PREPARATION OF SPECIMENS FOR LIGHT AND ELECTRON MICROSCOPY pdf

1: Sample Preparation For Microscopy

The electron beam inside a transmission electron microscope (TEM) causes problems for biological samples because of its high energy. It needs to have enough energy to pass right through the sample and out the other side.

Let us make an in-depth study of the electron microscope. After reading this article you will learn about: Principle of Electron Microscope 2. Transmission Electron Microscope Tem 3. Components of Electron Microscope 4. Preparation of Specimen 5. Image Viewing, Development and Recording Techniques 6. Use of Electron Microscope 7. High Voltage Modern Electron Microscope 8. Scanning Electron Microscope and 9. Introduction to Electron Microscope: In electron microscope, high-speed electron beam is used instead of light waves, which are used in optical microscope. Like light, the stream of electrons has a corpuscular and vibratory character. Electron microscope gives very high magnification and incredibly high resolution. The first transmission electron microscope was developed by Ernst Ruska and Max Knoll of Germany in EM is a remarkable research tool of twentieth century. It can magnify an object upto X one million times. The photomicrographs can be further enlarged and studied by using modern photographic techniques and computer aided techniques. The electrons can be focused by electro-magnetic lenses much like the light rays. Electron beam can vibrate like light rays but has very short wave length as compared to light rays. Resolution increases with the decrease of wave length. Resolution is dependent upon the wavelength of radiations. Smaller the radiation, greater will be the resolution. It is inversely proportional relation. The resolution determines the level of details that can be viewed from the specimen. It provides remarkable pictures with fine details. Light microscope can achieve a maximum resolution of about 0. Whereas EM can achieve a resolution of 0. Resolution is the ability of a lens to separate or distinguish between closely positioned small objects. Principle of Electron Microscope: Electrons are subatomic particles, which orbit around the atomic nucleus. When atoms of a metal are excited by heat energy, electrons fly off from the atom. In electron microscope, tungston is heated by applying a high voltage current, electrons form a continuous stream, which is used like a light beam. The lenses used in EM are magnetic coils capable of focusing the electron beam on the specimen and illuminating it. The strength of the magnetic lens depends upon the current that flows through it. Greater the flow of the current, greater will be strength of the magnetic field. The electron beam cannot pass through the glass lens. Transmission Electron Microscope Tem: It consist of a system of electromagnetic lenses mounted in a column. Components of Electron Microscope: EM is in the form of a tall column which is vertically mounted. It consists of the following main components: Image viewing and recording system. Electron gun is a heated tungsten filament, which generates electrons. Condenser lens focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam. To move electrons down the column, an accelerating voltage is applied between tungsten filament and anode. Now most EMs use accelerating voltages between kV kV. Electrons also function as a source of illumination for the specimen. High velocity electrons pass into the system of condenser lenses, which focus them on the specimen. The specimen to be examined must be extremely thin, at least times thinner than those used in optical microscope. Ultra thin sections of nm are cut. The specimen holder is an extremely thin film of carbon or collodion held by a metal grid. The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen. The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter. The electron beam coming out of the specimen passes down the second of magnetic coils called objective lens, which has high power and forms the intermediate magnified image. Finally, a third set of magnetic lenses called projector ocular lenses produce the final further magnified image. Each of these lenses acts as image magnifier all the while maintaining an incredible level of details and resolution. He whole image remains in focus. This image is projected on a fluorescent screen. Below the fluorescent screen is a camera for recording the image. These lenses provide immense magnification and

resolution. As the EM works in vacuum, the specimen should be completely dry. Air molecules present in the column of EM scatter the electrons causing flicker in the electron beam. Vacuum is created in two steps. Firstly, a mechanical vacuum pump is used to create vacuum. Secondly, a diffusion pump uses a fast downward moving liquid, either oil or mercury which traps air and gas in the column. In this way, ultra high vacuum is created. The specimen have to be specially prepared for EM studies. There are various techniques for studying the specimen under EM. Some of which are discussed here. The specimens are fixed in glutaraldehyde, osmium tetroxide to stabilize the cell structure. After fixation, dehydration is carried out slowly with organic solvents like acetone and ethanol. Resins such as araldite and epoxy are used for this purpose. Microbes are embedded in plastic resin. The specimen is soaked in un-polymerized, liquid epoxy plastic until it is completely permeated and then is hardened to form a solid block. To obtain extremely thin sections from this plastic block, Ultra-microtomes with diamond knife or glass knives are used. Specimens are stained with heavy metals such as lead, uranium, phosphotungstic acid. The thin sections soaked in solutions of heavy metals like lead citrate, uranyl acetate or osmium tetroxide is also used for staining. There are some additional techniques for preparation and study of various specimens and materials. Image Viewing, Development and Recording Techniques: The image formed in EM is real as compared to the virtual image in optical microscope. The highly magnified image is formed below the projector lens on a fluorescent screen. Below this screen, a camera or a film or light sensitive sensor such as charge-coupled device CCD camera are placed. The image can be displayed on computer or monitor. For direct viewing monocular or binocular viewing, lenses are used. The final image formed will always be in focus and needs no adjustments. The image recording and studying have undergone revolutionary changes. Digital cameras and computers have come to play a major role. Instead of one picture of one section, series of sections are studied and analysed. By computer aided averaging techniques of numerous images three- dimensional reconstructions of cell organelles of highest clarity are developed. Tilting of specimen also provides three-dimensional picture. Use of Electron Microscope: Invention of EM has come as a boon for biological sciences and industry. There is hardly any area of science that has not gained from the use of electron microscope. Immense magnification, high resolution has opened new vistas in research in cellular and molecular biology. Study of microorganisms like bacteria, virus and other pathogens have made the treatment of diseases very effective. Fields of medicine, pathology, human anatomy have gained immensely from electron microscope studies.

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2: Electron Microscope Sample Preparation: Leica Microsystems

Specimen Preparation for Light Microscopy â€¢ Sectioning a sample - must be careful not to significantly alter the microstructure during sectioning.

