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A REVIEW ON THE FORENSIC APPLICATIONS OF SCANNING ELECTRON MICROSCOPE

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ABSTRACT

The scanning electron microscope (SEM) is a magnification technique that utilises electron beams to interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition. Forensic scientists have made significant contributions using SEM in various areas like examining excavated human remains, comparing soil traces from different crime scenes, GSR analysis etc. The use of traditional techniques for magnification i.e. optical microscopes was earlier used but now, in the modern era with the advancement of technology, the way of microscopically analyzing samples has changed tremendously. Now microscopic techniques are mostly used along with EDS/EDX for composition and elemental analysis for evidence like soil, GSR, hair, diatoms etc. The technique gives more detailed and accurate information which has led to the extensive use of electron microscopes in the field of exhibit analysis from the crime scene. This paper outlines the basic sample preparation and examination of forensic evidences using SEM and its application in forensic science along with its contributions by various researchers from India and abroad.

Keywords: Scanning Electron Microscope (SEM), Energy Dispersive X-Ray Spectroscopy (EDS/EDX), Gunshot Residue (GSR), Sodium Dodecyl Sulphate (SDS), Focused Ion Beam (FIB), Electron Probe Micro-Analyser (EPMA), Energy Dispersive X-Ray Analysis (EDAX).

1. INTRODUCTION

In Forensic analysis electron microscopy is widely used for both initial screening and final assessment of suspicious samples. There is a wide range of samples either manmade or natural subjected for analysis in the forensic laboratory by forensic experts which is related to case investigations(Saeida Saadat, 2020). Electron microscopy is used to magnify the details of evidence with the use of an electron beam as the illumination source. Transmission electron microscope and Scanning electron microscope are the most frequently used microscopes to magnify micro details of the sample which helps in case investigation (Omidi et al., 2017). SEM is often used for geomorphological investigation as it is a high-powered microscope. The electron microscope is usually coupled with other microanalytical techniques. This high-powered microscope has the ability to become an essential step in the analysis of even submicron or proton levels of investigation (Grime et al., 1991).

In forensic practice the most commonly analyzed evidence are –soil, glass, paint, document, hair, blood etc. Scanning electron microscope (SEM) is one of the most frequently used research and forensic analysis equipment for imaging microscopic details for over decades because of its diverse application and advanced technique. Good quality images of microscopic samples are produced using focused beam of electron which is later captured by scattered electrons, backscattered electrons, two different types of detectors (Omidi et al., 2017). In scanning electron microscope, the electron beam is needed for scanning across the sample and the detector attached helps in building up an image by capturing the signals sent to it by the microscope. Usually, 5nm is the resolution limit of SEM and the images captured by SEM gives surface morphology only rather than the inner structure of the sample and it also produces 3D image of the sample, therefore scanning electron microscopy also known as focused ion beam technology and has gained immense opportunities in the field of forensic science.

The 3D surface of microscopic samples has gained high importance. Serial section transmission electron microscope and SEM/FIB are among the most widely used techniques and the procedure for imaging through these microscopy techniques involves sectioning of the sample example tissue into ultra-thin layers of tissue. Sectioning is performed manually in TEM while this procedure is automatically done in SEM using a diamond knife or focused gallium ion beam etc (Omidi et al., 2017). Using such a procedure one can have high-resolution image of the sample but as these techniques are destructive in nature for image resolution the full sample cannot be re-analyzed or revisited. Using SEM, 2D micrographs are captured and it is later utilized for 3D surface remodelling. One of the most favourable methods for 3D surface modelling of SEM images has been the multi-view class which is based on the acquisition of multiple images from different perspectives (Omidi et al., 2017). The magnification setting in electron microscopes can be adjusted thus facilitating the study of sample grains from very low to high magnification.

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2. DISCUSSION

2.1. Soil evidence

To obtain information about the shape, internal structure and chemical composition of soil (Pye, 2004) prepared the samples by embedding the particles in resin and followed by sectioning. The sections were polished and then imaged in SEM. To get the compositional information of the soil particles computer controlled elemental analysis or microprobe can be used but these techniques are expensive and time consuming. (Woods et al., 2014) compressed 10 mg of the soil sample to make a thin, indurate disc of soil. The sample discs were mounted on SEM stub using carbon tape for SEM/EDX analysis.

2.2. Forensic Medicine and Odontology

To identify charred bones, in certain studies the samples were prepared by cutting fresh sheep ribs into 4 cm long pieces. The cut samples were further divided into samples with flesh and defleshed samples from which all the flesh was manually removed. The samples were then burned using an electric muffle furnace at temperatures ranging between 100°C and 1100°C in 100°C increments. The samples were allowed to cool and the remaining fleshes on the fleshed samples were removed. All the samples were analyzed using a SEM. Backscatter electron detection was used to obtain morphological information. The elemental analysis was done using EDX measurements (Ellingham et al., 2015).

In forensic dentistry SEM was used to examine burnt and fragmented teeth. Several microscopic features were observed by directly placing the teeth under SEM at magnifications 20 and 2000 (Reesu et al., 2015) . In the use of SEM for the examination of exhumed bones by Melki et al an initial magnification of 35 was used to observe the usual bone tissue and damaged and unstructured regions. At a magnification of 63 multiple elements were observed which were not found in normal bone (Melki et al., 2011).

In the study carried out by (Erwin Vermeji, n.d.) to examine micro traces from bone samples using SEM, the samples were first excised from the victim during autopsy. The excised samples were macerated in ultra-clean water at 70°C to remove all soft tissue. The cleaned bone sample was then dried using a stove at 40°C. The dried samples were then analysed using SEM/EDS to study the morphological characteristics, elemental composition and location of the micro traces present in the bone matrix. The magnification was dependent on the particle to be examined.

