Forensic Applications of Scanning Electron Microscopy, a brief overview

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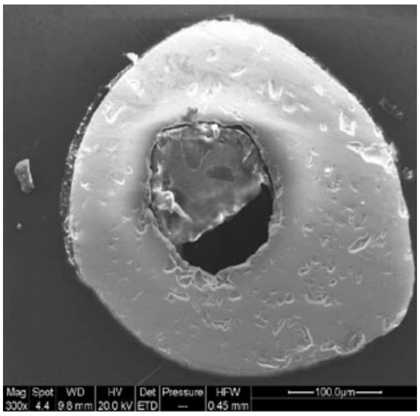
Optical microscopes are widely used for routine imaging tasks, are affordable and easy to operate. Unfortunately, they can only resolve to the micron level and have a limited depth of focus and contrast. Scanning Electron Microscopes (SEM) enable you to view morphology on the sub-micron scale. However it requires a significant investment and trained operators.

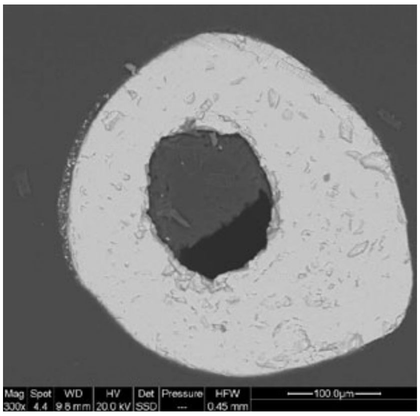
Principles of SEM

Basically a SEM is built-up of a column on a sample chamber. At the top of the column, electrons are generated. These electrons are focused on the sample by means of condensers and coils. The electron beam is scanned over the sample. Around the sample, detectors sense the different signals generated by the electron beam. The signals detected are used to generate an image on the computer screen. What basically happens is that the electron beam scans an array of pixels. Every pixel is filled with a grey-value from one of the detectors.

The most important signals that are detected are Secondary Electrons (SE) (relatively slow electrons that lost part of their energy in the sample), Backscattered Electrons (BSE) (electrons that retained most of their energy) and X-rays. SE gives topographic contrast, which is topographic information about the surface of the sample (Figure 1a). BSE gives compositional contrast, which is information about the composition of the sample: heavier elements appear brighter on the screen (Figure 1b).

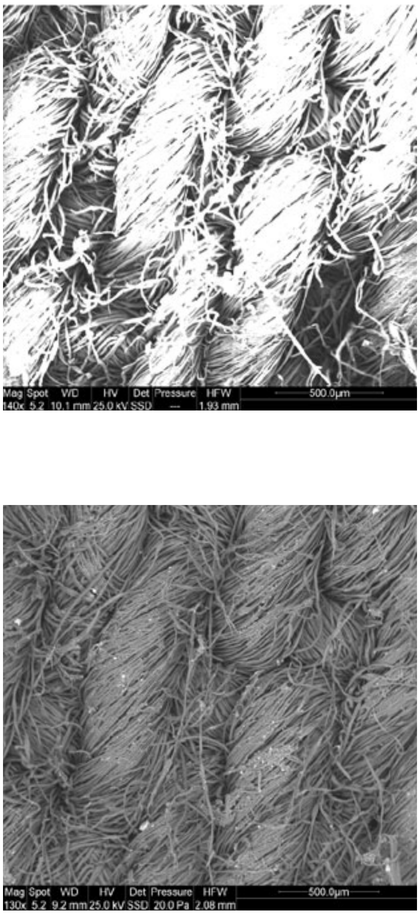


SE-image of a flat polished sample (Figure 1a).



BSE-image of a flat polished sample (Figure 1b).

In Low Vacuum Mode the pressure in A modern SEM can operate with different pressures in the sample chamber. In High Vacuum Mode the pressure is about 0,0003 mbar. The resolution in this mode is relatively high, but charging of non-conductive samples can occur, because electrons accumulate on these kinds of samples (Figure 2a).



A piece of cotton at High Vacuum Mode (upper image) and Low Vacuum Mode (lower image). The charging appears white (Figure 2).