Scanning electron micrograph SEM of various Pollen. Public domain image reference: Basic Differences There are not many things that these two microscope types have in common. Both electron and light microscopes are technical devices which are used for visualizing structures that are too small to see with the unaided eye, and both types have relevant areas of applications in biology and the materials sciences. And this is pretty much it. The method of visualizing the structures is very different. Electron Microscopes use electrons and not photons light rays for visualization. The first electron microscope was constructed in , compared to optical microscopes they are a very recent invention. The biggest advantage is that they have a higher resolution and are therefore also able of a higher magnification up to 2 million times. Light microscopes can show a useful magnification only up to times. This is a physical limit imposed by the wavelength of the light. Electron microscopes therefore allow for the visualization of structures that would normally be not visible by optical microscopy. Depending on the type of electron microscope, it is possible to view the three dimensional external shape of an object Scanning Electron Microscope, SEM. In scanning electron microscopy SEM , due to the nature of electrons, electron microscopes have a greater depth of field compared to light microscopes. The higher resolution may also give the human eye the subjective impression of a higher depth of field. Electron microscopes have a range of disadvantages as well: They are extremely expensive. Sample preparation is often much more elaborate. It is often necessary to coat the specimen with a very thin layer of metal such as gold. The metal is able to reflect the electrons. The sample must be completely dry. This makes it impossible to observe living specimens. It is not possible to observe moving specimens they are dead. It is not possible to observe color. Electrons do not possess a color. Sometimes the image is colored artificially to give a better visual impression. They require more training and experience in identifying artifacts that may have been introduced during the sample preparation process. The energy of the electron beam is very high. The sample is therefore exposed to high radiation, and therefore not able to live. The space requirements are high. They may need a whole room. Maintenance costs are high. When should one use optical light microscopes? One big advantage of light microscopes is the ability to observe living cells. It is possible to observe a wide range of biological activity, such as the uptake of food, cell division and movement. Additionally, it is possible to use in-vivo staining techniques to observe the uptake of colored pigments by the cells. These processes can not be observed in real time using electron microscopes, as the specimen has to be fixed, and completely dehydrated and is therefore dead. The low cost of optical microscopes makes them useful in a wide range of different areas, such as education, the medical sector or for hobbyists. Generally, optical and electron microscopes have different areas of application and they complement each other. Different types of electron microscopes There are two different types of electron microscopes, scanning electron microscopes SEM and transmission electron microscopes TEM. In the TEM method, an electron beam is passed through an extremely thin section of the specimen. You will get a two-dimensional cross-section of the specimen. SEMs, in contrast, visualize the surface structure of the specimen, providing a 3-D impression. The image above was produced by a SEM. Different types of light microscopes The two most common types of microscopes are compound microscopes and stereo microscopes dissecting microscopes. Stereo microscopes are frequently used to observe larger, opaque specimens. They generally do not magnify as much as compound microscopes around 40xx maximum but give a truly stereoscopic view. This is because the image delivered to each eye is slightly different. Stereo microscopes do not necessarily require elaborate sample preparation. Compound microscopes magnify up to about x. The specimen has to be sufficiently thin and bright for the microscope light to pass through. The specimen is mounted on a glass slide. Compound microscopes are not capable of producing a 3D stereoscopic view, even if they possess two eye pieces. This is

because each one of the eyes receives the same image from the objective. The light beam is simply split in two.

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3: Conventional transmission electron microscopy

Microscopes using visible light will magnify approximately times and have a resolution limit of about mm whereas a transmission electron microscope is capable of magnifying apÂ-proximately 2,00, times and has a resolution limit for biological specimens of about 1 nm.

The specimens are placed in the mounting press, and the resin is added. The specimens are mounted under heat and high pressure. The specimens are placed in a mounting cup and mounting material is then poured over the specimens. A vacuum impregnation unit photo is used for mounting of porous materials. Example of a reusable pad for use with diamond suspension. A single magnetic platen is positioned on the grinding and polishing machine to support the preparation pads. The surface of a metallographic specimen is prepared by various methods of grinding , polishing , and etching. After preparation, it is often analyzed using optical or electron microscopy. Using only metallographic techniques, a skilled technician can identify alloys and predict material properties. Mechanical preparation is the most common preparation method. Successively finer abrasive particles are used to remove material from the sample surface until the desired surface quality is achieved. Many different machines are available for doing this grinding and polishing , which are able to meet different demands for quality, capacity, and reproducibility. A systematic preparation method is the easiest way to achieve the true structure. Sample preparation must therefore pursue rules which are suitable for most materials. Different materials with similar properties hardness and ductility will respond alike and thus require the same consumables during preparation. Metallographic specimens are typically "mounted" using a hot compression thermosetting resin. In the past, phenolic thermosetting resins have been used, but modern epoxy is becoming more popular because reduced shrinkage during curing results in a better mount with superior edge retention. When specimens are very sensitive to temperature, "cold mounts" may be made with a two-part epoxy resin. Mounting a specimen provides a safe, standardized, and ergonomic way by which to hold a sample during the grinding and polishing operations. A macro etched copper disc After mounting, the specimen is wet ground to reveal the surface of the metal. The specimen is successively ground with finer and finer abrasive media. Silicon carbide abrasive paper was the first method of grinding and is still used today. Many metallographers, however, prefer to use a diamond grit suspension which is dosed onto a reusable fabric pad throughout the polishing process. Diamond grit in suspension might start at 9 micrometres and finish at one micrometre. Generally, polishing with diamond suspension gives finer results than using silicon carbide papers SiC papers , especially with revealing porosity , which silicon carbide paper sometimes "smear" over. After grinding the specimen, polishing is performed. Typically, a specimen is polished with a slurry of alumina , silica , or diamond on a napless cloth to produce a scratch-free mirror finish, free from smear, drag, or pull-outs and with minimal deformation remaining from the preparation process. After polishing, certain microstructural constituents can be seen with the microscope, e. If the crystal structure is non-cubic e. Otherwise, the microstructural constituents of the specimen are revealed by using a suitable chemical or electrolytic etchant. Analysis techniques[edit] Many different microscopy techniques are used in metallographic analysis. Prepared specimens should be examined with the unaided eye after etching to detect any visible areas that have responded to the etchant differently from the norm as a guide to where microscopical examination should be employed. Light optical microscopy LOM examination should always be performed prior to any electron metallographic EM technique, as these are more time-consuming to perform and the instruments are much more expensive. Further, certain features can be best observed with the LOM, e. Also, image contrast of microstructures at relatively low magnifications, e. LOM examination is fast and can cover a large area. Thus, the analysis can determine if the more expensive, more time-consuming examination techniques using the SEM or the TEM are required and where on the specimen the work should be concentrated. Each type has advantages and disadvantages. Most LOM work is done at magnifications between 50 and X. However, with a good microscope, it is possible to perform examination at higher

