We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,600
Open access books available

177,000
International authors and editors

195M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Chapter

Atomic Force Microscope in Forensic Examination

Niha Ansari

Abstract

Criminal activities have their footprints from time immemorial and nature of crime has drastically changed over a period of time. There is neither a geographical boundary, nor technical limitations. Moreover terrorist’s activities, drug trafficking eco-crimes, high-profile crimes, robbery hit and run cases, building collapse, petroleum products adulteration are some of latest forms of crimes. In last 20 years, scanning probe microscopes have emerged as an essential technique in various fields, and atomic force microscope (AFM) is most commonly used scanning probe technique which has shown its wide range of application in examination of various evidences encountered on crime scene. Major advantages of AFM involve its high resolution in three dimensions, and sample is not necessary to be conductive and it does not need to be operated within a vacuum. It helps in studying a large range of topographies and many types of materials can be imaged under it. Evidences such as blood, fibers, hair, soil, finger prints, gunshot residue, pollen, etc. found on crime scene at nano- or micro-level can be examined under AFM. The chapter describes applications of AFM with respect to its application in examination of evidences that can help in bringing justice.

Keywords: Atomic force microscope, forensic science, trace evidence, physical evidence, forensic examination

1. Introduction

Forensic science is an umbrella term incorporating an innumerable professions where they uses their skills to help law enforcement agencies in reaching to the truth during any investigations. Forensic scientist succors in investigating and adjudicating both criminal and civil cases. Criminal activities have their footprints from ancient time and the nature of crime has considerably changed over a time period. Criminal activities are neither limited to geographical boundary, nor technical limitations. Moreover terrorist’s activities, drug trafficking eco-crimes, high profile crimes, robbery hit and run cases, building collapse, petroleum products adulteration are some of the latest forms of crimes. In many cases, forensic evidence plays key role in obtaining conviction and often only trace evidences are existent on a suspect.

In past 20 years, scanning probe microscopes have emerged as an essential technique in various fields. The atomic force microscope (AFM) uses the most common scanning probe technique for materials characterization [1, 2]. Major advantages of
AFM involves its high resolution in three dimensions, the sample is not necessary to be conductive and it does not need to be operated within a vacuum. It help in studying a large range of topographies and many types of materials can be imaged under it. AFM is capable of imaging 3D topography information from the angstrom level to the micron scale with extraordinary resolution. In AFM, the Z-axis resolution (perpendicular to the surface) is better than the X–Y scan plane resolution of the sample surface. The Z-resolution in AFM is on the sub-angstrom level under ambient atmospheric condition while the resolution in X–Y scan is limited due to the diameter of the probe and is on the order of a few nanometers. In the X and Y axis, AFM images shows complication of the probe geometry and sample texture, however, if the probe is much smaller than the surface features, the image distortions lead by the probe are nominal. The AFM sensitivity is derived from a force sensor which measures the forces between the probe and target surface which is usually less than 1 nN/nm. 

Figure 1 shows a representation of the AFM [3]. 

Evidences such as blood, fibers, hair, soil, finger prints, gunshot residue, pollen etc. are found on the crime scene at nano or even at molecular level. At present, different nanotechnologies such as the application of nanoscale powders, high resolution scanning and transmission electron microscopy and atomic force microscopy are applied for the examination of various evidences for forensic investigations [4, 5]. Nevertheless, forensic trace depiction of forensic evidences at the nanoscale does not yield applicable forensic information as explained by Inman and Rudin [6, 7] with the principle of divisible matter. Examination of such type of evidences require combination of sophisticated instrumentation which can help in proving the facts and can provide a conclusive results which can provide justice to the society. As mentioned earlier AFM technique has showed application for the examination of such type of evidences which is centered with an extremely high resolution scanning probe microscope to sense intermolecular and interatomic forces between a sharp probe and the specimen. AFM is highly applied in forensic field as it has the biggest advantage of examination of evidences in minimal non-destructive manner as well as possess imaging capabilities to examine the sample in various environmental conditions. As it possess highly accurate piezoelectric scanners its lateral resolution is hundreds of times better than the diffraction limit of traditional optical microscope. The sample is scanned by the tip of the cantilever which results into the deflection because of the attractive or repulsive forces between sample and tip molecules. The cantilever’s deflection is measured by...
Atomic Force Microscope in Forensic Examination
DOI: http://dx.doi.org/10.5772/intechopen.104704

the laser beam which is later converted into an electrical signal by photodiodes, thus helping in imaging of the topography surfaces of the sample at the nano-level.