The charging appears white the chamber is about 0.1 - 1.3 mbar. Because of this higher pressure, a part of the electron beam will be scattered producing the so called “skirt effect”. As a result, resolution is lower but charging of non-conductive samples doesn’t occur (Figure 2b). Therefore, working in Low Vacuum Mode eliminates the need for coating the sample with a conductive layer of carbon or gold. In Environmental SEM (ESEM) Mode the pressure is 1,3 - 26 mbar. This is high enough to prevent wet samples from drying out. However in ESEM-Mode it is not possible to obtain good compositional contrast.

Spectrometers

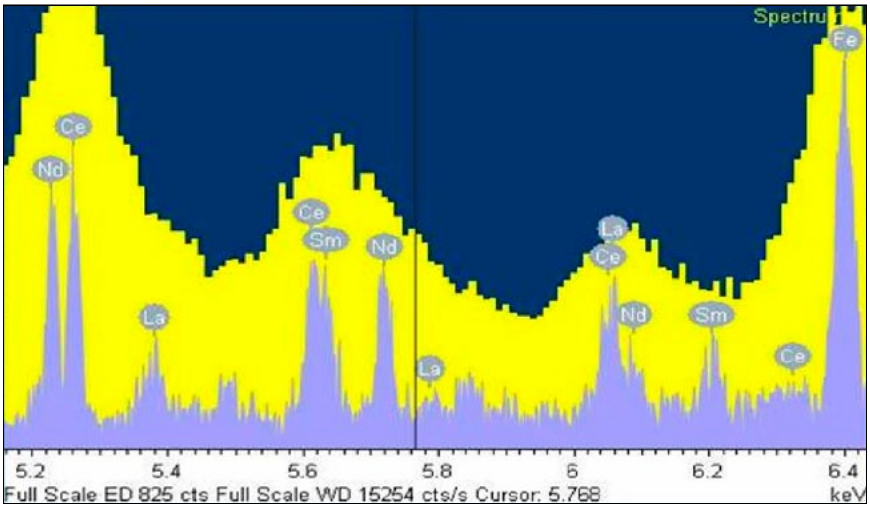
X-rays are detected by two kinds of detectors: the Energy-Dispersive Spectrometer (EDS) and the Wavelength-Dispersive Spectrometer (WDS). An EDS is much faster than a WDS, but the WDS detector has a much better analytical resolution (Figure 3). However, due to the higher price of the WDS it is not as widely used. Both EDS and WDS are able to detect elements heavier than Beryllium.

Other detectors

For some applications other detectors can be used. Sometimes Cathode Luminescence (CL) detectors are employed in the investigation of paints for the detection of visible light generated by the electron beam. Also it is possible to use Raman spectroscopy in conjunction of a SEM to investigate organic compounds like explosives. In general, these special detectors are rather expensive and the applicability is limited.

The concept of a Scanning Electron Microscope was first described by Knoll in 1935. Major improvements to the first designs were made during the ’40’s and ‘50’s at Cambridge University. The first commercial SEM, the Cambridgecientific Instruments Mark 1 Stereoscan was introduced in 1965. Today more than 50,000 SEM units have been sold, the vast majority of it with EDS capabilities.

The use of SEM as a forensic tool probably dates back to 1968 when the Metropolitan Police Forensic Laboratory in London started using the SEM for the



manual detection and analysis of gun shot residue (GSR) on a routine basis. A recent survey within the forensic community showed that today several hundreds of SEM are in use at forensic laboratories world wide and that the majority are used for the automated detection of GSR.

Hereafter, a brief overview will be given of the most important applications of SEM in forensic laboratories today. This overview will be illustrated with examples from recent casework at the Netherlands Forensic Institute (NFI).

Forensic Applications

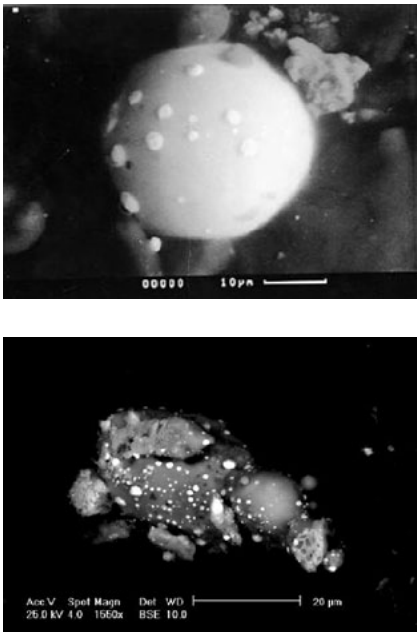
One of the most striking properties of SEM is its ability to combine imaging with elemental analysis together with its suitability for digitalization and automation of complete tasks. Forensic applications of SEM are found mostly in areas where there is a need for good imaging at relatively high magnifications in combination with elemental analysis. This is the case in areas where small particles of relatively heterogenic character and with a complex composition play a major part, for example gunshot residue and pyrotechnical post-explosion residues. In other areas where there is no special need for high magnification or elemental analysis, or where there is a need for information that SEM doesn't provide, for example color, SEM is not the preferred technique. For example in the forensic examination of fibers SEM is occasionally used to investigate fiber fracture and damage. Also for the forensic examination of biological samples SEM isn't used very often.