magnifications, e. However, the resolution limit of the LOM will not be better than about 0. Special methods are used at magnifications below 50X, which can be very helpful when examining the microstructure of cast specimens where greater spatial coverage in the field of view may be required to observe features such as dendrites. Besides considering the resolution of the optics, one must also maximize visibility by maximizing image contrast. A microscope with excellent resolution may not be able to image a structure, that is there is no visibility, if image contrast is poor. Image contrast depends upon the quality of the optics, coatings on the lenses, and reduction of flare and glare ; but, it also requires proper specimen preparation and good etching techniques. So, obtaining good images requires maximum resolution and image contrast. Bright-field illumination, where sample contrast comes from absorbance of light in the sample Dark-field illumination, sample contrast comes from light scattered by the sample Cross-polarized light illumination, where sample contrast comes from rotation of polarized light through the sample Bright- and dark-field microscopy[edit] Most LOM observations are conducted using bright-field BF illumination, where the image of any flat feature perpendicular to the incident light path is bright, or appears to be white. But, other illumination methods can be used and, in some cases, may provide superior images with greater detail. Dark-field microscopy DF , is an alternative method of observation that provides high-contrast images and actually greater resolution than bright-field. In dark-field illumination, the light from features perpendicular to the optical axis is blocked and appears dark while the light from features inclined to the surface, which look dark in BF, appear bright, or "self-luminous" in DF. Grain boundaries , for example, are more vivid in DF than BF. Polarized light microscopy[edit] Polarized light PL is very useful when studying the structure of metals with non-cubic crystal structures mainly metals with hexagonal close-packed hcp crystal structures. If the specimen is prepared with minimal damage to the surface, the structure can be seen vividly in cross-polarized light the optic axis of the polarizer and analyzer are 90 degrees to each other, i. In some cases, an hcp metal can be chemically etched and then examined more effectively with PL. Tint etched surfaces, where a thin film such as a sulfide , molybdate , chromate or elemental selenium film is grown epitaxially on the surface to a depth where interference effects are created when examined with BF producing color images, can be improved with PL. If it is difficult to get a good interference film with good coloration, the colors can be improved by examination in PL using a sensitive tint ST filter. Differential interference contrast microscopy[edit] Another useful imaging mode is differential interference contrast DIC , which is usually obtained with a system designed by the Polish physicist Georges Nomarski. This system gives the best detail. DIC converts minor height differences on the plane-of-polish, invisible in BF, into visible detail. The detail in some cases can be quite striking and very useful. If an ST filter is used along with a Wollaston prism , color is introduced. The colors are controlled by the adjustment of the Wollaston prism, and have no specific physical meaning, per se. But, visibility may be better. Oblique illumination[edit] DIC has largely replaced the older oblique illumination OI technique, which was available on reflected light microscopes prior to about In OI, the vertical illuminator is offset from perpendicular, producing shading effects that reveal height differences. This procedure reduces resolution and yields uneven illumination across the field of view. Nevertheless, OI was useful when people needed to know if a second phase particle was standing above or was recessed below the plane-of-polish, and is still available on a few microscopes. OI can be created on any microscope by placing a piece of paper under one corner of the mount so that the plane-of-polish is no longer perpendicular to the optical axis. Scanning electron and transmission electron microscopes[edit] If a specimen must be observed at higher magnification, it can be examined with a scanning electron microscope SEM , or a transmission electron microscope TEM. When equipped with an energy dispersive spectrometer EDS , the chemical composition of the microstructural features can be determined. The ability to detect low-atomic number elements, such as carbon , oxygen , and nitrogen , depends upon the nature of the detector used. But, quantification of these elements by EDS is difficult and their minimum detectable limits are higher than when a wavelength-dispersive spectrometer WDS is used. But quantification of composition by EDS has improved greatly over time. The WDS system has historically had better sensitivity ability to detect low amounts of an

element and ability to detect low-atomic weight elements, as well as better quantification of compositions, compared to EDS, but it was slower to use. Again, in recent years, the speed required to perform WDS analysis has improved substantially. An x-ray diffractometer X-ray diffraction techniques[edit] Characterization of microstructures has also been performed using x-ray diffraction XRD techniques for many years. XRD can be used to determine the percentages of various phases present in a specimen if they have different crystal structures. If a particular phase can be chemically extracted from a bulk specimen, it can be identified using XRD based on the crystal structure and lattice dimensions. For smaller particles, diffraction techniques can be performed using the TEM for identification and EDS can be performed on small particles if they are extracted from the matrix using replication methods to avoid detection of the matrix along with the precipitate. Quantitative metallography[edit] A number of techniques exist to quantitatively analyze metallographic specimens. These techniques are valuable in the research and production of all metals and alloys and non-metallic or composite materials. Microstructural quantification is performed on a prepared, two-dimensional plane through the three-dimensional part or component. Measurements may involve simple metrology techniques, e. Measurement may also require application of stereology to assess matrix and second-phase structures. Stereology is the field of taking 0-, 1- or 2-dimensional measurements on the two-dimensional sectioning plane and estimating the amount, size, shape or distribution of the microstructure in three dimensions. These measurements may be made using manual procedures with the aid of templates overlaying the microstructure, or with automated image analyzers. In all cases, adequate sampling must be made to obtain a proper statistical basis for the measurement. Efforts to eliminate bias are required. An image of the microstructures of ductile cast iron Some of the most basic measurements include determination of the volume fraction of a phase or constituent, measurement of the grain size in polycrystalline metals and alloys, measurement of the size and size distribution of particles, assessment of the shape of particles, and spacing between particles. For example, the amount of a phase or constituent, that is, its volume fraction, is defined in ASTM E ; manual grain size measurements are described in ASTM E equiaxed grain structures with a single size distribution and E specimens with a bi-modal grain size distribution ; while ASTM E describes how any grain size type or condition can be measured using image analysis methods. Characterization of nonmetallic inclusions using standard charts is described in ASTM E 45 historically, E 45 covered only manual chart methods and an image analysis method for making such chart measurements was described in ASTM E The image analysis methods are currently being incorporated into E A stereological method for characterizing discrete second-phase particles, such as nonmetallic inclusions, carbides, graphite, etc. References[edit] Wikimedia Commons has media related to Metallography. Metallography and Microstructures, Vol. Principles and Practice, G. Petzow, Metallographic Etching, 2nd Ed.

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4: Dimpler for TEM Specimen Preparation

Microscopy and Specimen Preparation. Analogous to procedures used for light microscopy For transmission electron microscopy, specimens must be cut very thin.

