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Interactions, Imaging and Spectra in SEM

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1. Introduction

In microscopy the question arises- Why employ electron beams instead of light beams to produce magnified images and the answer has to do with resolution. When doing microscopy to produce magnified image of objects, diffraction (bending of waves around narrow openings and obstacles) limits the resolution and hence the quality of image in terms of fine details one can see. The optical wavelengths from deep UV to IR are in range of hundreds of nanometers while electron beam of energy in keV have wavelengths in fractions of nanometers. The dependence of diffraction on the wavelength of the beam makes electron beam more suitable than beams of wavelengths in the optical region. The diffraction also depends on the size of the objects. A Scanning Electron Microscope (SEM) with electron beams in the keV range allows one to produce image (Fig. 1) of objects in the micro to nanometer range with relatively lower diffraction effects. Using a SEM to produce proper image requires a judicious choice of beam energy, intensity, width and proper preparation of the sample being studied. The electron beam in a SEM is nowadays generated using a field emission filament that uses ideas of quantum tunneling. Other methods are also available. The deflection of electron beam of certain energy E is accomplished by means of electromagnetic lenses. Typical E values for conventional SEM can range from as low as 2-5 keV to 20-40 keV.

A basic SEM consists of an electron gun (field emission type or others) that produces the electron beams, electromagnetic optics guide the beam and focus it. The detectors collect the electrons that come from the sample (either direct scattering or emitted from the sample) and the energy of the detected electron together with their intensity (number density) and location of emission is used to put together image. Present day SEM also offer energy dispersive photon detectors that provide analysis of x-rays that are emitted from the specimen due to the interactions of incident electrons with the atoms of the sample.

2. Interaction

Assume that an electron beam of energy E , with a circular cross-section A and a beam current I is incident on a sample with atomic number Z . We will assume that the energy E is typically much less than 100 keV in the following discussions. As the electron beam enters the sample it interacts with the atoms of the samples. This interaction of the electrons is not confined to the surface layers only but also with the atoms and molecules inside. The electron interaction with the atom consists of coulomb attraction with the nuclear positive

charge. The interaction of the electron beam with the electrons from the sample is of repulsive nature as the electrons are deflected by the target electrons. The electrons can undergo change in momentum and/or change in energy or both in these interactions. So an entering electron beam can scatter elastically and/or inelastically.

Fig. 1. Biological sample showing kT pores imaged with 20 keV electron beam using a quad backscattered detector. Scale shown by line of 100 μm .

2.1 Elastic scattering

If the scattering involves no loss of energy it is Rutherford scattering (Rutherford, 1911,1914) which is peaked in the forward direction with the probability of scattering decreasing dramatically with increase of angle of scattering and the electron trajectory is modified from some small angle elastic scattering to large angle deviation. Some of the electrons can travel laterally while others can even back scatter. After many of these events it is possible for

some of the electrons to leave the sample and these backscattered electrons provide one way of imaging the sample. Probability of elastic scattering depends on inverse square of energy E which means a higher energy beam will start to spread out much later in its path than a smaller energy beam. An electron can transfer energy to the conduction electrons or to a single valence electron – but this will not be important in SEM imaging as the mean free paths for both of these is large, the scattering angles are small and energy loss less than an eV.

2.2 Inelastic scattering

An electron can interact with the solid as a whole generating vibrations (phonon scattering). The energy of the electron goes into overall heating of the solid slightly. The overall energy loss is less than 1 eV and this channel is probably more important near the end of the path of the electron. The scattering results in electron being scattered by larger angle. This effect will be important for image resolution and contrast. The energy loss from inelastic scattering is related inversely with E therefore a higher energy incident electron will keep more of its energy at a depth than a lower energy incident electron at the same depth. If the scattering involves loss of energy then it cannot be described by Rutherford formula. There are many channels by which an electron can lose energy in a sample but here we will look at some that are more pertinent for SEM imaging.

