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Advancements in spectroscopic and microscopic techniques for modern analysis

Samuel N. Ndung'u[1*] https://orcid.org/0000-0002-3935-9593 **& Esther W. Kinuthia**[1]

[1*]Department of Chemistry and Biochemistry, Laikipia University, P.O BOX 1100-20300, Nyahururu, Kenya
Corresponding Author: samuelndungu530@gmail.com

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ABSTRACT

Nowadays, different well-established analytical techniques are employed for qualitative and quantitative analysis of a wide variety of samples. The use of instrumentation by chemists is ubiquitous in research. The material properties such as crystal structure, surface state, surface morphology, composition, optical and magnetic characteristics probed is an important step in understanding their chemical and physical performance. These instruments provide information about the surface characterization and structural elucidation of a sample. A detailed description of each instrumentation technique is usually linked with its associated theory, design and application.

The study entailed the important factors to consider when selecting suitable instrumentation for analysis, whether destructive or non-destructive and highlighted merits and demerits. The techniques such as Fourier Transformed Infrared (FT-IR) Spectroscopy, Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX), Atomic Absorption Spectroscopy (AAS), X- Ray Diffraction (XRD), UV-Visible Spectroscopy, Nuclear Magnetic Resonance (NMR) Spectroscopy, Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), Transmission Electron Microscopy (TEM) and X-Ray Fluorescence (XRF) Spectroscopy are reviewed. These insights will inform the researchers with a useful guide to assist them on the choice the appropriate instrumentation technique for their analytical research.

Keywords: analysis, elucidation, spectroscopy, instruments, characterization, microscopy

INTRODUCTION

Modern analytical chemistry is a dynamic field that deals with the principles and concepts of important spectroscopic, microscopic and spectrophotometric techniques for surface and structural analysis (Bisen and Sharma, 2013; Wang *et al.,* 2015). The techniques are pivotal in diverse fields such as medicine, food science, geology, nanotechnologies, materials science, and environmental analysis (Vickerman and Gilmore, 2009; Robinson *et al.,* 2014). Therefore, development, intercomparisons and applications of instrumentation techniques are valuable for analysis in scientific research, control or industrial laboratories.

A description of each technique is associated with its related theory, instrumentation design, and application in various fields (Rayson, 2004; Warner *et al.,* 2016). The instruments are usually interfaced with a computer, as a readout device with some instruments fully automated to perform all the functions (Kovarik *et al.,* 2020; Shah *et al.,* 2020). These instruments are highly selective and sensitive and are therefore important in different fields (Farmer and Jimenez, 2010). The nature of materials such as the composition, surface state, morphology, crystal structure, optical and magnetic characteristics probed is important in understanding their physical and chemical performance (Liu *et al.,* 2014). The instruments used can be coupled with other instruments to provide more vital information about the surface characterization and structural elucidation of the sample of interest (Kumar *et al.,* 2014). However, the main challenges for modern analysis that needs to be addressed is the pricy cost of the instruments, their complexity and accessibility.

The instruments such as Fourier Transformed Infrared (FT-IR) Spectroscopy (Tinega *et al.,* 2023), Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) (Hummadi *et al.,* 2022; Rosanti *et al.,* 2022), Atomic Force Microscopy (AFM) (Deng *et al.,* 2018), X- Ray Diffraction (XRD) (Njeri *et al.,* 2023), UV-Visible Spectroscopy (Oke and Mohan, 2022), Nuclear Magnetic Resonance (NMR) Spectroscopy (Jaime *et al.,* 2018), Atomic Absorption Spectroscopy (AAS) (Ndung'u *et al.,* 2020), Transmission Electron Microscopy (TEM) (Karthikeyan *et al.,* 2016), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Khan *et al.,* 2015) and X-Ray Fluorescence (XRF) Spectroscopy (Ndung'u *et al.,* 2023) among others are used for analysis. However, reports indicate limited information on their working principle, emerging innovations and applications of techniques and this formed the basis of this review work.

SPECTROSCOPIC TECHNIQUES

The techniques are employed to measure matter interaction with electromagnetic radiations of different frequencies (Akash and Rehman, 2020). The magnitude of the energies involved, magnetic field and instrumentation considerations has led to the rise of different spectroscopic techniques (Bisen and Sharma, 2013). The FT-IR, AAS, XRD, UV-Visible, XRF and NMR techniques are reviewed in the following sub-sections.

Atomic Absorption Spectroscopy (AAS)

The technique uses absorption spectrometry to examine the analyte concentration in a sample (Potts *et al.,* 2004; Ivanenko *et al.,* 2012). It entails absorption of electromagnetic energy of a certain wavelength, mainly ultraviolet or visible region, by ground atoms in their gaseous state (Farrukh, 2012). It involves absorption of light by free atoms of an element at a wavelength specific to that element therefore capable of quantifying sample chemical entities in trace elemental analysis (M Series, 2005; Wilberforce, 2016). The technique is very sensitive as its detection limit can be as low as 1 ppm using the flame procedure, with spectral lines extremely narrow (0.02-0.5A) and transition energies unique for each element (Paul *et al.,* 2014, Paul *et al.,* 2017). The technique is employed in the determination of metals in vast samples like biomaterials, food, nanomaterials, drugs, environmental, forensics and industrial wastes (Farrukh, 2012).

Generally, AAS instrument consist of a stable continuous radiation source, lenses, mirror and a slit, monochromator for selecting narrow wavelength band from the source; a sample compartment for holding the sample being analyzed; a detector and a readout device (Sudunagunta *et al.* 2012; Wilberforce, 2016) as shown in Figure 1. The sample is converted into atomic vapour and then absorption specific for a given element is measured (Paul *et al.,* 2017). The sample element is aspirated to a flame, then changed into atomic vapour (Yeung *et al.,* 2017). Some atoms are thermally excited by the flame whereas most of them remain in the ground state (Wilberforce, 2016). The ground state atoms absorb a specific wavelength radiation (Akash and Rehman, 2020). The amount absorbed corresponds to metal ions concentration (Sharma and Tyagi, 2013). It's worth noting that the hollow cathode lamp used is specific to the metal being analyzed.

