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1. Introduction to the Technique
Magnification and high resolution imaging are very important in materials science, especially at present when nanotechnology materials are being created. Magnification and high resolution imaging can help in the characterization, identification, and confirmation of the existence of the desired material. Magnification and high resolution imaging become problems when the size of the assessed material becomes too small; such as, they are beyond the micrometer scale. Ordinary techniques in microscopy such as light microscopy where in a traditional light microscope is used could not suffice. Fortunately, in 1931, two scientists named Ernst Ruska and Mx Knoll invented a technology that sets the limit for magnification even farther than what light microscopes are capable of – enter the transmission electron microscope or the technique transmission electron microscopy (TEM). The first transmission electron microscope allowed the used of the technique to magnify material specimen seventeen times light microscopes can do. In 1933 the invention was improved to provide magnifications more than a hundred times light microscopes can do. Since that time onwards several improvements have been made and magnifications up two thousands of times better than light microscopes have been achieved (Mogilatenko and Kirmse 21 – 22).
2. Theoretical Consideration
In order to understand how Transmission Electron Microscopy works, it is first necessary to understand the factors that limit magnification. In understanding magnification the concept of resolution must be considered. Accordingly, resolution is defined as the shortest distance between two unique points in the specimen that allows us to distinguish them as two separate elements. In other words resolution pertains to the capacity of a magnification technique to see the distance between two objects. Resolution is indirectly proportional to the capacity of the technique to magnify; that is, high resolution techniques (low resolution value) would allow higher degree of magnification. In optical microscopy – microscopy that makes use of light – resolution is defined by the equation called the Bragg’s Law:
d = λ / 2n sin α
where d is the resolution, λ is the wavelength of the incident light to the specimen being magnified, n is the medium’s refractive index between specimen and the objective lens, and α is the angle of the light rays which are emitted by the specimen and are collected in the objective lens. Since the resolution value must be small to achieve efficient magnification then the variable λ must assume a small value as well. In other words, the wavelength of the incident light must be small so as to achieve maximum magnification. Unfortunately, visible light, which is the one used in optical microscopes has a range of λ of 400 to 700 nm. This wavelength range has limited resolution values; hence, limited magnification capacities ((Mogilatenko and Kirmse 25)
In 1923 De Broglie proved that even particles have wavelengths associated with them. The relationship between the particle’s momentum and wavelength is described by the De Broglie equation:
λ = h / mv
where h is a constant known as the Plank’s Constant, m is the relativistic mass of the particle at velocity v. From this equation it can be seen that the wavelength of the particle could be manipulated by changing its velocity, or that wavelength is dependent in mass and velocity.
Four years after De Broglie described the relationship between wavelength and momentum, another scientist have proven another fact that contributed significantly to the invention of the TEM. Accordingly, in 1927 a scientist named Hans Bush showed that it is possible to focus a beam of electrons unto a point in the same manner as light could be focused by lenses. The De Broglie equation and Bush’s method for focusing electrons paved way for the making of first transmission electron microscope in 1931 and the first image was produced in 1932 (Mogilatenko and Kirmse 31).
Figure 1 illustrates how optical microscopy and electron microscopy works. Accordingly, both methods make use of lenses (three lenses) to focus the incident rays unto the specimen. The different between the two is that in TEM the electron beam is focused using magnets. When charged particles, such as electrons move, they generate a magnetic field of various magnitudes. This magnetic field interacts with the magnets (part of the magnetic lens) of the transmission electron microscope allowing the beam of electrons to be focused at a point desired. As aforementioned, the electrons need to be in high speed in order for them to produce the desired wavelength. De Broglie also explained that electrons could be accelerated (change in velocity) by manipulating the imposed voltage in them. This voltage is called the acceleration voltage is more visible in the expanded version of the De Broglie equation:
where V is the acceleration voltage, and c is a constant called the velocity of light.