The channels that are useful for imaging are the ones that results in radiative or non-radiative transitions to occur in the sample atom. This is when the electron transfers energy to one of the inner shell electrons and then this result in ionization or electronic rearrangement. The atom that absorbs the energy this way will either give out a photon (radiative process) or eject an electron from same or different shell (Auger process- non-radiative). The radiative photon is generally in the x-ray region of electromagnetic spectrum. The probability of radiative versus non-radiative process taking place defines the fluorescence yield ω . In energy dispersive analysis of a sample using SEM- ω plays an important role in conversion of x-ray intensities (from x-ray spectrum) into absolute numbers. These absolute numbers are related to sample elemental thicknesses and overall compositions.

2.3 Energy loss

The energy loss of the electron in scattering is dependent in a complex way on the atomic number Z of the sample atom, on their mass number A and the density ρ of those atoms. The energy lost by the electron can be transferred to the sample atoms in inelastic scattering. The rate of energy loss with the path length x , dE/dx , was described by Han Bethe (Bethe, 1930) mathematically. Calculations based on this formula suggest that dE/dx increases with Z while increasing E lowers this rate. The dependence on E is much more dramatic than with Z . Monte-Carlo type simulations (Metropolis & Ulam, 1949; Newberry & Myklebust, 1979; Rubinstein & Kroese, 2007) of trajectories of electrons (as they interact with the sample) suggest visualization in terms of an interaction volume. The size and depth of the volume is dependent on energy of the electron beam, their number density and the details about the interacting atoms. The probabilities of the electron interactions drops off by a large factor outside this volume.

2.4 Radiative and non-radiative mechanisms

The interaction between the incident electrons and the sample target atoms provides rich information about the chemical environment of the target atoms. This information is in the form of radiative and non-radiative transitions and subsequent emissions that take place in the atoms. The ion-atom collision results in transitions that involve energy transfer through the mechanisms (both radiative and non-radiative type). The radiative transitions in the atoms can lead to emission of photons mainly in the form of x-rays from K, L, M- shells. These x-rays are characteristics of the elements they come from and the x-ray spectra has signature to that effect. Recognizing these x-rays and then measuring them provides relative abundance of elements in the sample. To get an absolute value (e.g. # of atoms of one type as a fraction of all atoms) generally specified as parts per million (ppm)) normalization of the emission yields has to be done. This requires measuring the emission yields from the sample and from a standard sample under identical conditions so that ratios can be formed.. The standard must have been measured independently and sometimes with a different spectroscopic method (e.g. mass spectroscopy or infrared spectroscopy) and for it ppm needs to be available. The non-radiative transitions can result in emission of Auger electrons and Auger spectroscopy can provide information about the intensities there. Normally the standard SEM may not have capability of differentiating and measuring the auger electrons. What is done in that case is to use the value of fluorescence yield ω (which relates the radiative yields to non-radiative yields) and determine fraction of time an energy transfer to an atom will result in some form of radiative emission. The fluorescence yield then allows one to convert cross section for ionization into cross section for production of x-rays. The fluorescence yield factor F which is related to the ratio of radiative to non-radiative transition has to be carefully used or determined in the normalization procedure and plays a role in correction factors to get the absolute numbers. The correction factors take into account the fact that ratios of intensities are substantially different than the ratio of concentrations of elements in a sample. The atomic number Z and the mass absorption of x-rays in the volume of the sample A are the other two effects that go into the ZAF correction factor and they will be discussed in more detail later on..

2.5 Imaging

In usage the electron beam is incident on a target region from the specimen sample. The energy of the electron E , the mass density of the target, and the atomic number Z of the sample determines the relative intensities of various types of electron scattering. The penetration depth of the electrons, the mean free path and the strengths of different scattering (which are also dependent on both the Z and E) play a role in the information one gets (in the form of images) about the sample. Primarily the back scattering electrons provide an electronic signal that delineates the interaction volume and carries details about the scattering. In addition the information about the specimen is also comes from the production of secondary electrons from the sample.

