ELECTRON MICROSCOPY ANALYSIS 2001 pdf

1: Transmission electron microscopy for wood and fiber analysis â€“ A Review :: BioResources

Electron Microscopy and Analysis deals with several sophisticated techniques for magnifying images of very small objects by large amounts - especially in a physical science context.

Analytical techniques and sample preparation methods are used to localize substructures of the cell wall polymers and are discussed in this review. The ultrastructural features of the wood cell walls, the structures formed by microfibrils, and the distribution of cell wall polymers, as revealed by TEM, are covered. Research investigating the distribution of lignin in tension and compression woods using TEM is reviewed. Different kinds of wood biodegrading enzymes localized using TEM are mentioned. Lastly, a comparison between TEM and other imaging techniques used for wood and fiber research are made. Thus, this review provides insight into the contribution of TEM in wood research since its invention and demonstrates how to use it more effectively in the future. Box , FI Aalto, Finland; b: Box , FI Aalto, Finland; c: Electron microscopy and its supplementary techniques have been used extensively in most scientific fields. In addition, microscopy has been and is currently being used successfully in wood research to observe wood cells and their sub-cellular components. The invention of the light microscope led to the discovery and description of cells, and it remains influential in wood research. Bucur reviewed existing imaging methods for investigating wood structure; however, most of the techniques exhibited low resolution. Nevertheless, transmission electron microscopy, a technique that has been around for approximately 80 years, has one of the highest resolutions about 0. For wood materials, the characteristic dimension of ultrastructural features, which influence the effective material properties, is in the range of few nanometers. Moreover, the formation of three main components cellulose, hemicelluloses and lignin and their deposition in the wood cell is still fragmentarily known. Therefore, TEM, a package of 2D imaging, 3D tomography, and elemental analysis with high-resolution, can certainly be used more effectively to understand the sub-cellular structures of wood cells. TEM has already provided invaluable information on ultrastructure of wood cell wall following its development. However, sample preparation and stability under the electron beam have always been challenging steps to overcome for high-resolution imaging of wood specimens at the molecular level. In this review, we have assessed the majority of the information available for TEM analysis of wood materials necessary to overcome complications during analysis. Core areas of evaluation will pertain to the sample preparation, the ultrastructural features of the wood cell wall, and the distribution of cell wall polymers as revealed by TEM. The analytical potential of TEM can essentially be amplified using complementary instruments, and thus these will also be briefly reviewed. Briefly, TEM uses an electron beam to image the sample. This provides a higher resolving power than the visible light in optical microscopy. Because the wavelength of the energized electron beam is very short, the diffraction limit is correspondingly lower. Generally, in electron microscopy, high energy primary electrons hit the specimen and the same or different electrons deflect from the sample to form an image. In TEM, a stationary primary electron beam is transmitted through the ultrathin specimen and transformed into a non-uniform electron intensity after transmission or scattering by the specimen Fig. Schematic diagram showing the mechanism of image formation in bright-field imaging mode in TEM. Dashed lines show the scattered electrons. This non-uniform electron intensity hits the fluorescent screen or the electron detector and is translated into image contrast on the screen. Either the direct beam or a diffracted beam is used to form bright-field and dark-field images, respectively. Figure 1 illustrates the mechanism of image formation in the bright-field imaging mode using direct beam. In the bright-field mode, scattered electrons are blocked with an objective aperture in order to enhance the contrast. In addition, while interacting with the specimen, a wide range of secondary signals are produced. Many of them are used in analytical electron microscopy, providing the chemical composition and additional information about the specimens. A TEM analysis must be run under an ultra-high vacuum that prevents scattering of the electron beam by the gas molecules so that the electrons can move freely from the gun through the specimen and further to the detector. Because of the extraneous materials, low crystallinity, and tight association of cell wall materials it has always been challenging to image the structure and morphology of the wood cell wall. A comparison among different cellulose specimens and their sensitivity to

the radiation damage at different accelerating voltages is illustrated in Fig. It shows that the radiation damage caused by the energized electron beam can be reduced to an extent using the higher accelerating voltage of the microscope. The advancement of cryo-TEM also provided the opportunity to image beam sensitive specimens with heavy electron dose. Nowadays, it is possible to keep the specimen temperature below 20 K using liquid helium during the imaging.