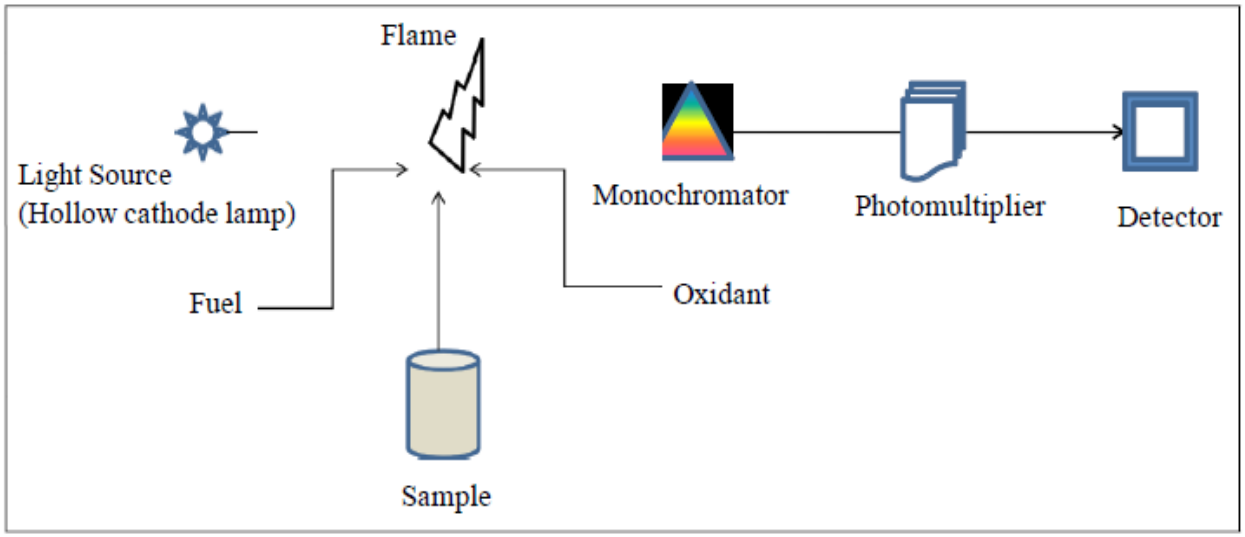

Figure 1: Components of Atomic Absorption Spectrophotometer (AAS)
Source: (Paul *et al.,* 2017)

Fourier Transform Infra-Red (FT-IR) Spectroscopy

The spectroscopic analysis in the mid-infrared spectral region (4000 cm^{-1}-400 cm^{-1}) provides quantitative structural, chemical, and compositional information on constituent molecules in the solid, gas and liquid phases, thus employed in vast field of industrial applications such as in medicine, materials science, biotechnology and environmental analysis (Haas and Mizaikoff, 2016). It is a non-destructive, rapid and time saving technique that is able to detect a range of functional groups and is sensitive to changes in molecular structures (Amir *et al.,* 2013; Kurniawati *et al.,* 2021; Rosanti *et al.,* 2022). FT-IR provides vital information on the chemical constitution and physical state of a sample (Cocchi *et al.,* 2004). The techniques determine the chemical bonding, molecular structure mainly functional groups of organic and inorganic materials (Munajad *et al.,* 2018; Al-Ghouti and Al-Absi, 2020). The technique has merits over other analytical techniques such as a small sample size use (milligram range), easy sample preparation, accurate, fast analysis, inexpensive and reliable and a simple sampling procedure (Sivakumar *et al.,* 2012; Chen *et al.,* 2014). It is widely used in many laboratories due to its improved signal-to-noise ratio and fast operational speed (Cui *et al.,* 2017).

The technique characterizes functional groups bond structure after IR radiation absorption causing dipole moment a change (Țucureanu *et al.,* 2016). This causes molecular bond vibrations and rotations resulting to a FT-IR spectrum at varying wavenumbers in the IR region (Faghihzadeh *et al.,* 2016; Perez-Guaita *et al.,* 2016). These FT-IR spectra exhibit characteristic absorption frequencies of varied intensities for different functional groups in a sample (Santhoshkumar *et al.,* 2017; Gonciarz *et al.,* 2020). In the modern analysis, advancements have resulted to rise of more digitalized FT-IR instruments that are faster and more sensitive as they can detect vast compounds (Simonescu, 2012).

The working principle of FT-IR (Figure 2) is that the light emitted is split to two beams (Valand *et al.,* 2019). Then, the light beams are re-combined again using fixed and movable mirrors (Cui *et al.,* 2017). The combined beams interact with the sample under study and the detector measures the radiation intensity moving into a sample and the radiation intensity transmitting through the sample (Munajad *et al.,* 2018).