3. X-ray imaging, analysis and other techniques

3.1 Elemental profile using SEM

Before one can do spectroscopy using a SEM, the sample has to be prepared correctly, mounted on special sample holders and oriented properly. Metallic stubs with sticky carbon

surface allows one to present the sample in a particular orientation to the beam. Samples that are placed on a goniometer can even be rotated to image the sample from a different direction. In a typical preparation of samples for SEM analysis: the sample has to be cleaned to remove contamination, dried in most cases and the surface to be analyzed prepared so that the analyzed surface is flat and electrically conducting. The cleaning starts with sample placed in ethanol baths. Part of this fixes the sample and also replaces the water content. For a biological sample -like a bone -first the bone has to be cleaned of most of soft tissues and then the remaining soft tissues are removed by placing the sample with dermestid beetles. The sample is observed under light microscope and if needed other techniques are used to remove any more soft tissue in the area of interest. More ethanol baths for different lengths of time and different concentration of ethanol may have be used. Cleaned samples are sectioned using high speed Dremil and other cutting tools. The surface to be analyzed has to be flat, smooth as possible and without any intruding parts in front of them. The samples are dried using the critical point dryer, if needed, and then sputter coated with Au to make them electrically conductive. For electron beam to be incident on the sample normally, the sample is placed on the mounting stub (with a sticky carbon tape exposed in the normal direction). The prepared flat cross section needs to be positioned correctly on the metal stub. This then ensures the proper orientation of the sample in the beam. The conductive gold layer allows the electrons a path to the local ground – absence of which will result in area of the sample acting as non-conductors (insulator). Electron beam incident, on the non-conductive area, will result in electrical charge getting collected. When seen in the SEM image, the area that is non-conducting will show up as whitish region with very less details to be seen. Over time the whitish area will get brighter losing even more details and also may grow in size (Figure 2). A layer of conducting metal like gold (few atom layer thick) will be sufficient to alleviate this charge clumping and in the SEM image the whitish appearance will disappear. If the image continue to show incomplete charge conduction from an area then a second layer of gold can help to minimize the charge clumping. In extreme cases, one has to use a lower energy and intense electron beam. One of the affect of an extra layer on a sample is to mask some of the features that are being imaged. Other difficulty that arises from a thicker coating of metal is x-ray interference. The metal coating (e.g. gold) emits characteristic x-rays from that metal. These x-rays can overlap partially the x-ray spectra coming from the sample being studied.

Samples that are to be studied in their original conditions have to be handled differently. Some of these are wet samples. Other samples that are not fixed and non- conductive create imaging problems that are tackled differently. These samples generally outgas in vacuum of the SEM chamber and have to be studied in a mode in SEM that allows for differential pumping in different sections of the SEM. For these samples high vacuum (like $\sim 10^{-6}$ Torr) cannot be achieved and so resolution is not as good and images are not as crystallized as a dry sample will do. But the SEM images will still provide details that are useful for the researcher.

Once the sample is placed in the SEM chamber and the detector is chosen (between secondary electron detector and/or backscattered electron detector) image is generated. The image details including the resolution are dependent on the energy of the electron beam type of sample, its geometry and atomic numbers of the atoms present. When the image

