OPT 254/PHYS 371 – Atomic Force Microscope Laboratory Report

Linh Vu

ABSTRACT

In three labs, three different samples are scanned with an atomic force microscope (AFM). Those are an unknown sample given by the company Nanosurf, a gold nanoantenna array sample, and a nano silver cubes sample. Through multiple image scans, we successfully practiced using AFM and the scanning software, and analyzing and understanding the structures of different samples.

INTRODUCTION AND OBJECTIVES

The primary goal of this lab is to scan the surfaces of different samples using AFM to study about their structures and how AFM works in scanning. AFM is a high-resolution scanning device with a resolution ranging from 10μm up to .1nm. Among its three main purposes of measuring forces, imaging topography, and manipulation, this lab focuses on topographic imaging. The AFM model used in the lab is the Easyscan-2. Its main components are a scanning head with a cantilever and a tip which are used to scan directly the sample surfaces, a scanner table where the samples are placed, an electric controller, a vibration isolation table, an additional vibration isolation table, and a connection to a scanning software. As scanning, the AFM provides data to the Nanosurf Easyscan 2 software. The software then converts the input data into topography and amplitude image. By looking at the topography and the corresponding amplitude image, it is possible to observe and study about the structures of the samples with a depth of view.

Three samples experimented during three labs are an unknown sample provided by Nanosurf company, the gold nanoantenna array, and the nano silver cubes. As easily being seen from the topography images, the unknown sample includes at least five structure patterns: rectangles, circles, stripes, bigger circles, and squares (with rounded edges). The second sample being studied is the gold nanoantenna array. It is observed to have a pattern of multiple nano rectangles in line together. Lastly, the silver nano cubes, as its name has shown, has a pattern of multiple nano cubes randomly distributed.

In order to obtain the best topography images, careful procedure of approaching and moving the AFM cantilever needs to be followed strictly. Additionally, adjusting the setting of scanning modes and parameters can improve the image quality in different ways.

Using the experiment results, we hope to successfully observe the samples structures as well as study the scanning procedure using AFM. Being able to understand the uncertainties and limitations of the AFM from the topography and amplitude images also plays an important role.

PROCEDURE

In this lab, a scanning procedure is applied on three different samples: an unknown sample provided by the company Nanosurf, the gold nanoantenna array, and the nano silver cubes.

To prepare for the scan, first turn on the electric controller and the additional vibration isolation table. Check the light signs to ensure that those two parts are activated. Make sure the white light in the AFM head is on. This light supports the camera inside the scanning head, so we are able to observe the distance between the cantilever and the surface, and also the movement of the cantilever while scanning. Next, ensure that the connection between the AFM system and the computer is connected in a safe condition. Interruption to this connection can cause lose of data and unfortunate damage to the tip or the sample surface.

Once the AFM system is secured, set up and adjust the probe using the Nanosurf Easyscan 2 software. Select ‘Acquisition’ tab on top of the tool bar, in the ‘Preparation’ section, make sure the scanning mode is in ‘Dynamic Force’. Dynamic force mode is the tapping mode, one of the most typical scanning mode since it provides the ultimate qualified image compared to the other two main modes: contact mode – static force mode and non-contact mode. Dynamic force mode – tapping mode can minimize the tip and sample damage by avoiding friction and adhesion as much as possible using an effective oscillating frequency. Each cantilever has its own suitable scanning mode; here, the Multi75Al-G cantilever is used.

The ‘Parameters’ and ‘Z-Controller’ play an important role in setting up the output image and its quality. Image size, scanning speed, image resolution, scanning angle, and more are set up in ‘Parameters’. Setpoint and gains are adjusted in ‘Z-Controller’. The ‘Video’ panel shows the camera view: top view to move the cantilever to desired positions and side view to adjust the distance between the tip and the sample.

The ‘Probe Status’ at the bottom of the software screen indicates the readiness of the probe (cantilever and tip) for scan. Since the probe has not been adjusted, the status is in red. When it is in red, it is safe to move the cantilever in the x-y directions. Using the camera top view and adjusting

the levers carefully to place the cantilever at the area of scanning.

Change to the camera side view to see the distance between the tip and the sample surface. Hold 'Advance' at the top toolbar to approach the surface. Only apply 'Advance' when the distance between the tip and the sample is still quite significant. Watch the shadow of the cantilever, once the shadow is close and clear, hit 'Approach'. This 'Approach' mode is different from the 'Advance' one; this is an automatic approach that is used when the cantilever is close enough to the surface for safety purpose. After hitting the 'Approach' button, wait for the process of setting a frequency to finish. Now, notice that the probe status at the bottom is in green. After setting up the 'Parameters' and 'Z-Controller' data, the AFM is ready to scan. Hit 'Start' to begin.

Pay careful attention to the scan process to detect any errors. The scan line starts from the bottom to the top of the image. Notice the black arrow outside of the right edge of the topography and the amplitude images, once it is close to the top, hit 'Stop'. If fail to do so, the scan will start again automatically. This does no harm but takes time since the scanning time of AFM is already not too fast (2 – 5 minutes per image).

The images and camera views must be saved once the scan is done. There are many ways to save the images and camera views after each scan, but the most effective way is to copy and paste into Powerpoints. Each scan process includes three slides: a screenshot of the overall setting including all parameters, scanning modes, … of the images, a copy of topography and amplitude images, and a copy of the two camera views at the end of the scan. With these three slides, it is clear to understand the images even without doing the experiment. Make certain to create new Powerpoints for each lab and save frequently.

To scan a smaller specific area within the area that has just been scanned, choose 'Zoom' option right above the topography. Make certain that when hover the mouse to the topography, the arrow shows up instead of the normal mouse symbol. If fail, try again. Once the arrow shows up, drag a square around the area that we want to scan. Double click on the new area to make sure that we have the new image size. For more certainty, check the 'Image size' in 'Parameters' on the left panel. Once the image size is smaller to the previous scan size, a new scan process is ready. Repeat again the whole process including 'Stop' and save images to the Powerpoints for each scan.

In measuring a specific length, for example, the radius of a circle, choose 'Analysis' tab on the top toolbar, then 'Measure Length'. Click on two points to create a line, the length of that line will show up in 'Tool status'. Take a screenshot and save as a new slide in Powerpoints.

To move the cantilever to another position, first have the camera view change to the side view. Click 'Withdraw' on the top toolbar to move up the cantilever automatically. Once the process is done, the probe status is in orange. Depends on the shadow showing up in the side view camera, withdraw for once to twice. Then, hold 'Retract' to withdraw mechanically. Only use 'Retract' once there is a safe distance between the tip and the sample surface. This is because 'Retract' uses a mechanical mechanism to lift the cantilever up. If the cantilever is close to the surface, the mechanical mechanism might make a small dip before actually move up, causing damage to the tip and the sample.

After maintaining a safe distance using 'Withdraw' and 'Retract', change camera to top view. For a clear vision, adjust the contrast bar on top of the camera panel. Carefully adjust the x-y levers to move the cantilever to a new position. Once satisfied, change to side camera view and begin the approaching process. If the sample size is small, for example, in the second lab with the gold nanoantenna array, it is difficult to move the cantilever to an exact area. Instead, move the sample table in x-y directions using different levers.

At the end of each lab session, process the withdraw procedure again, except that this time, hold 'Retract' for a significant longer time to ensure a much safer distance (until the shadow of the cantilever and the head are no longer on the screen). Observe the distance with human eyesight directly at the AFM head. Then, click on 'File' and 'Exit' to exit the software. Turn off the electric controller, unplug the connection between the AFM system and the computer to ensure that the software can not control the AFM by accident. Lastly, turn off the vibration isolation.

DISCUSSION

FIRST LAB SESSION: UNKNOWN SAMPLE FROM NANOSURF COMPANY

Overall

The first lab is an overall introduction of using AFM and the software Nanosurf Easyscan 2. In order to give the most basic and easy-to-scan examples, we use an unknown sample provided by Nanosurf company. This sample includes multiple structure patterns to observe and has an ideal sample size. As a result, we observe at least five patterns: rectangles, circles, stripes, larger circles, and squares (rounded edges). Since the nature of this lab is fundamental, we keep most of the parameters and Z-controller data constant throughout the whole process (fig. 1.1 and 1.2).

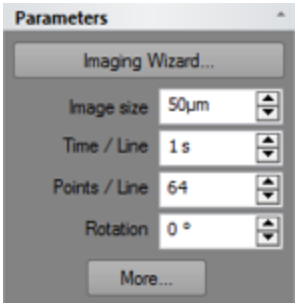

Fig. 1.1 Lab 1 Parameters

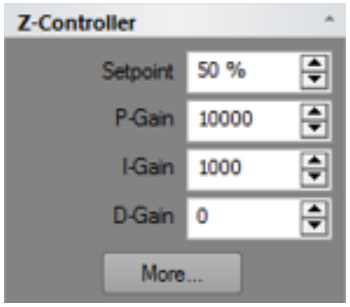

Fig. 1.2 Lab 1 Z-Controller

Details

The first pattern we observe is scanned on the lower left corner of the sample with respect to the top camera view (fig. 2.1), includes multiple rectangles with sharp edges in lines together. At 50µmx50µm, we are able to observe the overall picture (fig. 2.2 and 2.3). This is a pretty good quality image. There are some noises result in the black horizontal lines across the topography which are likely caused by dusts or particles (white dots). The amplitude image indicates the depth of the surface. Here, those rectangle blocks are higher than the flat surface.

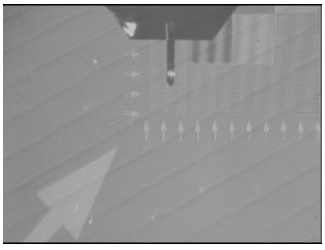
Fig. 2.1 Pattern 1 position

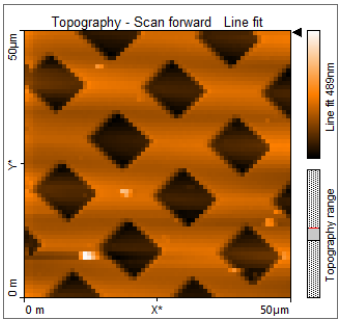

Fig. 2.2 Pattern 1 topography

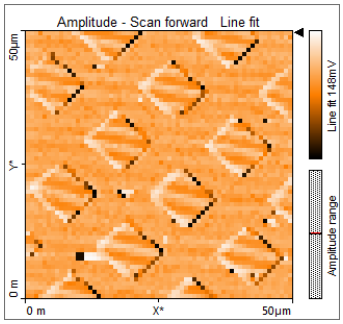

Fig. 2.3 Pattern 1 amplitude

The second pattern is located on the upper left corner of the four-squares area at the center of the sample (fig. 3.1). We observe multiple circle blocks in straight lines horizontally but interleaved vertically (fig. 3.3). Same as the first pattern, the image size of ~50µm is able to give an overall picture. Little noises are also recorded due to some dusts or particles. Another possible source of the horizontal distort lines is after each time the tip scan through a horizontal line of blocks, the change in the amplitude causes somewhat distortion since the cantilever might not be able to adjust fast enough.