2. Sample preparation

Sample preparation art is in fact a simple procedure of critical-path steps, where every single step makes a large difference. The sample preparation in AFM is easier as compared to the other electron microscope techniques [8]. Further, AFM provides advantage of operating in almost any environment conditions, such as aqueous solutions, in air, vacuum, or other gases. Typically, AFM is operated at three different modes namely contact mode, noncontact mode, and tapping mode. Contact is a static mode, and tapping and noncontact are dynamic modes, as the cantilever oscillates in tapping and noncontact modes. This is achieved by adding an extra piezoelectric element that oscillates up and down between 5 and 400 kHz to the cantilever holder. The contact mode is the mode where the tip of the cantilever scans the sample in close contact with the surface. This mode is used usually for surface force measurements. In noncontact mode, the tip flies about 5–15 nm above the sample surface. Whereas in tapping mode the tip of probe touches the sample, and moves completely away from the sample in each oscillation cycle. The tip usually taps the sample during each oscillation in tapping mode, hence it is often the most stable mode used in air. In noncontact mode the cantilever stays close to the sample all the times and possess much smaller oscillation amplitude. The contact mode imaging is heavily influenced by frictional and adhesive forces which may damage samples and distort image data. The non-contact imaging mostly provides low resolution and can get hindered by the contaminant thus producing interfere with oscillation. On the other side the tapping mode imaging overcome the disadvantages of the other two modes. It eradicates the frictional forces by spasmodically contacting the surface and oscillating with appropriate amplitude to avoid the tip from being trapped by adhesive meniscus forces from the contaminant layer.

In general, for particle analysis in AFM the smaller the size of the particles the flatter/smoother the substrate should be that is the size of the particles should be greater than the topographical features of the substrate. Commonly used substrates are glass cover slips, highly ordered pyrolytic graphite, silicon oxide wafers, mica and atomically flat gold. For biological samples like imaging DNA12 and proteins, atomically flat substrates are used while for fine-size features examination like bio-cells, colloids, quantum dots and carbon nanotubes, glass, mica and silicon substrate are used. If a sample comes in the form of a bulk material such as wood or epoxy-resin, metal discs are used as a substrate. The adhesive used in this case is typically carbon tape or thermal wax [3].

In case of biological samples, in order to observe biological structures in their native state, they are supposed to be attached to a smooth solid substrate to resist the lateral forces exerted by the scanning tip, in that reverence, mica, glass and silicon oxide have proved to be excellent substrates for the examination. Muscovite mica, is a non-conducting layered mineral composed of multiple 1 nm thick layers [9]. It can be cleaved simply with the help of adhesive tape to yield clean, atomically flat surfaces which are negatively charged. Mica is most normally used substrate for imaging double-stranded DNA, DNA-protein complexes, protein arrays, densely packed proteins, supported lipid films and animal cells. Also, the mica surface can be modified with silanes which helps in both to promote adsorption or to allow covalent binding of the
bimolecules [10]. Glass represents another suitable substrate for imaging biological samples. For imaging cells and other large structures glass cover slips are flat enough for imaging adsorbed molecules while in some cases, silicon oxide wafer scan also be used instead of glass. Though they are more expensive and difficult to handle, they offer much smoother surface than glass. Hydrophobic substrates, highly orientated pyrolytic graphite, which is atomically flat over large areas [11] are also preferred for biological sample preparation. Hydrophobic surface can be obtained by coating the mica surface with carbon for immobilizing DNA [12, 13].

For imaging document, adhesives and fibers sample under AFM, the frequently used substrate is microscope slide. Usually the samples are cut as per the required area to be imagined under the AFM and then double-sided adhesives are applied to affixed the sample at its fixed position as when the AFM tip is scanning it does not get deviated from the position. For soil sample analysis usually the grains are pressed into pressure-sensitive adhesive putty to provide suitable support during the scanning process thus this allows for retrieval of the grains afterwards or realignment if necessary during analysis [14–16].

The hair samples are priory washed before been examined under microscope using solvent namely sodium dodecyl sulfate solution or doubled distilled water. The substrate like metal discs or glass slide can be used as they are stable and shown eligible drift or creep. Adhesives such as conductive sticky carbon pads or double-sided tape are used to fix the sample at its position. If conductive sticky carbon pads are used as an adhesive then the hair sample are lowered onto the pads and pressed into place using tweezers, so that the fibers did not roll on the pad and hence pick up any contamination from the adhesive [14, 17, 18]. Researchers have also used epoxy as an adhesives at the sample ends to ensure that no interference with the top surface occurs and the adhesion between the hair sample and AFM disc keeps the middle of the sample fixed to the disc during AFM measurements [19].

3. Application of AFM in forensic science

This section recapitulates a number of AFM studies that illustrates applicability of AFM in relation forensic traces evidence analysis and its potential for crime investigations or reconstruction.

3.1 Determination of the age bloodstains

Blood stains are the most common type of forensic evidence found on the crime scene. The blood stains play very important role in the time determination of the actual criminal activity, hence determination of the age of bloodstains can prove to be highly effective in solving the crimes in shorter time duration. This information can provide a good perceptions regarding the victim time of death or to create a link between the suspects to the crime scene at the time when crime was committed. These area has attracted much attention worldwide over the years of various researchers since very few techniques such as electron paramagnetic resonance, high-performance liquid chromatography, quantification of RNA degradation and hyperspectral imaging [20–22]. In the review published by Bremmer et al. [23] they mention about the invasive techniques such as HPLC method, RNA analysis and EPR. Hyperspectral imaging are applied for the same problem. Even though HSI is a promising technology it has high error rate of about 2.7 days as per Edelman et al. [22].
Research has been done where the application of AFM is explored to study morphological changes of red blood cells (RBCs) to determine the relation with the time of death of individual. Wu et al. [24] has studied, the time-dependent, morphological changes of RBC in three different conditions such as room-temperature condition (controlled), outdoor environmental condition (uncontrolled) and low-temperature condition (controlled) using AFM on clean glass or newly peeled mica. They found that the substrate types have different effects on cellular morphology of RBC. Further, the RBC showed typical biconcave shape on mica and biconcave shape or flattened shape on glass, also the mean volume of RBCs on mica was significantly larger than that of cells on glass. In relation to the time, the changes in cell volume and adhesive force of RBC under the controlled room-temperature condition were similar to those under the uncontrolled outdoor-environmental condition as the time lapse. However, under the controlled low-temperature condition, the changes in cell volume happened mainly due to the RBCs collapse and the adhesive force curves exhibited the high alternations in RBC viscoelasticity. They concluded that AFM has significant application in forensic medicine or investigations, in relation to the estimation of age of bloodstain. Figure 2 shows the morphological comparison of RBCs on mica (a) and glass.

