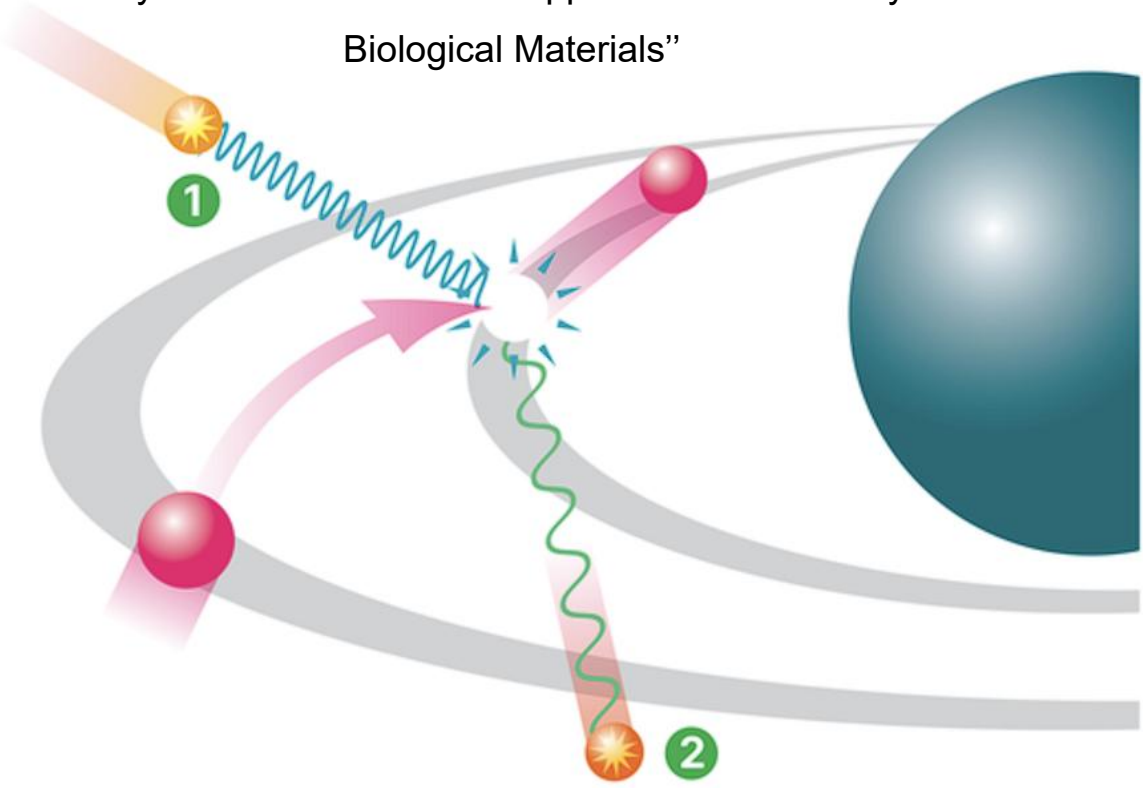
TECHNICAL UNIVERSITY OF CRETE

SCHOOL OF MINERAL RESOURCES ENGINEERING

Master Thesis

‘’X-Ray Fluorescence and Its Application in the Analysis of Biological Materials’’



Committee: Professor Nikolaos Kallithrakas-Kontos

Professor Emmanouil Manoutsoglou

Professor Nikolaos Pasadakis

Chania, 2025

Acknowledgments

I would like to express my sincere gratitude to all those who supported me through the journey of my master’s thesis.

First and foremost, I would like to thank my supervisor, Professor Nikolaos Kallithrakas-Kontos, for his guidance, encouragement and expertise. His insightful feedback and continuous support have been most helpful in shaping this research. I am grateful for the time you dedicated to reviewing my work and for the patience you showed during this time.

The past year and a half has presented numerous challenges for me in various aspects of my life, and I am truly proud to have completed this dissertation.

Lastly, I would like to thank my family for their unwavering support and constant encouragement. Their belief in me has been a constant source of motivation, and I could not have accomplished this without them.

Table of Contents

ABSTRACT

This Thesis explores the principles and applications of X-Ray fluorescence (XRF) in analyzing biological samples. The research begins with an overview of the X-Ray generation and the fundamental principles governing X-Rays, including their historical development and significance in various fields and particularly in medicine.

The study delves into different XRF techniques, such as Energy Dispersive X-Ray Fluorescence (EDXRF) and Total Reflection X-Ray (TXRF), highlighting their advantages and limitations in biological analysis. It emphasizes the unique capabilities of XRF to non-invasively detect trace elements in biological materials, which is crucial for clinical diagnostics, including the assessment of heavy metal exposure and the analysis of calcified tissues like bones and teeth.

Furthermore, the thesis compares XRF with other analytical methods, demonstrating its effectiveness in providing elemental composition data that can aid in understanding various health conditions. The research concludes by discussing future directions for enhancing XRF methodologies to improve sensitivity and accuracy in biological sample analysis, underscoring its potential impact on medical diagnostics and research.

Περίληψη

''Φθορισμός ακτίνων Χ και η εφαρμογή του στην ανάλυση βιολογικών υλικών''

Η παρούσα διατριβή, με τίτλο '' Φθορισμός ακτίνων Χ και η εφαρμογή του στην ανάλυση βιολογικών υλικών'', διερευνά τις αρχές και τις εφαρμογές του φθορισμού ακτίνων Χ (XRF) στην ανάλυση βιολογικών δειγμάτων. Η έρευνα αρχίζει με μια επισκόπηση της παραγωγής ακτίνων Χ και των θεμελιωδών αρχών που διέπουν τις ακτίνες Χ, συμπεριλαμβανομένης της ιστορικής τους εξέλιξης και της σημασίας τους σε διάφορους τομείς και ιδιαίτερα στην ιατρική.

Η μελέτη εμβαθύνει στις διάφορες τεχνικές XRF, όπως ο φθορισμός ακτίνων Χ με ενεργειακή διασπορά (EDXRF) και η ολική ανάκλαση ακτίνων Χ (TXRF), τονίζοντας τα πλεονεκτήματα και τους περιορισμούς τους στη βιολογική ανάλυση. Δίνει έμφαση στις μοναδικές δυνατότητες του XRF να ανιχνεύει με μη καταστροφικό τρόπο ιχνοστοιχεία σε βιολογικά υλικά, γεγονός που είναι ζωτικής σημασίας για την κλινική διάγνωση, συμπεριλαμβανομένης της αξιολόγησης της έκθεσης σε βαρέα μέταλλα και της ανάλυσης ασβεστιτικών ιστών όπως τα οστά και τα δόντια.

Επιπλέον, η διατριβή συγκρίνει το XRF με άλλες αναλυτικές μεθόδους, αποδεικνύοντας την αποτελεσματικότητά του στην παροχή δεδομένων στοιχειακής σύνθεσης που μπορούν να βοηθήσουν στην κατανόηση διαφόρων καταστάσεων υγείας. Η έρευνα ολοκληρώνεται με τη συζήτηση των μελλοντικών κατευθύνσεων για την ενίσχυση των μεθοδολογιών XRF για τη βελτίωση της ευαισθησίας και της ακρίβειας στην ανάλυση βιολογικών δειγμάτων, υπογραμμίζοντας το πιθανό αντίκτυπό της στην ιατρική διάγνωση και την έρευνα.

1. Basic Principles of X-Rays

1.1 Historical Flashback

In the late 1800s, Wilhelm Roentgen, Professor of Physics in Wurzburg, Bavaria made a groundbreaking and momentous discovery by accident: while testing whether cathode rays could pass through glass. His cathode tube was covered in heavy black paper, so he was surprised when an incandescent green light nevertheless escaped and projected onto a nearby fluorescent screen. Through experimentation, he found that the mysterious light would pass through most substances but leave shadows of solid objects. Because he did not know what these rays were, he called them ‘X,’ meaning ‘unknown,’ rays, thus the name of this type of radiation used today.

Röttgen noticed that these X-rays shared similarities with other known types of radiation at that time, such as light. However, what set X-rays apart from other known radiation forms was their extraordinary ability to effortlessly penetrate materials (e.g. tissues) that were impermeable to light.

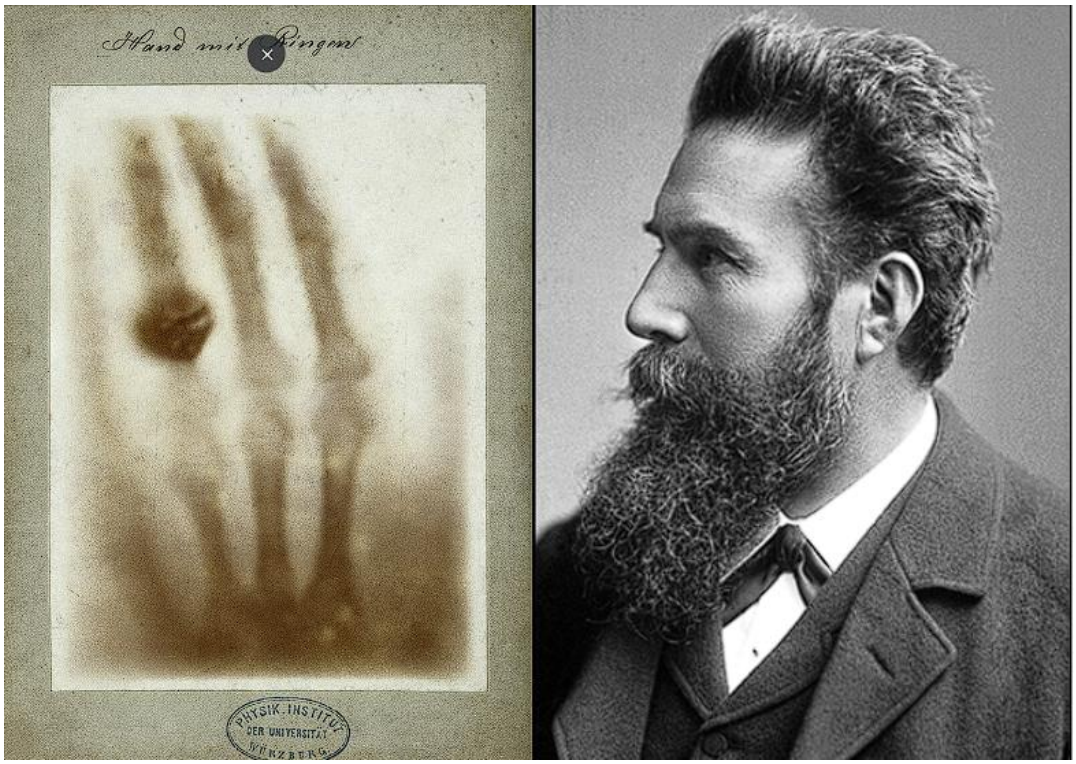


Figure 1.Print of Wilhelm Röntgen's first "medical" X-ray, of his wife Anna Bertha Ludwig's hand, taken on 22 December 1895 and presented to Ludwig Zehnder of the Physik Institut, University of Freiburg, on 1 January 1896 [1][2]

This distinctive feature quickly spread worldwide leading to the inevitable consequence of an extensive body of literature emerging shortly after the initial revelation. This comprehensive collection explored every facet of X-rays, as the early pioneers grappled with the challenge of unraveling the essence of these rays and devising methodologies to comprehend their characteristics and practical uses

This new form of radiation proved to be extremely valuable in a number of fields, as it allowed non-invasive examination of internal structures. As a result, the X-Rays expanded rapidly as a clinical diagnostic tool and within a year doctors in Europe and the United States were using X-Rays to locate gun shots, bone fractures, kidney stones and swallowed object.