shows the proper details and is magnified correctly one can open the energy dispersive system to do x-ray spectroscopy. The Energy Dispersive Analysis (EDS) mode of the SEM provides the x-ray spectra for elemental analysis. In order to quantify the elemental yield one needs standard samples. For example in the study of bones, standards representing Calcium Phosphate, are used. Also to get a good calibration of the detector's response in the energy region being studied, other standard elemental samples are employed. For example a pure copper sample has L-shell x-rays around 1 keV and K-shell x-rays around 9 keV. A pure gold or lead sample will give M-shell x-rays in 2-3 keV range and L-shell x-rays around 10 keV. It is essential that the range of x-ray energies being studied be understood in terms of the response of the detector. This response also needs to be established for the range of electron beam energies to be used. The x-ray spectra from standards and from the samples are analyzed using software that is specially developed for analysis needed with corrections built in for various effects that may be important at some energies and not at others. FLAME (fuzzy logic software for spectral analysis and elemental ratio determination) is one of those software. The software, with statistical capabilities provides identification of the elements, atomic and weight percent of elements, intensities of the x-rays and other parameters that are electron beam and elemental atomic number dependent. The software generates a table showing the elemental ratios (weight and atomic) among the elements detected: e.g. oxygen, phosphorus, and calcium in the bone samples.

Fig. 2. SEM image of a biological sample(cephalotes) using quad backscattered detector. The sample was not sputter coated resulting in excessive charging(white area) on the sample.

3.2 ZAF correction factors

Castaing (Castaing, 1951, 1966; Castaing & Henoc, 1966) showed that the k-ratio, which is the ratio of sample x-ray intensity to standard sample x-ray intensities, is proportional to the ratio of the mass fraction of the sample element to that for the standard sample. But experimentation has shown that there are deviation of this k-ratio from the actual concentration ratios. These differences arise from many parameters of the sample but mainly density, electron backscattering, x-ray ionization and production cross section (these are connected by the fluorescence yield), energy loss of the electron beam and the absorption in the sample matrix. In samples that contain many elements and the mixture is not very homogeneous the measured intensity may vary by a large factor on variation in elastic, inelastic scatterings, and the absorption of the x-rays though the elements of the sample before reaching the detector. In general these various effects coming from the sample matrix on the measured intensity can be lumped into correction due to atomic number (the Z-effect), the absorption of the x-rays in the sample (the A effect) and the F effect due to x-ray fluorescence yield. In total the correction is called ZAF factor and in a simplified equation it is given by eq. (1) as

$$C_i/C_{(i-std)} = \{ZAF\}_i \cdot (I_i/I_{(i-std)}) \quad (1)$$

where C_i and $C_{(i-std)}$ are the fractional sample weight of element i and for the same element in the standard sample. Here $(I_i/I_{(i-std)})$ are the intensities as measured for the same element in sample and in the standard sample. In order to understand the Z, A and F factors, one has to visually assimilate the various processes taking place as an electron beam traverses the sample, loses energy by scattering processes and excitation of the host atoms of the sample takes place.

Z-factor: When an electron beam is backscattered, the backscattering mechanism removes part of beam of electrons which then reduces the number of interactions that can lead to ionization and production of x-rays. In samples with many elements the kinematics of scattering results in greater spread of the beam. The scattering results in greater spread in the energy for the scattered electron. Kinematics suggests that a greater number of electrons backscatter when atomic number Z is greater. The higher Z elements then remove a larger fraction of electron energies. The energy loss from inelastic scattering tends to remove electron energy due to thickness (defined as a product of the thickness as measured along the path and the density). The low atomic number remove this energy at a higher rate than higher atomic number. A Monte Carlo simulation of the trajectory of electron suggests that as the electron traverses a sample it is losing energy. The ionization of an atom and subsequent production of x-rays is critically dependent on if the energy available is above the excitation energy for the particular atom. So the energy may be enough to excite L-shell x-rays but not excite higher K-shell x-rays or in the heavy elements like gold the energy may excite M-shell x-rays but not L-shell x-rays and definitely not K-shell x-rays. During elastic scattering, the kinetic energy conservation tends to scatter electrons at larger angles and hence deviate from its path more. These scattered electron would be less likely to produce ionization and x-rays then if it did not interact elastically. Thus the distribution of the electron in the sample, their energies at a point in the sample and the x-ray production depends strongly on the atomic number of sample atoms. This distribution can be defined in terms of a function $\phi(\rho Z)$. An area under the plot of this function $\phi(\rho Z)$ versus ρZ allows

one to integrate for the intensities that would be generated. The atomic number effect (the Z-factor) for each element is then the ratio of this function $\phi(\rho Z)$ for the sample versus for the standard sample.