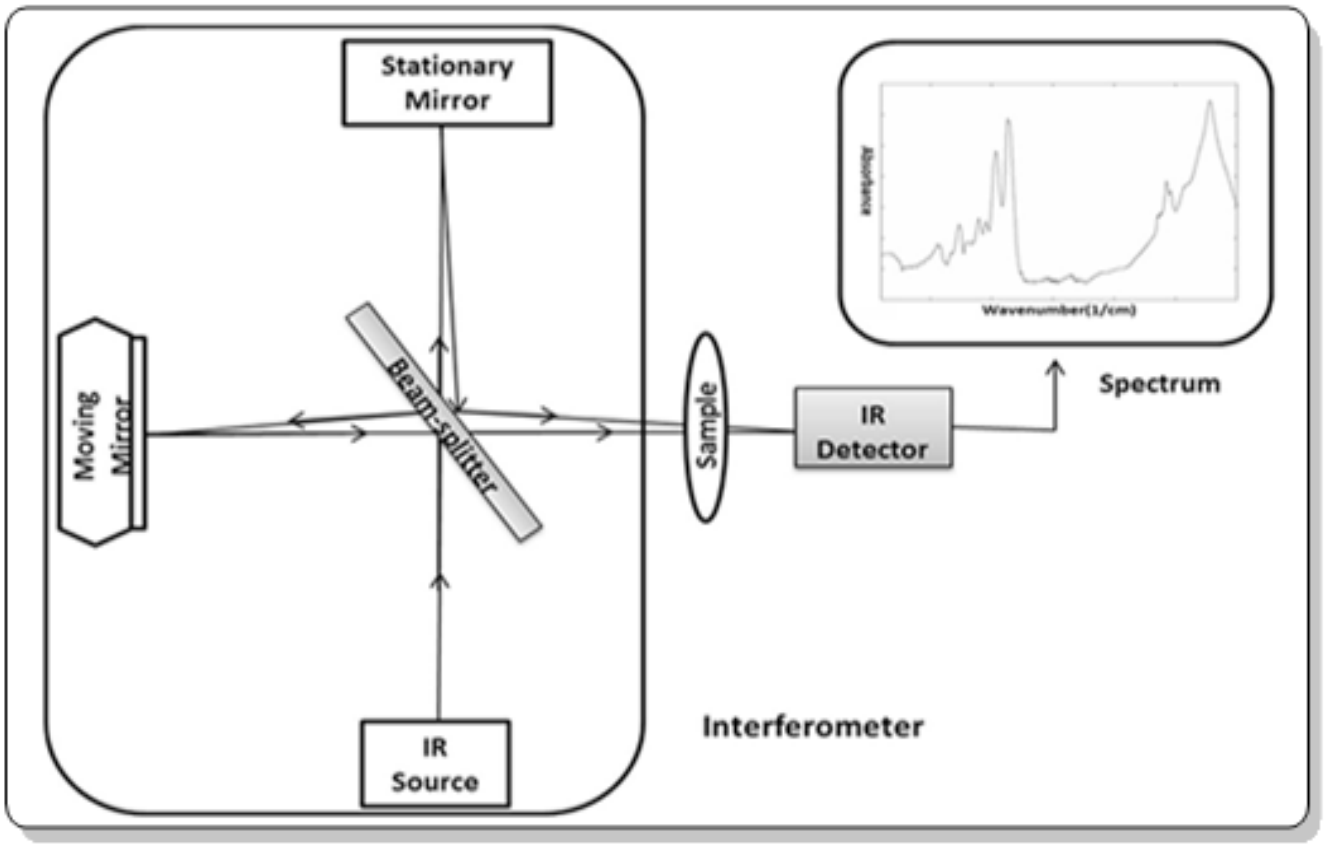

Figure 2: The FT-IR instrument components

Source: Faghihzadeh *et al.* (2016)

UV-Visible (UV-Vis) Spectroscopy

The technique measures the light intensity in the ultra-violet (10–400 nm) and the visible (400–800 nm) regions as a function of wavelength expressed in nanometers (Akash and Rehman, 2020). It is the most employed technique as an inexpensive, rapid, simple, flexible, simple, accurate, precise, non-destructive, specific and applicable to small quantities of compounds (Chakraborty *et al.,* 2018; Singh, 2020). The ultra-violet (UV) spectroscopy utilizes light in the ultraviolet, visible and Infra-Red ranges (Picollo *et al.,* 2018; Verma and Mishra, 2018). The technique measures the absorbance of light that passes through the test sample as a function of wavelength dependent on the molecules present (Singh, 2020).

This technique is centered on transition of valence electrons from their ground state to an excited state driven by electromagnetic radiation in the UV–Vis range (Haddi *et al.,* 2024). The light absorbed provides information about electronic transitions that occur within the sample material (Guo *et al.,* 2020). The radiation sources are deuterium or hydrogen discharge tube with 185 nm to 390 nm wavelength range, and tungsten-filament lamp which covers a visible region of 350 nm to 800 nm (Abu Bakar *et al.,* 2016). The UV-Vis spectrophotometers measures the light absorbed or transmitted as a function of wavelength (Rocha *et al.,* 2018).

The working principle of the technique (Figure 3) is based on passing a monochromatic radiation through the sample solution where the emitted radiation intensity depends on the thickness and the sample concentration (Shinde *et al.,* 2020). This is as explained by the Beer-Lambert law, which explains that the interaction between light that interacts with the sample (I, transmittance or reflectance) versus the incident intensity (I_0) depends on the concentration of the absorbing species in the solution and the path length (Verma and Mishra, 2018). Typically, the results are graphically presented as absorbance (no units) against wavelength in nanometers (nm) (Rocha *et al.,* 2018).

