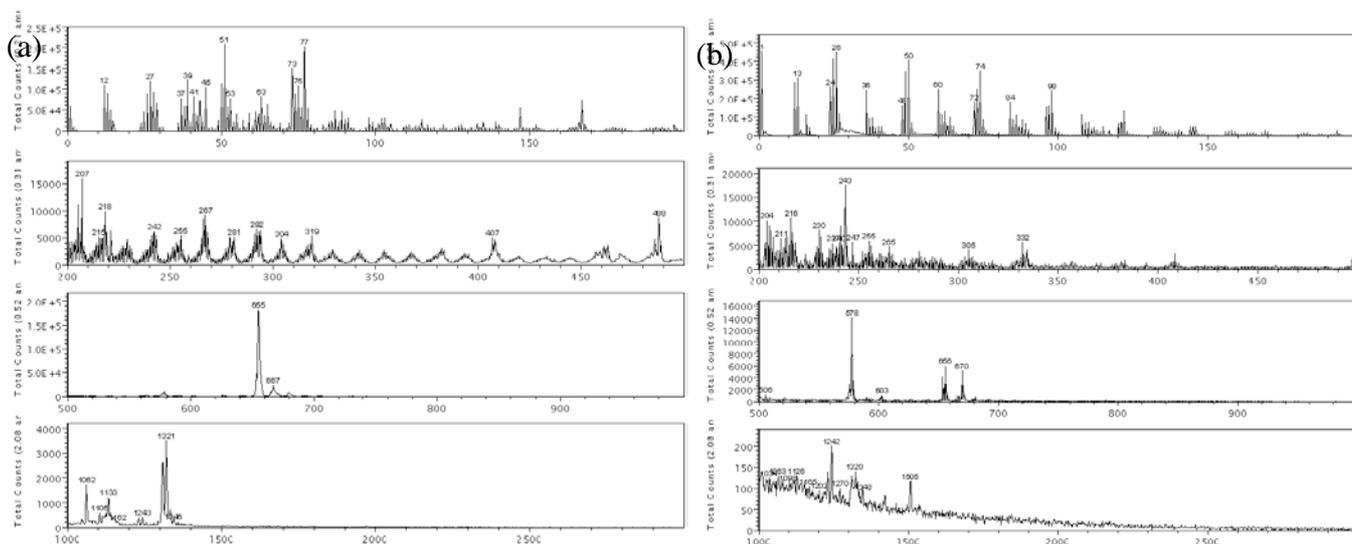
Figure 6. (a) Positive and (b) negative SIMS spectra of TPBi excited by 10-kV C_{60}^+ ions.

While providing excellent depth resolution, XPS and SIMS possess limited lateral resolution and only allow the vertical structure to be probed. Thus, ion-beam depth profiling has been combined with imaging techniques like scanning electrical potential microscopy (SEPM),[32] force modulation microscopy (FMM)[33] and scanning ToF-SIMS[34] to produce 3D volumetric images inside organic materials. Based on this novel imaging technique for soft matter, we are extending the application to biological specimens as well.

One of the established 3D imaging techniques that yields high resolution is electron tomography. These instruments are based on expensive TEMs and are usually operated at a high voltage. For organic materials that are amorphous and scatter electron beams weakly, the contrast in the resulting image is often limited and requires staining of the sample before observation. Furthermore, the contrast depends on coherent beam scattering, and the sample thickness is limited; hence, the chromatic aberration of the objective lens does not degrade the image quality significantly. Based on a SEM that is relatively cheap and operates at a lower voltage, the electron interacts with the specimens more strongly and could generate better contrast. In addition, the incoherent scanning technique could allow for the imaging of thicker specimens. Therefore, we are developing a SEM-based electron tomography technique. By using a home-made sample holder that holds a standard 3-mm TEM grid in the chamber and allows a multi-segment solid-state electron detector to be inserted behind the specimen (**Figure 7**), the SEM can be operated as a STEM. Although the current holder design only allows tilt angles between ±40° at a

5-mm working distance and 10-mm camera length that limits the spatial information, we are using this design as a proof of concept for a low-cost 3D imaging tool for biological applications.

Figure 7. Home-made specimen holder that converts a SEM to a STEM for development of the electron tomography technique.

References

(1) Lin, C.-C.; Yeh, Y.-C.; Yang, C.-Y.; Chen, C.-L.; Chen, G.-F.; Chen, C.-C.; Wu, Y.-C. *J. Am. Chem. Soc.* **2002**, *124*, 3508-3509.

(2) Wang, J. *Small* **2005**, *1*, 1036-1043.

(3) Katz, E.; Willner, I. *Angew. Chem. Int. Ed.* **2004**, *43*, 6042-6108.

(4) Salem, A. K.; Searson, P. C.; Leong, K. W. *Nat. Mater.* **2003**, *2*, 668-671.

(5) Sandhu, K. K.; McIntoshJ, C. M.; Simard, o. M.; Smith, S. W.; Rotello, V. M. *Biocojugate Chem.* **2002**, *13*, 3-6.

(6) Thomas, M.; Klibanov, A. M. *Oric. Natl. Acad. Sci. USA* **2003**, *100*, 9138-9143.

(7) Sundaram, P.; Xiao, W.; Brandsma, J. L. *Nucleic Acids Res.* **1996**, *24*, 1375-1377.

(8) Sullivan, M. M. O.; Green, J. J.; Przybycien, T. M. *Gen Therapy* **2003**, *10*, 1882-1890.