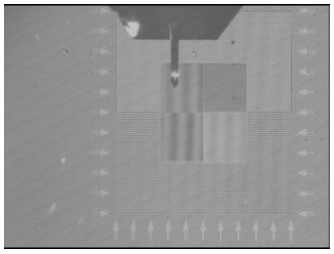
Fig. 3.1 Pattern 2 position

For this pattern, we continue to scan for smaller sizes in order to measure the diameter of the circle. At the image size of ~5µm (fig. 3.2), a single circle is captured, but the image is not clear enough due to small scan area. Therefore, to measure the diameter at its best, an image size of ~17µm is more effective (fig. 3.3). The recorded diameter is ~4.39µm.

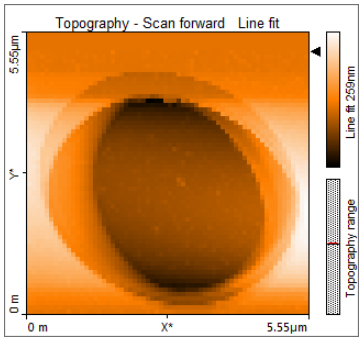

Fig. 3.2 Pattern 2 single circle

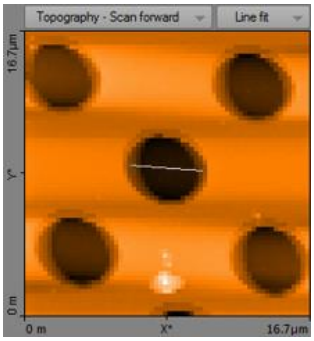

Fig. 3.3 Pattern 2 diameter

Moving to the upper right corner of that same four-squares area of the sample (fig. 4.1), we obtain a different pattern of stripes. There are some dusts that caused the distortions (fig. 4.2). The amplitude image represents exactly the depth of the surface in topography (the position of dusts is corresponding in two images). The multiple tiny orange dots result in the amplitude image are not the actual surface amplitude but the pixels (fig. 4.3). This is due to the quality of the software.

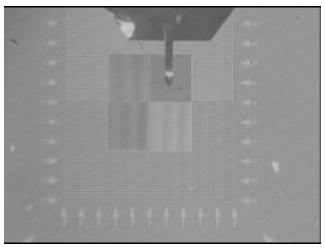
Fig. 4.1 Pattern 3 position

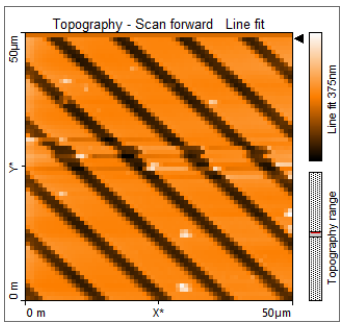

Fig. 4.2 Pattern 3 topography

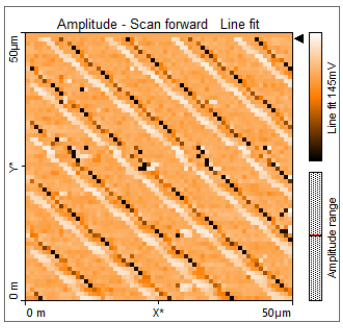

Fig. 4.3 Pattern 3 amplitude

The lower right corner of the four-squares area has a pattern of circle blocks (fig. 5.1), almost the same as pattern 2 (fig. 3.3) but with larger diameter circles. The diameter recorded is ~5.6µm (compared to ~4.39µm of the second pattern).

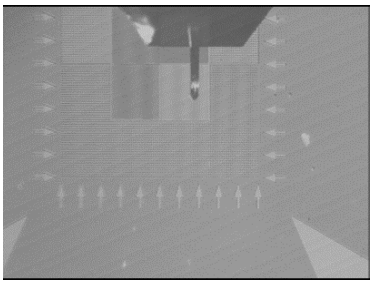
Fig. 5.1 Pattern 4 position

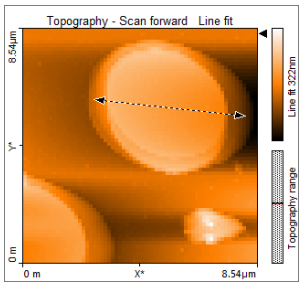

Fig. 5.2 Pattern 4 diameter

Lastly, we moved the cantilever to the upper right corner of the sample (fig. 6.1). The pattern observed is quite similar to the first pattern, except that the rectangle blocks have rounded edges, and are closer to each other (fig. 6.2 and 6.3).

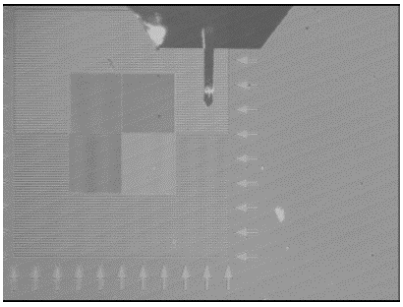
Fig. 6.1 Pattern 5 position

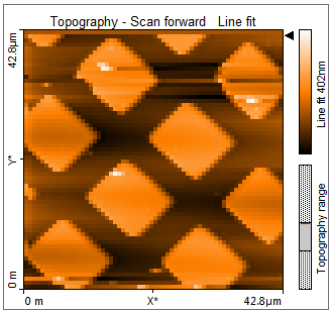

Fig. 6.2 Pattern 5 topography

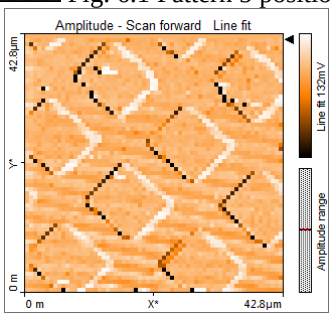

Fig. 6.3 Pattern 5 amplitude

We then take a closer look at the edge between two blocks with an image size of ~10µm (fig. 6.4). Changing the rotation angle from 0 to 45˚ also gives an interesting view of the edge (fig. 6.5).

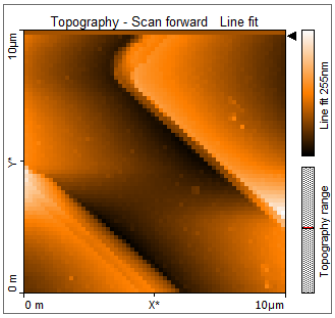

Fig. 6.4 Pattern 5 edge

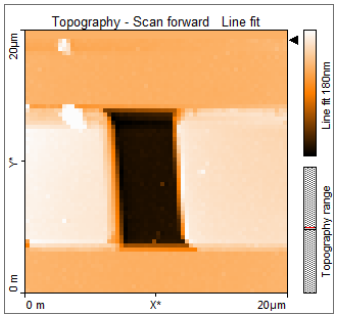

Fig. 6.5 Pattern 5 edge 45˚

SECOND LAB SESSION: GOLD NANOANTENNA ARRAY

Overall

It is great to study about gold nanoantenna array sample since it has various structures ranging from atomic clusters to nanocrystals of Au (1). These interesting structures make the gold nanoantenna array applicable in multiple aspects.

Just by looking at the camera top view, compared to the first lab session, the sample size of this second lab is much smaller (fig. 7.1 and 7.2). Due to the small sample size, we will move the sample table to scan different areas instead of moving the cantilever as in lab 1.

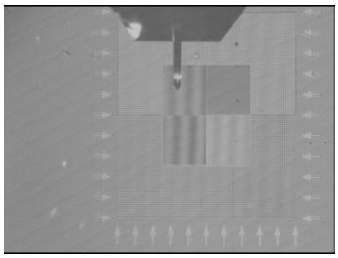
Fig. 7.1 Lab 1 sample size

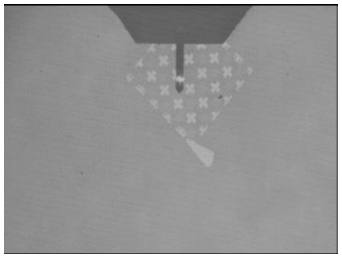
Fig. 7.2 Lab 2 sample size

Due to the difficulty level in scanning the gold nanoantenna array sample, parameters and Z-controller data are also kept constant throughout the whole lab: Time/line = 1s, Points/line = 100, Rotation = 0deg, Setpoint = 50%, P-Gain = 10000, I-Gain = 1000, and D-Gain = 0.

Details

Starting with an image size of 50µm, we observe a lot of noises through the whole scan (fig. 8.2 and 8.3). The obvious distortion can also be recognized in the amplitude image. When the tip meets particles or dusts on the way, there are black horizontal lines of distortion on the topography. Once passes the obstacles, a different direction of distortion occurs. It is shared that the other group witnesses a clean image after the tip passes the obstacles instead of a different type of distortion as seen in our group.

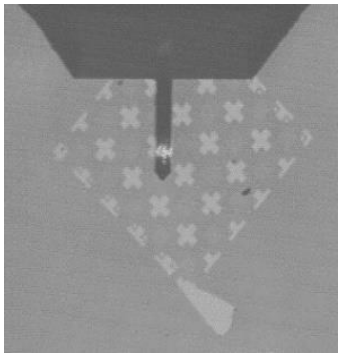
Fig. 8.1 Lab 2 scan 1 position

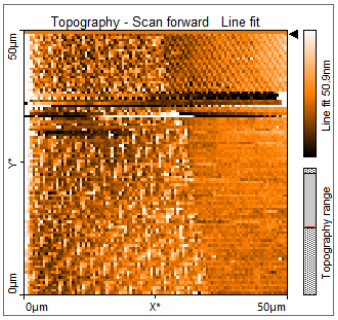

Fig. 8.2 Lab 2 scan 1 topography

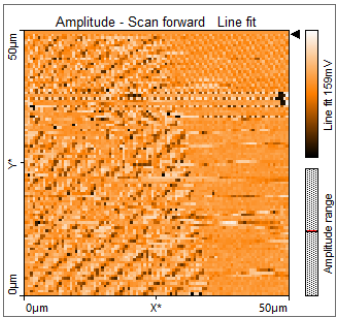

Fig. 8.3 Lab 2 scan 2 amplitude

Zooming into a smaller area where there are little to no distortions, we have an image size of ~16µm (fig. 8.4 and 8.5). Sometimes, it is hard to tell whether there are noises on the topography or not. Therefore, it is good to look at the amplitude image to make a conclusion. As the result, we obtain a quite clear topography from which it is possible to describe the overall sample's structure. The structure of the gold nanoantenna array is especially complicated.