Scanning electron microscope Image of bacillus subtilis taken with a s electron microscope The SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen raster scanning. When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms. The lost energy is converted into alternative forms such as heat, emission of low-energy secondary electrons and high-energy backscattered electrons, light emission cathodoluminescence or X-ray emission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition. The image displayed by an SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated. In the SEM image of an ant shown below and to the right, the image was constructed from signals produced by a secondary electron detector, the normal or conventional imaging mode in most SEMs. However, because the SEM images the surface of a sample rather than its interior, the electrons do not have to travel through the sample. This reduces the need for extensive sample preparation to thin the specimen to electron transparency. The SEM is able to image bulk samples that can fit on its stage and still be maneuvered, including a height less than the working distance being used, often 4 millimeters for high-resolution images. The SEM also has a great depth of field, and so can produce images that are good representations of the three-dimensional surface shape of the sample. Another advantage of SEMs comes with environmental scanning electron microscopes ESEM that can produce images of good quality and resolution with hydrated samples or in low, rather than high, vacuum or under chamber gases. This facilitates imaging unfixed biological samples that are unstable in the high vacuum of conventional electron microscopes. An image of an ant in a scanning electron microscope Color In their most common configurations, electron microscopes produce images with a single brightness value per pixel, with the results usually rendered in grayscale. This may be done to clarify structure or for aesthetic effect and generally does not add new information about the specimen. Examples are the Energy-dispersive X-ray spectroscopy EDS detectors used in elemental analysis and Cathodoluminescence microscope CL systems that analyse the intensity and spectrum of electron-induced luminescence in for example geological specimens. In SEM systems using these detectors, it is common to color code the signals and superimpose them in a single color image, so that differences in the distribution of the various components of the specimen can be seen clearly and compared. Such images can be made while maintaining the full integrity of the original signal, which is not modified in any way. Scanning transmission electron microscopy The STEM rasters a focused incident probe across a specimen that as with the TEM has been thinned to facilitate detection of electrons scattered through the specimen. The STEMs use of SEM-like beam rastering simplifies annular dark-field imaging , and other analytical techniques, but also means that image data is acquired in serial rather than in parallel fashion. Sample preparation An insect coated in gold for viewing with a scanning electron microscope Materials to be viewed under an electron microscope may require processing to produce a suitable sample. The technique required varies depending on the specimen and the analysis required: Negative stain â€” suspensions containing nanoparticles or fine biological material such as viruses and bacteria are briefly mixed with a dilute solution of an electron-opaque solution such as ammonium molybdate, uranyl acetate or formate , or phosphotungstic acid. This mixture is applied to a suitably coated EM grid, blotted, then allowed to dry. Viewing of this preparation in the TEM should be carried out without delay for best results. The method is important in microbiology for fast but crude morphological identification, but can also be used as the basis for high-resolution 3D reconstruction using EM tomography methodology when carbon films are used for support. Negative staining is also used for observation of nanoparticles. Cryofixation â€” freezing a specimen

so rapidly, in liquid ethane , and maintained at liquid nitrogen or even liquid helium temperatures, so that the water forms vitreous non-crystalline ice. This preserves the specimen in a snapshot of its solution state. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy of vitreous sections CEMOVIS , it is now possible to observe samples from virtually any biological specimen close to its native state. Embedding, biological specimens â€" after dehydration, tissue for observation in the transmission electron microscope is embedded so it can be sectioned ready for viewing. After the resin has been polymerized hardened the sample is thin sectioned ultrathin sections and stained â€" it is then ready for viewing. Embedding, materials â€" after embedding in resin, the specimen is usually ground and polished to a mirror-like finish using ultra-fine abrasives. The polishing process must be performed carefully to minimize scratches and other polishing artifacts that reduce image quality. Metal shadowing â€" Metal e. Replication â€" A surface shadowed with metal e. This is followed by removal of the specimen material e. Sectioning â€" produces thin slices of the specimen, semitransparent to electrons. Disposable glass knives are also used because they can be made in the lab and are much cheaper. Staining â€" uses heavy metals such as lead , uranium or tungsten to scatter imaging electrons and thus give contrast between different structures, since many especially biological materials are nearly "transparent" to electrons weak phase objects. In biology, specimens can be stained "en bloc" before embedding and also later after sectioning. Typically thin sections are stained for several minutes with an aqueous or alcoholic solution of uranyl acetate followed by aqueous lead citrate. The second coat of carbon, evaporated perpendicular to the average surface plane is often performed to improve the stability of the replica coating. The specimen is returned to room temperature and pressure, then the extremely fragile "pre-shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The still-floating replica is thoroughly washed free from residual chemicals, carefully fished up on fine grids, dried then viewed in the TEM. Freeze-fracture replica immunogold labeling FRIL â€" the freeze-fracture method has been modified to allow the identification of the components of the fracture face by immunogold labeling. Instead of removing all the underlying tissue of the thawed replica as the final step before viewing in the microscope the tissue thickness is minimized during or after the fracture process. The thin layer of tissue remains bound to the metal replica so it can be immunogold labeled with antibodies to the structures of choice. The thin layer of the original specimen on the replica with gold attached allows the identification of structures in the fracture plane. A subclass of this is focused ion beam milling, where gallium ions are used to produce an electron transparent membrane in a specific region of the sample, for example through a device within a microprocessor. Ion beam milling may also be used for cross-section polishing prior to SEM analysis of materials that are difficult to prepare using mechanical polishing. Conductive coating â€" an ultrathin coating of electrically conducting material, deposited either by high vacuum evaporation or by low vacuum sputter coating of the sample. This is done to prevent the accumulation of static electric fields at the specimen due to the electron irradiation required during imaging. Earthing â€" to avoid electrical charge accumulation on a conductively coated sample, it is usually electrically connected to the metal sample holder. Often an electrically conductive adhesive is used for this purpose. Disadvantages Electron microscopes are expensive to build and maintain, on the order of other complex machines such as airplanes. Microscopes designed to achieve high resolutions must be housed in stable buildings sometimes underground with special services such as magnetic field canceling systems. Operating the electron microscope requires specialized training and continuing practice and education. The samples largely have to be viewed in vacuum , as the molecules that make up air would scatter the electrons. Various techniques for in situ electron microscopy of gaseous samples have been developed as well. The low-voltage mode of modern microscopes makes possible the observation of non-conductive specimens without coating. Non-conductive materials can be imaged also by a variable pressure or environmental scanning electron microscope. Small, stable specimens such as carbon nanotubes , diatom frustules and small mineral crystals asbestos fibres, for example require no special treatment before being examined in the electron microscope.

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Samples of hydrated materials, including almost all biological specimens have to be prepared in various ways to stabilize them, reduce their thickness ultrathin sectioning and increase their electron optical contrast staining. These processes may result in artifacts , but these can usually be identified by comparing the results obtained by using radically different specimen preparation methods. Since the s, analysis of cryofixed , vitrified specimens has also become increasingly used by scientists, further confirming the validity of this technique.

PREPARATION OF SPECIMENS FOR LIGHT AND ELECTRON MICROSCOPY pdf

5: Sample Preparation for Scanning Electron Microscopy - Bitesize Bio

There are many different forms of microscopy but the one most commonly employed is "brightfield" microscopy where the specimen is illuminated with a beam of light that passes through it (as opposed to a beam of electrons as in electron microscopy).