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2: Electron Microscopy - A bridge between research and industry - STMicroelectronics

Summary Electron microscopy is now a mainstay characterization tool for solid state physicists and chemists as well as materials scientists. Electron Microscopy and Analysis presents a useful snapshot of the latest developments in instrumentation, analysis techniques, and applications of electron and scanning probe microscopies.

Ultramicrotome for ultrathin sectioning Leica, model: Immerse the pinched larvae in ml HL3 solution Recipe 1 to the Sylgard dish. Section the dorsal side longitudinally and remove the internal organs intestines, fat bodies and trachea. Stretch and pin the rest of the L3. Several muscles could be used. In this study experiments were performed at Ib boutons in muscles 6 and 7. Figure 1A Figure 1. High magnification image of muscle fibers 7, 6, 13, 12 and V-shaped; B. Image of the ventral muscles in L3 where a nerve black arrowhead is sucked into the suction electrode connected to a stimulator; C and D. Adjusting the illumination times of the pre-fixed sample loaded with FM WT larvae loaded for 30 sec. The DIC image shows that the illumination time of 9 min produces a successful photoconversion. Synaptic boutons can be identified as dark spots barely visible in the surface of the muscle white arrows. The illumination time of 15 min is excessive, as evident from strong DAB precipitation, observed as large dark spots over the length of the NMJ white arrows. Perform the following steps in low light conditions to protect the fluorophore component of FM dye. Cover the FM dye with foil or work in the dark at room temperature. For loading in the absence of stimulation spontaneous recycling pool leave the preparation for the time of loading 10, 30, , and sec were used in our studies. For active loading, using the head stage controllers pull the nerve into a suction electrode connected to a stimulator. Suck the end of the nerve. Use the stimulation protocol for low-frequency stimulation, 5 min, 5 Hz. Quickly remove the FM dye once the stimulation is complete Figure 1B. The technique as adopted from Verstreken et al. Fixation Prefix the preparation Recipe 2 for 15 min at room temperature. Leave the preparation covered in mM NH4Cl for 10 min to quench autofluorescence of the fixative. Place the sample under an epifluorescent microscope. Identify the area of interest under 60x water immersion objective, at room temperature. The dye will bleach with time. Observe the process of photoconversion by switching periodically to the bright field. As photoconversion takes place, dark brown DAB precipitate localizes at the illuminated area. The illumination time needs to be adjusted, as illustrated in Figures 1C and 1D. Immerse the preparation in the fixative solution Recipe 4 and fix the sample in BioWave for 2 min at W. Place the temperature insert close to the preparation to monitor temperature fluctuations. Unpin the preparation carefully from the Sylgard dish. Transfer the preparation into a sample bottle with a snap cap. Wash the sample in 90 mM sodium cacodylate buffer, pH 7. EM sample processing Note: The following steps unless specified are performed at room temperature. The unused solution can be stored refrigerated for up to 1 month. Perform all steps under a fume hood, using gloves and long sleeve lab coat. Note 4 Wash with 90 mM cacodylate buffer for 5 min. Wash with deionized water for 5 min. The unused solution can be stored refrigerated for up to 2 weeks. Wash with deionized water for 5 min twice. Add it to the preparation under continuous rotation for 1 h or overnight. Keep the sample in embedding resin for at least 18 h overnight. Under a stereomicroscope, place the preparation over ACLAR film, and then identify and trim the area of interest. Position an embedding capsule filled with polymerized resin upside-down on the top of the specimen. This will leave sample attached to the embedding capsule. Thin sectioning and EM imaging Remove the block from the embedding capsule and mount the block into a specimen holder of an ultramicrotome. Under a stereomicroscope, mark the area of interest with a razor blade. The marked area should be a trapezoid that would fit onto the slot grid. Trim the block over the outlined area. Insert specimen holder with the block in the ultramicrotome and align the long edge of the trapezoid to the trimming knife edge. Repeat the same for the short sides of the trapezoid Figure 2 and Videos Align the block face exactly parallel to the edge of the diamond sectioning knife. Cut thin sections nm. Collect individual sections or collect sections in series and place them on the 2 x 1 mm slot grids. Allow the sections to dry before taking images. Image the sections using conventional TEM. Sample block fine trimming. Upon rough block trimming with razor blade and elimination of resin the face of the block is shaped with trimming knife. The edge of the knife is aligned to the trapezoid top. Vertically positioned trapezoid with