Chen and Cai did study on the morphological changes in a whole erythrocyte and of the erythrocyte membrane surface ultrastructure using tapping mode atomic force microscopy (TM-AFM) on mica substrate exposed in air over a 5-day period. They observed that the erythrocyte showed deformation of whole cell and membrane surface of unfixed erythrocytes as the time lapse. After 0.5 days of exposure, the fissures and cell shrinkage was observed and at 2.5 days of exposure, the development
of nanometer-scale protuberances was observed, also the protuberances number increases with increasing time. Hence the present study presented the application that the changes of cell shape and cell membrane surface ultrastructure can prove to be helpful to estimate the time of death [25].

Lamzin and Khayrullin in their work studied the changes of RBC membranes stiffness in sRBC and the form and size of RBC probed using AFM by storing samples for 35 days at standard temperature conditions as shown in Figure 3. Their research revealed that statistically significant increase of YM values of RBC were observed as well as alteration of their form to echinocytes and spheroechinocytes of sRBC within 35 days at +4°C was noticed. They mentioned that this work can prove to be useful as an immediate criteria for applicability of sRBC for blood transfusion [26]. Marco Girasole et al. has exploited the full potential of atomic force microscopy (AFM) to investigate various characteristic of the erythrocytes’ life, death and interaction with the environment. As per Marco Girasole et al. [27] AFM is still a continuously growing technique which can be applied for studying more variant information in relation to the RBCs biochemical or biophysical status at different environmental conditions.

Threes Smijs et al. applied atomic force microscopy to investigate the elasticity of RBCs from the peripheral zone of 4–8 day old bloodstains. They observed that the elasticity of six RBCs from a 5 day old bloodstain seemed homogenous with a mean Young’s modulus of 1.6 ± 0.4 GPa. As the time lapse, a significant age effect was observed in RBC elasticity that is on 4 days: 0.8 ± 0.1 GPa; 5 days: 1.7 ± 0.9 GPa; 6 days: 2.3 ± 0.6 GPa; 7 days: 4.5 ± 0.6 GPa; 8 days: 6.0 ± 1.8 GPa; probe spring constants 25.16–67.48 N/m. They found that a bloodstain age determination with a 24 h precision only for 6–7 day old stains can be done. The silicon tip condition was regularly checked using scanning electron microscopy as an increase in bluntness was noticed after four to six cell indentations [28].

Cavalcanti and Silva studied biophysical properties that is morphology and elasticity of RBCs using atomic force microscopy. They aimed to investigate the time since death (TSD) from blood smears by analyzing changes occurring in the RBCs of a group of voluntary. Further, they also investigated that whether any difference in TSD analysis occurs on three different surfaces such as glass, metal, or ceramic after

![Figure 3. The AFM image of the dry specimen prepared from sRBC after 1 day (a) and 35 days (b) of storage.](image-url)
blood smears deposition occurs on these surfaces. They calculated force × distance curves obtained from RBCs membrane surface deformation as a function of time. They observed that there is no appreciable difference in the structure of RBCs over 28 days but significant differences were noticed on glass, metal, or ceramic surfaces. They concluded that the use of AFM in crime scenes still requires the development for accurate estimation of the TSD for blood spots [29]. Strasser et al. also explored erythrocytes in a blood sample to study elasticity changes in a fresh blood spot on a glass slide. At first they found presence of several RBCs in “doughnut-like” structure, which could easily be detected due to their typical “doughnut-like” appearance further the elasticity pattern showed a decrease over time which may be due to alteration of the blood spot during the drying and coagulation process. They concluded that these preliminary data can demonstrates the capacity for development of calibration curves, which have potential in estimation of bloodstain ages during forensic investigations [30]. Different body fluids are also been utilized for the extraction of DNA because of its use as a forensic tool during investigation. AFM can add in the characterization of the “trace DNA” deposited on various surface during any mutual contact. The stiffness of DNA’s double strand can be discriminated from its single strand and counting of the copied DNA can be done by using AFM [31].