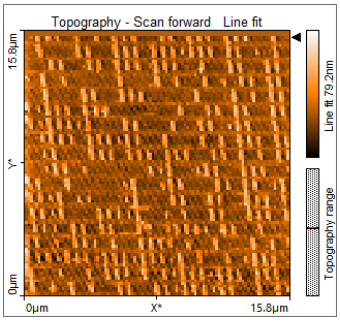

Fig. 8.4 Lab 2 scan 3 topography

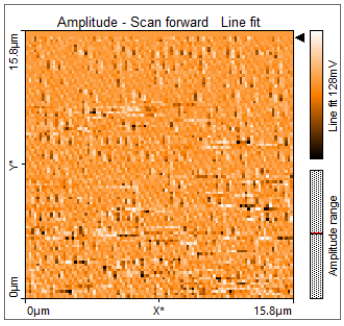

Fig. 8.5 Lab 2 scan 3 amplitude

Keep zooming in smaller areas with the least noises for twice, we are able to get the image of ~1µm with a single array (fig. 8.6 and 8.7). This is considered as a clear image. The width of the array is ~324nm, and its length is 808.1nm.

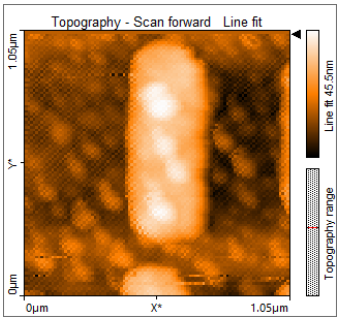

Fig. 8.6 Lab 2 scan 4 topography

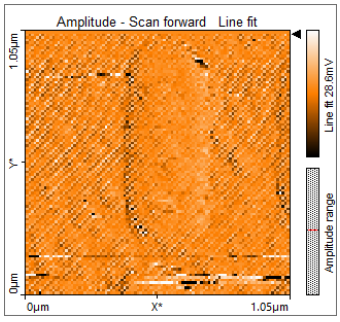

Fig. 8.7 Lab 2 scan 4 amplitude

In order to observe the edge, we keep zoom in the edge area. Eventually, when the image size is down to ~312nm (UV wavelength), it is too small that the tip is dragging the particles. Therefore, the image resolution is low. We then move on to another area to observe if there is any different pattern. As a result, the gold nanoantenna array seems to have one general pattern, but there are many that we have not discovered yet. Later attempts to scan different areas, zoom in and measure the dimension of a single array are not successful due to many reasons. One of the reasons that affects the image quality is the white light of the camera. Sometimes, in the middle of the scan, the light from the camera can distorts the whole image performance even if the image has been clear so far. Below is an example of distortion happening during the scan due to dusts or particles, together with the effect of light.

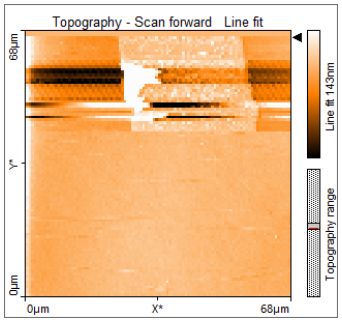

Fig. 9.1 Lab 2 scan 5 topography

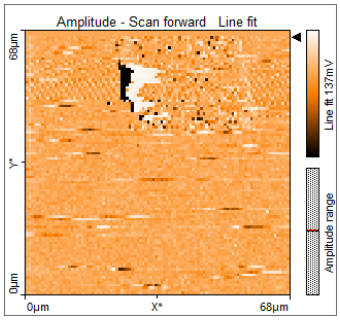

Fig. 9.2 Lab 2 scan 6 amplitude

THIRD LAB SESSION: SILVER NANO CUBES

Overall

From the conventional optical microscope top view, we are already able to see many white dots which could be polymer (fig. 10). The presence of poly (PVP) contributes to the stability of silver nano cubes, although it only acts as residuals (2). There are also some clusters with large nano cubes. These large-sized cubes can also be easily seen within ~60µm size image. Zooming down to ~1µm, we can observe the much smaller silver nano cubes (~40nm to ~100nm diameter) (3).

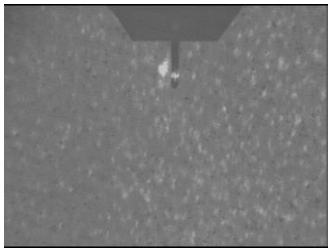
Fig. 10 Lab 3 overall sample

In this lab, the 'Parameter' and 'Z-Controller' data remain the same as the second lab session.

Details

Starting with the ~50µm scan size as usual (fig. 11.1 and 11.2), we observe many clusters and single particles in the topography, as expected from the first sight from the conventional microscope. The bigger white dots on the surface are likely to be poly particles.

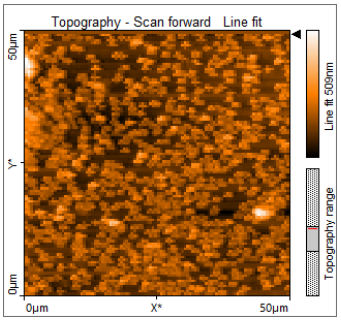

Fig. 11.1 Lab 3 scan 1 topography

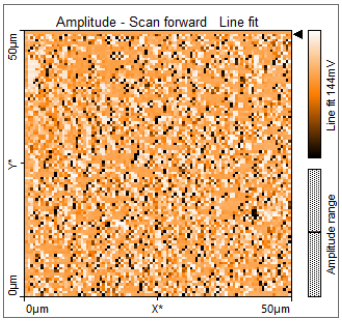

Fig. 11.2 Lab 2 scan 1 amplitude

At the top of the topography (fig. 11.1), there are some single particles. We might find it easier to spot the smaller cubes using zoom option in that area. Hence, a scan of ~10µm gives closer image of the large cubes or the clusters of cubes (fig. 11.3). They have pyramid shape might be because the tip was moving, and it can also move those particles.

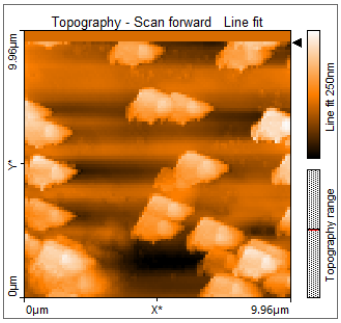

Fig. 11.3 Lab 3 scan 2 topography

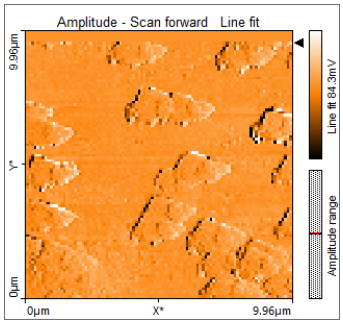

Fig. 11.4 Lab 3 scan 2 amplitude

Next, we zoom in to view a single large cube or a single cluster. The image size is down to ~2µm (fig. 11.5 and 11.6). At the first half, the topography was much clearer, however, it was then interrupted after passing the edge.

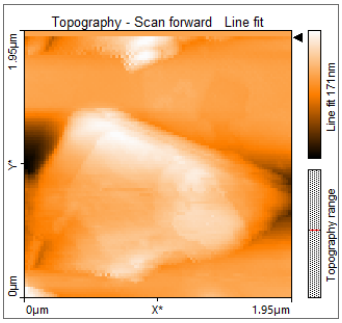

Fig. 11.5 Lab 3 scan 3 topography

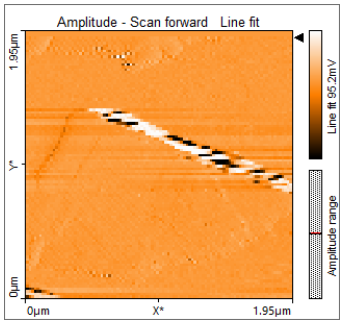

Fig. 11.6 Lab 3 scan 3 amplitude

Continue zooming in the top white particle. The size of the image is now ~600nm (red wavelength). The scan started with clear image, but then there was all orange (fig. 11.7 and 11.8). This might be caused by lose of contact between the tip and the sample surface.

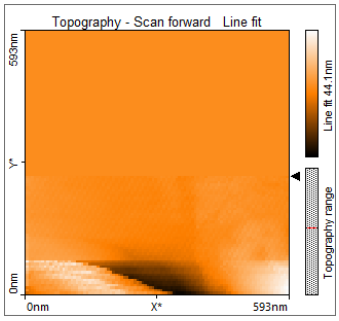

Fig. 11.7 Lab 3 scan 4 topography

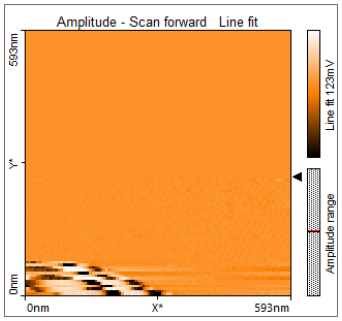

Fig. 11.8 Lab 3 scan 4 amplitude

We give another trial, it is certain that there is an image, but the feedback mechanism does not indicate so. In this trial, we gain better image yet still not qualified enough (fig. 11.9). However, the diameter of a particle at the top right corner of the topography is ~135nm which is approximately the diameter of a cube. Therefore, it is able to conclude that we have the image of a single cube and its diameter. We also check again by zooming only at the single cube, having an image size of ~185nm. With such a small area, there are a lot of noises, and the scan is unsuccessful.

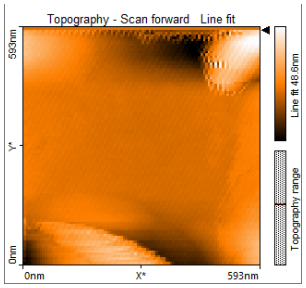

Fig. 11.9 Lab 3 single cube diameter

To address this issue, move to another area or adjust the gains data in 'Z-Controller'. Adjusting the gains can improve the image quality significantly, however, it is difficult to determine which gains are effective for each situation.

CONCLUSION

The three laboratories had successfully built us a foundation practical skills and knowledge in using the AFM and the scanning software. Moreover, we improved our understanding in reading and analyzing topography and amplitude images of different sizes and samples.

ACKNOWLEDGEMENTS

I acknowledge Professor Lukishova and my two lab partners from OPT 254 at the University of Rochester, Zihao Li and Yimin Xie, for the mentorship and support.