By Saraswathi Sadasivan Proper sample preparation plays an important role in obtaining the required information when using scanning electron microscopy SEM. Ideally, the smallest representative sample size is the one to use. Working Principles of Scanning Electron Microscopy An electron gun at the top of the microscope directs a stream of electrons vertically down a set of electromagnetic lenses inside a vacuum chamber. The lenses are placed in a vacuum chamber in order to avoid obstruction and contamination by other particles. The lenses help direct the electrons towards the sample. The electron imprint is converted to a three-dimensional image which is visualized digitally. Sample Preparation Starters Some samples need to be coated to make them conductive. Metals require no preparation due to their inherent ability to conduct electricity. However, non-metals need to be coated with a conductive material. This requires the use of a sputter-coater. Important parameters in preparing a sample for SEM imaging are as follows: Sample Cleaning A clean sample is essential for image clarity. For biological samples, use appropriate buffers or distilled water for cleaning the samples. Use a surfactant if the sample requires more vigorous cleaning. If the biological property of the sample is known, then you might be able to use proteolytic enzyme cleaning. To remove oils on the sample surface, wash with appropriate solvents. Additionally, you can use ultrasonic baths for cleaning the sample. However, ultrasonic baths require caution in order to avoid damaging the sample. Sample Fixation and Dehydration Use a fixative like glutaldehyde or osmium vapor to maintain the structural details of the sample. Drying Prior to placing the sample in a high vacuum environment, it must be totally dry. Otherwise, water vaporization will obstruct the electron beam and interfere with image clarity. When using biological samples, be careful when doing critical point drying or CPD , so as to not compromise the structural integrity of the sample. A suitable CPD instrument can help achieve this. Alternatively, you can try using freeze drying. In this regard, freeze drying causes the least amount of sample shrinkage in comparison to air drying or critical point drying. However, freeze drying carries the risk of ice crystal formation on the sample. Sample Preparation of Tissue Sections To observe details from tissue sections, remove the epoxy resin using organic solvents, ion beam etching, or plasma etching. You can also break the sample in the appropriate direction to reveal its internal details. Sample Stubs, Adhesives, and Mounting Approach Sample stubs or supports are available in different diameters making them convenient for imaging different kinds of samples. Be sure the stubs are clean and handle the sample with clean forceps. Also, use gloves during the entire sample preparation stage. The material you use as an adhesive to glue the sample to the stub should be non-toxic and should not tumble into the sides of the sample. Conductive double coated carbon tape is the most common adhesive for this purpose. You need to ensure that a conducting path exists as you mount the sample. If the sample requires a conductive coating, be sure to mount it before coating so that both the sample and plug receive the coating. Sample Storage Store the sample and stubs in a dry, clean environment. Use clean forceps and gloves while handling the stubs. In conclusion, I hope you now have a feel for what you need to start doing SEM.

PREPARATION OF SPECIMENS FOR LIGHT AND ELECTRON MICROSCOPY pdf

6: An Introduction to Specimen Preparation: Leica Biosystems

Electron microscopy preparation: Electron microscopic techniques are considerably different from light microscopy. They give higher resolution and magnification using extremely narrow wavelength electron beam in presence of magnetic field.

There are two types of samples, one are large cellular structures, which require further sectioning and the other are individual assemblies, which are small enough to be imaged in suspension. Any preparation method of these types of samples has to fulfil four basic requirements: The relative importance of each constraint varies with the type of specimen and with the level of resolution sought. Vitrification by rapid freezing converts liquid water into amorphous solid water. Vitrification perseveres ultra structures with the least artifacts cryo fixation. Large samples such as cells and tissues are stabilised by embedding in plastic resins or by vitrification. Thick objects are cut to appropriate thickness either with conventional ultramicrotomes diamond knifes or glass knifes or with a focused ion beam. Contrast can be enhanced by staining procedures, which use electron dense heavy atom derivates such as Uranyl acetate, Phosphotungstic acid, osmium tetroxide, ammonium molybdate. The grid has a diameter of 3 mm. The grid is somewhat translucent due to the holes and it has a wider rim arrow for handling. Schematic drawing of a negatively stained sample perpendicular to the direction of imaging and its projection image shown in the direction of imaging generated by the electron microscope. Top, the biological object white circle is supported by a carbon support film brown line and surrounded by the electron dense stain black. The stain accumulates at the edges of the object and is less thick further away from the object. The electron microscope generates a projection of the object. Stain is excluded by the object. Thus these areas appear bright. Stain accumulates close to the object giving a dark rim and is less thick further away from the object. This gives rise to a density gradient around the object. The humidification of the chamber is achieved with water soaked sponges. If hot water is used for soaking, the air inside the chamber is saturated with water. The grid is held by tweezers, which are mounted to a movable rod, which is accelerated by a spring not visible during plunging. The formation of a thin sample suspension is achieved by blotting with filter paper, which is mounted inside a chamber. A rod connects the filter paper to the outside and allows the user to handle it, without disturbing the humidity inside the chamber. The cryogen is placed in an insulating box outside the chamber. Blotting is done from both sides of the grid. The humidity inside the chamber is measured and adjusted with a nebuliser that generates small droplets of water with ultrasound. Viral capsids negatively stained left and vitrified right. The negatively stained capsids appear bright against a darker background, because the stain in the background is more electron dense than the capsids. The contrast is high and the capsids can be easily recognised. The spikes of the capsid appear to have collapsed on the body of the capsids. In the vitrified sample right capsids are dark against a brighter background, because the protein is more electron dense than the vitrified buffer. The contrast is low, which makes it more difficult to recognise the particles. The spikes are better preserved giving the particles a more spherical appearance. Schematic drawing of unidirectional metal shadowing. The object is shown as a grey circle, the carbon support film as a brown line and the metal coat as a red line. The Embo Journal Journal of Structural Biology Journal of Electron Microscopy Technique Brenner S and Horne RW A negative staining method for high resolution electron microscopy of viruses. Biochimica et Biophysica Acta Methods in Enzymology Dubochet J Vitrification of pure water for electron microscopy. Journal of Microscopy Quarterly Reviews of Biophysics Journal of Electron Microscopy Unwin PN and Henderson R Molecular structure determination by electron microscopy of unstained crystalline specimens. Journal of Molecular Biology Practical Methods in Electron Microscopy, vol. Griffiths G Fine Structure Immunocytochemistry. Berlin, Heidelberg and New York: Amsterdam and New York: Elsevier Science Publishing Co Inc. Staining Methods for Sectioned Material. Reid N and Beesley JE Sectioning and Cryosectioning for Electron Microscopy.