GSR

As mentioned before, one of the most well know applications of SEM in forensics is the automated detection and classification of gun shot residue (GSR). GSR is composed of burnt and unburnt particles from the propulsive charge and primer components from the bullet, the cartridge case and the fire arm itself (Figure 4). Because most primers today contain lead, barium and antimony, samples from suspect shooters are searched for particles that contain these elements. However in the near future primers will become increasingly free of lead and other heavy elements and this will put forward a new challenge to the automated detection of GSR by SEM.

The detection and analysis of GSR is reviewed in 2001 by Romolo e.a. [1]. The last five years a lot of effort has been put into quality assurance, which is the standardization of sets to sample shooters hands and the development of proficiency tests to validate the laboratory specific methods for the detection and classification of GSR. Every two years a new proficiency test is carried out within the framework of the ENSFI Expert Working Group "Firearms" [2].

The software used for the automatic detection and classification of GSR is not dedicated for GSR only and can be customized very easily for almost any application. Automated SEM has also been used for the classification of minerals in soil [3] and the detection of very small pieces of bone in fire debris.



Examples of gun shot residues (Figure 4).

Post explosion residues

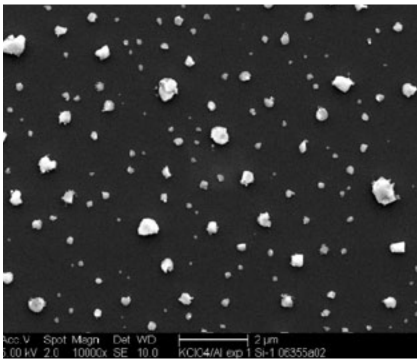
Improvised explosives may be based on pyrotechnic mixtures and these can leave behind distinctive explosive residues which in turn contain clues about the attack, such as the Bali- bombings [4]. Production, sampling an analysis of post explosion residues (PER) from pyrotechnic compositions the preferred technique for the detection and classification of post-explosion residues from these kind of compositions (Figure 5), while LC/MS (after swabbing) is the preferred technique for PER from organic explosives. Attempts to use SEM for the latter kind of PER are unknown. In the Enschede Fireworks disaster, SEM was used to detect PER on the clothing's of a suspect to prove they had been in the vicinity of the scene of crime, - the storage facility where the fire started.

Fibers

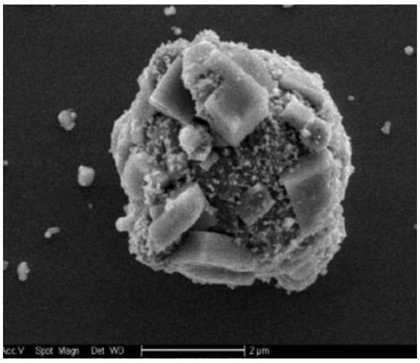
In the forensic examination of fibers SEM is sometimes used to investigate fiber fracture and damage. In the case of the death of a parachutist it was shown that the parachute was sabotaged. The fiber-end (Figure 6) has a flat top with a lip and it can be clearly seen that there is a tool mark in the end surface.

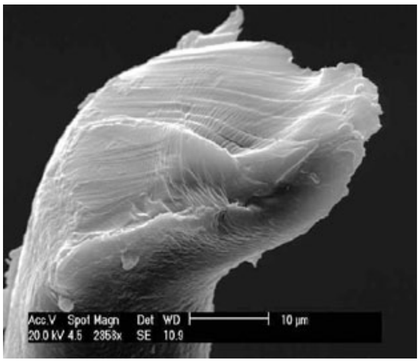
Ballistics

The examination of microtraces of foreign material embedded in or adhered to bullets provides critical information in the trajectory reconstruction of spent bullets. The verification of a ricochet by analysis of foreign material can have considerable legal implications because this verification can prove that it wasn't someone's intention to kill [5]. On the had been in the vicinity of the scene of other hand, traces of lead or brass found on damaged objects can prove that the object was hit by a bullet (Figure 7).