Figure 1: Comparison of the magnification process between optical or light microscopy and transmission electron microscopy
Another important theory or principle employed in TEM is that electrons tend to loose energy as they travel through different media. This means that electrons could energy from the moment that they are fired by the electron gun at the top most part of the electron microscope as shown in figure 1 up to the point where they are intercepted by spectrophotometer as shown in figure 2. The spectroscopy method for TEM takes advantage in the electron energy loss to create an image of the specimen under investigation. Electrons accelerated at a certain voltage would contain enough energy to pass through the sample. These transmitted electrons loose energy as they pass through the thickness of the sample. When the electron beam is passed upon the surface of the specimen, come of the electrons pass through with distinct energy loss in making their passage. This energy loss could be mapped by a computer program. This means that if a standard for energy loss for each measure of thickness then the specimen’s thickness in each area could also be measured. The said computer program does this automatically. Figure 2 illustrates the energy loss of electrons in high voltage electron beams before and after it hits the sample specimen. Accordingly, w thicker material would consume more energy for the electron to pass through and the reverse is true for the thinner parts. A spectrophotometer placed at the bottom intercepts the remaining electron beams, maps the energy of this electron beam and then generates the appropriate image. Note that there are diverse kinds of lines shown in figure 2, these lines represent the diverse fate of the incident electron beam as it hits the sample being analyzed. Accordingly, some the electrons are backscattered, some are diffracted, some knockout some of the inner electrons of the sample causing X-rays emission, and some transmit through the specimen. All these electrons could be used to create the image of the specimen, but for strictly TEM techniques (meaning no attachment is added to the transmission electron microscope) the electrons in focus are the transmitted electron (Mogilatenko and Kirmse 24).
Figure 2: Illustration of loss in energy of electrons in an electron beam as they pass through the sample
Note that the entire energy loss process is very complex for a detailed discussion, but it suffices to say that many of the electrons in the beam do not pass through the specimen, many of them are diffracted at different angles and these electrons will have no use in the creation of the specimen’s image by they must be taken into account. The amount of electrons lost can be best avoided using efficient sample preparation techniques, which usually involves coting the specimen with gold. Nevertheless, there are more complex and special TEM techniques that utilize the electron diffraction phenomena in order to add resolution and magnification to the specimen’s image. TEM also requires good expertise in microscopy, as it takes experience to handle the electron microscope well. Scorching of the sample could become a very serious problem if the electron microscope and the sample preparation are not handled well (Mikros Skopeo 13). 3. Advantages and disadvantages of the techniques
There are diverse advantage and disadvantages associated with the Transmission Electron Microcopy. One of the major advantages of TEM is its high magnification. It could magnify samples up to the nano level. This capacity of the TEM technique makes it highly useful in nanotechnology. Moreover, since the electrons could penetrate the sample being analyzed, the TEM technique could also be employed to study the inner morphology of samples. This is very important in studying biological samples and polymers that have multiple layers in them. Moreover, with juts few adjustments and attachment to the transmission electron microscope, TEM can be converted to STEM which is highly efficient in studying the surface morphology o diverse kinds of samples, from biological samples to objects used in the material science. The STEM is like feeling with one’s hands the surface of an object. Only this time, the things used for feeling are the electrons which are continuous sprayed over the surface of the ample material. E-dx is another mode of STEM which takes advantage of the X-Rays produced by the backscattered electrons. This backscattering of electrons releases X-rays from the nuclei of the sample. Each element has a distinct X-ray energy level. This means that at its E-dx mode and TEM could be used for elemental analysis. Hence, there are already three uses for TEM: surface morphology, internal morphology, and elemental composition of samples. Ad to these three uses is the possible use of the TEM for quantitative analysis. Not only can it identify the elemental composition of a sample, it could also identify the relative concentrations of these elements. Another important advantage of TEM is that it allows the three-dimensional imaging of the samples. This is made possible by the fact that the TEM sample holder can be tilted at different angles. At present computer programs are also being developed to further enhance the tree-dimensional images that could be produced using TEM. Perhaps, the fact that diverse kinds of samples can be analyzed can be considered as an advantage of TEM, as well. Note that the sample preparation technique, which coats the sample with different coatings such as gold allows the preservation of samples. This means that the biological samples, such as insect parts, may be analyzed without the fear of scorching them when they are subjected to the high energy electron beams. Another advantage in using TEM is that is allows much thicker samples to be studied (Mikros Skopeo 11).