the left edge of the trimming knife aligned to the top of the trapezoid. Fine trimmed trapezoid with parallel top and bottom, and slightly tilted sides. The paralleled top and bottom are important for forming a ribbon during serial sectioning. Rough trapezoid trimming Video 2. Fine trapezoid trimming, part one. Fine trapezoid trimming, part 2. Once sectioned the trapezoid top the trimming knife is retracted and positioned to trapezoid bottom without additional alignment. Fine trapezoid trimming, part 3. In contrast, the recycling pool observed in WT boutons during spontaneous loading was negligibly small data not shown, see Sabeva et al. Representative micrographs showing the recycling vesicle pools. Photoconverted labelled vesicles black , non-labelled vesicles grey. Detailed data processing analysis and replicates including applied statistical tests could be found in the original manuscript, see Sabeva et al. It works as a dye scavenger and reduces background fluorescence. Prepare in a new disposable ml beaker by mixing equal volumes of Embedding mix A Recipe 5 and Embedding mix B Recipe 6. Use a wooden tongue depressor to mix thoroughly. Degas eliminate the air bubbles for 5 min in a vacuum desiccator or leave 30 min at room temperature. All steps during EM sample processing are performed in shaker or sample rotator.

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3: Electron Microscopy

Electron microscopy is now a mainstay characterization tool for solid state physicists and chemists as well as materials scientists. Electron Microscopy and Analysis presents a useful snapshot of the latest developments in instrumentation, analysis techniques, and applications of electron and scanning probe microscopies.

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. 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.

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4: David McComb | Electron Microscopy and Analysis (CEMAS)

This book is a compilation of papers presented at the EMAG (Electron Microscopy and Analysis Group of the Institute of Physics) conference held September , at the University of Dundee in Scotland.

References Abstract Electron microscopy is a technology for examining the extremely fine detail or ultrastructure of biological specimens for use in research and medical situations. A scanning electron microscope SEM with capabilities for examining a hydrated specimen in a partial vacuum. Salmonella bacteria adhering to the surface of a rayon fibre. A chain of bacterial cells as viewed in the TEM. These bacteria, Streptococcus mutans, are responsible for causing tooth decay. These cells have been prepared and ultrathin sections of 70 nm have been cut through the cells. These bacteria are 0. A typical plant cell as seen in the TEM. Ultrathin sections reveal the interior of the cell in great detail. Courtesy of Microscopy Society of America. Sectioned mammalian cells as viewed in the TEM. These cells are infected with a tumour virus, with some virus particles visible in the space between the two cells arrow. N, nucleus; Nu, nucleolus. This membrane is derived from the host cell and is approximately 8 nm in thickness. A herpesvirus particle from a patient suffering from shingles. This virus is responsible for causing both chickenpox and shingles. The central part of the virus particle, the capsid, is approximately nm in size. Diagram showing the various parts of the virus particle in Figure. Principles and Techniques for Biologists, 2nd edn. Theory, Techniques, and Troubleshooting. WH Freeman and Co.

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5: Scanning electron microscope - Wikipedia

A comparison of the list of acronyms in the third edition with the list of abbreviations in the second edition gives an interesting guide to the way in which electron microscopy and analysis has developed since the late s.