PREPARATION OF SPECIMENS FOR LIGHT AND ELECTRON MICROSCOPY pdf

7: Electron microscope - Wikipedia

In the field of electron microscopy, perfect sample preparation is a prerequisite and crucial step. Leica Microsystems offers a comprehensive product portfolio for preparation of biological, medical and industrial samples.

The amount of detail depends on the resolving power of a microscope, which is the smallest separation at which two separate objects can be distinguished or resolved. The resolving power of a microscope is ultimately limited by the wavelength of light nm for visible light. To improve the resolving power a shorter wavelength of light is needed, and sometimes microscopes have blue filters for this purpose because blue has the shortest wavelength of visible light. Magnification is how much bigger a sample appears to be under the microscope than it is in real life. This is because when objects in the specimen are much smaller than the wavelength of the radiation being used, they do not interrupt the waves, and so are not detected. The wavelength of light is much larger than the wavelength of electrons, so the resolution of the light microscope is a lot lower. Using a microscope with a more powerful magnification will not increase this resolution any further. It will increase the size of the image, but objects closer than nm will still only be seen as one point. Different kinds of Microscopes: This is the oldest, simplest and most widely-used form of microscopy. Specimens are illuminated with light, which is focussed using glass lenses and viewed using the eye or photographic film. Specimens can be living or dead, but often need to be stained with a coloured dye to make them visible. Many different stains are available that stain specific parts of the cell such as DNA, lipids, cytoskeleton, etc. All light microscopes today are compound microscopes, which means they use several lenses to obtain high magnification. There has been a recent resurgence in the use of light microscopy, partly due to technical improvements, which have dramatically improved the resolution far beyond the theoretical limit. Preparation of Slide Samples Fixation: Chemicals preserve material in a life like condition. Does not distort the specimen. Water removed from the specimen using ethanol. Particularly important for electron microscopy because water molecules deflect the electron beam which blurs the image. Supports the tissue in wax or resin so that it can be cut into thin sections. Sectioning Produces very thin slices for mounting. Sections are cut with a microtome or an ulramicrotome to make them either a few micrometres light microscopy or nanometres electron microscopy thick. Most biological material is transparent and needs staining to increase the contrast between different structures. Different stains are used for different types of tissues. Methylene blue is often used for animal cells, while iodine in KI solution is used for plant tissues. Mounting on a slide protects the material so that it is suitable for viewing over a long period. This uses a beam of electrons, rather than electromagnetic radiation, to "illuminate" the specimen. This may seem strange, but electrons behave like waves and can easily be produced using a hot wire , focused using electromagnets and detected using a phosphor screen or photographic film. A beam of electrons has an effective wavelength of less than 1 nm, so can be used to resolve small sub-cellular ultrastructure. The development of the electron microscope in the s revolutionised biology, allowing organelles such as mitochondria, ER and membranes to be seen in detail for the first time. The main problem with the electron microscope is that specimens must be fixed in plastic and viewed in a vacuum, and must therefore be dead. Other problems are that the specimens can be damaged by the electron beam and they must be stained with an electron-dense chemical usually heavy metals like osmium, lead or gold. Initially there was a problem of artefacts i. There are two kinds of electron microscope. The transmission electron microscope TEM works much like a light microscope, transmitting a beam of electrons through a thin specimen and then focusing the electrons to form an image on a screen or on film. This is the most common form of electron microscope and has the best resolution. The scanning electron microscope SEM scans a fine beam of electron onto a specimen and collects the electrons scattered by the surface. This has poorer resolution, but gives excellent 3-dimentional images of surfaces. The electrons that pass through the specimen are detected on a fluorescent screen on which the image is displayed. Thin sections of specimen are needed for transmission electron microscopy as the electrons have to pass through the

specimen for the image to be produced.

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8: Metallography - Wikipedia

Microscopy I Light and Electron Microscopy Replica of van Leeuwenhoek's () Specimen Preparation A. Light Microscopy B. Scanning Electron Microscopy.

Dimpler for TEM Specimen Preparation Dimpler â€" Model An easy-to-use, state-of-the-art, mechanical thinning instrument designed for the reproducible preparation of high-quality electron microscopy specimens. Description The Model Dimpling Grinder is an easy-to-use, state-of-the-art, mechanical thinning instrument designed for the reproducible preparation of high-quality TEM specimens. The Model features grinding rate control which determines the rate at which material is removed. Microprocessor-based control circuitry automatically and precisely terminates the grinding process when the desired specimen thickness is achieved. The Model continuously indicates specimen thickness on an alphanumeric display with an accuracy of one micron. A 40x optical microscope facilitates precise specimen positioning as well as in-situ specimen observation. The specimen is illuminated by both transmission and reflected light. Features Prethin specimens for ion milling Polish specimens to electron transparency Controlled grinding force and rate Vibration-free grinding Precise indication of specimen thickness Easy to program Accepts mounted specimens from the Model Specimen Grinder Optional 40x microscope attachment allows direct observation of the specimen without the need to remove it Ideal specimen prep for ion milling The ultra-precise Model Dimpling Grinder is indispensable when ion milling is used for final specimen thinning. Because the specimen is prethinned by dimpling, ion milling must remove only relatively small amounts of material. This creates specimens free from uneven thinning, surface defects, and irradiation damage. Versatile By simply changing tools, the dimpling grinder can be used to flat grind bulk specimens, dimple, and then finally polish the specimen to electron transparency. Precise specimen positioning A key component of the dimpling grinder is the specimen stage that precisely rotates the specimen. The specimen is mounted with a low melting point polymer to the top half of a two-piece platen. The platen fits into a magnetic base, which is coupled to the specimen stage by a rare-earth magnet. This magnetic coupling allows the base to be positioned so that a particular area of the specimen can be precisely placed under the grinding wheel. Positioning can be observed through a 40 X optical microscope. The specimen can be readily removed for inspection and then precisely repositioned for continued grinding. Specimen rotation speed is continuously varied by a front panel mounted potentiometer. Optimized grinding control For effective preparation and to avoid specimen damage, an electromechanical stage lowers the grinding wheel at an optimized rate. This creates a very controlled reduction of specimen thickness. Wear on the grinding wheel is uniform, virtually eliminating eccentric tool-induced vibration and ensuring grinding without the risk of specimen fracture. At the conclusion of the grinding process, the advanced instrument control halts both the wheel and specimen rotation and then automatically lifts the grinding wheel from the specimen surface. Vibration-free grinding The precision fit of the grinding wheel to the shaft minimizes eccentricity. Both advanced rotary bearing technology and a specially designed drive system virtually eliminate wheel vibration. Grinding wheel rotation speed is continuously adjustable via a front panel mounted potentiometer. The dimpling grinder can incorporate different types of wheels: Grinding wheels for rapid material removal without scratching. Polishing wheels for an optimal surface finish. Wheels with different diameters for various dimple profiles. A variety of wheel materials are available and can be specially matched to given applications. Controlled grinding force and rate The grinding wheel stage is pivoted to present the grinding wheel to the specimen. It contains a micrometer-type, adjustable counterweight system to regulate the applied grinding force. The controlled grinding force and rate produces specimens with exceptional quality. Easy programming Programming is extremely easy via a keypad mounted on the front panel. Prompts guide you through quick and easy programming steps. A liquid crystal display continuously shows performance information. A zero position can be established on either the surface of the platen or the surface of the specimen. This enables you to either dimple to a given thickness or to remove a specific amount