(9) Jen, C.-P.; Chen, Y.-H.; Fan, C.-S.; Yeh, C.-S.; Lin, Y.-C.; Shieh, D.-B.; Wu, C.-L.; Chen, D.-H.; Chou, C.-H. *Langmuir* **2003**, *20*, 1369-1374.

(10) Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K. R.; Han, M. S.; Mirkin, C. A. *Science* **2006**, *312*, 1027-1030.

(11) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607-609.

(12) Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A.; Schatz, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 1643-1654.

(13) Shyue, J.-J.; De Guire, M. R.; Nakanishi, T.; Masuda, Y.; Koumoto, K.; Sukenik, C. N. *Langmuir* **2004**, *20*, 8693-8698.

(14) Gao, Y.; Koumoto, K. *Crystal Growth and Design* **2005**, *5*, 1983-2017.

(15) Shyue, J.-J.; Tang, Y. T.; De Guire, M. R. *J. Mater. Chem.* **2005**, *15*, 323-330.

(16) Shyue, J.-J.; De Guire, M. R. *Chem. Mater.* **2005**, *17*, 5550-5557.

(17) Shyue, J.-J.; De Guire, M. R. *Chem. Mater.* **2005**, *17*, 787-794.

(18) Lin, Y.-C.; Yu, B.-Y.; Lin, W.-C.; Chen, Y.-Y.; Shyue, J.-J. *Chem. Mater.* **2008**, *20*, 6606-6610.

(19) Lin, W. C.; Lee, S. H.; Karakachian, M.; Yu, B. Y.; Chen, Y. Y.; Lin, Y. C.; Kuo, C. H.; Shyue, J. J. *Physical Chemistry Chemical Physics* **2009**, *11*, 6199-6204.

(20) Lin , Y. C.; Yu, B. Y.; Lin, C. W.; Lee , S. H.; Kuo, C. H.; Shyue, J. J. *Journal of Colloid and Interface Science* **2009**, *340*, 126-130.

(21) Zorn, G.; Gotman, I.; Gutmanas, E. Y.; Adadi, R.; Salitra, G.; Sukenik, C. N. *Chem. Mater.* **2005**, *17*, 4218-4226.

(22) Yang, C. Y.; Hide, F.; Diaz-Garcia, M. A.; Heeger, A. J.; Cao, Y. *Polymer* **1998**, *39*, 2299-2304.

(23) Kim, J.; Lee, J.; Han, C. W.; Lee, N. Y.; Chung, I. J. *Appl. Phys. Lett.* **2003**, *82*, 4238-4240.

(24) Yimsiri, P.; Mackley, M. R. *Thin Solid Films* **2007**, *515*, 3787-3796.

(25) Chen, Y.-Y.; Yu, B.-Y.; Wang, W.-B.; Hsu, M.-F.; Lin, W.-C.; Lin, Y.-C.; Jou, J.-H.; Shyue, J.-J. *Anal. Chem.* **2008**, *80*, 501-505.

(26) Yu, B.-Y.; Chen, Y.-Y.; Wang, W.-B.; Hsu, M.-F.; Tsai, S.-P.; Lin, W.-C.; Lin, Y.-C.; Jou, J.-H.; Chu, C.-W.; Shyue, J.-J. *Anal. Chem.* **2008**, *80*, 3412-3415.

(27) Lin, W.-C.; Lin, Y.-C.; Wang, W.-B.; Yu, B.-Y.; Iida, S.-i.; Tozu, M.; Hsu, M.-F.; Jou, J.-H.; Shyue, J.-J. *Organic Electronics* **2009**, *10*, 459-464.

(28) Lin, W.-C.; Wang, W.-B.; Lin, Y.-C.; Yu, B.-Y.; Chen, Y.-Y.; Hsu, M.-F.; Jou, J.-H.; Shyue, J.-J. *Organic Electronics* **2009**, *10*, 581-586.

(29) Lin, Y.-C.; Chen, Y.-Y.; Yu, B.-Y.; Lin, W.-C.; Kuo, C.-H.; Shyue, J.-J. *Analyst* **2009**, *134*, 945-951.

(30) Yu, B.-Y.; Chen, Y.-Y.; Lin, W.-C.; Lin, Y.-C.; Shyue, J.-J. *Appl. Surf. Sci.* **2008**, *255*, 2490-2493.

(31) Cheng, J.; Wucher, A.; Winograd, N. *J. Phys. Chem. B* **2006**, *110*, 8329-8336.

(32) Yu, B.-Y.; Lin, W.-C.; Huang, J.-H.; Chu, C.-W.; Lin, Y.-C.; Kuo, C.-H.; Lee, S.-H.; Wong, K.-T.; Ho, K.-C.; Shyue, J.-J. *Analytical Chemistry* **2009**, *81*, 8936-8941.

(33) Yu, B.-Y.; Liu, C.-Y.; Lin, W.-C.; Wang, W.-B.; Lai, I.-M.; Chen, S.-Z.; Lee, S.-H.; Kuo, C.-H.; Kao, W.-L.; You, Y.-W.; Liu, C.-P.; Chang, H.-Y.; Jou, J.-H.; **Shyue, J.-J.** *ACS Nano* **2009**, (submitted).

(34) Yu, B.-Y.; Lin, W.-C.; Wang, W.-B.; Iida, S.-i.; Chen, S.-Z.; Liu, C.-Y.; Kuo, C.-H.; Lee, S.-H.; Kao, W.-L.; Yen, G.-J.; You, Y.-W.; Liu, C.-P.; Jou, J.-H.; Shyue, J.-J. *ACS Nano* **2010**, *4*, 833-840.