A-Factor: Inner shell ionization followed by x-ray production takes place over a range of thickness in the sample. The volume from which x-rays come from increases with energy of the incident electrons and scattered electrons can come from deeper region and overall a larger volume. Ionization followed by a radiative transfer of energy leads to the production of x-ray. The x-rays on their way to the detector gets absorbed by the matter they have to pass through. This absorption can be defined in terms of an exponential function. This exponential decrease is given as eq. (2)

$$I = I_0 e^{-\mu \rho t} \quad (2)$$

where I and I_0 are the intensity of the x-ray at the detector versus intensity when produced, μ is the mass absorption coefficient, ρ is the density of matter the x-ray passes through and t is the path length of this matter layer and ρt gives the thickness in units of mass per unit area. The exponential term representing the fraction by which incident intensity is reduced is calculated for each of the layers the x-rays have to pass through. The direction in which a generated x-ray has to travel to get to the detector defines the path length. This is related to the takeoff angle, the angle between the incident electron beam and the direction of the x-rays. The incident energy of the electron beam and the takeoff angle can affect the fraction absorption by a large factor. X-ray absorption factor A generally is the largest factor in the ZAF factor. Again the plot of $\phi(\rho t)$ versus with ρt is used to determine the A-factor from difference in area under the curves of ϕ for generated x-rays and for emitted one. The emitted x-ray intensity contains the absorption effect using the exponential law.

F-factor: In addition to x-rays being produced following ionization of the atoms by the electron beam, the x-rays themselves can fluoresce more x-rays from the atoms of the sample they pass near. The x-rays fluoresced have energies less than the energy of the x-ray (E_0) that fluoresced them. This has to do with the threshold excitation energy E_c needed for fluorescence. The fluorescing becomes negligible if E_0 is greater than E_c by 5 keV or more.

3.3 Comparative techniques

The x-ray spectra obtained from an SEM is analyzed with special software to determine the yield of x-rays. The spectra is generally shown as intensity versus the energy of the x-rays (Figure 3 and 4). The detector normally used in a SEM is a (Si(Li) detector with a resolution of about 140 eV at 5.9 keV for ^{54}Mn x-rays. This resolution is enough to resolve x-rays from adjacent elements and also can differentiate some of the individual transitions within the x-rays from the same element. Si(Li) detectors use a Silicon crystal which is Lithium doped (has to be cooled below liquid nitrogen temperatures for it to work). The response of the crystal to photons in the 1- 100 keV region is generally depicted with an efficiency curve. This curve shows the percent detection of the photons arriving in the active region of the detector. Other than the geometry of the detection system, a typical efficiency may be 1 out of 10000 (or 1% or less). The physical region between location where x-ray photons are generated and their passage through the in-between matter before reaching the active

silicon region of the detector determines the attenuation fraction of the original x-ray signal. In a typical SEM, this attenuation takes place in the layers of air (in the high vacuum chamber), beryllium window layer as the front window of the detector, the gold contact layer and the dead layer of silicon. This absorption and attenuation depends on the energy of the x-ray photon and also the thickness of each layer. For energies above 3-4 keV, the efficiency is smoothly varying (fairly constant in the 5-20 keV range). There are many calibrated photon sources available to measure the efficiency in this region. Experimentally measured efficiencies, together with that predicted and calculated from models are compared. The calculated efficiency includes the attenuation of photon intensities in the layers described above. measured and calculated efficiencies are normalized to each other using the measured energy point (Gallagher & Cipolla,1974; Lennard & Phillips, 1979; Papp, 2005; Maxwell & Campbell, 2005, Mehta et al., 2005)). This procedure results in normalized efficiency curves. The efficiency in the 5-20 keV region can be determined to uncertainties of few percent but for energies of x-rays in the 1-3 keV efficiency is lot more uncertain especially below 1 keV and there lies the problem.