of material from the specimen. Process termination Final specimen thickness is readily programmed for accurate, unattended operation. However, at any time, the process can be either paused for specimen inspection or stopped. During grinding, specimen thickness is continuously displayed. The dimpling grinder also features a time-based polishing mode. At the conclusion of an elapsed set time, the process automatically terminates. Transmitted or reflected illumination The platen that holds the specimen has a glass center section that allows light to be transmitted through the specimen from a source located beneath the specimen stage. The intensity level of the transmitted light is adjustable for optimal specimen observation. This is particularly important when dimpling silicon, which undergoes changes in color as the specimen approaches electron transparency. Microscope for direct observation A 40x microscope attachment allows direct observation without the need to remove the specimen. Specifications Grinding Control Automated grinding rate control, Grinding force adjustable with micrometer counterweight system, Independent control of grinding wheel and specimen rotation speeds Specimen Stage Precise specimen stage rotation, Magnetic mount allows easy specimen positioning User Interface All program inputs via front panel keypad, Specimen thickness indicated on a liquid crystal display Specimen Illumination Specimen observation in either transmitted or reflected light Enclosure Weight:

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9: Learn & Share: EM Sample Preparation: Leica Microsystems

To achieve the best results during examination in the Electron Microscope (EM), the perfect EM Sample Preparation (for TEM, SEM, AFM) is a www.enganchecubano.com required techniques depend on the samples (biological samples, material samples) as well as on the application.

Miscellaneous What is Electron Microscopy? The electron microscope is a type of microscope that uses a beam of electrons to create an image of the specimen. It is capable of much higher magnifications and has a greater resolving power than a light microscope, allowing it to see much smaller objects in finer detail. They are large, expensive pieces of equipment, generally standing alone in a small, specially designed room and requiring trained personnel to operate them. Hertz suggested that cathode rays were a form of wave motion and Weichert, in , found that these rays could be concentrated into a small spot by the use of an axial magnetic field produced by a long solenoid. But it was not until that Busch showed theoretically that a short solenoid converges a beam of electrons in the same way that glass can converge the light of the sun, that a direct comparison was made between light and electron beams. Busch should probably therefore be known as the father of electron optics. In the German engineers Ernst Ruska and Maximillion Knoll succeeded in magnifying and electron image. This was, in retrospect, the moment of the invention of the electron microscope but the first prototype was actually built by Ruska in and was capable of resolving to 50 nm. Although it was primitive and not really fit for practical use, Ruska was recognised some 50 years later by the award of a Nobel Prize. The first commercially available electron microscope was built in England by Metropolitan Vickers for Imperial College, London, and was called the EM1, though it never surpassed the resolution of a good optical microscope. The early electron microscopes did not excite the optical microscopists because the electron beam, which had a very high current density, was concentrated into a very small area and was very hot and therefore charred any non-metallic specimens that were examined. When it was found that you could successfully examine biological specimens in the electron microscope after treating them with osmium and cutting very thin slices of the sample, the electron microscope began to appear as a viable proposition. This was an effective, high-resolution instrument, the design of which eventually led to what was to become known as the RCA Radio Corporation of America range of very successful microscopes. Unfortunately, the outbreak of the Second World War in held back their further development somewhat, but within 20 years of the end of the war routine commercial electron microscopes were capable of 1 nm resolution. Glass lenses, used in light microscopes, have no effect on the electron beam. The basic design of an electromagnetic lens is a solenoid a coil of wire around the outside of a tube through which one can pass a current, thereby inducing an electromagnetic field. The electron beam passes through the centre of such solenoids on its way down the column of the electron microscope towards the sample. Electrons are very sensitive to magnetic fields and can therefore be controlled by changing the current through the lenses. The faster the electrons travel, the shorter their wavelength. The resolving power of a microscope is directly related to the wavelength of the irradiation used to form an image. Reducing wavelength increases resolution. Therefore, the resolution of the microscope is increased if the accelerating voltage of the electron beam is increased. The accelerating voltage of the beam is quoted in kilovolts kV. It is now possible to purchase a 1,kV electron microscope, though this is not commonly found. The electron microscope is an integral part of many laboratories such as The John Innes Centre. Researchers can use it to examine biological materials such as microorganisms and cells , a variety of large molecules, medical biopsy samples, metals and crystalline structures, and the characteristics of various surfaces. Nowadays, electron microscopes have many other uses outside research. They can be used as part of a production line, such as in the fabrication of silicon chips, or within forensics laboratories for looking at samples such as gunshot residues. In the arena of fault diagnosis and quality control, they can be used to look for stress lines in engine parts or simply to check the ratio of air to solids in ice cream! Transmission Electron Microscope TEM The original form of electron microscopy,