REFERENCES

1. Drachev et al., "Engineered nonlinear materials using gold nanoantenna array", Scientific Reports, para. 3 (2018)
2. Rycenga et al., "Controlling the Assembly of Silver Nanocubes through Selective Functionalization of Their Faces", Advanced Materials, p. 2417 (2008)
3. "nano Composix.", https://nanocomposix.com/pages/silver-nanocubes

OPT 254/ PHYS 371 (S21): Nanometrology Laboratory

Electron Microscopy Essay

Transmission and Scanning Electron Microscope

Linh Vu

Table of Contents

I. Introduction of Transmission and Scanning Electron Microscope

An electron microscope (EM) is a high-resolution device that uses accelerated electron beams as an illumination source[1]. There are different types of electron microscope: transmission electron microscope (TEM), scanning electron microscope (SEM), reflection electron microscope (REM), scanning transmission electron microscope (STEM), and scanning tunneling microscope (STM). This essay focuses on the transmission electron microscope (TEM) and the scanning electron microscope (SEM).

In general, the electron microscope has a better resolution than the optical microscope due to its working principle. As electron microscopes use electron beams, there is no diffraction as observed in a light microscope (optical microscope). Due to its high resolution, an electron microscope is used widely in various studies in microorganisms, bacteria, metals, crystals, …

The electron microscope has the highest resolution among all microscopes. To compare, normal human eyes can observe objects at size 0.1mm without support; a typical optical microscope has a limit of resolution of 0.2μm; and electron microscopes' resolution can range from 50pm to > 20nm. Specifically, the limit of resolution of the SEM is 1nm, and of the TEM is 0.2nm. This high resolution is achieved by using accelerated electron beams instead of visible light. Electrons have a wavelength of 1Å, about 4000 to 7000 times shorter than a visible photon's wavelength (400 – 700nm).

The optical microscope was invented during the 17th century, and its resolution improves gradually over four centuries, reaching a limit of ~ 0.2μm. Despite being invented recently, since 1931, the electron microscope's resolution has been improved rapidly in just almost a century. Molecules, atoms, bacteria, organs, … are seen clearly under electron microscopes with the current limit resolution of electron microscopes.

II. Transmission Electron Microscope (TEM)

1. Working Principle and Components in TEM

The transmission electron microscope (TEM) transmits a beam of electrons through an ultrathin specimen (less than 100nm thick) to form an image through the interaction between the electrons and the sample[2]. "The image is then magnified and focused onto an imaging device, such as a fluorescent screen"[2].

From the top to the bottom, a TEM consists of an illuminating source (electron gun), a condenser lens, a specimen port, an objective lens, an objective aperture, an intermediate lens, an intermediate aperture, projector lenses, and a fluorescent screen with an image recording system below. In other words, a TEM has multiple subsystems from top to bottom: vacuum subsystem, electron gun subsystem, electron lens subsystem, sample stage, electron lenses, viewing screen, and camera chamber.

[1] "Electron microscopes", https://en.wikipedia.org/wiki/Electron_microscope
[2] "Transmission electron microscopy", https://en.wikipedia.org/wiki/Transmission_electron_microscopy

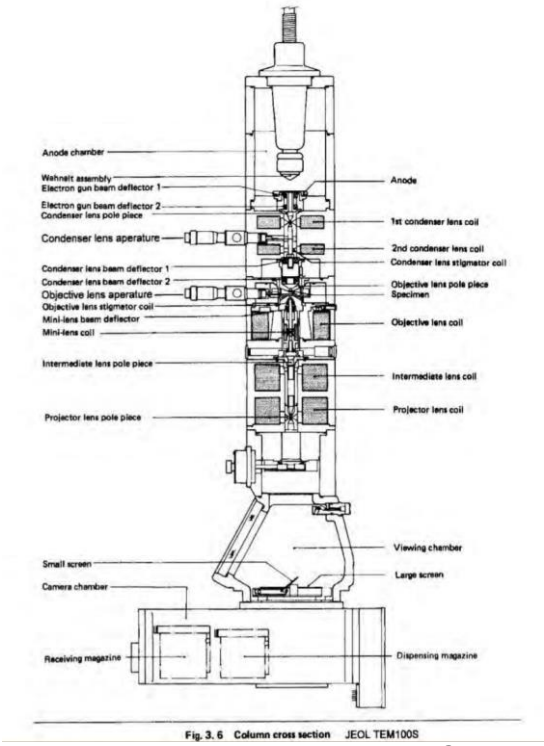

Fig 2.1.1 TEM components[3]

2. Electron Lens in TEM

TEM is an optical instrument that uses a lens system to form an image. There are two types of electron lenses: electrostatic and electromagnetic. An electrostatic lens assists the transport of charged particles by magnifying or converging the electron trajectories[4]. It also helps focus electron beams, using a gun cap (Wehnelt cylinder) as support. The electrostatic lens or lens system must be completely inside a vacuum environment. The most common lens type in electron microscopes is electromagnetic. The working principle is similar to the electrostatic lens, but here the electron beam is converged by a magnetic field. This magnetic field is generated by a solenoid magnet[5]. The electromagnetic lens does not need to be but is partially outside of the vacuum environment.

3. Imaging Modes in TEM

There are three imaging modes in a TEM: bright field mode, dark field mode, and diffraction mode. Areas that scatter or absorb electrons appear to be darker. Other areas transmit electrons and appear brighter. The most common imaging mode in TEM is the bright field mode. In this mode, the objective aperture is used to select the un-scattered electron beams[6]. Therefore, the scattered beams are excluded from the image. The beams used to form images are transmitted beam, 000 beam, and zero-order beam.

---

[3] OPT 254, EM Lecture 2, Slide 2
[4] "Electrostatic lens", https://bit.ly/2PQp4xu
[5] "Electromagnetic lens", https://bit.ly/3xNtiXS
[6] "Image types", https://bit.ly/2ShJ4tA

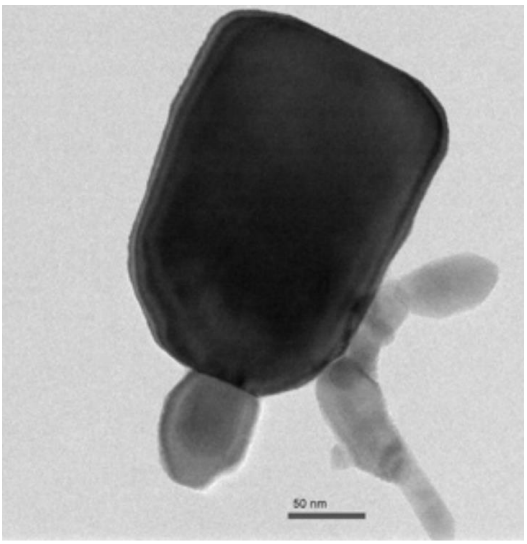

Fig 2.3.1 Zinc oxide crystals in bright field imaging mode[6]

On the other hand, dark field mode produces images using scattered electrons. Areas where the primary (un-scattered) electron beam passes straight through appear to be black. There are two methods of dark field imaging: off-axis imaging and tilted beam imaging.

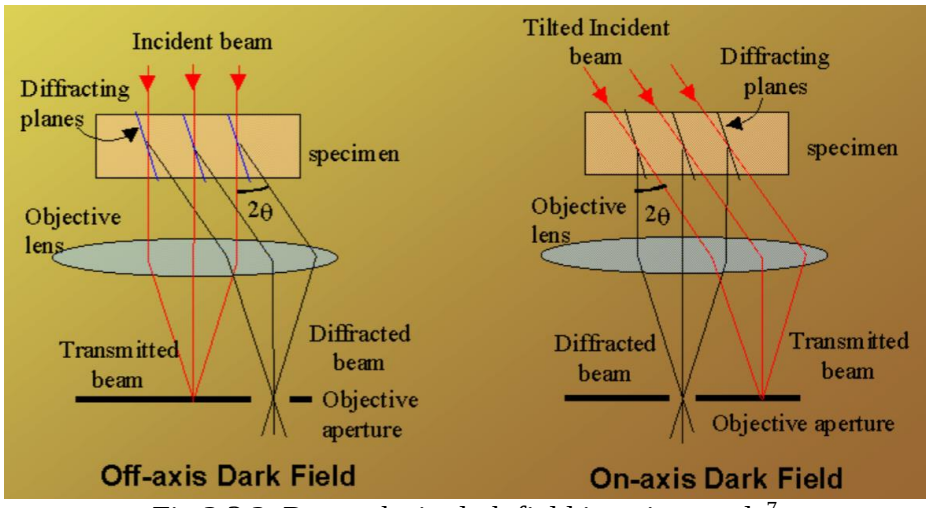

Fig 2.3.2. Ray paths in dark field imaging mode[7]

[7] OPT 254, EM Lecture 3, Slide 8

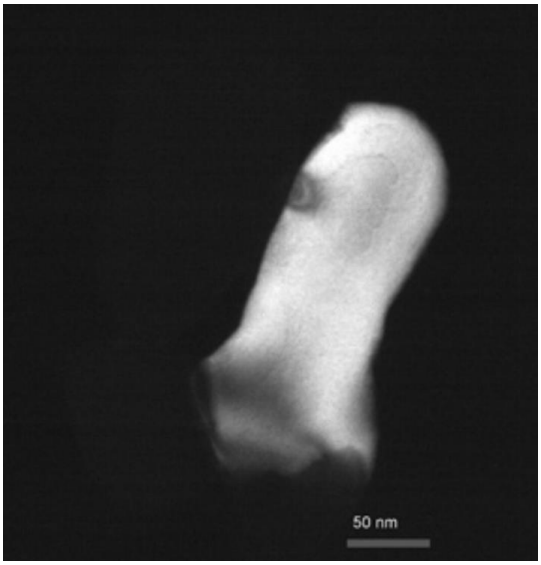

Fig 2.3.3 Zinc oxide crystals in dark field imaging mode[7]

Diffraction imaging mode applies when a beam passes through a crystallized sample, therefore the Bragg scattering effect results. The Bragg diffraction equation is $n\lambda = 2dsin\theta$ where n is the diffraction aperture. The image created below the sample (back focal plane) appears as an array of dots or a set of diffused rings. This gives information of the crystal structure of the sample. There are four conditions in the back focal plane of the objective lens. If there is no sample, there is no reflection. If the sample is amorphous, the image observed has random scattering; if polycrystal, rings; and if single crystal, then spots. The equation of $Rd = \lambda L$ calculated the relationship between the lattice spacing and the distance from the transmitted spot[8].