3.2 Document forgery

Document examination involves techniques which causes less or no damage to the documents and allows maximum retrieval of data from it. The determination of the sequence of strokes is still a big problem in the field of forensic document examination. Till today the optical microscope are used with different illumination methods and magnifications in determination of sequence of strokes. But the use of same does not provide a reliable results in every cases because of the interaction of the light with crossing ink lines, the depth of focus, low resolving power as well as low magnification range of the optical microscopes. Kasas et al. [32] studied line crossing problem on paper printed form dot matrix printers and different ball-point pens on plain paper. They found that AFM produces qualitatively similar results and overcomes some of the scanning electron microscope limitations, i.e., vacuum and specimen’s conductive coating. Figure 4 shows the cut-outs of crossings of ball-point pen strokes on dot matrix printed letters in newer printer ribbon and worn printer ribbon. They concluded that AFM is a powerful alternative to the SEM for line crossing problem. Brandao et al. in their work has focused on the problem of counterfeiting which involves making an imitation or copy manufactured without the legal sanction of the government. They explored AFM and Raman techniques for the examination of both authentic and counterfeit Brazilian driver licenses, and national and international banknotes. The AFM results showed that the parameters, such as roughness and topographic profiles of the chalcographic region of banknotes and Brazilian driver licenses, can be successfully visually discriminate between authentic and counterfeit documents. They also showed the application of statistical analysis using the Student’s t-test which showed that the asymmetry values obtained from series numbers and micro-letter regions can help in identifying the counterfeiting. They also indicated that the paper used to counterfeit the Brazilian driver license and the real banknote was an “office” type with inkjet printing by the use of the AFM technique [33]. Further the combination can also help to recognize the crossing lines between ball-point pens, and ballpoint pens and printers, to discriminate between genuine and counterfeit medicines, to identify counterfeit documents produced from washing
methods, to determine the microstructural information on textile fibers (discriminate between carpets, clothes, cars, etc.) in a crime scene investigation. The combination provides fast, very reliable, and reproducible analysis.

Chen et al. in their work highlighted the quality of AFM compared to SEM for forensic forgery investigations in relation to crossing lines. They examined topographic features of four papers namely duplicator, copper printing, glassine and kraft paper on which crossing lines were done with three different types of oil-based pens as shown in Figure 5. For all pens they establish similar differences in height profiles analogous to the inks accumulations at the places where the first pen stroke overlay with the edge of second pen stroke. The work do showed the usefulness of AFM imaging to detect crossing lines under the selected test conditions [15]. As per Ellen, AFM imaging technique can provide high potential in forensic document examination especially in cases to study crossing lines and document forgery cases which can further be explored [34]. Although the many research is been done to prove the usefulness of AFM imaging to detect crossing lines but the overall paper surface roughness hampers the detection of erased, partially erased lines or slightly printed ink patterns on the pages. The height profiles of ink streaks on documents differs on the different types of the paper as the absorption differs. These hinder the correct interpretation of the height images. Though if AFM imaging is applied in these types of investigations the confirmation can only be achieved by usage of other instruments such as Raman spectroscopy to convey the final crucial decisive information.

3.3 Hair analysis

Hair can prove to be a useful evidence in crimes in relation to determine the history of drug intake and abuse as well as exposure to toxins as the chemical composition of hair does not change by the external environment. Hair is the most encountered evidence in a forensic investigation and can act as a good source of DNA. The mitochondrial DNA present in the hair shaft and nuclear DNA is mostly within
the root sheath plays an important role in DNA examination [35]. AFM offers unique advantages for analysis of hair surface, primarily due to the high image resolution as well as an ease of sample preparation. Durkan and Wang employed atomic force microscopy in a forensic approach to distinguish between different hair care products on the basis of the deposits left behind. They studied AFM techniques on hair samples that were washed/treated with a number of different shampoos/conditioners and 2-in-1 products as shown in Figure 6. They found that the exocuticle carries a negative charge and gets deposits on unwashed hair with a mean roughness of up to 50 nm. Further they found that washing hair with shampoos reduces the roughness of hair + deposits to typically below 10 nm also the 2-in-1 products, conditioners or shampoos shows deposits that cover the entire surface, with roughness up to 30 nm. They concluded that the measurement of surface roughness combined with images of the resulting surface deposits can prove effective to distinguish between the effects of different hair care products [17].

The surface topography of human hair is defined by the cuticles which helps in cosmetic properties determination of the hair. The cuticles condition has the potential to aid in the medical diagnosis and forensic sciences. AFM offer unique advantages in hair surface analysis as it provides high resolution image and the simplicity of sample preparation. Gurden used an algorithm for the automatic examination of AFM images of human hair. By using a series of descriptors such as tilt angle, step height and cuticle density, the cuticular structure of hair was characterized and quantitatively investigated. They studied 38 AFM images consisting of hair samples untreated and bleached hair samples along with examination of the root and distal ends of the hair fiber. The multivariate classification technique partial least squares discriminant analysis was used to test the capability of the algorithm for further characterization of the images according to the hair properties. They were able to classify 86% hair images correctly. They study the classification of hair properties based on several cuticular descriptors by calculating it form the height images of various hair parts. The cuticular descriptors provided information on hair surface properties which can be correlate between the hair structure characteristics and environmental conditions.
the hairs are exposed to. Though the direct forensic relevance of this work was not established but the study do created extensive database of hair image along its mechanical properties [36].