Figure 2. Early X-Ray Machine [1][2][9]

Clinical use of the X-ray flourished in the starting years, but with little regard to potential side effects from radiation exposure. During 1896, the dangers of excessive exposure to X-rays became apparent. Yet, establishing safe operating procedures was hindered by the fact that the effects of X-radiation on matter, whether living or not, were an entirely new area of study. Scientists like Nikola Tesla and William J. Morton had a few early suspicions regarding the safe use of X-Rays resulted by small injuries they had themselves when

experimenting, but overall, early use of X-Rays was widespread and unrestrained, even to the degree that during the 1930's and 1940's, shoe stores offered free X-Rays so that customers could see the bones in their feet. [3]

Nowadays, we have a far better comprehension of the risks associated with X-Ray radiation and have developed protocols to minimize unnecessary exposure. And while X-Rays remain a milestone of modern medicine, their discovery paved the way for the development of today's broad rage of imaging techniques, including magnetic resonance imaging (MRI), computed tomography (CT), ultrasound, echocardiography, and many others.

1.2 X-Ray Generation and Principles

X-rays are a form of electromagnetic radiation similar to visible light. Their Fundamental principles involve the generation, the transmission and detection of X-Ray photons.

X-Rays belong to the electromagnetic spectrum characterized by their short wavelengths -that range between 10 to 0,01 nanometer- in comparison with visible light, which allows them to penetrate dense structures like bones and teeth.[4]

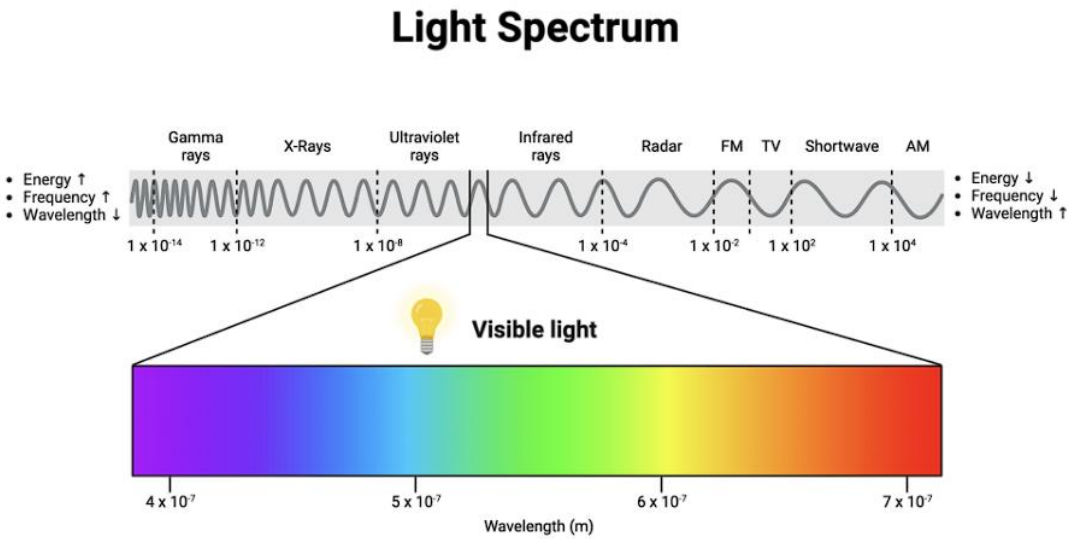


Figure 3.Light Spectrum [11]

An X-ray tube, with its respective components placed in a vacuum, and a generator, make up the basic components of X-ray production. Essential components of an X-ray tube include a cathode, and an anode separated to short distance from each other, a vacuum enclosure, and high voltage cables forming the X-ray generator attached to the cathode and anode components. .[5] [6]

The generation of X-rays occurs through the application of high voltage in the vacuum tube where high-energy electrons are emitted from the cathode and are directed toward a tungsten anode. When striking the anode, the electrons interact with atoms, resulting in the release of w-ray photons through processes like *thermionic emission* and *photoionization*. The energy of the X-rays can be adjusted by varying the voltage applied across the cathode tube, influencing the intensity and quality of the emitted radiation.

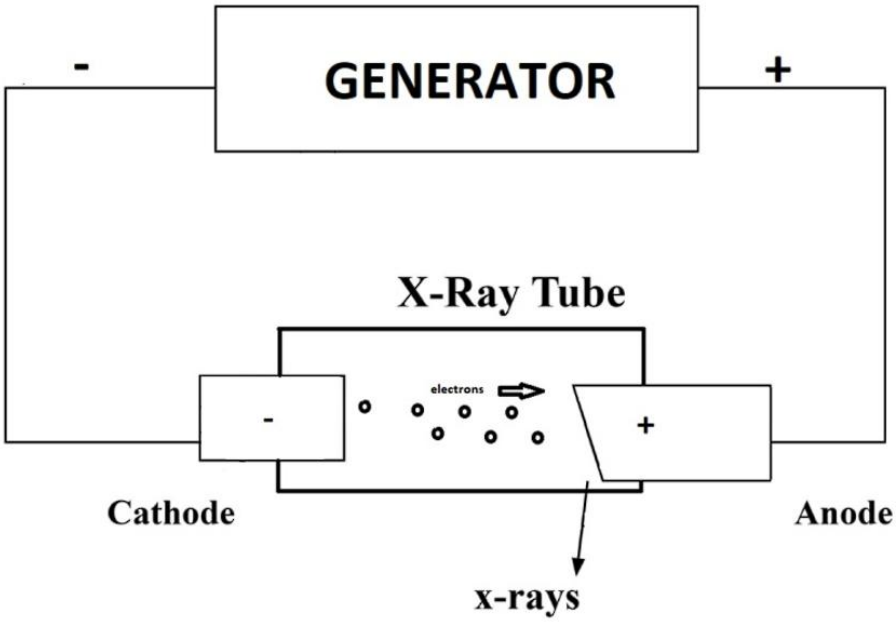


Figure 4.X-ray Generator Contributed by D Tafti, MD [7]

When the X-Ray passes through a patient, different tissues absorb them to varying degrees. Hight density tissues like bone absorb more X-Rays and

appear white on the film, while low-density tissues- like for instance the lungs- allow more X-Rays to pass thought and thus they appear darker. This differential absorption creates an image which can be interpreted by medical professionals.

1.3 Bohr's theory

Bohr’s model posits that Atoms consist of a nucleus and electrons arranged in layers, with the outermost layer being the valence electrons. These levels- shells are classified as K, L, M, etc. Electrons can only exist in specific orbits with defined energies. When an electron transitions between these energy levels, it can either be absorbed or emitted in the form of electromagnetic radiation (Including X-Rays).

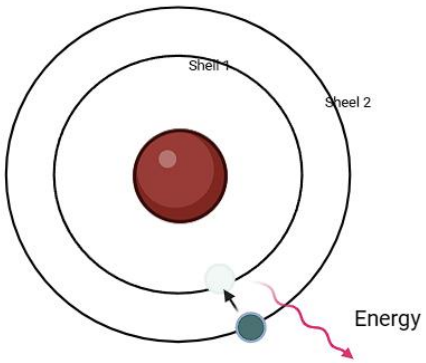


Figure 5. The transition of the electron resulting in the transmission of energy

To identify an element, it is essential to analyze the energy of the electron that can emit X-Ray photons thought a characteristic radiation. Since protons are more massive and heavier than electrons, they produce continuous X-Rays that are generally less intense. Sharp peaks in the radiation patterns correspond to characteristic X-Rays emission from the specific element (characteristic photon).

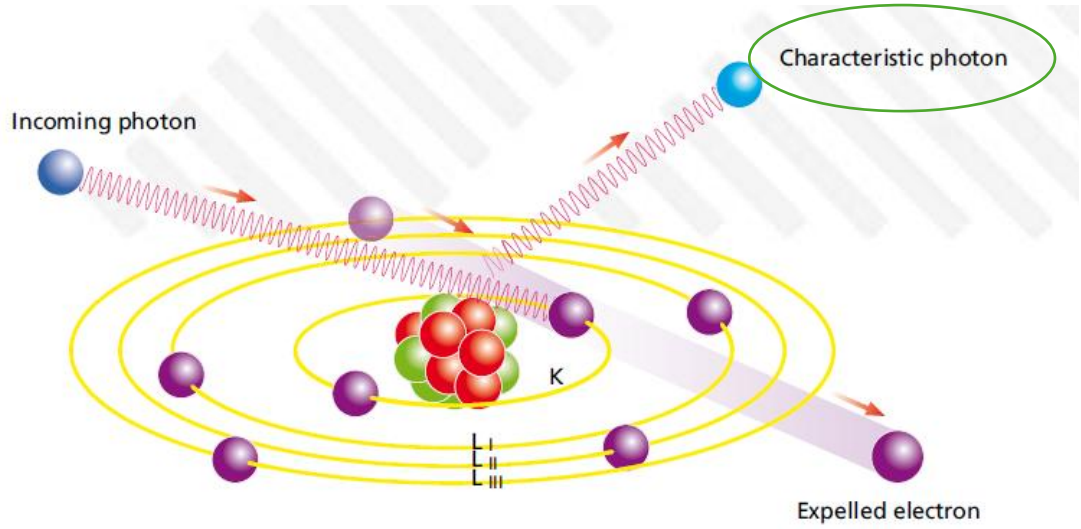


Figure 6. Production of characteristic radiation [13]

1.4 Excitation Sources

The main way to produce X-rays is by colliding high-speed electrons between metal targets in an X-ray tube. These tubes are equipped with electron sources, high accelerating voltages and metal targets. Filament tubes use filament that is heated as source that provides electrons, while gas tubes produce electrons by ionizing the gas inside the tube.