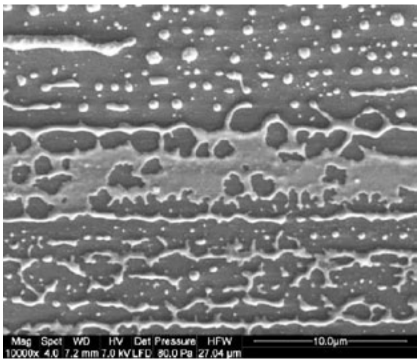


Overview (upper image) and detail (lower image) of post explosion residue from flash powder (Figure 5).



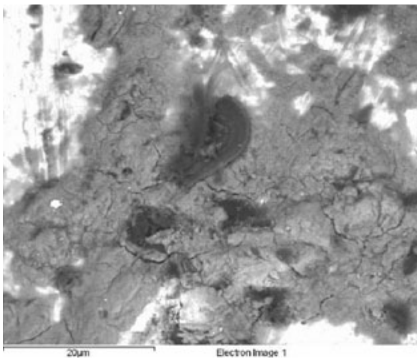


Fiber-end of a parachute cord that probably has been cut with a knife (Figure 6).



Surface of a glass pane that was hit by a ricocheting brass (FMJ) bullet (Figure 7).

In a recent study at our institute it was shown that softer bullets (lead) are more susceptible to transfer of material than harder bullets (Full Metal Jacket, t.i. brass). From the results it also follows that it is possible to divert the order of impact from the stratification of the foreign material are all very similar to that for GSR. SEM analyzed (Figure 8).



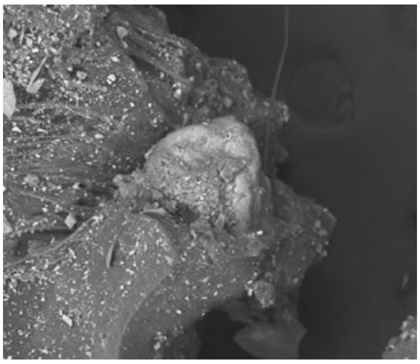
Surface of a bullet that hit MDF after hitting Greenboard (gypsum). MDF (black) is found on top of gypsum (grey). (Figure 8).

Somewhat related are cases in which bricks were thrown at passing cars. In one of these cases the brick smashed the window screen and eventually killed the driver. Both bricks and glass are brittle materials that are not very susceptible to becoming embedded with foreign materials. However modern window screens are composed of two layers of glass with a sheet of foil between. The foil is soft and readily picks up pieces of concrete and stone (Figure 9).

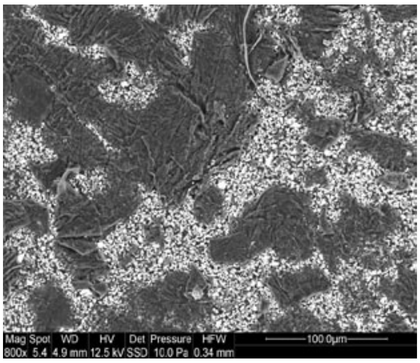
Biology

Biological samples consist solely of organic material and therefore don't give much compositional contrast. Epidermal cells are especially difficult to distinguish from debris and dirt (Figure 10). It is also not possible to view through samples and therefore it is difficult if not impossible to detect separate cells. For these reasons SEM isn't used very much for the forensic detection and visualization of biological samples although it has been proven that the electron beam doesn't damage DNA [6]. Sometimes SEM can be used to detect small bloodstains in order to reconstruct the trajectory of bullets (Figure 11).