Jeong et al. [18] have given an interesting contribution by studying the effects of aging on normal Korean hair diameter and surface features using AFM. They
examine 60 Korean volunteers of various ages who had no hair diseases and studied hair diameter, hair surface, cuticular descriptors and micro-scale mechanical properties to determine their associations with aging. They found that hair diameter increases for the first 20–30 years of life and later showed decrease. AFM images of most of the younger subjects showed clear scale edges of hair while of older subjects revealed dilapidated structures, poorly defined scale edges and undulated surfaces. The cuticular descriptors, surface roughness showed increase significantly with age. Also the force to distance analysis confirmed its dependence on age. They concluded that aging causes changes in hair diameter and surface structure. These work done by Jeong et al. do contribute in estimating the age from forensic trace evidences like hair. The hair surface area studies were done by Tomes et al. using both SEM and AFM which showed little difference in quality of surface profiles obtained. For forensic hair imaging, the minimally invasive AFM technique can be preferred over SEM [37].

AFM is also used to investigate the effects of ethnicity, fatigue and water absorption on the tensile strength of hair and found in different ethnic hair types namely Caucasian, Asian and African and the results indicated that they have different mechanical properties [38]. Seshadri did the similar study on the tensile strength of hair by imaging the cuticular structure of hair. They found that hair shows stress-strain curve for keratinous fiber. Also the chemical, mechanical damage and conditioner treatment does not have any effect on the stress-strain curve or its tensile properties [39]. DelRio and Cook [19] provided interesting data of hair samples untreated virgin hairs and conditioned and bleached hairs. They stated an indentation modulus of 2.4 ± 1.1 GPa and 1.8 ± 0.9 GPa respectively for virgin and the bleached hairs samples while for the conditioned hairs, the indentation modulus varied between 0.05 and 0.5 GPa. They performed all the measurements on a 5 by 5 μm area.

### 3.4 Diatom test

Diatoms are a group of algae found in oceans and fresh waters possessing tough silica wall (SiO₂) which is resistant to decay. Diatoms plays very crucial role in cases of drowning to determine whether it is antimortem or postmortem drowning, hence proving useful in forensic investigation. The recovery of diatoms from different organs, their quantitative and qualitative composition examination prove to be very trustworthy proof to determine the place and time of drowning in many cases [40]. They are studied in forensic geoscience in relation to transfer from different environments to clothing to obtain information of the crime scene and the perpetrator [41]. Newer techniques namely nuclear magnetic resonance, AFM, inductively coupled plasma (ICP) hyphenated technologies, fluorimetry and automatic diatom identification and classification are also been used for diatom study. AFM is used to study the diatoms morphological characteristics which can act as an indicator of its location, its growth cycle henceforward demonstrating its usefulness in forensic application. AFM has the potential to differentiate diatoms on the basis of its feature and can individualized atoms by scanning the objects that are 8” long and having a diameter of 0.5”. Even the largest diatoms can be scanned in this range also the technique has the additional advantage of scanning the object in vertical and horizontal axis [42].

Almqvist et al. explored the possibilities of AFM to study diatoms in relation to its biominerlization and micromechanical properties. They studied the silica shell of the diatom Naviculapeliculosa (Bréb.) Hilse. The structure was imaged and the shell’s micromechanical properties were studied in semi-quantitatively manner. The results indicated that the diatom’s overall hardness and elasticity are same as that of silicas.
Electron Microscopy

Figure 7 shows the separated epitheca and hypotheca of one cell. They also showed that certain areas of the shell were significantly harder or more elastic which can be detected in different crystalline phases [43].

3.5 Finger print

In most the crime, fingerprints are the most common type of evidence found on to the crime scene. A fingerprint is an impression of friction ridges on human finger. The discovery, visualization of latent fingerprints constitutes an important part of any crime investigation. Fingerprints consist of exogenous and endogenous compounds. The endogenous part mainly includes the skin remnants, sweat gland and sebaceous secretions along with many different inorganic and organic substances. The fingerprints remain unchanged throughout the life of an individual hence they play a very important role in person identification. Usually visible and latent fingerprints are found at the crime scene. The visible prints do not require any aid to be visualized while the latent prints are invisible thus require physical, chemical and instrumental techniques to be visualized [44]. Very few researchers have tried to explore the use of AFM in fingerprint investigation.

Atomic force microscopy technique highlights its use to study the deposition characteristics and detection efficiency of fingerprint details. Direct application of the AFM is not soon in the examination of comparison of the fingerprint but the use of AFM is shown in the fingerprint cases by some researchers. Jones et al. used AFM to characterize the various substrates whilst of fingermark deposition in relation to the surface roughness, maximum height variation, skew and kurtosis. The fingermarks were developed using iron oxide powder on formica, polyethylene and unplasticised polyvinylchloride surfaces [45]. As per Goddard et al. the limitation of the AFM height imaging to study the fingerprint ridge is the surface roughness when it is in the same order of magnitude as the height of the ridges as shown in Figure 8 [46]. The same problem available with lifted fingerprints as well as the prints present on the metal surfaces. The roughness of the surface on which the fingerprint is present is the main obstacle for routine applications of AFM in fingerprint analyses. In case were the surface roughness can be reduced atomic force microscopy can be useful in recovering...
the missing details that are essential to reconstruct a fingerprint. This problem was overcome by using scanning Kelvin probe force microscopy performed by Williams and McMurray. They studied the fingerprints deposited on metallic surfaces. They were able to retrieve sufficient ridge detail of fingerprint which were physically removed. Furthermore they demonstrated the use of Volta potential mapping to examine the fingerprint present on planar brass substrates [47].