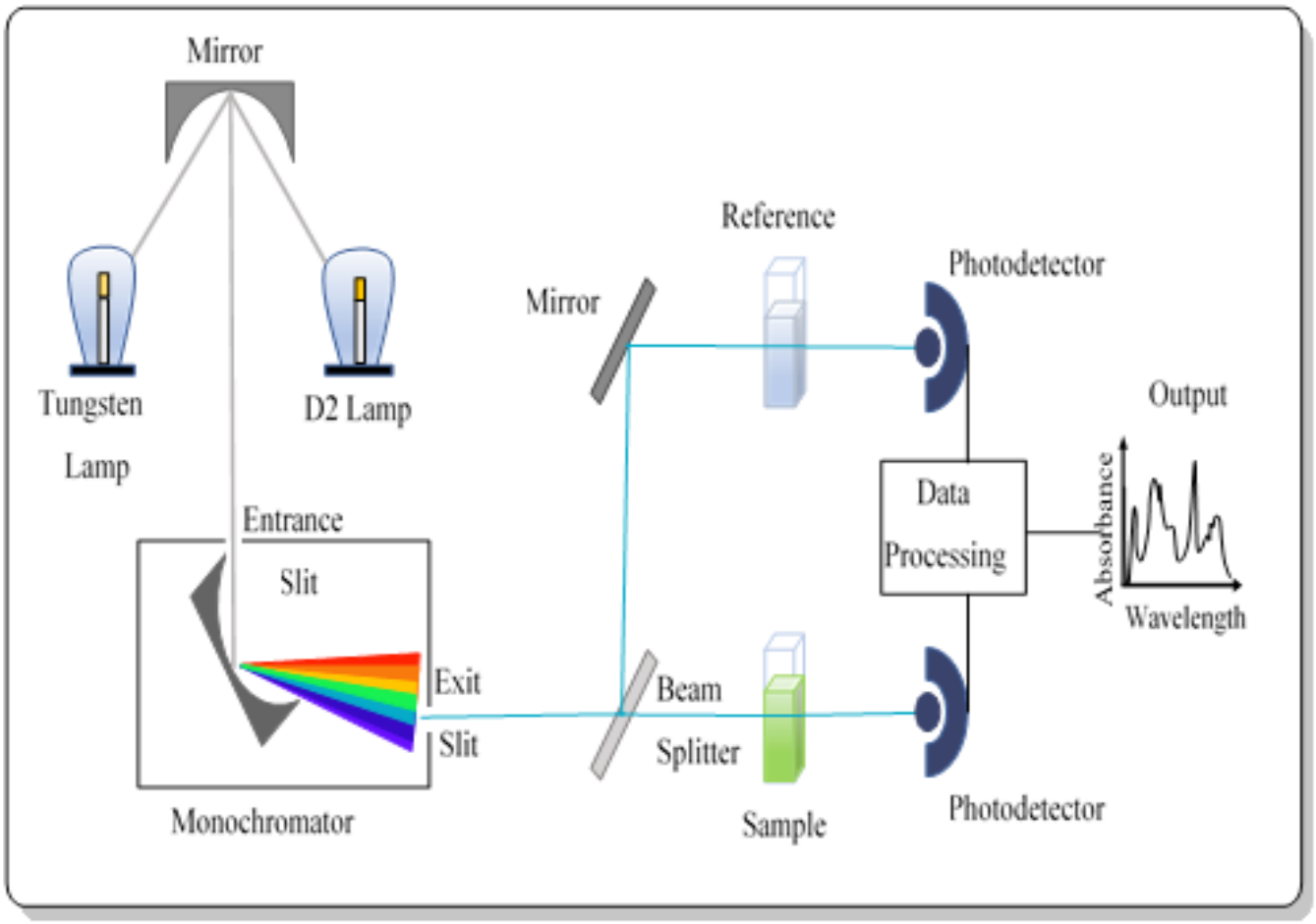

Figure 3: Components of UV-Visible spectrophotometer
Source: Rocha *et al.* (2018)

X-Ray Diffraction Spectroscopy

The spectroscopy is a non-destructive and a simple technique that offers a quick quantitative and qualitative analysis of pure and multi-component compounds that requires minimal sample preparation (Khan *et al.,* 2020). It is used in determining the elemental proportions of

the sample under study and its structural state (Bishnoi *et al.,* 2017; Titus *et al.,* 2019; Fakhirah *et al.,* 2024). The technique determines the crystalline structure, phase nature and crystallite grain size of sample materials (Melkamu and Feleke, 2022; El-Azazy *et al.,* 2023; Pandey *et al.,* 2021; Ermrich and Opper, 2011).

The principle is founded on constructive interferences of the monochromatic X-rays with the sample (Bunaciu *et al.,* 2015). The X-rays from the cathode ray tube are filtered producing monochromatic radiations which are then collimated and directed to the sample being analyzed (Figure 4) (Epp, 2016). The positions of the diffracted incident X-Ray beams are ruled by atoms positions in the crystal unit cell (Kim *et al.,* 2013). This phenomenon is explained by Bragg's law which describes the relationship between the incident X-Ray light and its reflection from the crystal surface (Figure 5) (Cornelius and Thomas, 2018). The X-ray beam scatters off a planes at a certain angle, θ, which equals the incident angle with respect to crystalline planes (George and José, 2021). The diffracted X-rays are detected, processed, analyzed and counted (Bunaciu *et al.,* 2015).

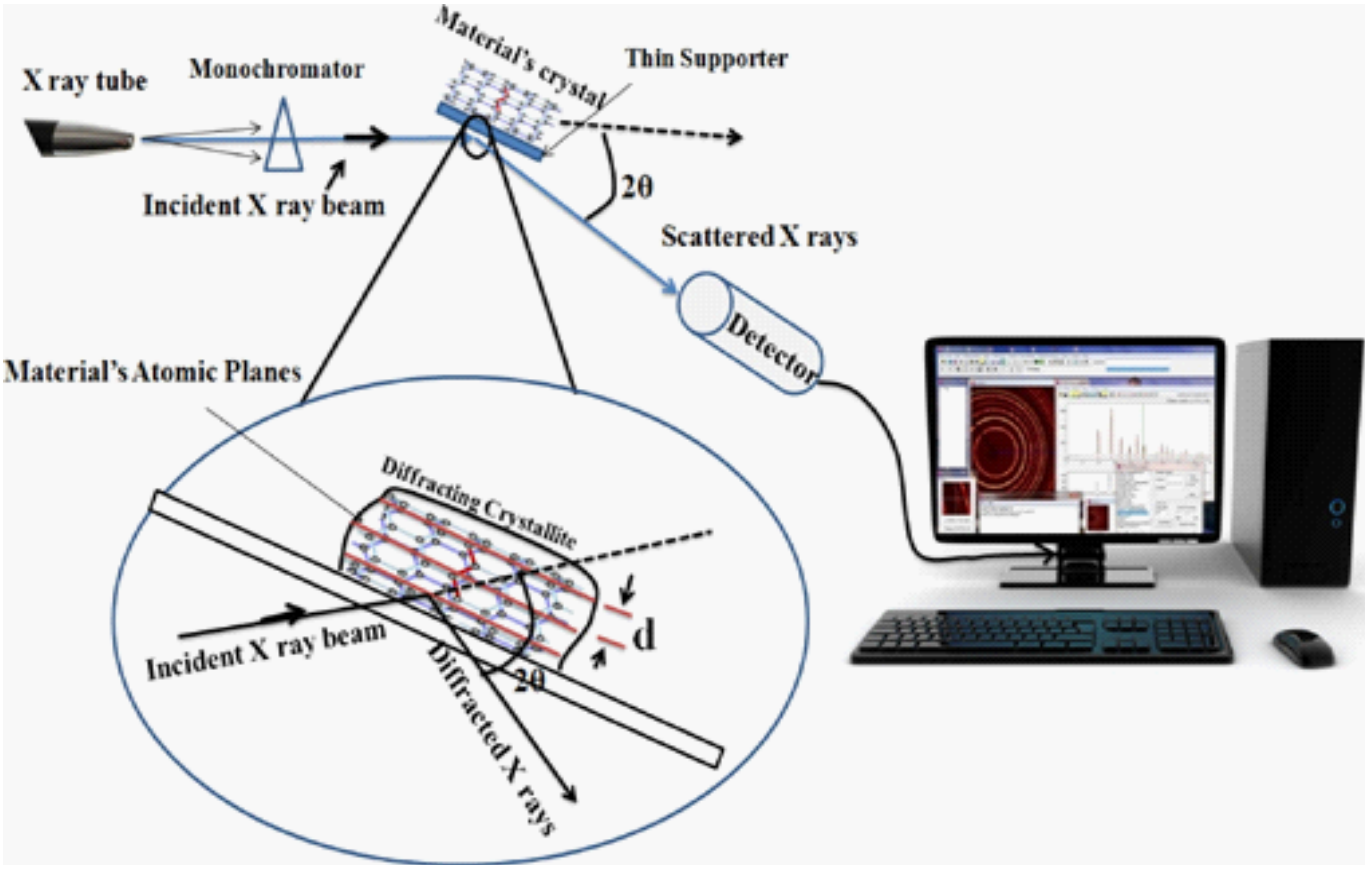

Figure 4: Components of an XRD instrument
Source: Das *et al.* (2014)