Historically, threaded pipe was the most commonly used. Today, all x-ray tubes are made from filament tubes. These devices have evolved significantly over time, resulting in two designs that are considered the best for every day use: *end window tubes* and *side window tubes*. The basic operating principle of X-ray tubes is to accelerate electrons in an electric field and decelerate them at the anode. To avoid collisions with gas molecules, a vacuum is created inside the casing.

As mentioned X-ray tubes are the most common sources of X-rays. They work by accelerating high-speed electrons towards a metal target, typically tungsten. The production of X-rays is mainly issued by two main mechanisms:

The ‘’Characteristic Radiation’’, which occurs when an electron collides with an inner-shell electron of the target atom, and ejects that electron. An outer-shell electron then falls into the vacancy, resulting into the emission of energy in the form of X-rays specific to the target material. And the ‘’Bremsstrahlung Radiation’’ which occurs when electrons are decelerated upon approaching the nucleus of the target material. The loss of their kinetic energy is emitted as X-ray photons, resulting in a continuous spectrum of X-ray wavelengths.

1.5 X-Ray Detectors

In the field of X-ray detection, there are many widely used methods, such as Photographic film, Digital X-ray Detectors, Semiconductor Detectors, Scintillation Detectors, Ionization Chambers, Energy-Dispersive X-ray Spectroscopy (EDS) Detectors etc.

Photographic film is the first detector ever used and is still widely used for X-ray inspection because it responds to X-rays in the same way as visible light, making it ideal for taking X-ray diffraction images.

In contrast *Digital X-Ray detectors* utilize advanced technology to produce high-quality images without the need of film processing. They usually consist of a Scintillator, that converts X-Rays into visible light, a Photodiode, that transforms the light into an electrical signal and a Read-Out Electronic that processes the electrical signal and creates a digital image. These detectors offer advantages such as faster image acquisition, lower radiation doses and the ability of enhance images with computed tomography (CT).

Another ‘’family’’ of detectors that provides a high detection efficiency and energy resolution are the *Semiconductor Detectors*. Which use materials like Silicon or Germanium to detect the X-Rays directly by converting them into electron-hole pairs. The two mainly used types are the Silicon (Si) Detectors an the Cadmium Telluride (CdTe) Detectors. The Si detectors are highly effective for low energy rays and CdTe detectors are more suitable for higher energy levels.

Scintillation detectors use materials such as sodium iodide doped with thallium to absorb X-rays and emit light. This light is then detected by photomultiplier tubes or photodiodes. They are commonly used in medical imaging and radiation detection due to their sensitivity and ability to provide energy resolution

There are also the Ionization Chambers which work by passing X-rays through gas between two electrodes in a tube (ionizing the gas). The resulting electrons produce an electric current in an external circuit. When an X-ray photon enters the chamber through a thin window, it ionizes the gas inside, and an ion current is established between the two electrodes. The gas is chosen to absorb strongly in the desired wavelength region. With increased voltage applied across the electrodes, the ionization chamber becomes a proportional counter, which produces an amplified electrical pulse when an X-ray photon is absorbed within it. At still higher voltages, absorption of an X-ray photon with consequent ionization of many atoms in the gas initiates a discharge breakdown of the gas and causes a large electric pulse output. This device is known as a Geiger-Müller tube, and it forms the basis for radiation detectors known as Geiger counters

Yet the most used detectors for energy dispersive X-ray spectroscopy are the EDS detectors that analyze the elemental composition of the materials by measuring the energies of characteristic X-rays emitted when excited by an electron beam or X-ray beam. Modern EDS systems often employ silicon drift detectors (SDDs), which offer high count rates and improved resolution compared to traditional silicon-lithium (Si(Li)) detectors

Manufactured from silicon wafers, SDDs have become the first choice for most EDS applications, replacing the Silicon (Lithium) detectors. Although SDD and Si(Li) have similar X-ray photon absorption and charge generation, their charge collection differs. SDDs have lower sequential noise, allowing them to operate at higher temperatures, and are easier to work with than Si(Li). However, SDD can only support thicknesses less than 1mm.

The main disadvantage of Si(Li) detectors is the demand for the presence of liquid nitrogen or cooling. Even though they provide good energy resolution,

their use and management are limited because of the liquid cooling requirements. SDD detectors, on the other hand, can operate at temperatures only a few degrees under 0, using Peltier devices to cool the SDD sensors. In addition, SDD detectors provide very good energy resolutions even at high count rates, positively impacting analytical performances. [8]

1.6 X-Ray Absorption and Scattering- General Principles

When X-rays encounter an object, interactions occur with matter such as absorption and scattering will occur resulting into photo-absorption, Compton's scattering and Rayleigh scattering. [9][10]

As an X-ray travels through a homogeneous material, its intensity gradually decreases with the distance traveled. The differential relationship is given by:

$$-\frac{dI}{I} = \mu dx$$

where μ is a constat. The negative sign is introduced because dI is a decrease in the intensity if the dx represents an increase in distance. The constant μ is specific for ach material and is called *linear absorption coefficient* of the material. The linear absorption coefficient μ depends on the density of the substance and the wavelength of the X-ray beam.

After integration, the equation becomes:

$$I = I_0 e^{-\mu x}$$

Since μ is proportional to the density ρ, μ/ρ is a constant known as the mass absorption coefficient. This coefficient doesn't depend upon the physical state and thus, this equation can be written as:

$$I = I_0 \; e^{\left(-\frac{\mu}{\rho}\right)\rho x}$$

In this equation, *I* represents the intensity of the beam after a distance x, and I_0 is the initial intensity μ of the X-ray beam. The density ratio ρ gives the mass absorption coefficient (μ/ρ).

In photoelectric absorption, an electron attached firmly to an atom in one of the atomic shells is ejected by the complete energy transfer of a photon. The electron begins to move and loses its energy, but the energy that is transferred by the photon is stored near the site of the photoelectric interaction.

These interactions are only possible when the photon energy is slightly higher than that of the electron. The energy of the photon must be high enough to overcome the binding energies of the electron in order to remove it from the atom. The probability of these interactions may be increased by using low energy photons and materials with high atomic numbers. Photoelectric interactions majorly contribute to the absorption of X-rays. The mass absorption coefficient steadily decreases and energy of incident X-rays increases. [9]

2. X-Ray Fluorescence Technique

2.1 Introduction – Basic Principles

X-ray fluorescence (XRF) is a spectroscopic method used to analyze the chemical composition of various materials regardless of their physical state. The materials-samples can be in solid, liquid, powder, filtered or any other form. This technique can also measure the thickness and structure of the layers of the materials-samples, making it very flexible and very widely used for many scientific purposes. When materials are exposed to an X-Ray beam, they emit fluorescent radiation of varying energies, allowing their composition to be identified and characterized.

XRF is fast, accurate and non-destructive, and usually requires only minimum sample preparation. Applications are very broad and include the metal, cement, oil, polymer, plastic and food industries, along with mining, mineralogy and geology, and environmental analysis of water and waste materials. XRF is also a very useful analysis technique for research and pharmacy.

In XRF analysis a different energy is equivalent to a different color. By measuring the energies (determining the colors) of the radiation emitted by the

sample it is possible to determine which elements are present. This step is called qualitative analysis. By measuring the intensities of the emitted energies (colors) it is possible to determine how much of each element is present in the sample. This step is called quantitative analysis. [11]

Spectrometer systems can be divided into two main groups:

- Energy Dispersive X-Ray Fluorescence (EDXRF), that measures the energies of emitted X-Rays directly, allowing for rapid analysis. EDXRF is very suitable for a wide range of elemental analysis.
- Wavelength Dispersive X-Ray Fluorescence (WDXRF), which separates the emitted ray based on their wavelengths -as the name suggests- using diffraction crystals, providing higher sensitivity for complex samples.

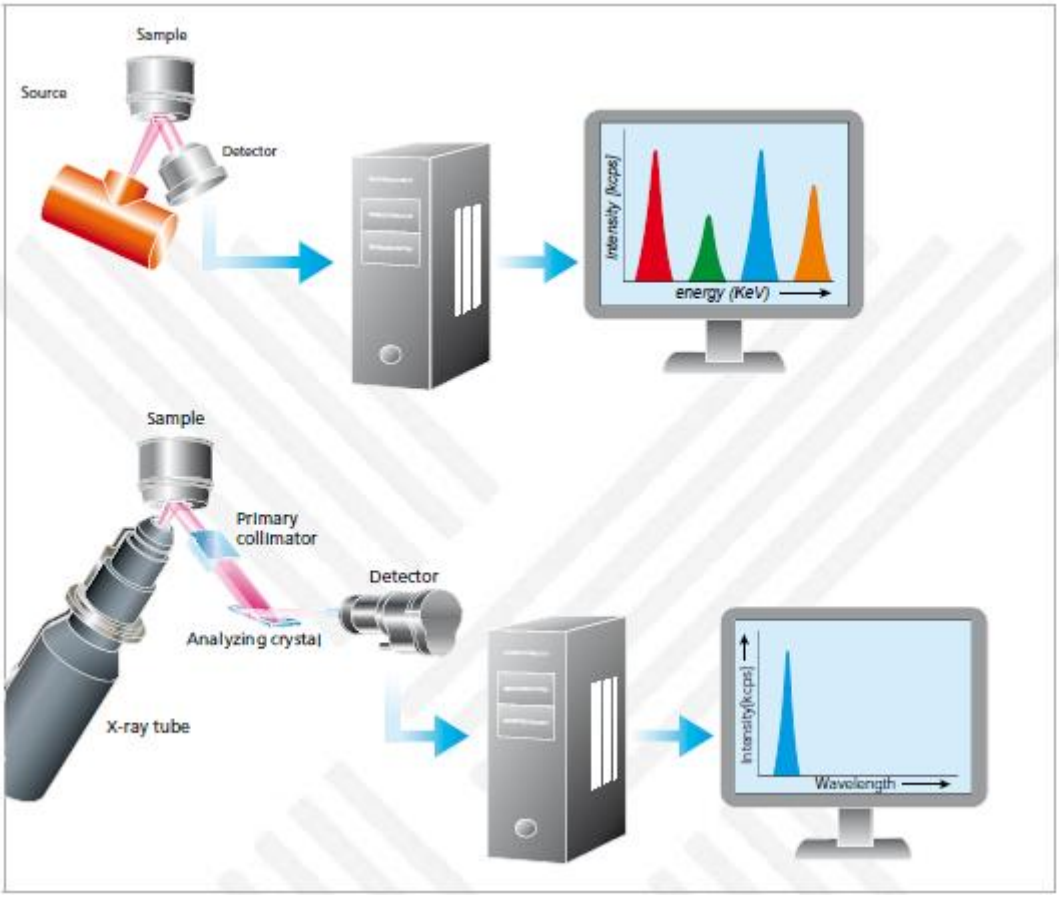


Figure 7. Most common EDXRF & WDXRF spectrometer layouts [11]