Ardenne applied the scanning principle not only to achieve magnification but also to purposefully eliminate the chromatic aberration otherwise inherent in the electron microscope. He further discussed the various detection modes, possibilities and theory of SEM, [6] together with the construction of the first high magnification SEM. Principles and capacities[edit] The signals used by a scanning electron microscope to produce an image result from interactions of the electron beam with atoms at various depths within the sample. Various types of signals are produced including secondary electrons SE , reflected or back-scattered electrons BSE , characteristic X-rays and light cathodoluminescence CL , absorbed current specimen current and transmitted electrons. Secondary electron detectors are standard equipment in all SEMs, but it is rare for a single machine to have detectors for all other possible signals. In secondary electron imaging SEI , the secondary electrons are emitted from very close to the specimen surface. Consequently, SEI can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. Back-scattered electrons BSE are beam electrons that are reflected from the sample by elastic scattering. They emerge from deeper locations within the specimen and, consequently, the resolution of BSE images is less than SE images. However, BSE are often used in analytical SEM, along with the spectra made from the characteristic X-rays, because the intensity of the BSE signal is strongly related to the atomic number Z of the specimen. BSE images can provide information about the distribution, but not the identity, of different elements in the sample. The energy or wavelength of these characteristic X-rays can be measured by Energy-dispersive X-ray spectroscopy or Wavelength-dispersive X-ray spectroscopy and used to identify and measure the abundance of elements in the sample and map their distribution. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. A wide range of magnifications is possible, from about 10 times about equivalent to that of a powerful hand-lens to more than , times, about times the magnification limit of the best light microscopes. No conductive coating was applied: SEM samples are prepared to withstand the vacuum conditions and the high energy beam of electrons, and have to be small enough to fit on the specimen stage. Samples are generally mounted rigidly to a specimen holder or stub using a conductive adhesive. Nonconductive specimens collect charge when scanned by the electron beam, and especially in secondary electron imaging mode, this causes scanning faults and other image artifacts. For conventional imaging in the SEM, specimens must be electrically conductive , at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge. Metal objects require little special preparation for SEM except for cleaning and conductively mounting to a specimen stub. Non-conducting materials are usually coated with an ultrathin coating of electrically conducting material, deposited on the sample either by low-vacuum sputter coating or by high-vacuum evaporation. The improvement arises because secondary electron emission for high-Z materials is enhanced. An alternative to coating for some biological samples is to increase the bulk conductivity of the material by impregnation with osmium using variants of the OTO staining method O-osmium tetroxide , T- thiocarbohydrazide , O-osmium. The high-pressure region around the sample in the ESEM neutralizes charge and provides an amplification of the secondary electron signal. To prevent charging of non-conductive specimens, operating conditions must be adjusted such that the incoming beam current is equal to sum of outcoming secondary and backscattered electrons currents a condition that is more often met at accelerating voltages of 0. This technique is achieved in two steps: The main preparation techniques are not required in the environmental SEM outlined below, but some biological specimens can benefit from fixation. Biological samples[edit] For SEM, a specimen is normally required to be completely dry, since the specimen chamber is at high vacuum. Hard, dry materials such as wood, bone, feathers, dried insects, or shells including egg shells [19] can be examined with little further treatment, but living cells and tissues and whole, soft-bodied organisms require chemical fixation to preserve and stabilize their structure. Fixation is usually