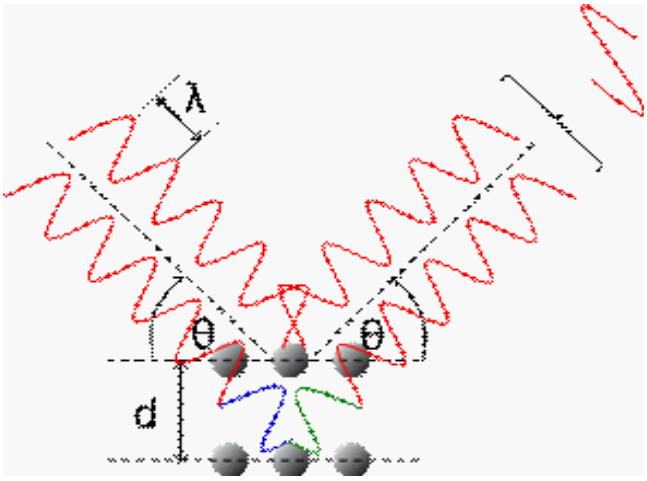

Figure 5: Bragg's law diagram
Source: Plaipichit *et al.* (2018)

The crystallite size of crystalline samples is usually determined using Scherrer equation (Muniz *et al.,* 2016; Chen *et al.,* 2023) expressed by Equation 1.

$$L = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

Where L is the average crystal size (nm), k is the shape factor constant (0.9), λ is the specific wavelength of the X-ray used (1.5406), θ is the diffraction angle and β is the angular width in radius.

X-Ray Fluorescence (XRF) Spectroscopy

The analytical tool is employed for qualitative and quantitative analysis of multi-elemental components

(Grieken and Marguı´, 2013). It is a versatile tool in trace elements analysis (Potter and Brand, 2019). The technique is based on the wavelength-dispersive principle, which entails emission of X-Ray photon energy of individual atoms that can be measured (Oyedotun, 2018). It is an established technique in application in many fields, such as in the biological, archeological, environmental and forensic sciences as well as in industry (Tsuji *et al.,* 2015; Wilberforce, 2016).

The principle is based on the use of an X-ray beam to produce ionisation in the atoms inner shells present in the sample due to photoelectric absorption (Marguí and Grieken, 2013). It is centered on the effect of X-ray interactions with the matter, causing excitation of certain electrons, which when relax to their ground state, emitting X-rays of a wavelength characteristic to a particular element (Gupta, 2014). The energy calibrations are first carried out to ensure that peak energies were accurately tied to specific elements. The XRF analysis is done by measuring the energy of fluorescent photons emitted by a sample after being excited on X-ray beam (Montanha *et al.,* 2020). The emitted X-Ray energy or wavelength is then employed to identify the different elements present in the sample which is proportional to its concentration (Marguí *et al.,* 2014). The Figure 6 is a schematic diagram of XRF spectrometer.

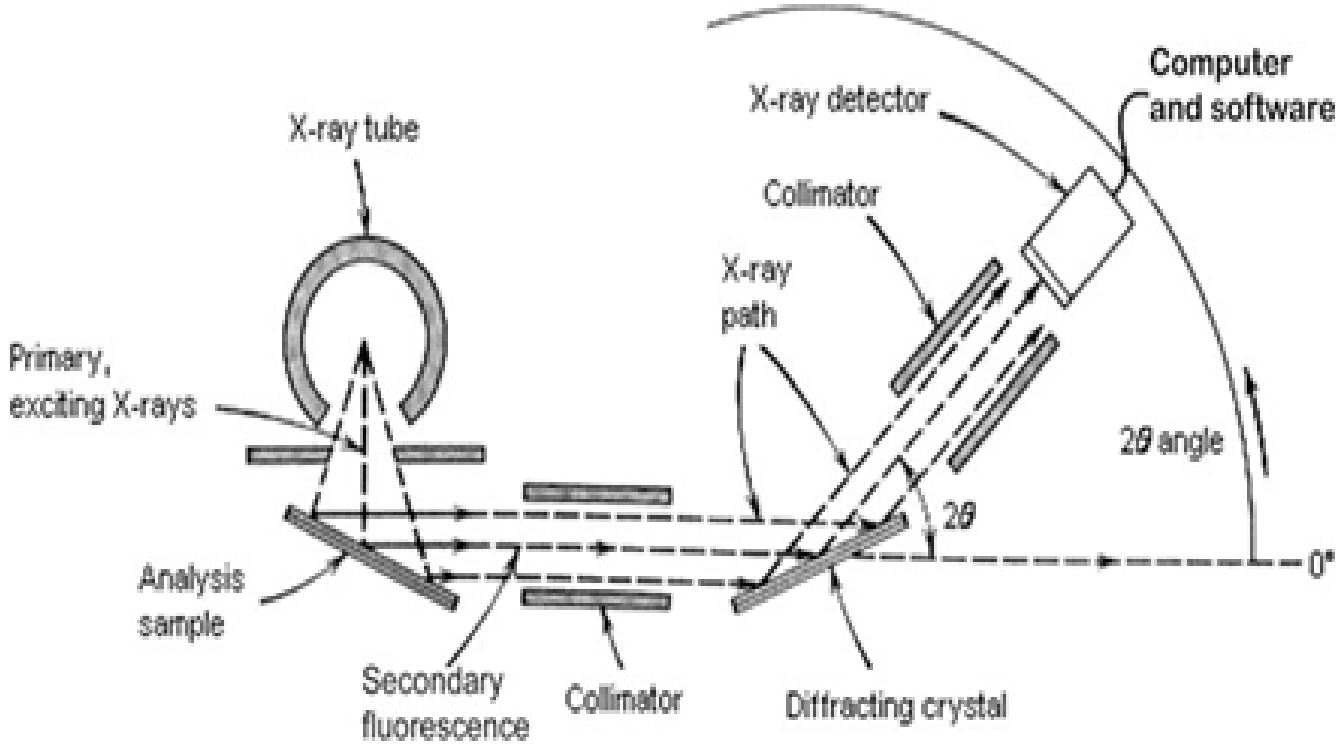

Figure 6: X-Ray Fluorescence spectrometer
Source: Shackley (2011)