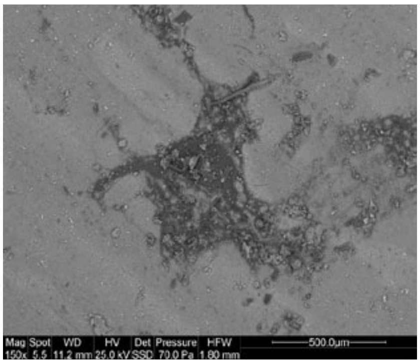
Another area in which SEM is used is the identification of animal hairs. Hairs shed by domestic pets like cats, dogs and hamsters are often found on clothing or in household dust. SEM is very useful to visualize the characteristic scale patterns on hairs (Figure 12). SEM is also used in others field of non-human biology, for example in the examination of diatoms in drowning cases and the examination of pollen grains.



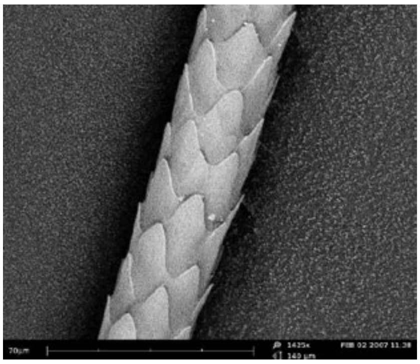
Foil from a window screen with embedded pieces of sand and concrete (Figure 9).



Epidermal cells on a background of fine divided silver (Figure 10).

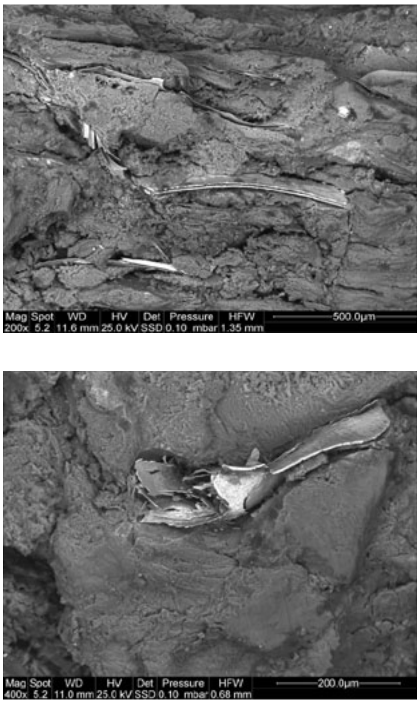


Bloodstain with some tiny fragments of bone near damage in a doorpost saying that the victim was hit first (Figure 11).



Scale pattern on hair of a civet (Figure 12).

Left side of the skull with an incomplete circular fracture (G3). The other circular fractures are on the right side and are not shown. Traces of metal were found at S2 and S3 (Figure 13).



Traces of metal found at location S2 in figure 14 (Figure 14).

The murder weapon: a bicycle lock (Figure 15).

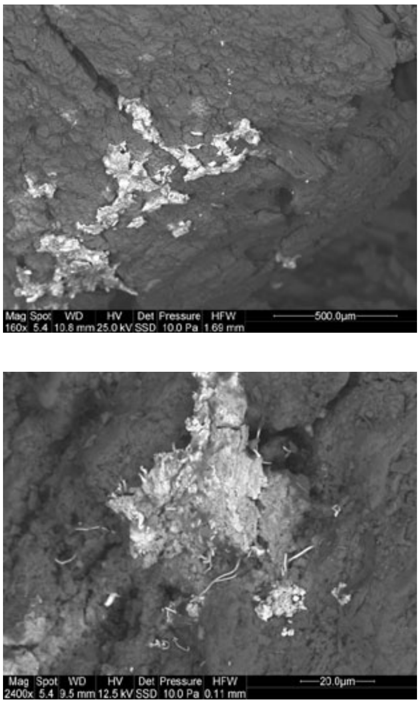
Forensic pathology and anthropology

At the NFI the SEM is often used in the area of pathology and anthropology to reveal microtraces of metals and other materials from murder weapons in the invasive traumas of victims and to visualize saw and tool marks on bone. In one particular case a missing person was eventually found buried. A large part of the skull was missing, specifically, the frontal part (Figure 13).

Three incomplete circular fractures were found. The forensic anthropologist believed that the woman was shot with large caliber ammunition, like a Brenneke. However no gunshot residues, traces of lead or bullet fragments were found. Only a chip of chromium was found in the hair of the victim. After a while it was decided to also investigate the non-circular fractures. Small traces of metal were found in the lines of fracture (Figure 14). Both the chip and the traces of metal in the skull had the same structure and composition. Probably chip and traces had the same origin. Later, a suspect confessed that he murdered the person with a bicycle lock (Figure 15). The lock was found near the scene of crime and appeared to have an upper layer with in the fractures. The round knobs on the lock could have caused the circular fractures in the skull. In another case the skeleton of a missing person was found under a concrete floor during a renovation of the building.