Some of the advantages of the XRF is that it is a non-destructive technique which has vital significance for sensitive samples. The technique provides quick results with minimal sample preparation and it Is capable of analyzing a wide range of materials and elements, specifically:

- bulk chemical analyses of major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) in rock and sediment
- ulk chemical analyses of trace elements (>1 ppm; Ba, Ce, Co, Cr, Cu, Ga, La, Nb, Ni, Rb, Sc, Sr, Rh, U, V, Y, Zr, Zn) in rock and sediment

On the other hand, one of the XRF limitations is that the commercial instruments are very limited in their ability to precisely and accurately measure the abundances of elements with atomic number lower than 11 (Z<11) in most natural earth materials, further more they cannot distinguish variations among the isotopes of an element and also they cannot distinguish between ions of the same element in different valance state.

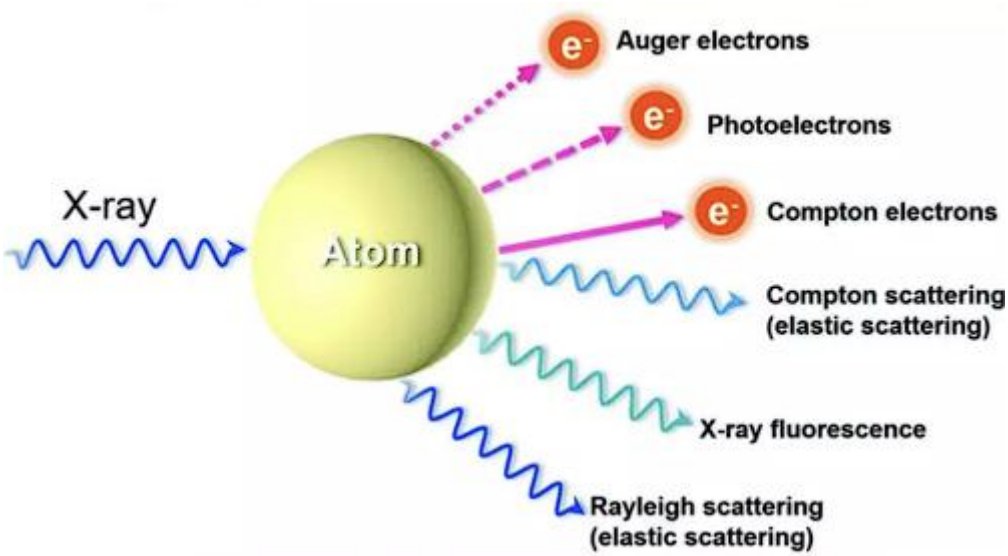


Figure 8. The interaction of X-Rays with matter.[13]

2.2 Small spot instrumentation - Micro XRF

Micro X-ray fluorescence (μXRF) is a specialized elemental analysis technique that enables the examination of very small sample areas. Unlike traditional X-ray fluorescence, which analyzes larger areas, μXRF utilizes advanced X-ray

optics to focus the excitation beam to a *small spot* on the sample surface, allowing for detailed analysis of small features. Modern μXRF instruments are equipped with features that not only facilitate small spot analysis but also mapping of larger areas. For instance, some spectrometers can measure spots as small as 0,5 mm in diameter while also allowing for the analysis of larger areas up to 30 mm.

In most XRF applications, the sample size is typically about one centimeter, allowing an average representation of the composition. However, when a specific region of interest needs to be analyzed, such as a chip wafer or a spot on a disk, local analysis is required. In this case, the available samples can be very small, ranging from 50 μm to a few millimeters in diameter.[14]

Traditional μ-XRF instrumentation employs a basic pinhole aperture to limit the size of the incident beam on the sample surface. This setup allows only the X-rays aligned with the hole to pass through. However, this approach significantly reduces the amount of X-Ray flux emitted by the source, leading to a low incident flux on the sample. As a result, this limitation negatively impacts the sensitivity of the method for detecting trace elements.

To generate small focal spots with high X-Ray flux on the sample surface for μXRF applications we use Polycapillary and doubly curved crystal focusing X-Ray optics. μXRF with X-Ray optics have been successfully used for applications including small feature evaluation, elemental mapping, film and plating thickness measurements, detection of micro-contamination, evaluation of multi-layered coatings for advanced circuit boards, small particle analysis, and even forensics.

Polycapillary focusing optics gather the X-Rays form a divergent source and concentrate them int small, focused bean at the sample surface, achieving the small spot focus points. Doubly curved crystal optics, on the other hand, direct a concentrated micro0sized monochromatic X-Ray beam to the sample, facilitating superior elemental analysis. The main advantage of this technique is the reduced X-Ray scattering background beneath the fluorescence peaks, which enhances measurement sensitivity.

μXRF can be achieved using polycapillary or doubly curved crystal optics using EDXRF or WDXRF geometries.

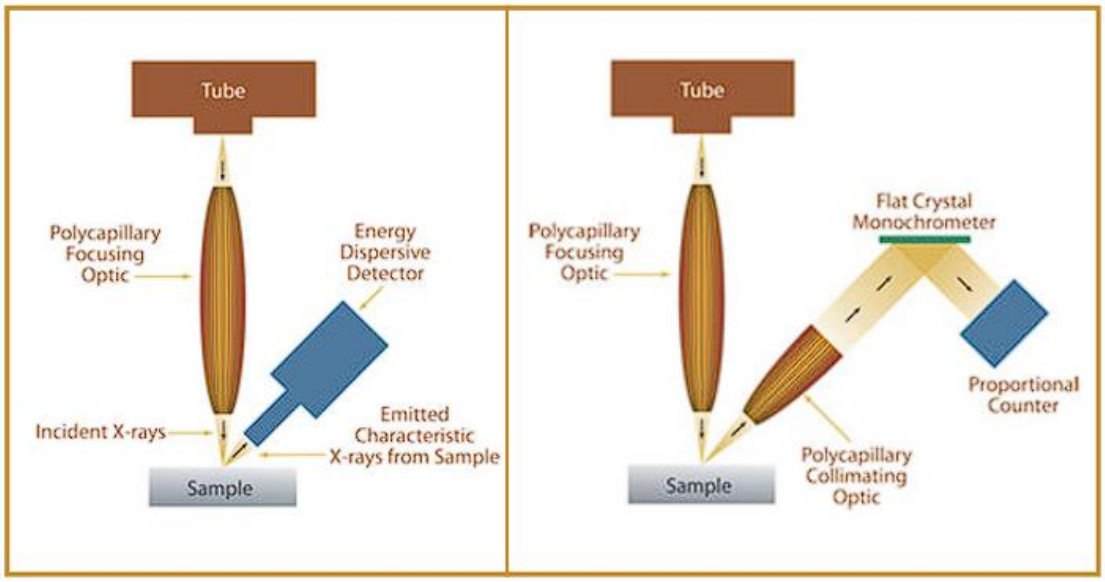


Figure 9. Standard EDXRF and WDXRF geometries [15]

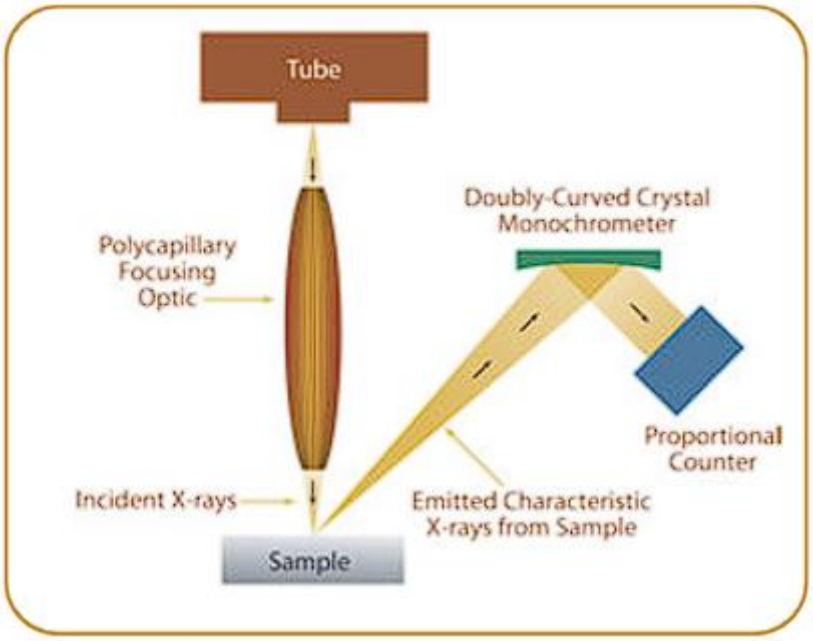


Figure 10.Micro WDXRF instrumentation using a polycapillary focusing optic [15]

2.3 Application of synchrotron radiation to XRF (SR-XRF)

Strong X-rays and other electromagnetic waves, such as ultraviolet, visible, and infrared light, are produced by synchrotron radiation (SR). It is several orders of magnitude more intense than laboratory X-ray sources. Monochromatized X-rays can be used as incident X-rays in XRF thanks to SR X-rays. A comparison of the XRF spectra produced by monochromatized X-ray (SR-XRF) and continuous X-ray (conventional XRF) irradiation is presented in Fig. 11. The characteristic X-rays of the target material and the X-ray source provide a high background in the XRF spectrum from continuous X-ray irradiation (Fig. 12(a)), whereas "Bremsstrahlung" causes a broad background. Consequently, the background obscured the specimens' weak characteristic X-rays, making it challenging to analyze trace elements with traditional XRF. On the other hand, the Compton scattering peak is the only background visible in the XRF spectrum of a monochromatized X-ray (Fig. 11(b)), making it easy to identify trace elements (~ppm). The ability to focus SR X-rays into diameters ranging from μm to nm has made it possible to analyze the distribution of trace elements at the intracellular or cellular level in recent years.