Nuclear Magnetic Resonance (NMR) Spectroscopy

This is the most powerful technique in the modern science that utilizes radiofrequency waves to reveal information about magnetic nuclei of a compound (Bothwell and Griffin, 2011). It is a simple and a non-destructive technique that requires a small sample size and provides structural information that is quantitative (Claridge, 2016). The NMR technique has a broad scope in application such as in pharmaceuticals, organic chemistry, food science, biochemistry and material science (Yu *et al.,* 2021). It is an informative tool that is employed to determine the chemical makeup of organic molecules (Muller *et al.,* 2014). It is employed in vast fields due to its versatility in structural elucidation and the interactions in a range of molecular systems (Simpson *et al.,* 2018).

The technique utilizes permanent magnets that generate magnetic fields and therefore offering advantages of simplicity in operation and cost-effective, and this offers a viable alternative to high-field NMR spectroscopy (Yu *et al.,* 2021). The frequencies of NMR transitions are in the range of 10-1000 MHz which probe the nuclei of atoms. The nuclei show an energy state change when the sample analyte is placed in an intense magnetic field (Mishra *et*

al., 2017). The NMR signal produced reflect the chemical environment and the dynamics of a nuclear spin (Sugiki *et al.,* 2017). This is referred to as the "chemical shift" and it identifies different chemical groups within a molecule and their chemical bond environment (Simpson *et al.,* 2012). The NMR analyst can easily determine the degree of purity in a sample being analyzed, and this practice is used to validate the purity of different synthetic standards (Espina *et al.,* 2009).

The basic principle is that the chemical and structural constitution of a sample is determined by their nuclei which have distinctive magnetic fields (Kong *et al.,* 2015). The NMR spectrometer performs analysis using magnetic fields and a special detector is utilized to assess the changes (Figure 7) (Zia *et al.,* 2019). The external magnetic field strength makes the electrically charged nucleus to transition from a lower to a higher energy level with the difference depended on the magnetic field power and the nuclear field moment (Keeler, 2011).

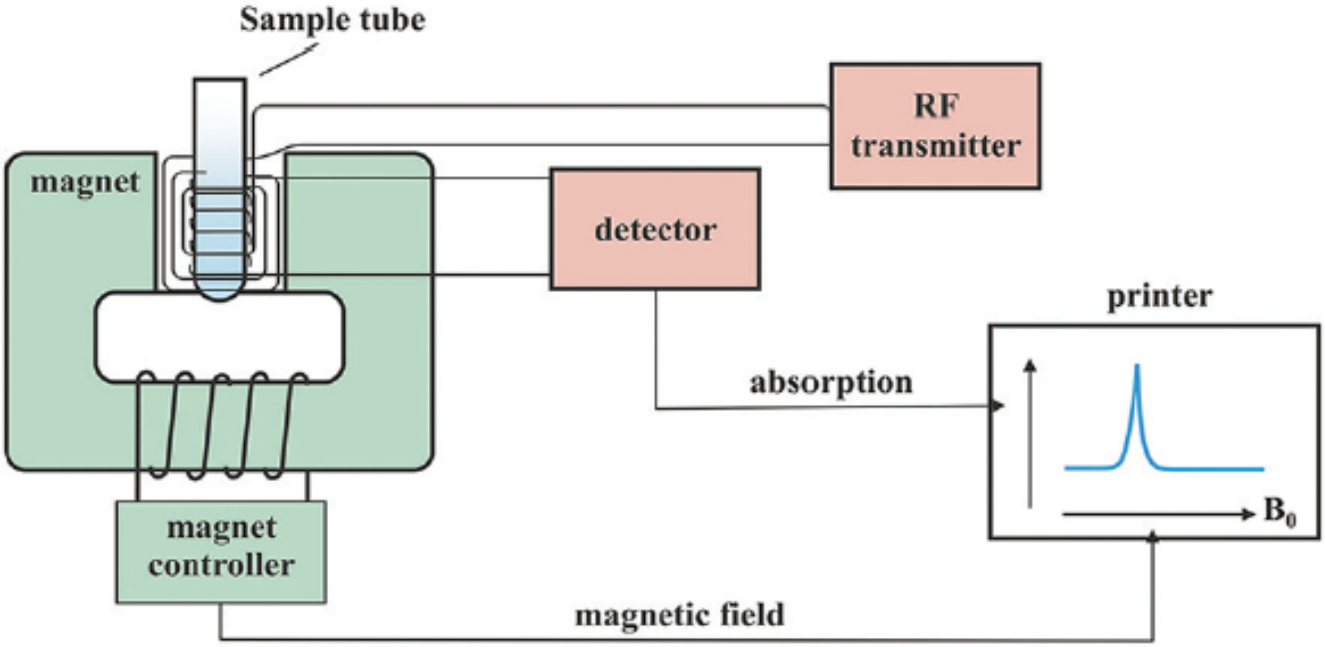

Figure 7: A schematic diagram of Nuclear Magnetic Resonance (NMR) spectrometer

MICROSCOPIC TECHNIQUES

Microscopy entails a "direct" imaging of the sample surface in visualizing the macro and/or microscopic structure and therefore provides the best resolution than the light-based imaging techniques (Kaech, 2013). The electron microscopy is on the basis of a circular electromagnetic field which acts on electron beam in a way related to the action of a glass lens on a photons beam (Bisen and Sharma, 2013). The SEM-EDX and TEM techniques are reviewed in the following sub-sections.

Transmission Electron Microscopy (TEM)

The technique provides structural information on the materials (Franken *et al.,* 2017). The TEM image provides a detailed information about the nano-structural characteristics of a sample material (Geetanjali *et al.,* 2024). The technique depends on the electron beam energy (eV), microscope resolving power (smaller than 0.3 nm), specimen thickness (less than 1 µm), stability and composition (Goodhew, 2011).