In the back of the skull a so-called gutter wound was found. In the upper two vertebrae, traces of lead were found at several locations, see Figure 16. Probably the victim was killed by a gunshot to the back of the neck. In some of the lead found at the vertebrae also a little glass microfiber was found (figure 17), indicating that the shooter has used some kind of improvised silencer.

Locations at which lead was found in one of the upper vertebrae (Figure 16).

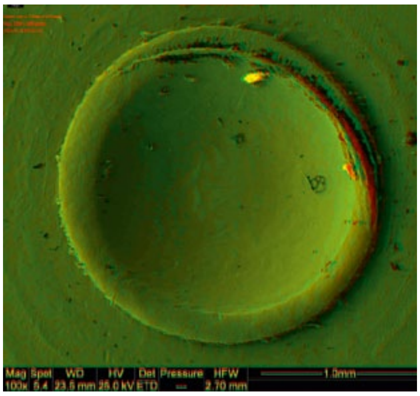


Traces of lead in one of the upper vertebrae (top and below) of which one with glass microfiber (image below) (Figure 17).

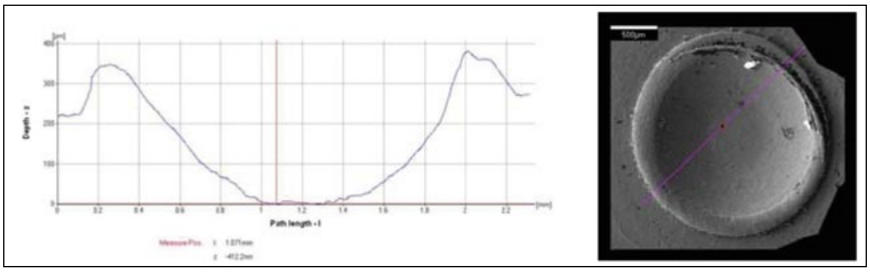
3D-applications

Our eyes see slightly different views of an object in front of them. The apparent position of an object in the two views is different by an amount, known as the parallax P. Our brain converts this parallax P to depth information. Viewing an object from two different viewpoints is equivalent to taking two images from a single viewpoint, but rotating the object. This procedure is usually employed in the SEM: two images are recorded from the same area of a specimen, but the sample is tilted between exposures. To visualize the depth in the images one image is printed in green and the other in red on top of each other. The combined images are called an anaglyph and this is viewed through colored glasses to separate the images at the eyes (Figure 18).

When the parallax P is measured, the depth Z can be calculated using $MZ = P/\{2\sin(\alpha/2)\}$, with M being the magnification and α the tilt angle. The resulting depth profiles (Figure 19) can be used for comparison. However the software for 3D-applications that is available today is not very suitable for forensic applications, because it is not possible to make overlays to compare the 3D-profile of a imprint of a tool mark found at the crime scene with a 3D-profile of a imprint from a test mark made with a tool found at the suspect. This also applies to imprints in cartridge casings.



Anaglyph of a cartridge case bottom (Figure 18).



Depth-profile of a cartridge case bottom (left) at the purple line (right) (Figure 19).

The Phenom (Figure 20).

New Developments

The Phenom™

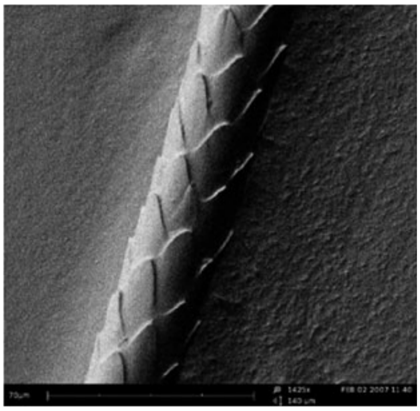
Scanning Electron Microscopes can be quite expensive and sometimes difficult to operate. Recently Phenom-World marketed a completely new SEM that is fool-proof and so easy to operate that everyone can use at a price that is comparable with that of a light microscope. The Phenom™ (figure 20) is meant to close the gap between optical microscopes and electron microscopes. The configuration consists of an imaging module, a 17″ touch screen, a vacuum pump, a rotary knob, a power supply and a memory stick. All software is embedded and there is no external computer. The Phenom is designed to handle a wide range of samples with minimal preparation. Samples are mounted onto a sample holder that can accommodate samples up to 26 x 30 mm. It is not possible to mount larger samples and this disadvantage can be overcome by reducing the sample size. The sample is loaded by simply inserting the sample holder and closing the door. After loading the sample an optical overview is generated by an integrated color CCD camera. This optical overview is used for navigation. The electron optical image is formed by a four quadrant BSE-detector (Figure 21). By switching of two of the four quadrants some shadowing can be created resulting in a better topographical contrast (Figure 22).