3.6 Gunshot residues

Gunshot residues (GSR) mainly contains unburned or partially burnt propellant powder, particles from the ammunition primer, grease, smoke, metal residues and lubricants from the fired cartridge while the organic compounds in GSR originate from propellant and firearm lubricants [48, 49]. The analysis of the inorganic GSR can evidence to be useful in forensic reconstruction of shooting incidents. Techniques such as neutron activation analysis, ICP, atomic absorption spectrometry (AAS), and SEM combined to energy dispersion analysis are used for inorganic GSR analysis [50–53]. Neutron activation analysis are used for analysis of barium and antimony and for lead analysis conventional AAS and ICP are useful. High-resolution ICP-MS are reported to identify lead, barium and bismuth concentrations upto 1 ng/mL [54]. SEM-EDX is considered as golden standard of forensic GSR analysis as it has the ability to characterize GSR both chemically and morphologically. The SEM analysis is a time-consuming process. The organic GSR analysis are done by using gas chromatography, HPLC or GS-MS [55]. For both inorganic and organic GSR characterization time-of-flight secondary ion mass spectrometry, Raman micro-spectroscopy and ablation-ICP/MS are reported [56, 57]. Apart from these, AFM technique have shown a great applicability in forensic GSR analysis on the basis of its morphological structure in relation to solving the crime [58].

The estimation of shooting distance plays a vital role in firearm cases also when combined with other evidence it helps in reconstructing shooting events. The bullet entrance hole appearance and the GSR patterns around the wound are usually used

Figure 8.
AFM images from the polished and printed brass surface showing 3D image of part of ridge detail.
to estimate the firing distance [59–61]. Most commonly used color test Griess test along with series of modified and improved Griess tests are used to determine the presence of nitrates and hence for estimation of muzzle to target distance. Mou et al. reported the application of atomic force microscopy and Fourier transform infrared attenuated total reflectance spectroscopy. They use the techniques for firing distance estimation or muzzle-to-target shooting distance as well as the manufacturers of the cartridge and its powder. In their work, standard procedures contain test firing at various distances along with the evidence pattern comparison. They observed that for the samples the Winchester SuperX and CCI cartridges GSR particle sizes increased as the shooting distance decreased. From the AFM images of GSR they found that particles size distribution is inversely proportional to the shooting distance. AFM can be applied for the investigation of various materials unrelatedly to their conductivity. AFM is a non-destructive technique which helps in measurements in either air, liquid, or controlled atmospheres thus allowing the intact sample to be characterized without any pretreatments of the samples. The AFM images of GSR particles showed with different shapes like spherical, twins-like, irregular, boomerang-like, non-spherical, heart-like, rod-like and cube/rectangular-like as shown in Figure 9. The results indicated that the particles size distribution was inversely proportional to the shooting distance [62]. As per Jones when AFM is used for the GSR particles analysis the powder get stuck on the probe tip, thus drastically changing the shape and size of the powder particles resulting into the newer shape formation hence significantly alters the subsequent analysis [63]. This could be considered as a drawback of AFM for the analysis of fine GSR particles. But these same was overcome by Mou et al. which prove to be useful in firing distances determination.

D’Uffizi et al. in their work examine the GSR particles deposited on the bullet and on the shooter hands using combination of scanning electron microscopy + energy-dispersive spectroscopy, atomic force microscopy and selected-area X-ray photoelectron spectroscopy. The GSR samples were collected using double-sided tape. They studied the micromechanical and micromorphological features of gunshot residue particles. Of importance in this investigation the use of AFM itself (Nanoscope IIIa Digital Instruments microscope, tapping mode, frequency: 250–390 kHz) was done to examine the height and phase imaging [64]. Some research has shown the applicability of AFM in context to forensic gunshot and explosive investigation with regards to physicochemical characterization that can be detected on hairs and in between the ridges of fingermarks.

The mechanical properties of the organic and inorganic particles present in GSR and explosives, were studied by Xu et al. They showed the application of AFM techniques, including force volume mode, phase imaging as well as Kelvin probe force microscopy with resonance enhancement for dielectric property mapping was used to map the local physical properties of mock explosive materials. These work will allow the identification of sub-micrometer heterogeneities in relation to their electrical and mechanical properties [65].