2.3. Forensic Serology

To detect the persistence of spermatozoa on decomposing human skin, the samples which were preserved in formalin was immersed in alcohol solutions and air dried. The treated samples were fixed to slides using graphite adhesive tape and were coated with graphite. The prepared samples were analysed using SEM to study the morphological characteristics of spermatozoa by (Gibelli et al., 2013).

In the study by (Orr et al., n.d.) to determine the efficiency of extracting pure DNA from different types of swabs, SEM was used to study the swab morphology. No sample preparation was required and the magnification was fixed at 509.

2.4. Paint and Glass evidence

For the examination of paint using SEM small piece of paint chip was directly examined at a maximum magnification of 1,000,000 times by (Zeng et al., 2010). The various layers of the paint chip were clearly visible in SEM.

In the study to examine glass, the sample fragments which had smooth and flat surfaces were placed on adhesive carbon tabs on an aluminium stub and then coated with carbon. The elemental analysis was done using SEM with an EDX detector. The magnification ranged from 1000-2000X (Michalska et al., 2016).

2.5. Fingerprints

In the study conducted to examine fingerprints left on different metallic surfaces SEM/EPMA analysis was performed using cathodoluminescence detector. Elements such as sodium, chloride and oxygen were simultaneously detected. The analysis was carried out under vacuum conditions at a minimum pressure of 6 x 10-6 Pa (Challinger et al., 2018).

In the study to develop an effective visualization method for latent fingerprints with red fluorescent La 2 (MoO 4) 3:Eu 3+ microcrystals, the prepared microcrystals were washed using distilled water twice and once with ethanol, and then dried. The dried samples were observed using SEM to study the surface morphology (Li et al., 2017).

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2.6. Gun Shot Residue (GSR)

To demonstrate the presence of Selenium and other components in GSR particles, (Romolo & Margot, n.d.) collected the samples using aluminium stubs with adhesive carbon tape. Ethanol dipped cotton swabs were used to collect samples from the breech face and barrel of the firearm. The stubs were observed under SEM which was equipped with Si:Li SUTW detector by EDAX (Brozek-Mucha, 2011). In the study of gunshot residue distribution for close-range shots, micro traces were obtained from regions around the gunshot hole using aluminium stubs of 12mm with adhesive carbon tabs. The stubs with the micro traces were coated with a layer of graphite and carbon thread in order to prevent electrostatic charging. In studies carried out by (Tucker et al., 2017) and (Dalby et al., 2010), the GSR samples were collected using double-sided adhesive carbon tape.

For estimating the shooting distance using variable pressure SEM images of the surroundings of bullet holes in textiles by (Hinrichs et al., 2017), the samples were clothing cut from the vicinity of the bullet hole and placed on the SEM sample holder using double-sided adhesive tape.

Adhesive stubs coated with carbon for the collection and examination of organic gunshot residues were used by (Maitre et al., 2018). To collect GSR from a bullet wound for examination under SEM a thin layer of inert sticky latex was used. On the entrance wound, the sticky latex was pressed to collect the particles (Fabbris et al., 2020).

2.7. Diatoms

For the examination of diatoms from liver, lungs and kidney tissue, the samples were first washed using ultrapure water (Zhao et al., 2013). The inner tissue of the cleaned samples was then cut and placed in a vessel of a microwave digestion system. The samples were digested with concentrated nitric acid and hydrogen peroxide. The digested samples were then filtered by Millipore membrane and the membrane was viewed under SEM.

In another study, 2 ml of water samples and 2 grams of the organs were placed in a vessel of a microwave digestion system. The digested samples were filtered using nylon membrane which was washed using ethanol and water. The nylon membrane with the sediment was placed on adhesive carbon tape of a metallic stub and coated with a layer of gold for analysis under SEM (Zhao et al., 2017).

2.8. Hair

In the study for the examination of hair samples under SEM, the samples were prepared by coating them with heavy metal for imaging (Koch et al., 2020). Furthermore, in the study to examine Caucasian and Afro hair samples, the samples were placed in the metallic sampler which came with the SEM equipment. No sample preparation was required (dos Santos et al., 2019).

In the study to examine the hair samples from drug abusers using SEM the collected samples were first fixed using gluter aldehyde for a period of 24 hours. The samples were then washed using phosphate buffer, post fixed using osmium tetroxide, washed again using phosphate buffer and finally dehydrated using acetone. The dried hair samples were then mounted on metal stubs with double-sided adhesive band (Turkmenoglu FP et al., 2015).

For the examination of lion, tiger and leopard hair by SEM, sample preparation involved washing the samples with absolute alcohol and in SDS three times. The samples were dried at room temperature and were then cut into 5mm pieces. The cut samples were coated with gold-palladium and examined under SEM (Dahiya & Yadav, 2013).

2.9. Forensic Document Examination

In the study conducted on the examination of intersecting strokes using SEM, the uncoated paper samples containing different types of sealing inks and writing inks, were coated with palladium alloy to remove the electrostatic effects (Kim et al., 2016). (E Brito et al., 2017) in their review of forensic investigations of crossing inks, have mentioned(Singla et al., 1994) where the sequence of strokes was successfully determined from the embossed area behind the writings using SEM without any sample preparation. Furthermore, for the examination of toner particles in printing inks, the samples were analysed under SEM with EDX detector with no sample preparation (Corzo, 2018).

3. CONCLUSION

The use of electron microscopes in the field of forensic science has given rise to new opportunities. SEM has been extensively used in both academic and applied research over the decade. By using SEM one can examine vital evidence to obtain information about

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the surface morphology of the respective evidence. The results obtained from SEM analysis have been reliable in most of the cases in the examination of GSR, hair and sequence of strokes.

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