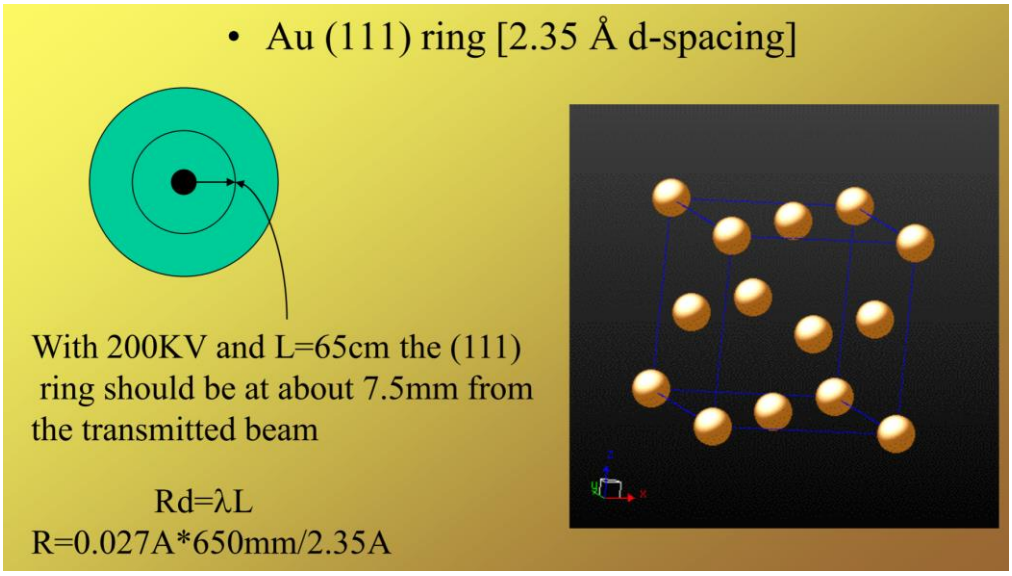

Fig 2.3.4 How the crystal structure of Au is imaged using the reflected ring patterns in the back focal plane[9]

[8] OPT 254, EM Lecture 3, Slide 12

III. Scanning Electron Microscope (SEM)

1. Working Principle and Components in SEM

A scanning electron microscope (SEM) scans a sample's surface with a focused beam of electrons to form images of the sample[9]. The interaction between the electrons and the sample's atoms generates various signals containing information about the sample's surface topography and composition. The signals generated include secondary electrons, back-scattered electrons, characteristic X-rays and light, absorbed current, and transmitted electrons.

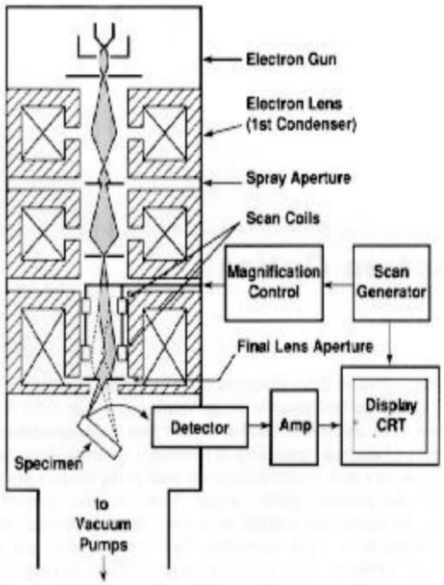

Fig 3.1.1 SEM components[10]

The SEM's system consists of a vacuum subsystem, an electron gun subsystem, an electron lens subsystem, a scan generator subsystem, scattered signal detectors, an observation CRT display, and a camera CRT or digital image store. The scan generation system is the core component of an SEM.

a. SEM Scan Generation System

The main purposes of the scanning system are to "set up beam sweep voltage ramp in x and y directions" and to synchronize the beam on the sample and the beam on CRT display.

[9] "Scanning electron microscope", https://en.wikipedia.org/wiki/Scanning_electron_microscope
[10] OPT 254, EM Lecture 2, Slide 3

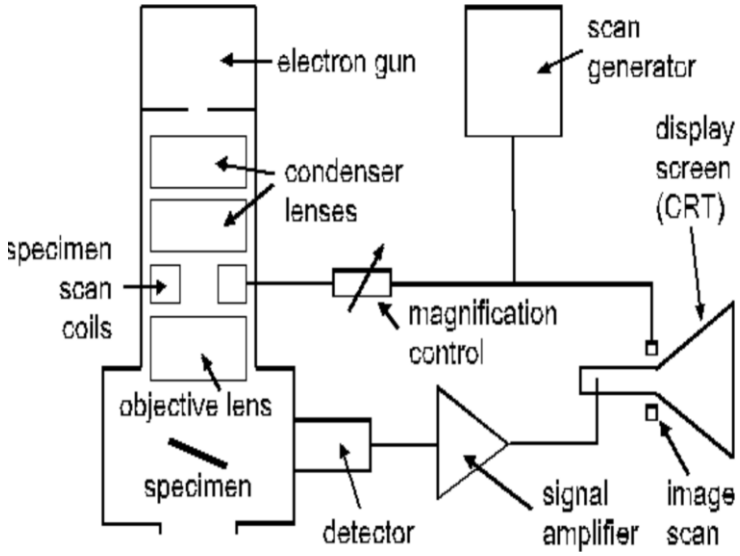

Fig 3.1.2 Scan Generator interface[11]

b. Magnification Control in SEM

The magnification in SEM can be controlled in a range of 6 orders of magnitude, from 10 to 3,000,000 times[12]. Even though an SEM has condenser and objective lenses as in TEM or OM, the principle is not to image the whole specimen, but to focus the electron beam to a spot. Therefore, an SEM can function without the condenser and objective lens subsystem. The magnification result is the ratio of the CRT size and the rater size.

c. Depth of Field in SEM

As the SEM focuses an electron beam to a spot, the beam is very narrow, therefore the SEM has a large depth of field (DOF). To adjust the depth of field, the aperture diameter, the lens subsystem, and the distance between the sample and the imaging tool are the crucial factors[13].

The DOF is inversely proportional to the aperture angle. The smaller the semi-aperture angle, the larger the DOF.

Moving the sample (focal plane) far away from the imaging tool does not necessarily decrease the quality of the scan. A longer distance gives a smaller aperture angle, therefore increases the DOF. As the aperture angle decreases, the beam diameter also decreases providing better resolution.

d. Spot Size Control in SEM

The final spot size of an electron beam limits the resolution of an SEM. Focal lengths of the lenses and the working distance also affect the resolution. Specifically, shorter focal lengths and shorter working distance produce smaller spot size, and hence, better resolution.

---

[11] OPT 254, EM Lecture 2, Slide 5

[12] "Scanning Electron Microscope", https://en.wikipedia.org/wiki/Scanning_electron_microscope#Magnification

[13] "Optimizing the Depth of Field in a SEM", https://bit.ly/3xLPx0g

e. Signal Detectors for SEM

When the electron beam hits the surface of the sample, it penetrates the sample to a depth of a few microns[14]. This depth is based on the accelerating voltage and the density of the sample. This interaction produces different types of signals, which are further collected by detectors to form images. The interaction volume can also be estimated using the Monte Carlo simulations. This method depends on the energy of the beam electron, the likelihood of interaction, the change in direction and energy of the electrons, the mean free path of the electrons, and a random factor for every interaction[15].

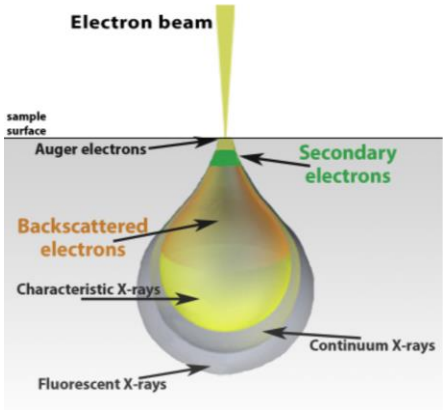

Fig 3.1.3 Schematic of electron beam interaction with sample surface[14]

2. Electron Optics in SEM

The SEM is not an optical device: it does not use but uses electron optics to form an image. In other words, the SEM is a probe-forming, signal-detecting device[16]. Electron optics is the calculation of electron trajectories along electromagnetic fields[17]. When an illuminating beam enters a medium of a different environment (different refractive index), refraction or bending occurs. In SEM, the two environment changes are from vacuum condition into glass and in return. The refractive index of the vacuum environment is 1.0 while glass has a much higher value. The refractive index changes gradually between surfaces, therefore beams are curved continuously rather than being bent abruptly as in classical optics. Refraction of electrons only occurs around charged electrodes or solenoids.

Theoretically, a beam can not enter a conventional lens with a different refractive index[18]. Therefore, to bend the beam, a force must be applied. In SEM, 'force' lenses are used, which are electrostatic and electromagnetic lenses. An electrostatic lens requires a very clean and high vacuum environment to avoid arcing cross plates. An electromagnetic lens will pass a current through a solenoid such that it produces a strong magnetic field within the coil's center.

---

[14] "Scanning Electron Microscopy", https://bit.ly/3nPR8h8
[15] OPT 254, EM Lecture 2, Slide 21
[16] OPT 254, EM Lecture 1, Slide 43
[17] "Electron optics", https://en.wikipedia.org/wiki/Electron_optics
[18] OPT 254, EM Lecture 1, Slide 46

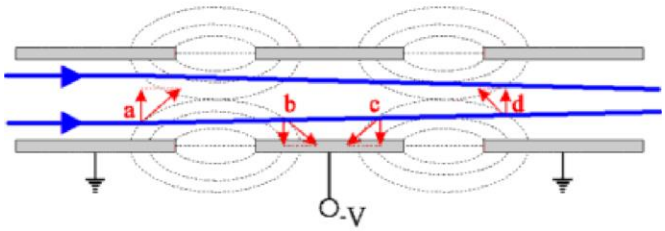

Fig 3.2.1 Electrostatic lens[19]

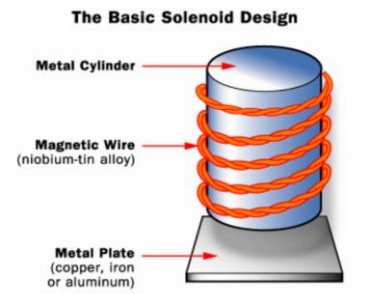

Fig 3.2.2 Electromagnetic lens[20]

When a current is run through the coil, there are two force vectors: one follows the direction of the electron trajectory, one is perpendicular to it. These two force vectors lead the electrons to move helically through the magnetic field within the coil.