The principle is that matter interacts with an electron beam producing X-rays which are then used for elemental analysis using specialized detectors (Kaech, 2013). The transmitted electrons are focused by lenses and collected by parallel detectors to form an image (Inkson, 2016). The electron gun in a TEM instrument accelerates electrons of upto 300 kV so as to provide sufficient energy for them to penetrate through the sample material of up to 1 µm (Reimer and Kohl, 2008). The TEM microscope comprise of an electron gun, the electrostatic lenses which focusses electrons and a transmitted electron detection system (Figure 8).

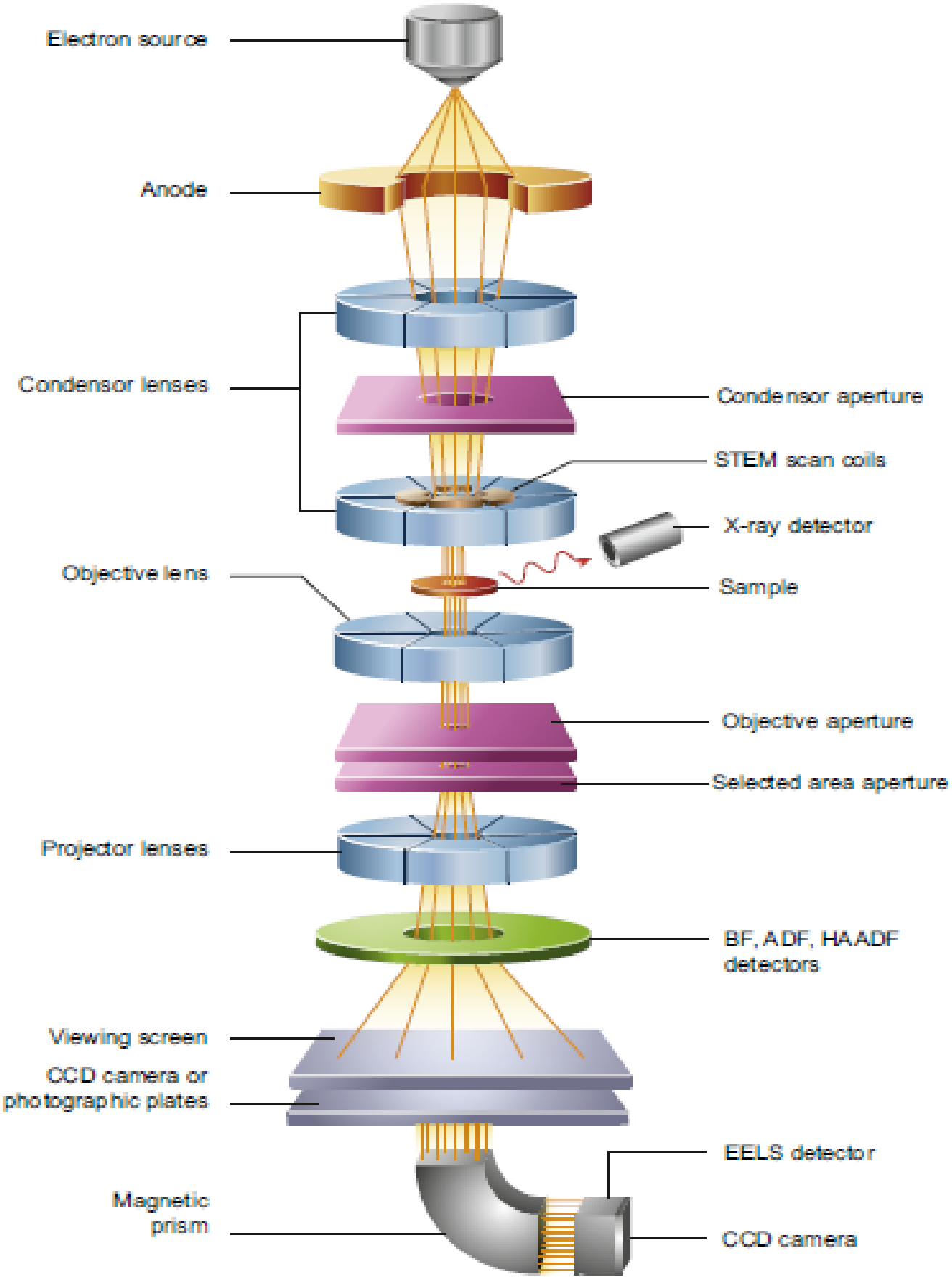

Figure 8: Schematic diagram of TEM components
Source: Inkson (2016)

Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX)

The microscopy uses beam of electrons which interacts with the sample and scatters to produce a signal which provides information about topological image and relative composition (Nie *et al.,* 2015; Han *et al.,* 2018). SEM images of various magnifications are employed to evaluate the morphology and sample size (Sharma *et al.,* 2016). The SEM instrument is mostly coupled together with Energy Dispersive X-Ray (EDX) detectors as an additional tool for semi-quantitative analysis (Cardell and Guerra, 2015; El-Zahhar *et al.,* 2019).

The working principle is that SEM uses a focused electron beam from an energy cathode which the upon interacting with a sample produces a topological image and it relative chemical constitution (Haddi *et al.,* 2024). The focused electron beam produces secondary electrons, backscattered electrons and characteristic X-rays upon interaction with the sample material, which then generate SEM signals that are collected by proper detectors to form images (Abd Mutalib *et al.,* 2017; Han *et al.,* 2018). The SEM instrument constitute an electron source, column containing electromagnetic lenses, sample chamber, electron detector and the computer display (Figure 9).