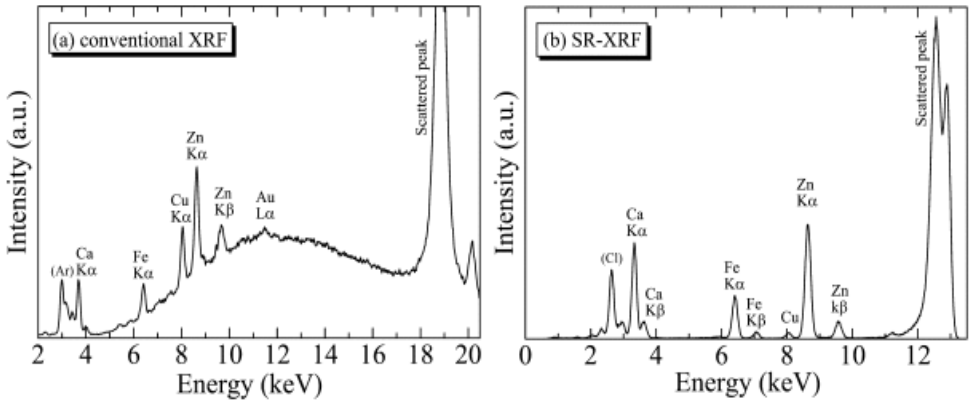


Figure 11.A comparison of XRF spectra obtained by conventional XRF (a) and SR-XRF (b). [23]

Wilson disease causes copper buildup in tissues, which leads to liver damage and neurological or mental problems. Copper concentration measurements in tissue (liver) should be done in the early stages since early detection and treatment determine the subsequent convalescence. SR-XRF was used by Matsuura et al. to diagnose Wilson illness. The distribution of copper in liver

tissue was shown, and knowledge of the liver's copper concentrations could help with treatment planning.

Furthermore, there have been reports of variations in trace element concentrations in different cancer tissues. Oral squamous cell carcinoma (SCC) specimens embedded in paraffin and thin-sliced (10 μm thick) were measured. A pathological image of an SCC specimen and elemental distribution images produced by SR-XRF are displayed in Fig. 12. At the Photon Factory, High-Energy Accelerator Research Organization, BL-4A, SR-XRF measurements were carried out. Such thin-sliced specimens cannot be imaged using conventional XRF elemental imaging because the fluorescence X-ray produced by the thin specimen is very feeble. Fluorescence X-rays from thinly sliced specimens with low backgrounds can be detected using SR-XRF. The pathological and elemental distribution images can then be compared using this method, which allows elemental distribution images to be acquired from successive slices of the diseased material. Ni, Co, and Cu were found in the epidermal layer of an SCC location in Fig. 12. Although it was uncertain how the location of metallic elements related to the pathogenesis of SCC, it is anticipated that the imaging of metallic trace element distributions in these histological regions will offer valuable diagnostic information.

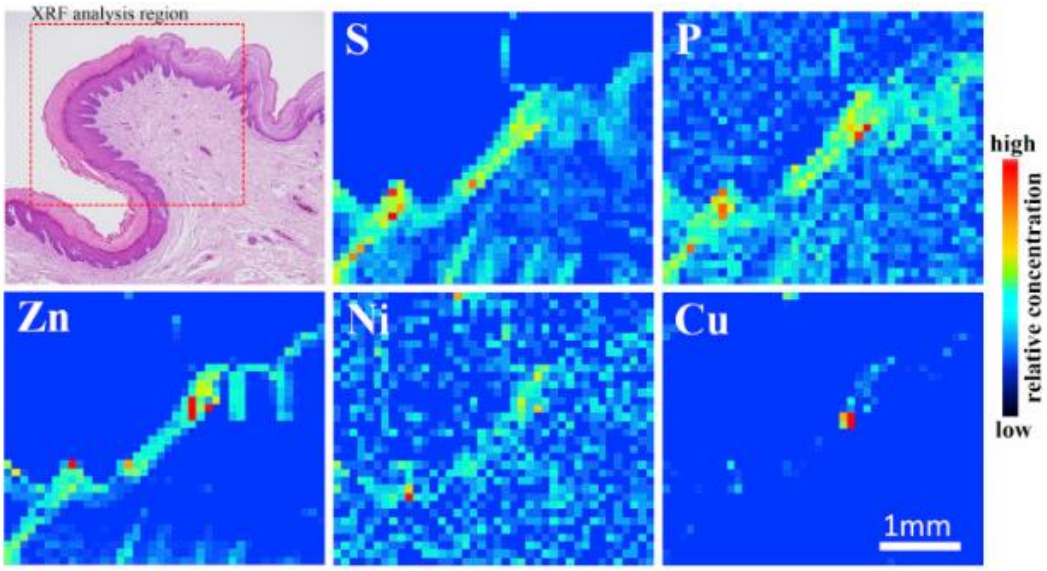


Figure 12. A histological image and elemental distribution images of a SCC specimen using SR-XRF. [23]

3. X-Ray Fluorescence Compared with Other Analytical Techniques

3.1 Introduction

The fundamental ideas used by many practical techniques involving the interaction of electron beams, X-rays, and materials forms the basis of the X-ray fluorescence (XRF) approach.

These techniques include X-ray diffraction (XRD), wavelength dispersive spectroscopy (e.g., microprobe wavelength dispersive spectroscopy), and X-ray spectroscopy (e.g., scanning electron microscopy with energy-dispersive X-ray spectroscopy).

- The atomic response to radiation makes it possible to use X-ray fluorescence to examine major and trace elements in geological rocks.
- Material ionization is possible when exposed to high-energy, short-wavelength radiation, such X-rays.
- The atom becomes destabilized, and an outer electron replaces the missing inner electron when the radiation's energy is sufficient to displace an inner electron that is firmly bound within the atom.
- When this process takes place, energy is released, which can be explained by the inner electron orbital's lower binding energy compared to an outer electron orbital.
- Fluorescent radiation is the term for the radiation that is emitted, which has less energy than the original X-rays.
- The distinctive energy of released photons, which correlates to transitions between particular electron orbitals within the element, can be used to determine element abundances in a sample.
- Fluorescent X-rays can be used for this because of this occurrence.

In general, there are two types of X-ray fluorescence analysis instruments: those that employ energy-dispersive X-ray spectroscopy (EDX) and those that utilize wavelength-dispersive X-ray spectroscopy (WDX). Because fluorescent

X-rays generated in a sample need to be distributed using a goniometer and an analyzing crystal, the WDX apparatus is extremely large. The EDX detector, on the other hand, has a more compact design and provides superior energy resolution without the need for a large dispersion system.

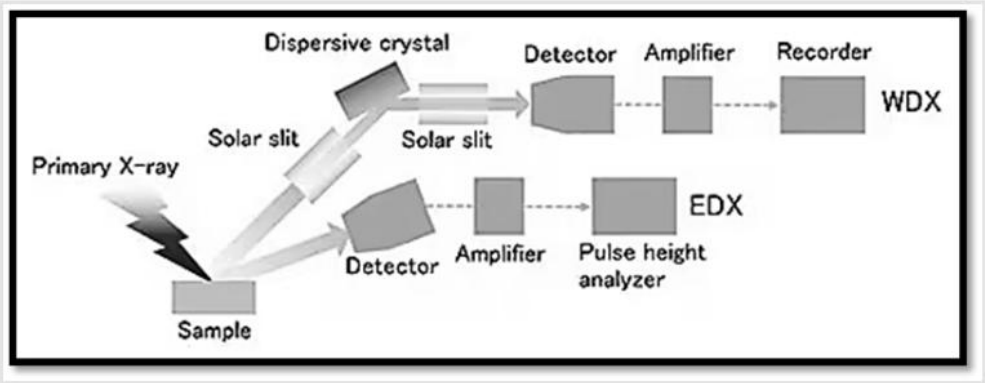


Figure 13. General EDXRF and WDXRF geometry [11][15]

X-RAY GENERATION

- In order to create X-rays, the X-ray tube accelerates electrons at a high voltage and then bombards them against a metal anode, also known as the anti-cathode. There are two primary types of X-ray tubes: side window and end window. Both are designed to evenly disperse powerful X-rays throughout the sample's surface.
- In most cases, incident X-rays are collected using a window composed of beryllium foil. Tungsten, rhodium, molybdenum, and chromium are examples of anti-cathodes. The anti-cathode can also be referred to as the target. The anti-cathode to employ depends on the kind of samples that will be analyzed. Using X-ray tubes with anti-cathodes composed of the same components under study is not a smart idea.