performed by incubation in a solution of a buffered chemical fixative, such as glutaraldehyde , sometimes in combination with formaldehyde [20] [21] [22] and other fixatives, [23] and optionally followed by postfixation with osmium tetroxide. Because air-drying causes collapse and shrinkage, this is commonly achieved by replacement of water in the cells with organic solvents such as ethanol or acetone , and replacement of these solvents in turn with a transitional fluid such as liquid carbon dioxide by critical point drying. If the SEM is equipped with a cold stage for cryo microscopy, cryofixation may be used and low-temperature scanning electron microscopy performed on the cryogenically fixed specimens. The preparation method reveals the proteins embedded in the lipid bilayer. This section does not cite any sources. Please help improve this section by adding citations to reliable sources. Unsourced material may be challenged and removed. February Learn how and when to remove this template message Back-scattered electron imaging, quantitative X-ray analysis, and X-ray mapping of specimens often requires grinding and polishing the surfaces to an ultra smooth surface. In general, metals are not coated prior to imaging in the SEM because they are conductive and provide their own pathway to ground. Fractography is the study of fractured surfaces that can be done on a light microscope or, commonly, on an SEM. The fractured surface is cut to a suitable size, cleaned of any organic residues, and mounted on a specimen holder for viewing in the SEM. Integrated circuits may be cut with a focused ion beam FIB or other ion beam milling instrument for viewing in the SEM. Special high-resolution coating techniques are required for high-magnification imaging of inorganic thin films. Scanning process and image formation[edit] Schematic of an SEM In a typical SEM, an electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. Tungsten is normally used in thermionic electron guns because it has the highest melting point and lowest vapor pressure of all metals, thereby allowing it to be electrically heated for electron emission, and because of its low cost. Other types of electron emitters include lanthanum hexaboride LaB 6 cathodes, which can be used in a standard tungsten filament SEM if the vacuum system is upgraded or field emission guns FEG , which may be of the cold-cathode type using tungsten single crystal emitters or the thermally assisted Schottky type, that use emitters of zirconium oxide. The electron beam, which typically has an energy ranging from 0. The beam passes through pairs of scanning coils or pairs of deflector plates in the electron column, typically in the final lens, which deflect the beam in the x and y axes so that it scans in a raster fashion over a rectangular area of the sample surface. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons by elastic scattering, emission of secondary electrons by inelastic scattering and the emission of electromagnetic radiation , each of which can be detected by specialized detectors. The beam current absorbed by the specimen can also be detected and used to create images of the distribution of specimen current. Electronic amplifiers of various types are used to amplify the signals, which are displayed as variations in brightness on a computer monitor or, for vintage models, on a cathode ray tube. Each pixel of computer video memory is synchronized with the position of the beam on the specimen in the microscope, and the resulting image is, therefore, a distribution map of the intensity of the signal being emitted from the scanned area of the specimen. Older microscopes captured images on film, but most modern instrument collect digital images. Low-temperature SEM magnification series for a snow crystal. The crystals are captured, stored, and sputter-coated with platinum at cryogenic temperatures for imaging. Magnification[edit] Magnification in an SEM can be controlled over a range of about 6 orders of magnitude from about 10 to , times. Unlike optical and transmission electron microscopes, image magnification in an SEM is not a function of the power of the objective lens. SEMs may have condenser and objective lenses, but their function is to focus the beam to a spot, and not to image the specimen. Provided the electron gun can generate a beam with sufficiently small diameter, an SEM could in principle work entirely without condenser or objective lenses, although it might not be very versatile or achieve very high resolution. In an SEM, as in scanning probe microscopy , magnification results from the ratio of the dimensions of the raster on the specimen and the raster on the display device. Assuming that the display screen has a fixed size, higher magnification results from reducing the size of the raster on the specimen, and vice versa. Magnification is therefore controlled by the current supplied to the x, y scanning coils, or the voltage supplied to the x, y deflector plates, and not by objective lens power. Due to their low energy, these electrons originate within a few nanometers from the