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Transmission electron microscopy TEM involves a high voltage electron beam emitted by a cathode and formed by magnetic lenses. The electron beam that has been partially transmitted through the very thin and so semitransparent for electrons specimen carries information about the structure of the specimen. The spatial variation in this information the "image" is then magnified by a series of magnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or light sensitive sensor such as a CCD charge-coupled device camera. The image detected by the CCD may be displayed in real time on a monitor or computer. Transmission electron microscopes produce two-dimensional, black and white images. Resolution of the TEM is also limited by spherical and chromatic aberration, but a new generation of aberration correctors has been able to overcome or limit these aberrations. Software correction of spherical aberration has allowed the production of images with sufficient resolution to show carbon atoms in diamond separated by only 0. The ability to determine the positions of atoms within materials has made the TEM an indispensable tool for nano-technologies research and development in many fields, including heterogeneous catalysis and the development of semiconductor devices for electronics and photonics. In the life sciences, it is still mainly the specimen preparation which limits the resolution of what we can see in the electron microscope, rather than the microscope itself. We have two digital cameras on it, one is higher resolution than the other, so that the need for developing and printing film has been negated. Our TEM is designed for use with biological samples and is capable of resolving to better than 1nm. It is also capable of 3-D tomography which involves taking a succession of images whilst tilting the specimens through increasing angles, which can then be combined to form a three-dimensional image of the specimen. Scanning Electron Microscope SEM Unlike the TEM, where the electrons in the primary beam are transmitted through the sample, the Scanning Electron Microscope SEM produces images by detecting secondary electrons which are emitted from the surface due to excitation by the primary electron beam. In the SEM, the electron beam is scanned across the surface of the sample in a raster pattern, with detectors building up an image by mapping the detected signals with beam position. Our TEM can easily resolve details of 0. Our two SEMs at JIC are both relatively recent acquisitions and are high-resolution instruments capable of about 2 nm resolution on biological samples. Because the SEM image relies on electron interactions at the surface rather than transmission it is able to image bulk samples and has a much greater depth of view, and so can produce images that are a good representation of the 3D structure of the sample. SEM images are therefore considered to provide us with 3D, topographical information about the sample surface but will still always be only in black and white. In the SEM, we use much lower accelerating voltages to prevent beam penetration into the sample since what we require is generation of the secondary electrons from the true surface structure of a sample. At JIC we currently have two SEMs, both with high-resolution capabilities, digital imaging facilities and cryo-systems which enable them to be used for looking at frozen-hydrated specimens. Sample Preparation Materials to be viewed in an electron microscope generally require processing to produce a suitable sample. This is mainly because the whole of the inside of an electron microscope is under high vacuum in order to enable the electron beam to travel in straight lines. The technique required varies depending on the specimen, the analysis required and the type of microscope: Cryofixation - freezing a specimen rapidly, typically to liquid nitrogen temperatures or below, that the water forms ice. This preserves the specimen in a snapshot of its solution state with the minimal of artefacts. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy, it is now possible to observe virtually any biological specimen close to its native state. Fixation - a general term used to describe the process of preserving a sample at a moment in time and to prevent further deterioration so that it appears as close as possible to what it would be like in the living state, although it is now dead. In chemical fixation for electron microscopy, glutaraldehyde is often used to crosslink protein molecules and osmium tetroxide to preserve lipids. Dehydration - removing water from the samples. The water is generally replaced with organic solvents such as ethanol or acetone as a stepping stone towards total drying for SEM specimens or infiltration with resin and subsequent embedding for TEM specimens. Embedding - infiltration of the tissue with wax for light microscopy or a resin for electron microscopy such as

araldite or LR White, which can then be polymerised into a hardened block for subsequent sectioning. Sectioning - the production of thin slices of the specimen. For light microscopy, the sections can be a few micrometres thick but for electron microscopy they must be very thin so that they are semitransparent to electrons, typically around 90nm. These ultra-thin sections for electron microscopy are cut on an ultramicrotome with a glass or diamond knife. Glass knives can easily be made in the laboratory and are much cheaper than diamond, but they blunt very quickly and therefore need replacing frequently. Staining - uses heavy metals such as lead and uranium to scatter imaging electrons and thus give contrast between different structures, since many especially biological materials are nearly "transparent" to the electron beam. By staining the samples with heavy metals, we add electron density to it which results in there being more interactions between the electrons in the primary beam and those of the sample, which in turn provides us with contrast in the resultant image. Freeze-fracture and freeze-etch - a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The fresh tissue or cell suspension is frozen rapidly cryofixed , then fractured by simply breaking or by using a microtome while maintained at liquid nitrogen temperature. For the SEM, the sample is now ready for imaging. A second coat of carbon, evaporated perpendicular to the average surface plane is generally performed to improve stability of the replica coating. The specimen is returned to room temperature and pressure, and then the extremely fragile "shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The floating replica is thoroughly washed from residual chemicals, carefully picked up on an EM grid, dried then viewed in the TEM. Sputter Coating - an ultra-thin coating of electrically-conducting material, deposited by low vacuum coating of the sample. This is done to prevent charging of the specimen which would occur because of the accumulation of static electric fields due to the electron irradiation required during imaging. It also increases the amount of secondary electrons that can be detected from the surface of the sample in the SEM and therefore increases the signal to noise ratio. Disadvantages of Electron Microscopy Electron microscopes are very expensive to buy and maintain. They are dynamic rather than static in their operation: As they are very sensitive to vibration and external magnetic fields, microscopes aimed at achieving high resolutions must be housed in buildings with special services. A significant amount of training is required in order to operate an electron microscope successfully and electron microscopy is considered a specialised skill. The samples have to be viewed in a vacuum, as the molecules that make up air would scatter the electrons. This means that the samples need to be specially prepared by sometimes lengthy and difficult techniques to withstand the environment inside an electron microscope. Recent advances have allowed some hydrated samples to be imaged using an environmental scanning electron microscope, but the applications for this type of imaging are still limited. Artefacts It must be emphasised from the outset that every electron micrograph is, in a sense, an artefact. Changes in the ultra-structure are inevitable during all the steps of processing that samples must undergo: The best thing we can do is to keep these changes to a minimum by understanding the processes involved so that we make informed choices of the best preparative procedures to use for each sample. Artefacts present themselves in many ways: With experience, microscopists learn to recognise the difference between an artefact of preparation and true structure, mainly by looking at the same or similar specimens prepared in the same or a different way. Scanning electron microscopes usually image conductive or semi-conductive materials best. Non-conductive materials can be imaged, either by an environmental scanning electron microscope or more usually by coating the sample with a conductive layer of metal. A common preparation technique is to coat the sample with a layer of conductive material, a few nanometers thick, such as 10nm of gold, from a sputtering machine. This process does, however, have the potential to disturb delicate samples and cover some detail. When using chemical fixation and dehydration as part of the sample preparation, there is often much shrinkage and collapse of delicate structures and so, especially for our interests at JIC in botanical specimens which are highly hydrated, we tend to use the cryo-fixation technique which is far less prone to artefacts. For the TEM, samples are generally prepared by exposure to many nasty chemicals, in

order to give good ultra-structural detail which may result in artefacts purely as a result of preparation. This gives the problem of distinguishing artefacts from genuine structures within the specimen, particularly in biological samples. Scientists maintain that the results from various preparation techniques have been compared, and as there is no reason that they should all produce similar artefacts, it is therefore reasonable to believe that electron microscopy features correlate with living cells. In addition, higher resolution work has been directly compared to results from X-ray crystallography, providing independent confirmation of the validity of this technique. Recent work performed on unfixed, vitrified rapidly frozen, without the use of any chemicals, to form ice without any crystallisation specimens has also been performed, further confirming the validity of this technique.

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