Fig. 3. SEM Image (magnification x6670 and scale as shown) and x-ray spectrum showing L-shell (~1 keV) and K-shell (~ 9 keV) x-rays from zinc in a zinc oxide Nanowire. Also chlorine K_{α} and K_{β} can be seen as just resolved. The K-shell x-rays of zinc clearly show separated K_{α} and K_{β} peak with a peak intensity ratio of 4:1. Right side table show relative percentages of the elements in the sample (not corrected with k-ratio).

Fig. 4. SEM Image(from a box < 2 μm on the side) and x-ray spectrum showing L-shell (~1 keV) and K-shell (~ 9 keV) x-rays from zinc in a zinc oxide nanowire. The k-shell x-rays clearly show separated K_α and K_β peak with a ratio of 4:1. The image clearly shows the wires of ZnO.

The x-rays from K-shell of carbon, oxygen, up to sodium are all ~ 1 keV or less. L-shell x-rays below 1 keV come from elements Calcium ($Z=20$) through Zinc ($Z=30$) while M-shell x-rays are all less than 3.5 keV (highest M-shell x-rays for Uranium $Z=92$). For lanthanum ($Z=57$) the M-shell x-rays are less than 1 keV. The x-rays generated in an SEM are limited by the maximum energy the electrons can have. For a typical SEM that has a maximum voltage available for accelerating of say 20 kV – the electron beam has maximum possible energy of 20 keV. The x-rays generated from samples by such beams can then only be up to 20 keV. So depending upon the elements present in the sample, the x-ray data can give yields that are uncertain by above uncertainties. Yields can be converted to absolute numbers if the number of electrons involved in the generation of x-rays can be determined and standard samples for the elements are available. This leads to the realization that any absolute numbers have to be checked against absolute numbers from other comparable technique. Any normalization procedure among the techniques have to find a unique common point.

3.3.1 X-ray fluorescence (XRF)

For large Z elements ($Z > 45$) XRF (Bundel et al., 1992) can provide information about x-rays greater than 20 keV that the SEM cannot. XRF is used in that situation and again normalize K-shell x-ray production using XRF with L- or M-shell x-ray production by the electron beam of an SEM. Some of the analyzed samples are fluoresced using radioactive sources of Fe-55, Cm-244 and Am-241 in the XRF. EDS analysis from SEM is energy limited by the electron beam energy used, while XRF is not. XRF spectra is measured to provide x-ray measurements that are outside of the energy range of the SEM measurements. In addition, the lower energy L and M-shell x-rays are measured to provide another set of elemental ratio data. This allows for comparison between elemental ratios determined using SEM and XRF.

3.3.2 Neutron activation analysis

A standard neutron source (Pu-Be in a Howitzer or a neutron generator) can be used to do neutron activation work. The energy of the neutron beam and the flux coming from the source may determine if this technique can allow one to analyze a sample also analyzed with SEM. The incident energy of the neutrons from the source will determine if neutron-atom interaction can lead to compound nucleus formation. In order to see any particular decay mode from this compound nucleus, there has to be appropriate isotopes formed with half lives of transitions in that isotope suitable for decay measurement. Also the yield of these newly made isotopes will depend upon the cross section for absorption of the neutrons in the sample. In order to do neutron activation analysis (NAA), the table of isotopes is used to determine the isotopes that can be produced in activation of the samples. The suitability of the radiation these isotopes produce for analysis has to be established too. Once this is established the uncoated samples are prepared for neutron activation and activated for an optimum length of time. The activated samples are analyzed using gamma ray spectroscopy using a combination of Geiger counter, Sodium iodide detector and/or germanium type high resolution gamma detectors. Intensities of photo peaks can be used to form ratios in a particular photon energy range. This divides out any effect due to efficiency variation. Next taking into account other parameters (like neutron cross section, atomic number, branching ratio etcetera) and comparing the ratios of intensities from a standard sample and from the measured sample, a normalized absolute intensities can be

determined. For example standard samples can be used to provide a baseline for radioactivity measurements and dose dependent measurement of other standards to be used. This baseline can provide a scheme for normalizing the intensities from different samples. Comparison among elemental ratios determined using SEM, XRF and NAA is possible then.