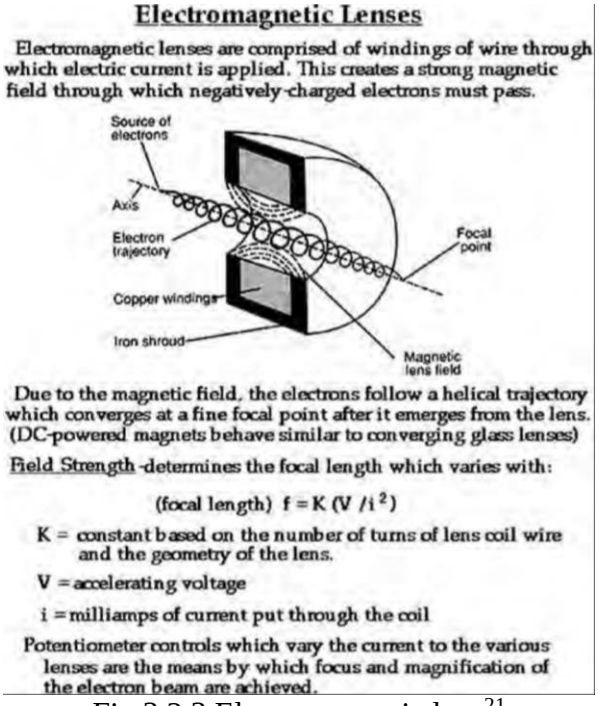

Fig 3.2.3 Electromagnetic lens[21]

[19] OPT 254, EM Lecture 1, Slide 50
[20] OPT 254, EM Lecture 1, Slide 51
[21] OPT 254, EM Lecture 1, Slide 57

3. Special Imaging Mode in SEM

There are two imaging modes in SEM: backscattered-electron (BSE) imaging and secondary electrons (SE). The BSE imaging mode applies the elastic collisions between electrons and the sample's atoms[22]. Larger atoms create higher signals. The number of backscattered electrons that reaches the detector is proportional to its Z number. Therefore, this dependence on atomic numbers helps distinguish different samples. The BSE mode is helpful in studying about the sample's topography, crystallography, and magnetic field.

On the other hand, secondary electrons originate from the sample's surface or near-surface regions. The SE imaging mode applies the inelastic interactions between scattered electron beam and the sample, therefore contains less energy than the BSE one. The SE imaging mode provides information about the sample surface's topography.

IV. Common concepts and components in both TEM and SEM

1. Electron Gun

The electron gun is connected to a high voltage of 100 – 300kV to emit electrons. There are two types of electron sources: thermionic emission sources and field emission forces. The thermionic emission sources are commonly made of a tungsten filament, a LaB_6, or CeB_6 filament. The filament is heated by an electrical current so the material's "work function" exceeds. Therefore, the electrons are able to leave their outermost orbital. This can generate an electron cloud.

The tungsten hairpin filament is the most common type of filaments in electron gun because of its low cost (~ $20) and its multiple-beam current. However, it is not a very intense illuminator, its work function is high (4.5eV), and its emission temperature is also relatively high (~ 2700K). Due to high emitted temperature, a tungsten filament can only last about 100 hours.

The LaB_6 and CeB_6 filament, on the other hand, has a higher cost (~ $700). But this filament has a lower work function of 2.4eV, emits a lot brighter light source than the tungsten one; the emission temperature is much lower (~ 1700K), and it can last for hundreds of hours. Therefore, the LaB_6 and CeB_6 filament can directly replace the tungsten type. However, there are some concerning disadvantages regarding the LaB_6 emitter. A LaB_6 filament needs a higher vacuum condition to reduce reactivity, and it is more complicated to assemble. Additionally, since it is a brittle material, the heating and cooling process must occur slowly (the heating process must be indirect through a graphite well).

In conclusion, to optimize a thermionic emitter lifetime, the vacuum system needs to function well, the gun area is clean, the filament is not oversaturated, and the number of heating/cooling cycles should be minimized.

[22] "Different Types of SEM Imaging", https://www.azom.com/article.aspx?ArticleID=14309

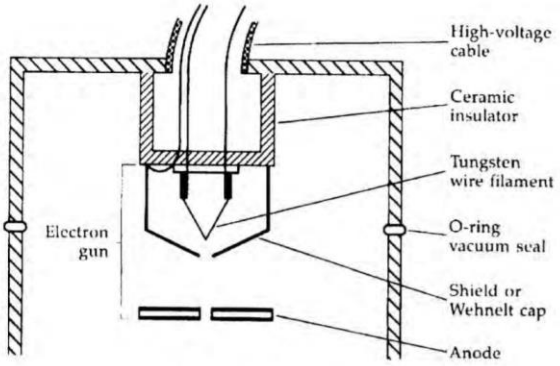

Fig 4.1.1 Thermionic Gun Layout[23]

The second type of electron source is the field emission source, which includes the cold and Schottky field emitters. Overall, the field emission sources are more ideal than the thermionic ones. These sources are usually made of a single crystal, sharpened and shaped tungsten wire with a tip radius of < 1.0um. If there is any heating, a ZrO2 component assists the emission. Since the emission is supported, this source can be 10,000 times brighter than the tungsten thermionic filament. The illuminating source has a small apparent (tip), therefore it "helps obtain small probes with high temporal coherence". This will also decrease the energy spread in the beam. One of the most impressive advantages is that this field emission electron source can last many thousands of hours (~ 20,000 hours).

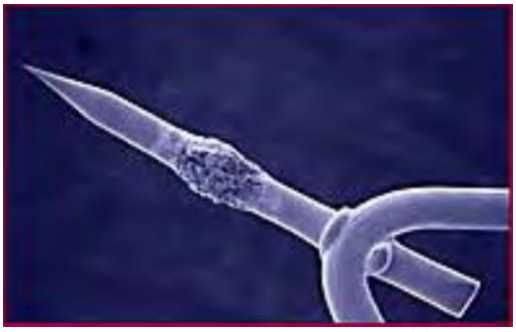

Fig 4.1.2 Tip in FESEMs[24]

The first type of field emitter is the cold field emitter. This is the most intense electron source therefore it requires a very high electric field intensity and an ultrahigh vacuum condition in the gun. However, the tip can be contaminated so it needs to be cleaned with the "flashing" technique frequently. The other type of field emitter: Schottky field emitter. This one is more stable than the cold field emitter. As ions impact the tip, it can be self-annealed. Schottky field emitter does not require a very high vacuum environment as in the cold field one, but it has a lower work function and a lower extraction field intensity. Both types of field emitters are extremely expensive (~ $4000).

[23] OPT 254, EM Lecture 1, Slide 31
[24] OPT 254, EM Lecture 1, Slide 40

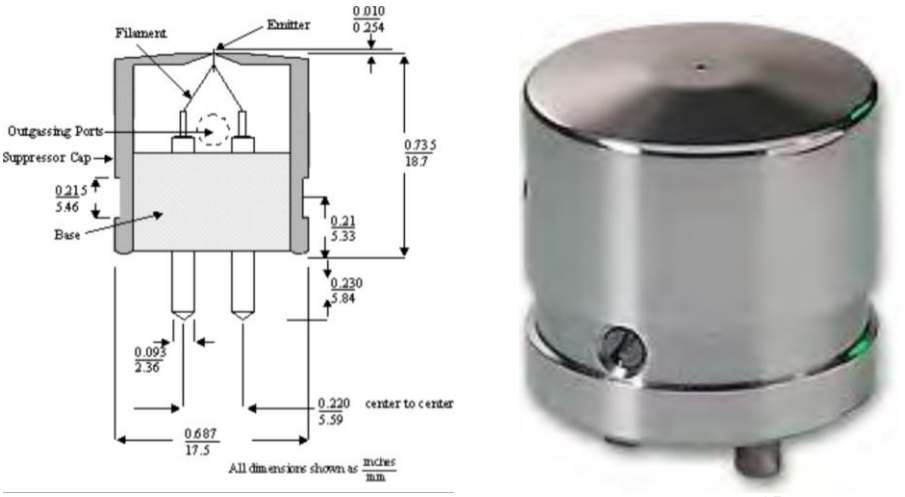

Fig 4.1.3 Typical Schottky Field Emission Source[25]

The suppressor cap in Schottky field emitters is helpful in limiting the electron emission to the desired area of the tip. Therefore, it can block the electrons from the heater and the shaft. When the tip emits electrons, a voltage of 1.8 – 7 keV is applied from an extractor anode (outside of the Schottky emitter) to extract electrons.

In conclusion, to optimize a field emission emitter's lifetime, similar to the thermionic one, the vacuum subsystem needs to be in good working order; the emitter must be heated, and no over-extraction or overheat should occur to avoid the tip getting melted.

2. Sample Preparation

Only certain types of samples can be imaged or scanned with electron microscope: electrically conductive samples, electrically insulating samples, biological samples, and "odd" samples. The samples need to be prepared because the EM only works in vacuum and charged particle environment. Additionally, the samples must be within size limit, and they can not move in response to the beam. Generally, there are two sample types: bulk samples (for SEM only) and thin samples (for both SEM and TEM). For TEM, the samples must be cut very thinly to allow electrons to pass right through the sample. For SEM, because it visualizes the surface of 3D objects, therefore there is no need to cut the sample into thin specimen. However, samples need to be coated in metal (commonly gold or gold-palladium) so they can be conductive.

V. Conclusion

The invention of electron microscopes in 1931 overcame the resolution limit of visible lights. Additionally, the magnification of electron microscopes can reach 10,000,000 times, the highest magnification among all microscopes. The depth of field in electron microscope, especially in the scanning electron microscope, is also improved significantly.

Therefore, the cost of electron microscopes is extremely high, ranging from $50,000 to $27 million. Following is the complicated maintenance and repair process. Additionally, the samples must satisfy certain requirements such as conductive.

[25] OPT 254, EM Lecture 1, Slide 36

Nanometrology Laboratory, University of Rochester

Spring 2021

OPT 254/PHYS 371 – Optical Microscope Laboratory Report

Linh Vu

ABSTRACT

In four labs, nanoantenna arrays and silver nanocubes were scanned with the Olympus BX51 conventional (wide-field) optical microscope and the VIVASCOPE 3000 confocal optical microscope. Additionally, the silver nanocubes' spectrum was collected using a spectrometer and its special CCD camera. Through multiple image scans, intensity scans, and photos of the spectrum, we successfully practiced using two types of optical microscopes, many corresponding scanning softwares, and understood the structures of the samples as well as the photon bunching effect in silver nanocubes.

INTRODUCTION AND OBJECTIVES

The primary goal of this lab is to observe the structure surface of the nanoantenna arrays and silver nanocubes, to measure the spectrum of the silver nanocubes, and to observe the silver nanocubes' photon bunching effect. Optical microscope is a scanning device using visible light and objective lenses to observe magnified images of samples. Optical microscope is one of the very first microscope systems, therefore its resolution ranges from a simple (200nm) to a very high-resolution scanning system (up to 2.4nm). The optical microscope models used in the lab are Olympus BX51 (conventional optical microscope) and VIVASCOPE 3000 (confocal optical microscope). The main components of the confocal microscope are laser, objective, sample table, scanning system, pinhole, and detector. Additionally, the dichroic mirror and the interference filters are also important.