Nevertheless, just like any other technologies of microscopy, TEM, is not without its disadvantages. The first disadvantage is that is relatively more expensive compared to other types of microscopy. TEM uses high voltage and wattage of electricity in order to operate, meaning, it is needs much energy to run. Another cause of the high expenses is the sample preparation associated with it. The sample preparation usually involved multiple steps, such as, dispersion, free drying, and then coating with expensive coats such as gold-based or palladium-based coats. Thirdly, there are very few experts that know how to use the TEM, in fact there limited number of schools and service institutions that have transmission electron microscopes with them. Another disadvantage of TEM is that it could not be used to analyze live samples, which is possible for light microscopy. The reason for this is that the sample preparation itself – which involves freeze drying and coating – kills the biological sample, much more does the high energy electros that will heat it upon analysis. Still another disadvantage is that Tem could analyze a very small portion of the sample at a time. The size is just usually a few nanometers in length and width. This means that if an analyst is analyzing a sample with inconsistent or varying composition throughout its length, then the analyst will need to cut the sample in many pieces, determine the representative parts, and then subject each part to TEM. This process if often rigorous, which leads us to another disadvantage – the TEM employs complex procedures, especially for the sample preparation (Mikros Skopeo 12). The next disadvantage is that since it emits X-rays which potentially leak through the main body of the transmission electron microscope, TEM also pose health risks to the technicians and analysts using it. X-rays are proven to cause disruptions in the DNA causing mutation. The same danger is associated with the sample preparation chemicals – some of them are highly carcinogenic and have high teratogenicity. Another disadvantage of TEM is that it could never be used to sample that decompose very easily at high temperatures, materials with volatile components. These samples would be decomposed upon their contact with the high energy electron beams (Ficher). 4. Examples
In order to fully appreciate the use of TEM, one must take a look at the actual examples. The following images are actual images produced through TEM. The three example images are proofs that TEM have broad application. It can be applied in material science (polymer and metal industries), and in biology. It is the versatility of the method that allows it to be used in various fields of scientific studies.
The image above is a cross sectional area of a composite polymer. This image was used by the researchers to confirm whether the polymeric layers have been successfully intercalated with the bulk material. In this particular image, it can be seen that there are layers upon layers of the polymer and in-between them is the more porous bulk material, which is sand.
The above image shows how TEM as used to visualize them and determine whether there are dislocation in steel. These dislocations are at the atomic level and would have not been obvious if inspected visually by materials engineers. Identifying such dislocations allows engineers to avoid accidents upon construction of reaction vessels, which usually require millions of dollars for funding and claims lives when accidents a happen in them. Vessels for nuclear reactors are typically inspected for such dislocations in order to make sure that no nuclear leaks ensue in the future. In the image, the horizontal lines are the dislocations.
The image above is a biological sample of polio virus. This TEM image was used by the researchers to determine the three dimensional shape of the virus. This image is used both for simple imaging and confirmatory analysis for the polio virus sample.
5. Applications
Transmission Electron Microscopy finds significant and important application material science. In material science, the internal and surface morphologies of synthetic materials are very important as such determines their purposes and functionalities. At par is the importance of material composition. The E-dx mode allows the confirmation of polymer composition. Note that in the polymer industry, the amount of the different types of monomers is essential to record. In an industrial scale of production any deviation from the preferred composition could result to the creation or loosing of the preferred characteristics of the polymer even its functions. The same is true for semiconductor industries, where the dopant and the doped material composition must be strictly maintained at particular relative compositions.
TEM has also important applications in nano technology. Note that nano technology has been very and is still a very important sub-topic in material science. The ability of the TEM to magnify up to the nano-level (approximately 0. 05nm) allows the confirmation of the synthesis of nano particles, or the intercalation of nano fibers in a particular sample. At present, only TEM techniques could be employed to magnify materials up to the nano-level making it indispensible in nano-technology and other affiliated topics.
Lastly, TEM could be applied in biological studies. Most plants, and animal samples require the use of TEM to study their surface morphologies at the nano or micro level. Moreover, studying the internal systems of microorganisms such as viruses and bacterial samples could only be done using TEM and its diverse modes of analysis. Only TEM allows the imaging of internal structures, this is something that is impossible for light microscopy to do. In conclusion, TEM is indispensible in material science and biology research or in life sciences in general. The information that it confirms through its high resolution images serve as evidence for theories applied in a particular study. With the advent and growing popularity of nano technology, which finds its application on different fields – from material science, biology, chemistry, physics, and medicine, it can only be concluded that TEM will become an indispensible tool on all these fields of expertise. It is therefore very important for the students and experts of today to understand how this technology works, and obtain every opportunity to have an experience in using this technology. Moreover, TEM is continuously being improved by scientists and inventors. The TEM itself, therefore would be an interesting topic for research.
5. List of References
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