3.7 Explosion

One of the recent advancements showed the use of AFM as a characterization technique for explosives detection. The surface morphology of explosives such as triamino-trinitro-benzene, plastic-bonded explosives, ammonium perchlorate was analyzed through AFM [66–68]. The surface morphology of such explosives helps in understanding the different characteristics of explosives which can help in identification [69].
Accumulation of explosives namely 2,4,6-trinitrotoluene (TNT) and triacetone triperoxide (TATP) in chemically treated hair sample was studied by Oxley et al. [70] using AFM and SEM. The interaction of TNT and TATP as a function of chemical pretreatment with acetonitrile, neutral and alkaline hydrogen peroxide, methanolic potassium hydroxide and potassium permanganate was studied and further the morphological changes which resulted from these treatments were studied. Hair examination surface showed different degrees of smoothening. Density functional theory calculations were employed to known the possible nucleation sites of TATP microcrystals on the hair samples. From their calculations study they concluded that the dark hair adsorbs explosives better than light hair. The authors have showed the use of AFM on their previously described applications of AFM in hair structure investigations [17, 36, 71]. Studied reported shows that AFM play a vital role in trace evidence analysis in post-explosion cases. These studies indicate that recently the potential of the AFM technology has been explored in relation to the forensic evidences analysis and the full potential of technology is yet to be discovered. The possibility of mapping a number of physical and chemical material properties prove to be a worthy contribution in distinguishing the different components in complex heterogeneous structure of explosive residues samples. The AFM technology is only a complementary technique its use can be enhanced if combined with other analytical technique which can prove to be of great importance in forensic context for not only examination of GSR or post explosives residues but also for other trace evidences found on to the crime scene.

Figure 9.
AFM images of GSR particles showing various particle shapes, twins-like (a), heart-like (b), boomerang-like (c), and rod and cube like (d). The bullet type is CCI and the shooting distance is 10 ft.
Valle et al. [72] used AFM to investigate and identify several characteristics of firearms. Replica molding of the head of these cases was done using the fired cartridge cases and the surface morphology of replicated areas at the breech faces were studied. In this framework, the method showed reproducibility of different copies of the similar sample indicating that they are indistinguishable over all the accessible length scales.

Researchers have also shown the utility of AFM in fire investigation cases. In fire cases the determination of source of fires plays very important role in order to validate [73, 74]. In fire cases, molten electric marks are found on the electric arc bead. Examination of these marks can help to determine the source of the fire. Gao et al. used OM and AFM to examine a molten mark on copper wire by artificially creating the molten mark inflicted on the wire under laboratory conditions. The AFM results showed that the technique is an brilliant add-on to examine the copper molten mark and thus provide excellent data to confirm the actual causes of fire [75].

3.8 Soil sample analysis

Soils vary among different areas and possess characteristics due to their natural effects and transfers made by human being and other living beings with time. Examination of soil in forensic context can help in determination of crime location. Investigative and interpreting the soil or sediment can help in their origin determination [76]. Konopinski et al. studied the grain surface texture of quartz sand using AFM. AFM analysis provide topographical data from the grain surface that permits statistical analysis, 3D reconstruction and quantitative valuations of the microscopic surface textures. AFM offers numerous statistical methods which can discriminate between grain surface textures and also helps in creating automated database to compile and generate reports. AFM has great potential to be used for forensic analysis where sample preservation is extremely valuable. As per Konopinski et al. using AFM helps in quantifiable measurement of quartz grain surface textures which opens up a number of possibilities for forensic quartz grain surface texture analysis as it provides a corroborative independent verification of quartz type classifications as shown in Figure 10 [14].

Sullivan et al. in their work investigated the surface characteristics of plastic wrapping materials of forensic interest in soil environments in order to determine the environmental factors that influence the degradation process of such polymers. They buried polyethylene bags and poly (vinyl chloride) sheeting in model environments surrounding different soil types, moisture content, pH and temperature. Atomic force microscopy was used to study the changes which results on the polymer surface at a nanometre level. They found that over a 2-year burial period, the degradation of polyethylene was greater by an increased moisture content and a raised soil pH. The plasticizer content of poly (vinyl chloride) was got affected by burial, thus leaching of the same was observed in all environments continually over the burial period. The surface roughness measurement of plastics using atomic force microscopy was sensitive to the burial environment and demonstrates the potential of technique to measure relatively subtle changes to burial items when exposed to different environments conditions [77].

3.9 Pressure sensitive adhesives analysis

Pressure sensitive adhesive tapes are utilized for various purposes in criminal activities such as packaging of controlled drugs, the restraint of an individual during robbery and offences against a victim, the enclosure of explosive devices and for
concealment. To identify chemical constituents techniques such as Fourier transform infrared spectroscopy and pyrolysis–gas chromatography–mass-spectrometry are applied in forensic science laboratories for the discrimination of PSAs. However, AFM can offer supplementary and useful analytical data on PSAs as it has the capability to map the adhesives surface morphological and mechanical properties also AFM can give nanoscopic information. With respect to forensic application it holds the ability to interpret the physical data obtained from evidence found at a crime scene and linking it to a particular suspect [16]. Figure 11 shows the AFM phase images for transparent cello, brown packaging tape and electrical insulation tape.

3.10 Forensic analysis of fibres

Fibers are an important trace evidence that can provide valued evidence to support an association of individual to a crime scene. Standard forensic examinations of man-made fibers usually involves microscopic techniques such as visible, polarized light and fluorescence microscopy as well as micro-spectrophotometry. Infrared spectroscopy is also used to identify the fiber polymer type present if two fibers are indistinguishable by microscopic techniques. Man-made fibers namely polyamides, polyacrylics and polyesters are analyzed using techniques such as FTIR, circular dicroism, Raman spectroscopy, differential scanning calorimetry, transmission electron microscopy and wide angle X-ray diffraction [78]. Forensic comparison of fibers is mainly focused on morphological analysis and spectral analysis. Shady Farah et al. in their study, analyzed polyethylene terephthalate (PET) fiber on three different materials such as plain fibers of pet, a common textile fiber and plastic material. They studied the morphological feature of the fiber using AFM [79].