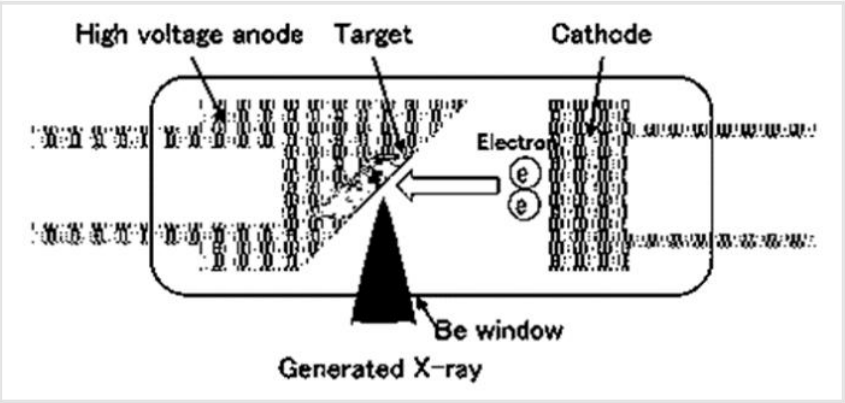


Figure 14. X-Ray generation scheme [8]

DETECTORS

- The Si (Li) detector uses a diode with a p-i-n junction. The diode acts as a rectification mechanism, restricting electric current to flow in just one direction.
- When a voltage is applied in the opposite direction of the electrons' motion (referred to as reverse bias), the current is blocked; however, when light is permitted to pass through, the electrons in the forbidden band are stimulated into a conductive band, and only the excited electrons' current flows.
- Measurements of the individual current pulses produced by each single X-ray photon are necessary for X-ray detection.
- The X-ray energy can be found by measuring the pulse height of the current pulse since the incident X-ray energy is proportional to the instantaneous current value of a single pulse.
- The Si (Li) semiconductor detector is a diode with Li drifting over a high-purity single Si crystal that is chilled by liquid nitrogen. The FET is cooled by liquid nitrogen.
- Due to a scarcity of liquid nitrogen and an increase in temperature, the first semiconductor detector was harmed by excessive voltage.
- To avoid unintentional harm, modern detectors keep an eye on their surface temperature and cut off the high voltage if it increases above a predetermined point.

- The detector can be used 30 minutes after liquid nitrogen at low frequencies.

Samples are irradiated with X-rays from various angles in both top-surface and bottom-surface sample chambers. While both types can be detected, top-surface irradiation facilitates sample observation and measurement while moving across a stage.

Most X-ray fluorescence analysis equipment allows for the sample chamber environment to be reduced to vacuum conditions. This is because the environment weakens X-rays, making them less useful. As a result, a vacuum is required to measure lighter materials.

When a sample is exposed to a powerful X-ray beam (the incident beam), some of the energy is scattered and some is chemically absorbed by the sample. This is the basis for how an XRF spectrometer operates. Although other materials including W, Mo, and Cr may be employed, a Rh target is often used to create the incoming X-ray beam. When subjected to this basic X-ray radiation, the sample is referred to as "excited." The stimulated sample reacts by releasing X-rays with a wavelength spectrum unique to the types of atoms it contains. Why does this happen?

- After absorbing X-rays, the sample's atoms ionize, releasing electrons from their lower energy levels, which are typically K and L.
- Higher energy electrons from a more distant orbital are drawn in to replace evicted electrons.
- Energy is released during this process because the binding energy of an inner electron orbital is lower than that of an outer one.
- Each sort of atom emits its own unique X-rays as a result of this heat. A complicated emission X-ray spectra can be broken down into unique wavelengths for each element in a sample that contains numerous elements, as is typical for most minerals and rocks, using a Wavelength Dispersive Spectrometer like the one found in an EPMA.
- Among other instruments, scintillation detectors and gas flow proportional detectors are used to measure the intensity of the emitted beam.

- The K spectra of elements lighter than zinc, which generally generate X-rays with a large wavelength (>0.15 nm), are commonly measured using the flow counter.
- Shorter wavelengths of the X-ray spectrum (K spectra of elements from Nb through I; L spectra of Th and U) are typically examined using the scintillation detector. At intermediate wavelengths, two detectors are usually employed in tandem for X-ray measurements.
- This is due to the various detection limits of the L spectra generated from Ba and the rare earth elements and the K spectra generated from Zn to Zr.
- The energy levels that these detectors measure are proportionate to the number of elements present in a sample. The precise value of this proportionality for each element is derived from mineral or rock standards whose composition has already been examined.

XRF tooth analysis: caries estimate and trace element detection: A new approach to treating dental caries called "minimum intervention" calls for keeping as much healthy tooth as feasible while removing as little volume as possible. Accurate identification of the carious region is required for this method. Traditional caries diagnosis relies primarily on X-ray transparency and visual and probing examinations. One obvious sign of caries is a decrease in calcium brought on by demineralization. Hiraishi et al. [46] evaluated the Ca concentration of demineralized tooth surfaces using scanning XRF microscopy. The gold standard for assessing demineralization is contact microradiography. However, other parameters, such as the amount of other minerals and organic material, can have a significant impact on X-ray transmission in addition to the concentration of Ca. It is possible to directly assess the Ca concentration using XRF microscopy to estimate tooth demineralization more precisely.

Environmental contamination can cause heavy metals to accumulate in teeth and hair. Additionally, substances from cigarettes, food, and dental restorations can build up in the teeth. Thus, tooth trace element analysis would be a suitable indicator of the impact of different heavy element contaminants in the environment. XRF measurement of trace elements in teeth taken from residents of Poland's most and least polluted regions was reported by Baranowska et al.

This study found a positive relationship between environmental pollution levels and the quantities of Zn, S, and Pb in teeth. Furthermore, smokers' teeth had noticeably greater levels of Zn and Pb than non-smokers' teeth.

Applications of XRF for diseased and soft tissue samples: Application of XRF for pathological and soft tissue specimens: Pathological specimens often contain foreign materials; however, they may infrequently include calcified or precipitated solid entities. To facilitate diagnosis, these unidentified entities must be recognized. Every case and patient has a unique pathology. XRF analysis is suitable for this application as it may be conducted without damaging or pre-treating the specimens. Elemental distribution photos in Fig. 15 illustrate the oral mucosa in contact with a pure titanium cover screw from a dental implant. The external morphology of the specimens is illustrated in the sulfur dispersion images (Fig. 15(a) and (c)). The position of Ti in these specimens is illustrated in the Ti distribution images (Fig. 15(b) and (d)).

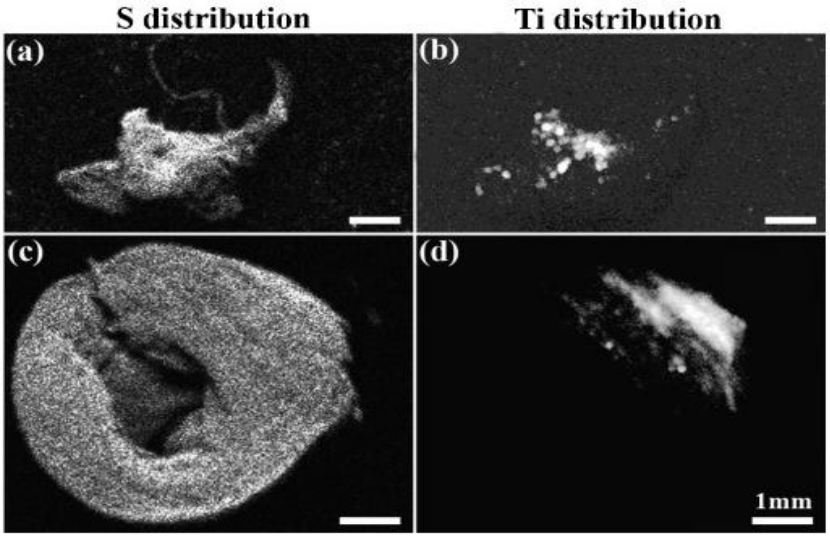


Figure 15. S and Ti distribution images from oral mucosa in contact with a pure titanium cover screw of a dental implant. [36]

Ti was concentrated in some regions in Fig. 15(b), indicating the presence of Ti-based particle-like materials. Using X-ray absorption fine structure (XAFS) analysis, these Ti particles were identified as metallic Ti. They were subsequently proposed to be abrasion-generated debris after implant surgery. On the other hand, Ti was evenly distributed throughout the specimen

in Fig. 15(d). Using the XAFS approach, the chemical state of the Ti was identified as TiO2 (anatase). Regarding the origin of the TiO2, it is thought that Ti may have oxidized and localized after eroding and dissolving into the surrounding tissue.

Typically, pathological specimens are encased in paraffin. Because paraffin has a low melting point and a high volatility, pathological specimens embedded in paraffin cannot be examined with EPMA or SEM/EDS without first undergoing a deparaffinization procedure. Specimens encased in paraffin can be subjected to XRF examination without suffering radiation damage. The XRF spectrum of a paraffin-embedded lung biopsy sample from tungsten carbide pneumoconiosis is displayed in Fig. 16. "Tungsten carbide pneumoconiosis" is a severe form of pneumoconiosis that can be brought on by fine particle dust from cemented tungsten carbide (WC) cutting tools. Both a histologic estimate and the identification of tungsten in lung tissue are required for the diagnosis of this illness. The lung biopsy samples obtained from inhaled WC in Figure 16 clearly shows peaks attributed to tungsten L lines, indicating the presence of tungsten or a tungsten compound in the lung tissue. Therefore, the identification of the source material in pneumoconiosis was aided by elemental information from XRF.

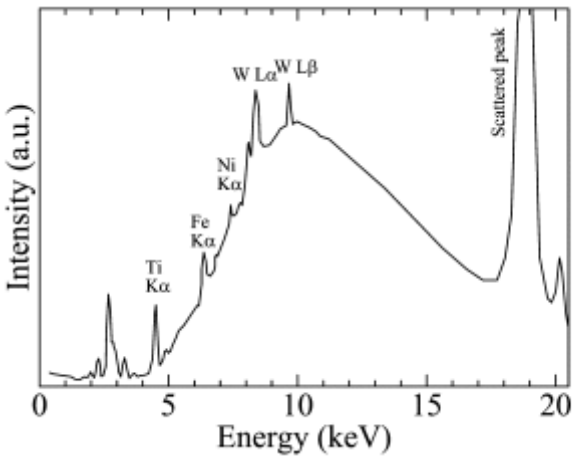


Figure 16. The XRF spectrum of a paraffin-embedded lung biopsy specimen derived from tungsten carbide pneumoconiosis (measurement conditions: 50 kV, 1 mA with Rh target, 600 s/point). [48]

3.2 General Considerations

Complex structures can be efficiently analyzed using reliable methods nowadays. Numerous analytical methods have played an important role in order to determine the chemical composition and structure of these substances.