3.3.3 Other comparative methods

Another technique that provides absolute weight and atomic percent of the elements in the samples is Particle Induced X-ray Emission (PIXE) (Flewitt & Wild, 2003). This is performed at an accelerator lab facility. PIXE analysis at an accelerator lab can be used to study biological samples using microprobe beam. The samples and standards are mounted as targets on special sample holders. Proton or alpha particle beams interacting with the targets provides an absolute value for weight percent and atomic percent of the elements in the samples. Again an elemental ratio from this technique can be compared to ratios from other techniques described earlier. The goal is to determine a normalization procedure that can be used to efficiently determine a normalized absolute weight or atomic percent of the elements in the sample. The reliability of the results and efficiency of the technique allows researchers to choose one of these techniques to produce reliable results using the normalization procedure established. The goal of any normalization technique is to decrease the uncertainties in the measurements including those done with SEM.

3.3.4 Statistical analysis

A crucial factor in coming to any conclusion in all these techniques is appropriate application of Statistical analysis. It is imperative to the researcher that they analyze the data using statistical package (e.g. student t-test or ANOVA) after establishing normal distribution of data and homogeneity of variances.

4. Conclusions

SEM is suitable to look at micro- and nano- structural characteristics of solid objects. Visual images obtained from electron detectors combined with characteristic x-rays mapping allow for detailed micro- and nano-compositional analysis. SEM combined with XRF,NAA and PIXE provide a platform to quantify and produce absolute numbers related to compositional elemental and molecular structures.

The sample that is to be investigated has to be specially prepared so as to provide images and spectral information meaningful to the investigation. Many factors play a role here: the type of sample (say biological sample versus a sample for material science study has to be prepared differently at some stage of preparation), the appropriate energy of the beam, angle of incidence, beam intensity (resolution will be affected greatly from this), the counting time and statistics and others. SEM imaging is done differently for a wet cell sample than a critically dried and sputter-coated solar cell slides.

The other crucial factor is the methodology or methodologies adopted for data analysis and the subsequent results determination. Once the images and the spectra have been collected, the data has to be sorted, analyzed and mathematical functionality recognized and

established. Statistical analysis then provides the basis for the eventual conclusions and their validity. For example topographical and/or compositional images can be used to generate structural patterns leading to understanding of type of crystalline lattice underlying a bone. This can then provide the basis for determining the strength of a bone or its elasticity or the reason a bone under microgravity conditions leads to Osteoporosis. SEM spectra that can be analyzed to determine the elemental composition of a certain bone have inherent uncertainties. When studying changes in bone composition these uncertainties will affect the determination of the conclusion.

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Today, an individual would be hard-pressed to find any science field that does not employ methods and instruments based on the use of fine focused electron and ion beams. Well instrumented and supplemented with advanced methods and techniques, SEMs provide possibilities not only of surface imaging but quantitative measurement of object topologies, local electrophysical characteristics of semiconductor structures and performing elemental analysis. Moreover, a fine focused e-beam is widely used for the creation of micro and nanostructures. The book's approach covers both theoretical and practical issues related to scanning electron microscopy. The book has 41 chapters, divided into six sections: Instrumentation, Methodology, Biology, Medicine, Material Science, Nanostructured Materials for Electronic Industry, Thin Films, Membranes, Ceramic, Geoscience, and Mineralogy. Each chapter, written by different authors, is a complete work which presupposes that readers have some background knowledge on the subject.

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