sample surface. The accelerated secondary electrons are now sufficiently energetic to cause the scintillator to emit flashes of light cathodoluminescence , which are conducted to a photomultiplier outside the SEM column via a light pipe and a window in the wall of the specimen chamber. The amplified electrical signal output by the photomultiplier is displayed as a two-dimensional intensity distribution that can be viewed and photographed on an analogue video display, or subjected to analog-to-digital conversion and displayed and saved as a digital image. This process relies on a raster-scanned primary beam. The brightness of the signal depends on the number of secondary electrons reaching the detector. If the beam enters the sample perpendicular to the surface, then the activated region is uniform about the axis of the beam and a certain number of electrons "escape" from within the sample. As the angle of incidence increases, the interaction volume increases and the "escape" distance of one side of the beam decreases, resulting in more secondary electrons being emitted from the sample. Thus steep surfaces and edges tend to be brighter than flat surfaces, which results in images with a well-defined, three-dimensional appearance. Using the signal of secondary electrons image resolution less than 0. Detection of backscattered electrons[edit] Comparison of SEM techniques: Since heavy elements high atomic number backscatter electrons more strongly than light elements low atomic number , and thus appear brighter in the image, BSEs are used to detect contrast between areas with different chemical compositions. Dedicated backscattered electron detectors are positioned above the sample in a "doughnut" type arrangement, concentric with the electron beam, maximizing the solid angle of collection. BSE detectors are usually either of scintillator or of semiconductor types. When all parts of the detector are used to collect electrons symmetrically about the beam, atomic number contrast is produced. However, strong topographic contrast is produced by collecting back-scattered electrons from one side above the specimen using an asymmetrical, directional BSE detector; the resulting contrast appears as illumination of the topography from that side. Semiconductor detectors can be made in radial segments that can be switched in or out to control the type of contrast produced and its directionality. Backscattered electrons can also be used to form an electron backscatter diffraction EBSD image that can be used to determine the crystallographic structure of the specimen. The high-energy electrons from the SEM beam will inject charge carriers into the semiconductor. Thus, beam electrons lose energy by promoting electrons from the valence band into the conduction band , leaving behind holes. In a direct bandgap material, recombination of these electron-hole pairs will result in cathodoluminescence; if the sample contains an internal electric field, such as is present at a p-n junction , the SEM beam injection of carriers will cause electron beam induced current EBIC to flow. Cathodoluminescence and EBIC are referred to as "beam-injection" techniques, and are very powerful probes of the optoelectronic behavior of semiconductors, in particular for studying nanoscale features and defects. The blue and green channels represent real colors, the red channel corresponds to UV emission. Cathodoluminescence , the emission of light when atoms excited by high-energy electrons return to their ground state, is analogous to UV -induced fluorescence , and some materials such as zinc sulfide and some fluorescent dyes, exhibit both phenomena. Over the last decades, cathodoluminescence was most commonly experienced as the light emission from the inner surface of the cathode ray tube in television sets and computer CRT monitors. In the SEM, CL detectors either collect all light emitted by the specimen or can analyse the wavelengths emitted by the specimen and display an emission spectrum or an image of the distribution of cathodoluminescence emitted by the specimen in real color. X-ray microanalysis[edit] Characteristic X-rays that are produced by the interaction of electrons with the sample may also be detected in an SEM equipped for energy-dispersive X-ray spectroscopy or wavelength dispersive X-ray spectroscopy. Analysis of the x-ray signals may be used to map the distribution and estimate the abundance of elements in the sample. Resolution of the SEM[edit] Play media A video illustrating a typical practical magnification range of a scanning electron microscope designed for biological specimens. Unlike in an optical system, the resolution is not limited by the diffraction limit , fineness of lenses or mirrors or detector array resolution. The focusing optics can be large and coarse, and the SE detector is fist-sized and simply detects current. Instead, the spatial resolution of the SEM depends on the size of the electron spot, which in turn depends on both the wavelength of the electrons and the electron-optical system that produces the scanning beam. The resolution is also limited by the size of the interaction volume, the volume of specimen material that interacts with the

electron beam. The spot size and the interaction volume are both large compared to the distances between atoms, so the resolution of the SEM is not high enough to image individual atoms, as is possible with transmission electron microscope TEM. The SEM has compensating advantages, though, including the ability to image a comparatively large area of the specimen; the ability to image bulk materials not just thin films or foils ; and the variety of analytical modes available for measuring the composition and properties of the specimen. Environmental scanning electron microscope Conventional SEM requires samples to be imaged under vacuum , because a gas atmosphere rapidly spreads and attenuates electron beams. As a consequence, samples that produce a significant amount of vapour , e. Processes involving phase transitions , such as the drying of adhesives or melting of alloys , liquid transport, chemical reactions, and solid-air-gas systems, in general cannot be observed. Some observations of living insects have been possible however.

6: Electron Microscopy and Analysis - CRC Press Book

Electron microscopy is a mainstay characterization tool for solid state physicists and chemists as well as materials scientists. This book presents a useful snapshot of the developments in.

7: David McComb | Center for Electron Microscopy and Analysis (CEMAS)

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*Electron Microscopy and Analysis [Peter J. Goodhew, John Humphreys, Richard Beanland] on www.enganchecubano.com *FREE* shipping on qualifying offers. Electron Microscopy and Analysis deals with several sophisticated techniques for magnifying images of very small objects by large amounts - especially in a physical science context.*

9: Michael Mills | Electron Microscopy and Analysis (CEMAS)

Transmission electron microscopy (TEM) is the original form of electron microscopy and produces two-dimensional, black and white images. Unlike the light microscopes that use glass lenses, electromagnetic and/or electrostatic lenses are used in all electron microscopes to control the path of electrons.

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