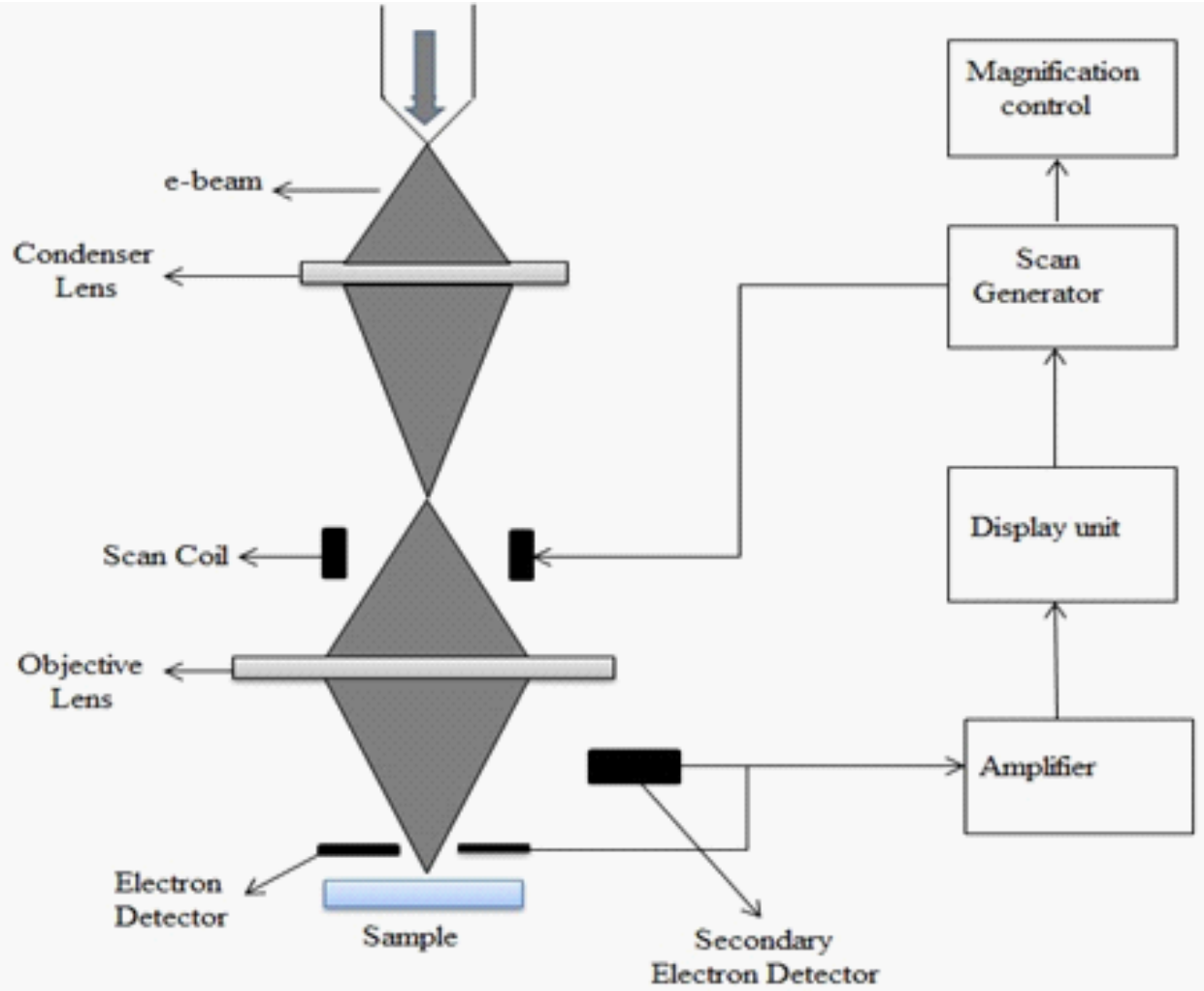

Figure 9: Schematic view of SEM components
Source: Han *et al.* (2018)

CONCLUSIONS

Spectroscopic and microscopic techniques are reported to be versatile and highly informative analytical tools for characterization and structural elucidation. The study reviewed FT-IR, AAS, XRD, UV-Visible, XRF and NMR techniques because of their increased usability by scientists in different fields such as in geology, medicine, food science, materials science, nanotechnologies, and environmental analysis amongst others. The techniques provide vital information on the chemical composition of organic and inorganic moieties making them unique and ideal in analysis. The existence of more diverse techniques that complement spectroscopic and microscopic studies further attract a more advanced review to enhance their potential impact to analysts. The Table 1 shows a summary of the reviewed spectroscopic and microscopic techniques highlighting their merits, demerits and some of the applications.

Table 1: Summary of reviewed spectroscopic and microscopic techniques

S/No	Technique	Merits	Limitations	Applications
1	AAS	Low operational costs, simple and fast working, highly selective and sensitive, performs quantitative analysis of metals in trace amounts.	Cannot detect non-metals, sample is destroyed, spectral interference, cannot analyze metal oxides, not suitable for complex matrices	Environmental analysis, pharmaceuticals, geological analysis, food and beverage analysis, biochemical analysis
2	FT-IR	Rapid and speedy, accurate, non-destructive, high sensitive, small sample required, high resolution, rapid reproducibility of data, ease of use, spectral quality, sensitive	Limited surface sensitivity, expensive equipment in purchase and maintenance, requires careful sample preparation, water interference, limited identification	Food analysis, medical analysis, environmental analysis, drug analysis, mineral analysis
3	UV-Visible	Cost-effective, non-destructive, quick measurements, easy to use, versatile, can perform quantitative analysis of compounds	Limited structural information, solvent interference, sensitive to temperature and pressure, can only analyze liquid samples, lower selectivity and sensitivity	Drug identification, quality control in a chemical research, food and beverage analysis
4	XRD	Fast, non-destructive, cost-effective, versatile, powerful, minimal sample preparation	Time consuming especially when analyzing large samples, limited to reference patterns, requires only a homogenous sample	Geological analysis, nuclear science, Nanotechnology, Cement industry
5	XRF	Speedy, accurate, non-destructive, portable, easy in ease	Interferences, background noise, matrix effects, limited depth of analysis	Medical applications, food inspection, archeological applications in stone analysis
6	NMR	Reproducible, quantitative, detailed, non-destructive, dynamic	Low magnetic moments for some elements, limited isotope abundance, equipment interference, magnetic field drift effect	Biomolecular analysis, Environmental analysis, pharmaceutical industry in drug analysis,
7	TEM	Detailed imaging, 3D mapping, high magnification and resolution.	Expensive in purchasing and maintenance, specialized experts required, inability to visualize living specimens	Nanotechnology, drug discovery, forensic analysis, semiconductor research
8	SEM-EDX	Rapid and high-resolution imaging, non-destructive, fast and cheap	Lack of specificity, limited to solid samples, poor accuracy to quantitative measurements of light elements	Materials science, forensics, pharmaceutical sciences, nanotechnology

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