While XRF is a widely used analytical technique for various elemental analysis in multiple scientific fields, there are also many other analytical methods with multiple advantages compared to the XRF, such as Inductively coupled plasma mass spectrometry (ICP-MS), Inductively coupled plasma atomic emission spectrometry (ICP AES), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Atomic Absorption Spectroscopy (AAS), Proton Induced X-Ray Emission (PIXE), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM-EDS), and many others. [16]

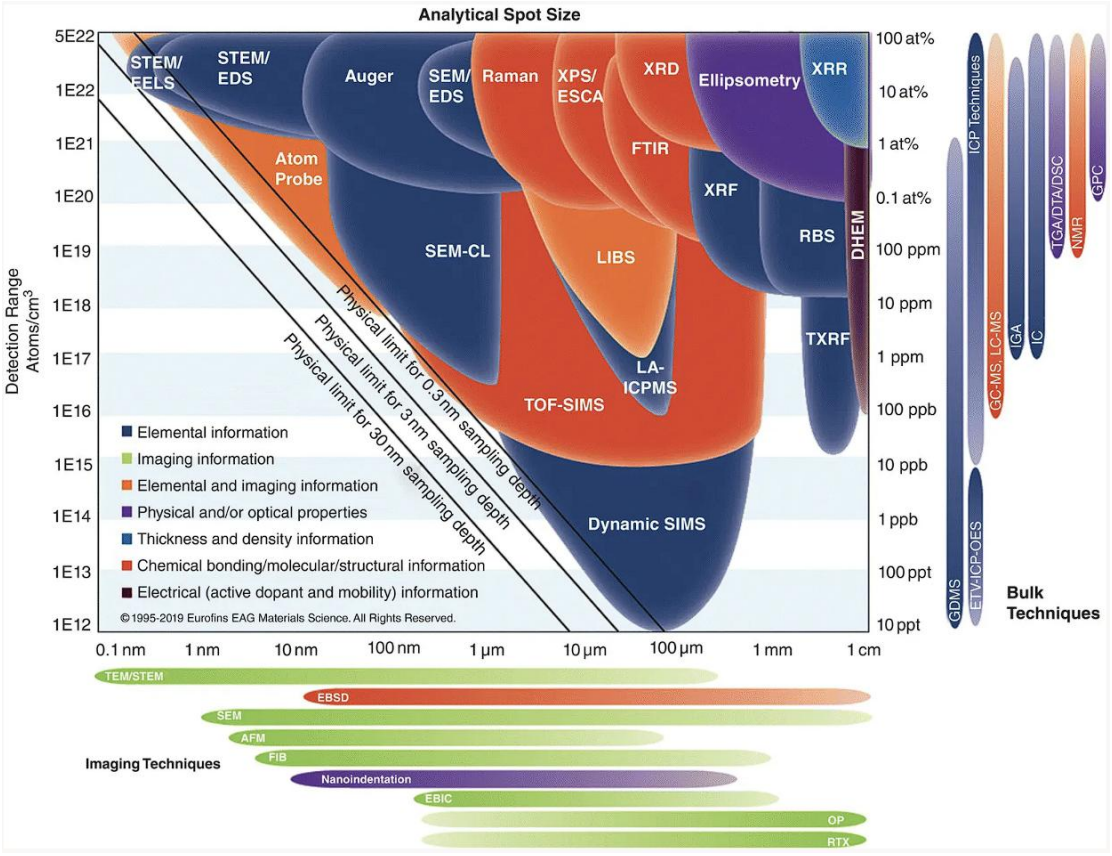


Figure 17. Comparison of the Detection Range of various Analytical Methods. [11][16]

It is highly important to mention that using multiple methods when analyzing samples is crucial for obtaining reliable and accurate results. By comparing results from different techniques, analysts can confirm findings and reduce the likelihood of errors or anomalies that may arise from a single method. It is also possible to eliminate the error detection. Discrepancies between results can indicate potential issues such as sample contamination, instrument calibration errors or methodological limitations. Furthermore, different methods have varying sensitivities and detection limits for specific elements. By using multiple techniques, we ensure a more complete elemental profile of the samples. Another aspect to consider I that some methods provide qualitative data (e.g. phase identification (XRD)), while others offer quantitative analysis (e.g. XRF, ICP-MS, AAS etc.).

Each analytical technique has its strengths and its weaknesses. For instance, while XRF is excellent for heavier elements, it may not detect lighter elements effectively. Using complementary methods like ACP-OES or AAS we can fill these elemental analysis gaps. (Figure 17)

Lastly, the aspect of regulatory compliance and quality assurance is critically important, especially when dealing with samples from industrial products rather than solely for research purposes. Many industries require validation of analytical results through multiple methods in order to meet standards and ensure product safety. Thus a multi-method approach enhances quality assurance protocols, helping to maintain high standards and industrial applications.

3.3 Inductively coupled plasma mass spectrometry (ICP-MS)

Detection and quantification of chemical elements in samples is often achieved using ICP spectroscopy, which remains the preferred analytical method due to fact that it is the mot sensitive elemental analysis technique for trace and ultra-trace elements.

ICP-MS allows for the analysis of multiple elements in a single run, increasing it's efficiency as an analytical method. ICP-MS is applicable to a variety of sample types including liquids, solids and slurries after appropriate preparation.

ICP-MS like other methods has it's own limitations one of these is the presence of interfering factors such as atmospheric gases, plasma argon, and impurities in glassware and cones which can create difficulties during the analysis process.

The technique is based on ionizing the sample using a very hot plasma, usually produced from argon gas. Plasma is often called the fourth state of matter after solid, liquid, and gas, and refers to a gas of charged particles generated by ionization. Plasma exists in a very high-energy (high temperate) state and the temperature of the Ar plasma used in ICP-MS ranges between 6,000 K and 10,000 K. [17]

The plasma is used to atomize the sample, creating polyatomic ions, which are then detected. The ions from the plasma are separated through a series of cones into a mass spectrometer, usually a quadrupole. The extraction of ions is based on their mass- to charge ratio (that is why it is calls Ms). An ionic signal is received by a detector which is correlated to the concentrations of atoms in the sample. These concentrations are then obtained by drawing -the calibration curves - by using some certified reference materials called CRMs. The the CRMs are used to construct the calibration curves and consequently to determine concentrations. [16] [18]

The technology is capable of analyzing metals and non-metals in liquid samples ranging from milligrams to nanograms per liter. In addition, it is used for isotope analysis of elements. ICP-MS is widely recognized as a key analytical technology in various industries such as biomedical analysis, pharmaceutical industry, metallurgical mining, geochemical analysis, environmental monitoring and semiconductor manufacturing.

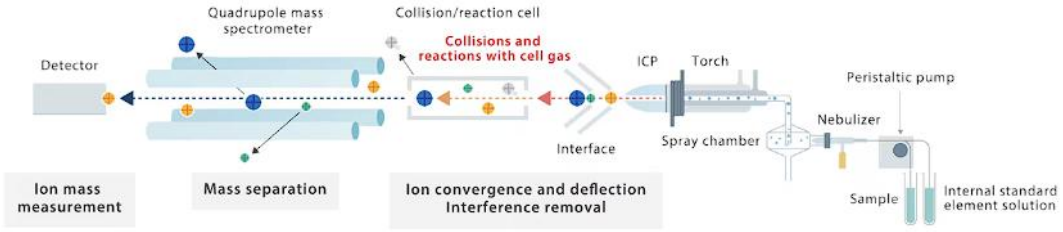


Figure 18. Typical Geometry of ICP-MS

In order to compare the detection limits and spatial resolution of the ICP-MS (LA-ICP-MS) and micro-XRF technique Gholap, D.S., Izmer, A., Samber, B.D. et al. (2010) [19] conducted an experiment on Daphnia Magna (a small planktonic crustacean) -commonly referred to as a water flea- which is mainly used as an indicator of aquatic ecosystems health and is also used as a model organism in ecotoxicology.

Section of the planktonic crustacean had been used to analyze the elemental localization of some elements, in particular Ca, P, S, and Zn which allowed elemental correlation with the tissues. RGB maps were then created to visualize the simultaneous presence of metallic elements in the sample. Fig. 19 display the RGB representation of the calcium (Ca), iron (Fe) and phosphorus (P) distributions using the micro-XRF and the distribution of Ca, P and Zinc (Zn) analyzed through laser ablation inductively couped plasma mass spectroscopy (LA-ICP-MS) in both the sagittal and the dorsoventral sections of the Daphnia Magna.

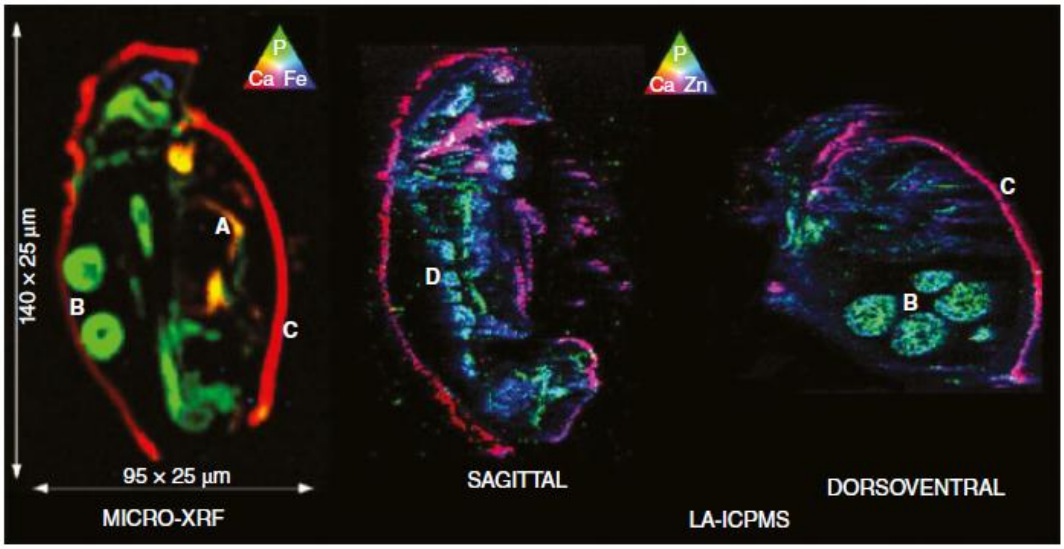


Figure 19. RGB mapping with both micro-XRF and LA-ICP-MS. [19]

The findings indicate that Ca and P coexist in the thoracic appendages, P and Zn are present in the gut, and Ca and Zn are found in the exoskeleton. The simultaneous presence of Ca/P and Zn/P is attributed to the formation of intracellular and membrane-bound phosphate granules, which in turn may serve as storage for metallic ions such as Ca^{2+} and Zn^{2+} within the living tissues.