In the first lab, nanoantenna array and silver nanocubes were observed through magnified objective lenses of the Olympus BX51 wide-field optical microscope. The magnification of 5x, 10x, 20x, 50x, and 100x were operated respectively with both samples. The most challenging procedure was to find the correct scanning area on the whole sample. This process can take a lot of time.

During the last three labs, silver nanocubes was scanned under the VIVASCOPE 3000 confocal microscope. Two detectors were used to record the intensity spikes based on time throughout the sample's surface. Additionally, the photon bunching effect of silver nanocubes was observed using the spectrometer and its special CCD camera.

To obtain the best images, a careful procedure of setting up the microscope, as well as the spectrometer, was followed strictly. Additionally, adjusting the setting of the CCD camera modes and parameters can improve the image quality in different ways.

PROCEDURE

Different microscopes require different procedures. In the first lab, the Olympus BX51 conventional (wide-field) optical microscope has a simple procedure. First, turn on the electric controller, check the light signal to make sure it is turned on. There is no scanning table for this conventional microscope, so the process of finding the desired scanning area takes time. To have a better sense, start focusing on the edges. Use hands to move the sample piece slowly until the area shows up on the computer screen. The magnifications of 5x, 10x, 20x, 50x, and 100x were operated with both nanoantenna array and silver nanocubes. The higher the magnification is, the harder it is to find the sample area. If there are a lot of difficulties in using hands to find the sample area, when it is likely that the edge of the sample area is found, use the screw to move for a much smaller distance. In addition, adjust the brightness in the computer software for better contrast. To check whether the magnification makes sense or not, measure the edge of the sample or the ruler marked around the sample.

In the second lab, the VIVASCOPE 3000 confocal microscope was used. This device can be used both as a conventional and confocal microscope. First, make sure that the electric controller is turned on. Choose a port mode from 1 to 5; switching between these modes allows us to switch between the eye slit view, conventional view, confocal view, and so on. Here, first we chose port number 2. Take a direct look at the sample to check if there is a laser point on its surface. Then, plug in two detectors. These detectors are very sensitive with light, so make sure to turn off the lights before proceeding any scan.

For this lab, the scanning software SCOM_v8_luke was used. To change the scanning area, simply change the value of x and y in the parameters. Place the green dot on the detectors' scanning image and click "Goto xy" to specifically record the intensity change in time where the green dot is placed.

In lab three, the same procedure was repeated, but we additionally learnt how to use the spectrometer to capture the spectrum of silver nanocubes. This procedure requires three different softwares: Laser Quantum, Monochromator Control, and Solis Acquisition and Analysis. In the Laser Quantum software, make sure the power mode is showing the appropriate value of 2.8mV. Change the wavelength between two values of 0nm and 532nm in Monochromator Control. Adjust settings in Solis Acquisition and Analysis until the spectrum is clearly seen on the camera view. The settings include exposure time, shift speed (3.3), electron multiplier gain, shutter control, contrast, temperature (-65C), fan control (on), …

If there is nothing shows up in the camera view, first use the room light to prove that the spectrometer and the system are working properly. Turn on the light, start the process, the camera should show multiple lines since the natural light consists of multiple wavelengths. Although it is proved that the spectrometer works precisely, the spectrum of the sample might not show up in the camera view. Adjust the settings or restart the programs are good ways to resolve this issue. Errors might also occur because the softwares and the computer cards are not compatible. Both the camera view and the graph of counts versus wavelengths are used to discuss about the silver nanocubes' spectrum. During the whole process of taking the spectrum of any sample, except for the room light, lights must be turned off.

To turn off the system, make sure to follow these steps: disable the electron multiplier in Solis Acquisition, choose "Permanently Closed" in the shutter control, then turn the lights on. The temperate control is also needed to be turned off. Now, it is good to exit all the softwares and turn off the power supply.

The last lab was to study about the photon bunching effect of silver nanocubes. To achieve the result, the computer cards need to be calibrated. Here, the TimeHarp software is used to create the graph of counts versus time. To calibrate the computer cards, we changed the detectors' orders. Normally, the two detectors are connected in series. Now, we connect the two detectors in the 3-cable system, so they are parallel to each other. The voltage is divided into half for each detector, one detector is connected to the start, the other one is connected to the end.

Next, to observe the photon bunching effect, connect the oscilloscope with the control box. Make sure to turn the lights and the laser off. The graph will show up directly on the oscilloscope (o-scope) screen. After the observation, unplug the o-scope.

DISCUSSION

FIRST LAB SESSION: Conventional (wide-field) optical microscope with nanoantenna array and silver nanocubes

The first lab is an overall introduction of using the conventional (wide-field) optical microscope. The conventional microscope allows users to observe a wide range of areas, from overall to molecule level. It also has a very simple procedure. On the other hand, finding the sample area with the conventional microscope takes a lot of time. Sometimes this can not be achieved.

To give the most basic examples, we use nanoantenna array and silver nanocubes samples, which their patterns are already known. With the magnification of 5x, 10x, 20x, 50x, and 100x, we were able to measure the overall size of the sample area and the single particles of both samples.

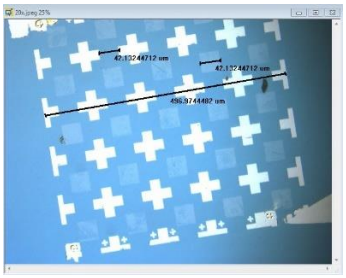

Fig. 1.1 20x Nanoantenna array

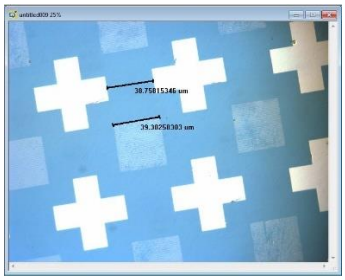

Fig. 1.2 50x Nanoantenna array

With different magnifications (figure 1.1 & 1.2), the distance between the crosses was found to be consistent (~40μm). This verifies that we used the conventional microscope's objectives properly.

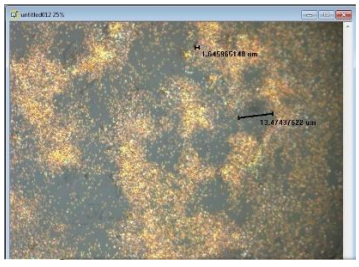

Fig. 2.1 100x Silver nanocubes

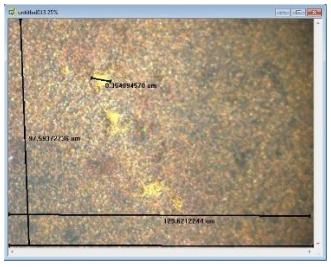

Fig. 2.2 100x Silver nanocubes

It is very clear to observe silver particles with a magnification of 100x. The diameter of a silver particle is roughly 8μm (figure 2.2).

SECOND LAB SESSION: Confocal optical microscope with silver nanocubes

Compared to the conventional microscope, the confocal microscope can have a better resolution due to the confocal (focus laser points) mechanism. The pinhole helps focus the beam into the detector, therefore the confocal microscope gives better images. Comparing the images from the conventional and the confocal microscope, it is obvious that the images from the confocal have better contrast. This also gives the advantage of creating 3D images in confocal microscope.

The microscope is connected to Hanbury Brown and Twiss interferometer. This device measures the intensity fluctuation between two light detectors by measuring photon counts. The detector has a dead time, therefore to record all the voltage changes, another detector is needed to cover that dead time interval.

To measure the intensity through time at a specific point, move the green dot to the desired location. Changing the glass filter does create changes in the intensity at a specific location. Below are the differences in the intensity between different orders of glass filters. All three cases had the same location, same setup of 2.7mV laser output.

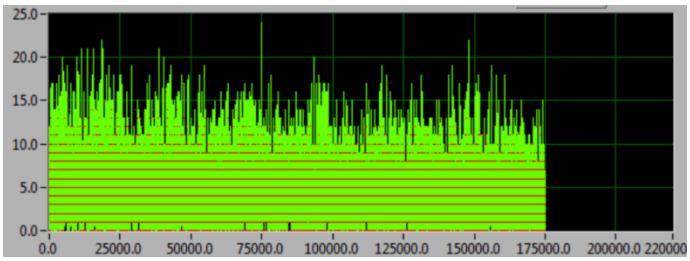

Fig. 3.1 Intensity with 2 orders of magnitude glass filters

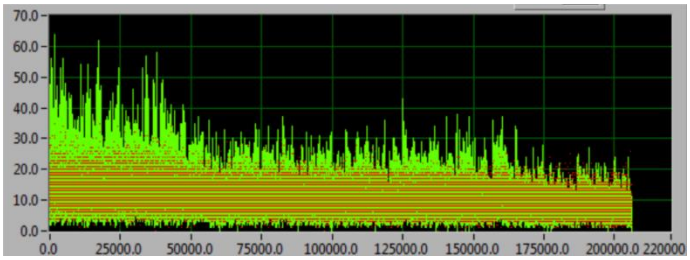

Fig. 3.2 Intensity with 1 order of magnitude glass filter

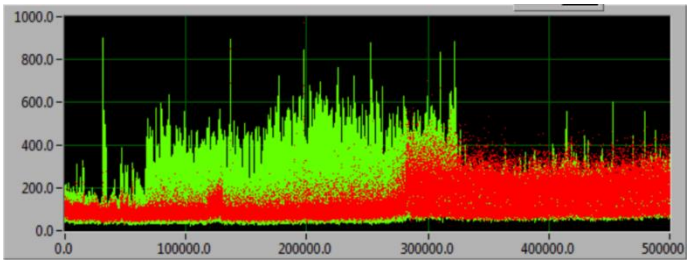

Fig. 3.3 Intensity with 0 order of magnitude glass filter

THIRD LAB SESSION: Spectrometer with silver nanocubes

A spectrometer is a device to measure the wavelength of a light source. Light beams go through a very narrow slit, into a mirror, then reflected into a diffraction grating. The light beams go through another mirror before exit to the camera. This special camera is made to capture the unique spectrum of each light source. It goes with a cooling and noise cancellation system.

During the first half of the lab, we observed very interesting intensity spikes through the detectors. These unusual fluorescence intensity spikes could be caused by the silver nanocubes or the polymer particles.