The Phenom makes it possible for forensic scientists who are not used to working with SEM to do their own imaging.

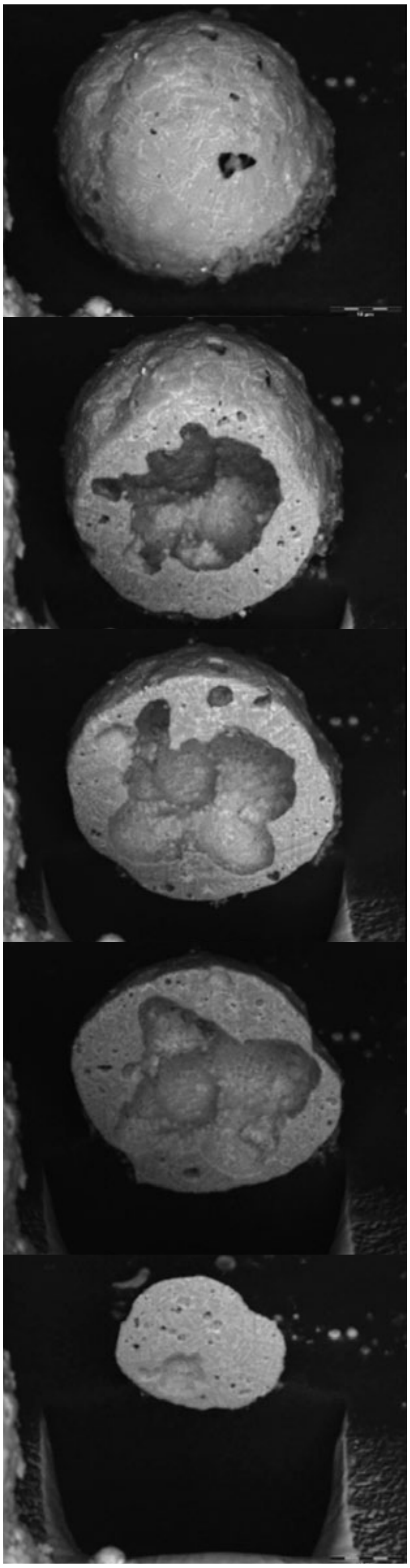
It is expected that this will further increase the forensic applications of SEM in the near future. Unfortunately it is not possible to conduct elemental analysis with the Phenom, but maybe future versions of the Phenom will include an EDS detector.

Image of diatom (Figure 21a).

Bulb wire (Figure 21b).



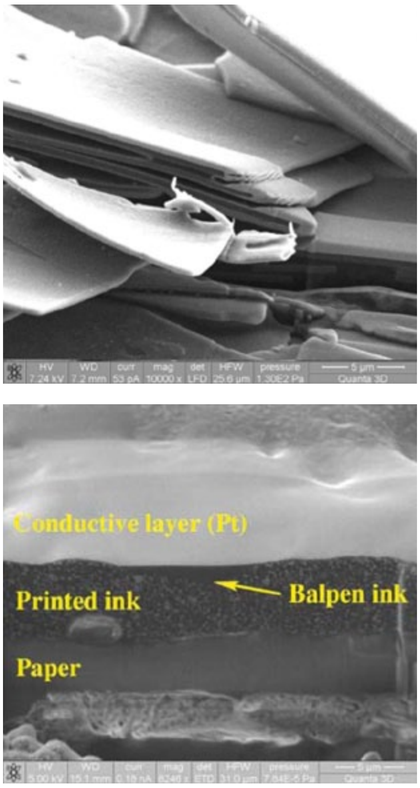
The same hair as in figure 12 by switching off two of the four quadrants (Figure 22).