The ability of the AFM to reconnoiter the nanoscopic morphological changes in the surfaces of fabrics was studied by Canetta et al. This study was focused on two natural namely cotton and wool and a regenerated cellulose (viscose) textile fibres. All the fiber samples were exposed to different environmental stresses for different lengths of times. The surface texture parameters of the environmentally stressed fabrics was measure quantitatively as a function of the exposure time from the obtained AFM images. In the AFM images the nanoscale the finest details of the surfaces of three weathered fabrics was clearly distinguishable between the detrimental
Electron Microscopy

effects of the executed environmental conditions. The heights and roughnesses of the unexposed and exposed fiber surfaces was measured by analyzing the obtained AFM images. Figure 12 shows the AFM height images of cotton fibre exposed to loam and riverside soils, and pond and sea waters for 2 and 6 weeks. This study confirmed that the AFM can prove to be a very powerful tool in forensic examination of textile fibers to provide significant fiber examination as an evidence due to its proficiency of distinguishing between different environmental exposures or forced damages to fibers [80].

3.11 Data recovery from damaged SIM cards

In crimes involving digital evidences the data recovery plays very crucial role. Damaged SIM cards are highly useful evidence in such cases. The data obtained from such SIM cards give insights about the link between criminal and aids in future investigation. Nardi et al. used AFM for the enhancement and characterization of a forensically authenticated technique for sample processing and data extraction from a damaged SIM card. They develop a process to view the underside of the embedded EPROM/flash memory arrays present in smart card microcontrollers [81–83].
4. Advantages and limitations

Atomic force microscopy works by running a sharp tip attached to a cantilever and sensor over the sample surface and measures the surface forces between the probe and the sample. As the cantilever runs laterally the sample surface, it moves up and down due to the surface features and the cantilever deflects accordingly. This deflection is computed using an optical sensor, with the laser beam being reflected on the back of the cantilever onto the light detector. AFM provides various advantages over other
Electron Microscopy

techniques. AFM can operate in ambient air or under liquid, it does not need to be operated in a vacuum hence it is increasingly being used to image biological samples as well as nanoparticles. AFM has resolution in the order of fractions of a nanometer and provide a 3D imaging technique. The AFM allows the topographic characterization of surfaces at resolutions not attainable by optical microscopy. The lateral resolution of the AFM is limited by the tip size and shape and is typically on the order of a few nanometers. The height ($z$) resolution in AFM is nearly 1 Å, limited only by electronic and thermal noise in the system. FM can only scan a single nanosized image at a time of about $150 \times 150$ nm and possess chances of damaging the tip and the sample during detection. Further it has a limited magnification and vertical range. Furthermore the speed of scanning of AFM is very slow compared to SEM and sample analysis cannot be done for areas greater than 100 μm. The images also gets affected by non-linearity, hysteresis, and creep of the piezoelectric material. Another drawback is that the images are generated because of the interaction of probe with sample which might not be the true topography of the sample. Also the probe tip can result in shape changes in samples like fine powder. The surface roughness over which a forensic traces are deposited may obstruct a proper examination of an image height. This problem could partly be overcome by use of phase imaging. Nevertheless, the addition of optical microscopy and Raman spectroscopy or surface enhanced Raman spectroscopy along with the AF can prove to be useful for forensic examinations.

5. Conclusion

Undeniably, the AFM power to measure topography, morphology, adhesion forces, elastic modulus, dielectric properties and energy dissipation characteristics via minimal invasion. Furthermore, the 3-Dimentional multi-parameter function provide information add-on in cases of trace fusion imaging. Considering the practicality, sampling and sample logistics are still remains desirable in AFM, though with respect to SEM the tedious work of sample preparation as well as high vacuum settings are not required. AFM has its advantages while studying, optimizing, understanding and validating techniques for examination of trace evidences found at the scene of crime. Also, microtraces evidences physiochemical features imaging can be done which can assist in classification and comparison. Though it has such advantages, roughness of substrate do hamper one or other way while studying the sample height measurements. Certain researchers have answer to this solution by accompanying surface roughness along with larger scan areas in supplementary phase imaging. In practice, AFMs can image rough surfaces as long as the roughness does not surpass the limit of scanner in vertical, Z-direction. But the probe will crash if the surface roughness surpasses the scanning limit. To grip the forensic applicability of AFM in real case work superfluous research as well as laboratory and crime scene authentication studies are prerequisite. AFM could yield surplus possibilities for forensic association and reconstruction, assisting in forensic analysis at activity level.

Conflict of interest

The authors declare no conflict of interest.
References


[34] Ellen D. Scientific Examination of Documents Methods and Techniques. Boca Raton, USA: CRC Press; 2006


[67] Kumari A, Jain MS, Jain MK, Bhattacharya B. Nano-ammonium perchlorate: Preparation,


[74] Shea JJ. Identifying causes for certain types of electrically initiated fires in residential circuits. Fire and Materials. 2011;35:19-42


[82] Jose S. EEPROM failure analysis methodology–Can programmed