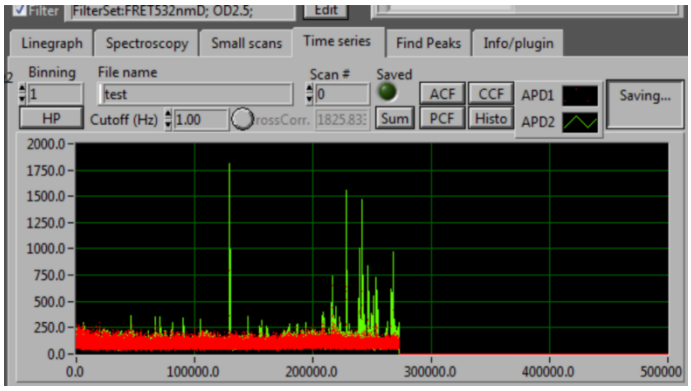

Fig. 4.1 Intensity spikes

In the second half of the lab session, we successfully measured the spectrum of silver nanocubes. Using a combination of three softwares (Laser Quantum, Monochromatic Control, and Solis Acquisition), the spectrum recorded was around 532nm. It was not possible to get a clear image of the spectrum at the first try. A lot of adjustments needed to be done to capture the clearest picture.

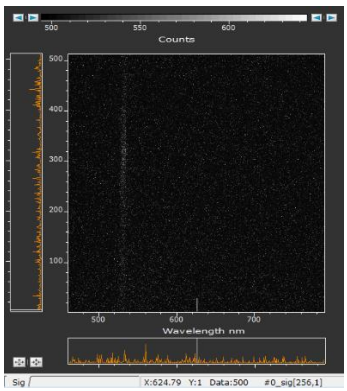

Fig. 4.2 Zero order

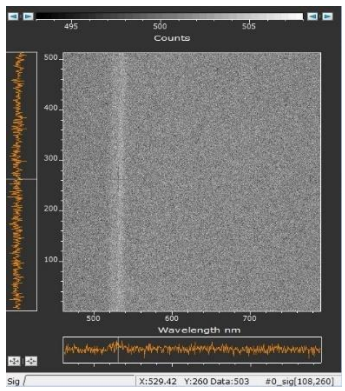

Fig. 4.3 No electron multiplier

Figure 4.2 shows the spectrum image when the wavelength in Monochromator Control is 0nm. Figure 4.3 is the image of the spectrum when the electron multiplier (EM) is disabled. Therefore, to get the best image, we set

the wavelength to 532nm, enable the EM, and other default settings (temperate on, shift speed 3.3, exposure time, …).

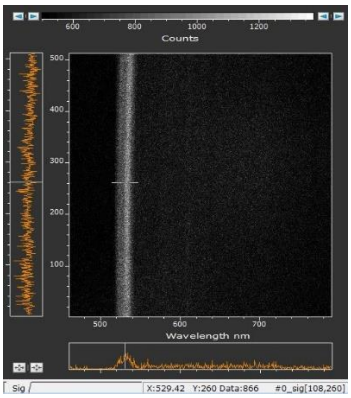

Fig. 4.4 Spectrum image

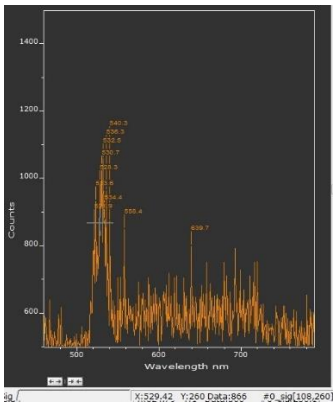

Fig. 4.5 Wavelength graph

Based on figure 4.5, the wavelength of silver nanocubes is ~532nm.

FOURTH LAB SESSION: Photon Antibunching, Calibrating computer card with silver nanocubes

For this lab, to study about the photon bunching effect of silver nanocubes, we use a software called TimeHarp to graph the photon counts versus time. The graph made by this software is different from the graph made by Solis Acquisition in the previous lab because TimeHarp graphs single photon counts over time.

To calibrate the computer cards, we change the connection between two detectors into a parallel circuit. Details have been discussed in the procedure.

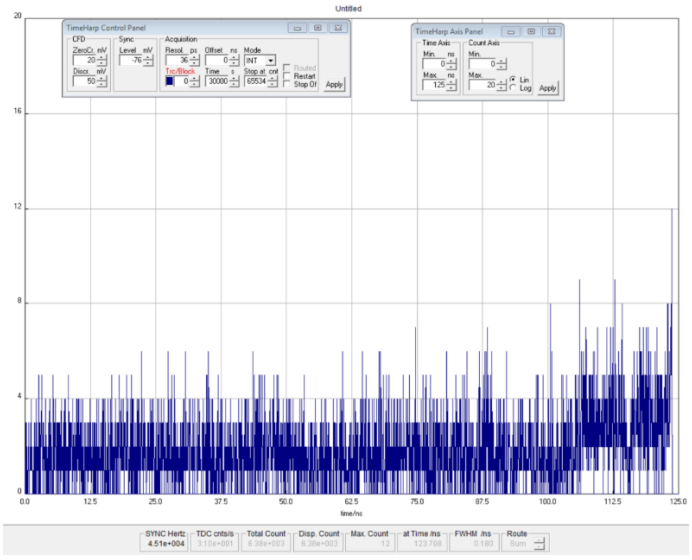

Fig. 5.1 TimeHarp measurement before calibration

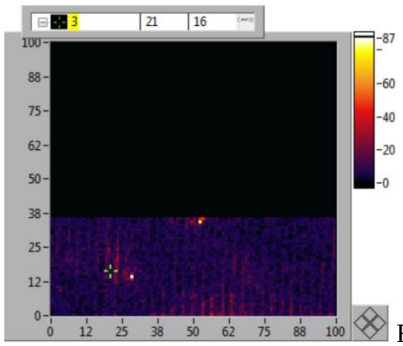
Fig. 5.2 Location of interest

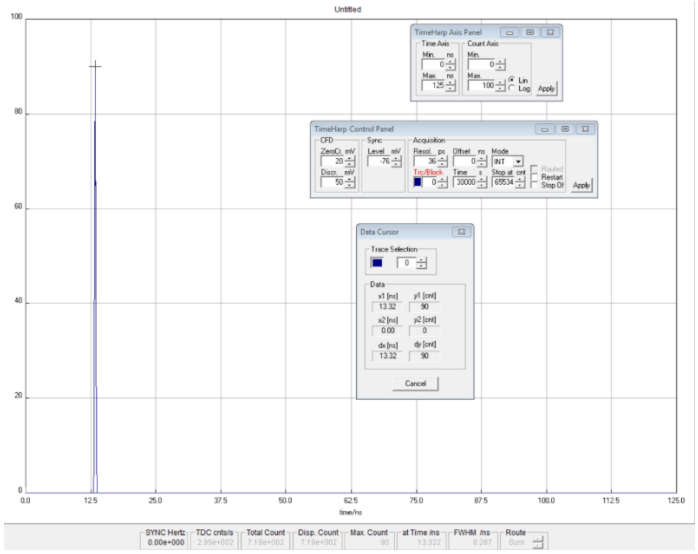

Fig. 5.3 TimeHarp measurement after calibration

We only observed one single spike because the data had been calibrated (figure 5.3). The time interval is now 0 within a spike.

Photon antibunching is a light field with more equally spaced photons than the random state[1]. To observe photon antibunching, a laser beam is focused into a small area that contains "a very low concentration of emitters". From there, only one photon is emitted at once. In contrast, photon bunching occurs when the emitted photons field is contaminated with single photons, pairs, and triplet of photons. Looking at the graph of the second order correlation versus time (figure 5.4) and compare with figure 5.3, we concluded that silver nanocubes have the photon bunching effect.

[1] Photo Antibunching, https://en.wikipedia.org/wiki/Photon_antibunching

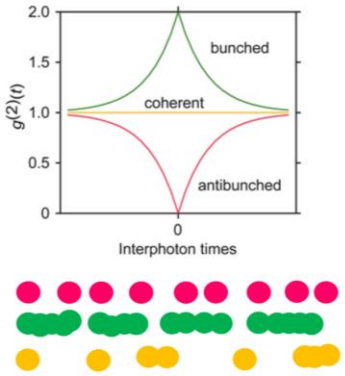

Fig. 5.4 2nd correlation order

Additionally, we used the oscilloscope to observe the calibrated voltage signals over time. These voltages signal will be converted into photon counts, and the shape of this wave gave us an idea of how the graph of photon counts versus time looks like. From figure 5.5, we confirm that silver nanocubes have the photon bunching effect.

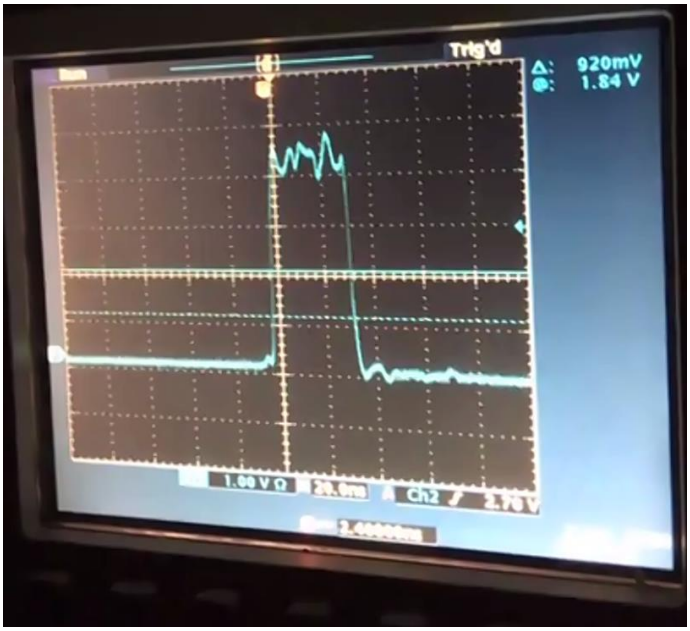

Fig. 5.5 Oscilloscope calibrated voltage signals

CONCLUSION

The four laboratories had successfully built us a foundation of practical skills and knowledge in using the conventional and confocal optical microscope, the spectrometer, and the corresponding softwares. Moreover, we learnt about the definition of the photon bunching and antibunching effect and were able to analyze that effect in silver nanocubes.

ACKNOWLEDGEMENTS

I acknowledge Professor Lukishova and my two lab partners from OPT 254 at the University of Rochester, Zihao Li and Yimin Xie, for the mentorship and support.

REFERENCES

1. OPT 254/PHYS 371, Lecture and Laboratory Notes

2. Photo Antibunching, https://en.wikipedia.org/wiki/Photon_antibunching