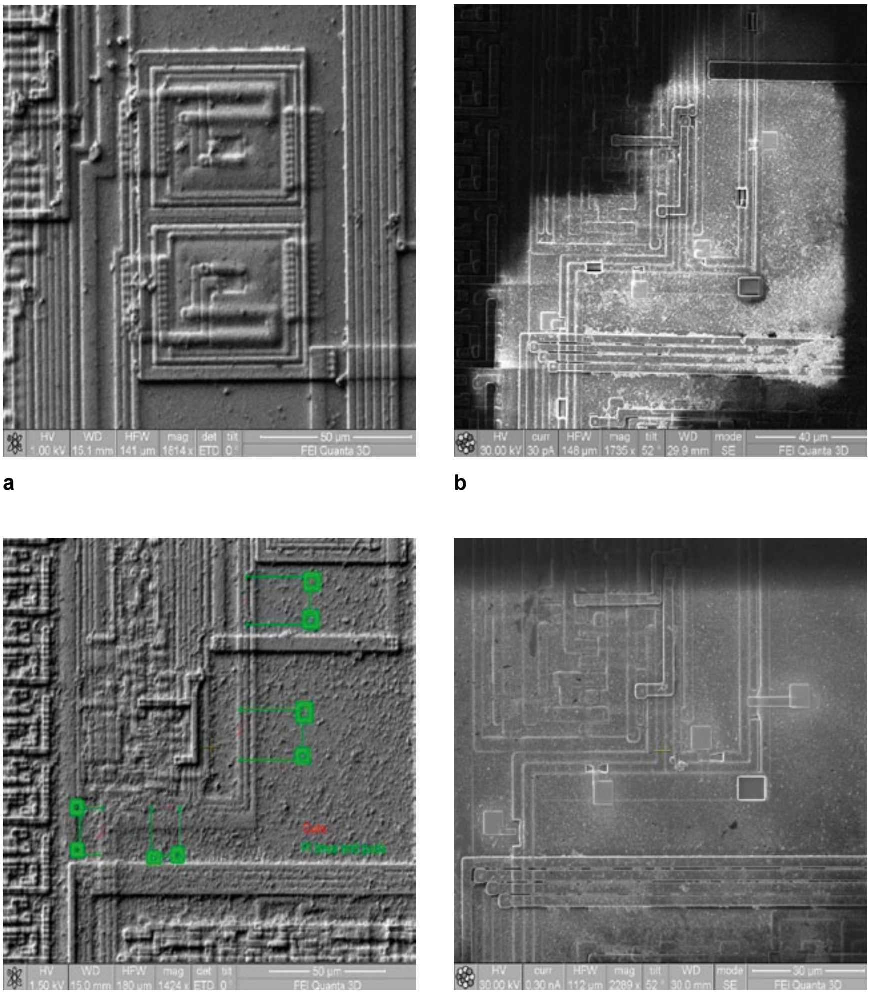
The milling of a GSR particle (Figure 23).

Focused Ion Beams

A Focused Ion Beam (FIB) is a beam of Ga+ ions focused on the sample. The greater mass of the ion permits the FIB to become a microsurgical tool that removes material from the sample (Figure 23). The FIB is complementary to SEM and is most commonly used to mill through the surface of the sample to reveal a cross sectional view of its underlying structure to allow SEM imaging of the internal structure. In forensics FIB in combination with SEM (SEM-FIB, also called DualBeam) is especially useful for the investigation of samples with a layered structure, for example crossing ink lines and paint (Figure 24). The DualBeam is also very useful in the area of computer crime, because a FIB allows us to modify computer chips and to edit devices (Figure 25). With a DualBeam system it is not only possible to remove contacts but also to make new contacts by means of beam-induced deposition. Today, several FIB/SEM combinations are in use at forensic laboratories, for example the Nova Nanolab system at the BKA in Wiesbaden, Germany.



The layered structure of paint (top) and crossing lines (below) (Figure 24).



a b c d

The ion beam is used to remove the passivation layer (a) and to expose the metal lines at different levels (b). The metal lines can be cut and rewired in a different layout (c), by depositing new metal strips (Platinum in this case). Also metal pads can be deposited to create test points (d) (Figure 25).

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