Both techniques showed comparable limits of detection (LOD) for Ca and P, validating the imaging results. While LA-ICP-MS effectively detected Zn with an LOD of 20 ppm at a 15 µm spot size, µ-XRF was less effective for this purpose. Conversely, LA-ICP-MS was inadequate for analyzing Sulfur (S) distribution, which µ-XRF could visualize more effectively (LOD 160 ppm over a five-second lifetime). Although LA-ICP-MS provided better spatial resolution than µ-XRF, issues such as wash-out effects and spikes slightly compromised image quality. µ-XRF successfully mapped elemental distribution for S, while LA-ICP-MS excelled in Zn distribution.

3.4 Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

ICP-AES is also a high valuable analytical technique that allows researchers to accurately determine the elemental composition of different types of samples, including solids, liquids and powders. The method utilizes a high-temperature plasma to atomize and excite the sample -just like the ICP-MS. The excited atoms return to their ground state and thus they emit light at a characteristic wavelength. The intensity of this emitted light is directly proportional to the concentration of the element present in the sample .

The method is widely used in various fields, like Environmental Monitoring (analyzing water, soil, air samples etc.), Geological Analysis (rocks, minerals), Industrial Quality Control (in metallurgic processes), in the Food Safety Sector (detects heavy metal in the food) and also in the Pharmaceutical domain.

ICP-AES generally exhibits lower detection limits and higher sensitivity, making it better for trace-level analysis while XRF typically has higher detection limits, making it more appropriate for qualitative and semi-quantitative analysis, especially for bulk samples and it may struggle with detecting light elements effectively.

The downside of the ICP-AES is that the spectrum of emission is intricate and is dependent on the presence of other components. Additionally, the matrix effect has a number of problems when attempting to accurately measure the desired components.

3.5 Proton Inducted X-Ray Emission (PIXE)

The PIXE (Proton Inducted X-Ray Emission) technique involves detecting X-Rays emitted form a samples as a result of it's bombardment with high energy ions. Various types of excitation beams exist and generate X-Rays with energies that are characteristic of the elements present in the sample (EDXRF -WDXRF). In PIXE spectroscopy, charged particle beams are used, such as He^{2+} or H^{+} ions. As a non-destructive analytical technique, PIXE provides signal levels comparable to those obtained from electron beam methods but offers

better signal-to-background ratios, as a result it can analyze elements from sodium to uranium.

In all these spectroscopic methods, the excitation bean removes a core electron, creating a vacancy that prompts the outer shell electrons to transition to fill the inner shell vacancy (Fig. 20) [21]. This process results in the emission of X-Ray – as already mentioned- with specific energies that are unique to the elements present in the sample. [16]

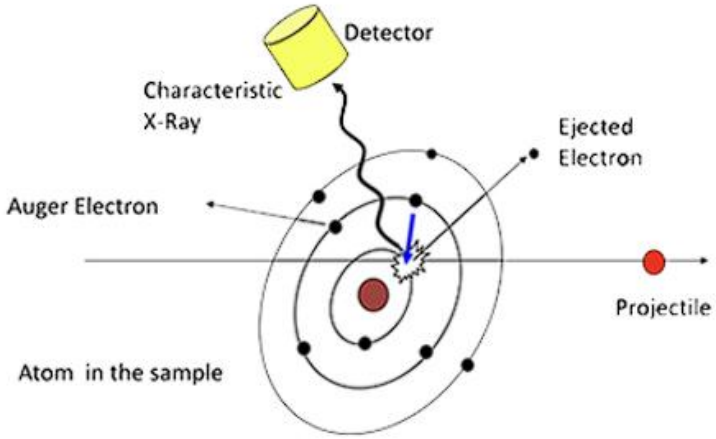


Figure 20.The PIXE schematic principles [21]

PIXE in particular is advantageous for identifying heavy elements when used along side with Rutherford's backscattering spectrometry (RBS). Although heavy elements may exhibit only slight differences in RBS backscattered energies due to their similar masses, PIXE spectral studies reveal distinct differences among them. While XRF is also capable of detecting a wide range of elements, it may have higher detection limits compared to PIXE.

However, there are some limitations. The analysis area is typically restricted to 1-2 mm and accurate information can generally be obtained only at depths of approximately 1μm within samples. Recent advancements in this technique have introduces tightly focused ion beams to enhance microscopic analysis capabilities, this refined technique is called micro-PIXE and is employed for determining trace distributions across a wide range of samples. [20]

3.6 Scanning Electron Microscopy -Energy Dispersive X-Ray Spectroscopy (SEM-EDS)

SEM is also a widely used analytical tool know for providing high-resolution images of sample surfaces. Its is particularly popular because it can quickly deliver detailed and high-quality images. SEM can be equipped with an Energy Dispersive Spectroscopy Detector -thus the SEM-EDS- allowing for the identification of most elements across the periodic table.

SEM-EDS is employed in situation where optical microscopy fails to achieve resolution or magnification. Additionally, SEM produces intricate surface topography images.[16]

In this technique the sample is scanned by an electron beam , which interacts with the samples to generate images using secondary or backscatteres electrons. (Fig.21) [22]. Elemental analysis is conducted through the excitation of X-Ray fluorescence, enabling direct comparison between the obtained images and the elemental content.

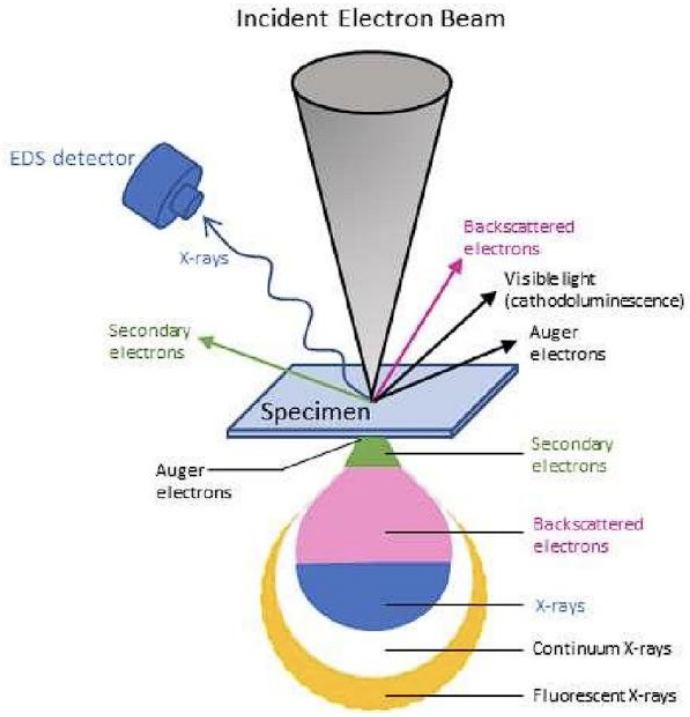


Figure 21. Schematic representation of the SEM-EDS geometry [22]

3.7 Differences in XRF and SEM-EDS

Distinguishing features between X-ray fluorescence (XRF) and Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy (SEM-EDS) include differences in sample handling, experimental conditions, sample voltage, and excitation sources. In terms of sample handling, the procedures for XRF are significantly simpler compared to those of SEM-EDS.

Unlike SEM-EDS, μ-XRF analysis does not require samples to be conductive. SEM analysis utilizes electrons to excite the sample and create charge, which must be managed; thus, the conductivity of the sample is crucial due to the low penetration depth of electrons inside the materials. If a sample is not conductive, it can adversely affect the image quality and quantification process. Non-conductive materials such as glasses, minerals, and plastics must be coated with carbon or gold in order to be analyzed. In contrast, μ-XRF can analyze these types of samples directly, greatly reducing the preparation time.

When comparing the analytical capabilities of XRF and SEM-EDS, it is evident that there is a significant difference in sensitivity, especially when analyzing trace elements. The sensitivity of trace element analysis is primarily determined by the peak/background ratio.

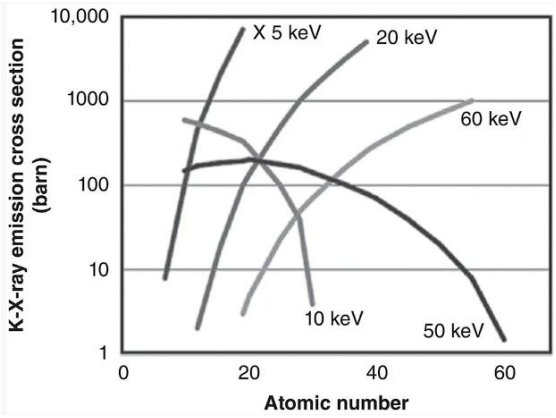


Figure 22. K Shell X-Ray emisison cross section compared to to the atomic number of the components [23][24]

The relationship between the atomic number and the electron shell cross section along with X-ray excitation is clearly illustrated in Fig. 22 [23]. It indicates that lighter elements exhibit more efficient electron excitation, resulting in a larger effective cross section; even elements like boron (B) and

beryllium (Be) can be identified using an electron microscope. Conversely, heavy elements are more efficiently detected through X-Ray excitation, which leads to improved detection limits for these heavier elements, as demonstrated in Fig.23 [23, 24].

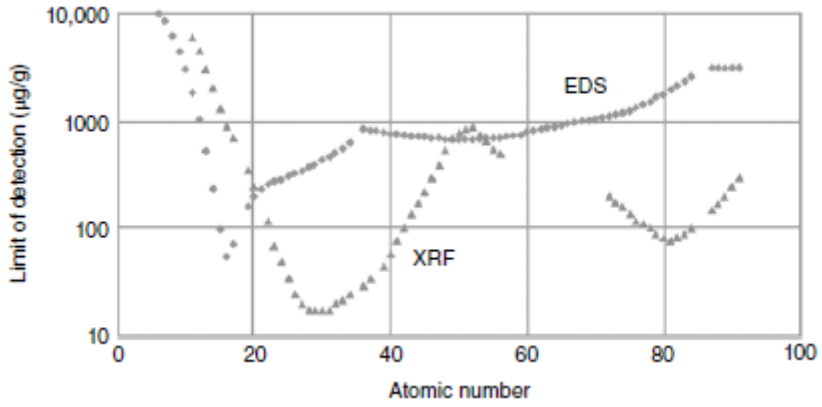


Figure 23.Limit of detection for electron and X-ray [23] [24]

3.8 Combination of SEM-EDS with µ-XRF

By combining µ-XRF with other established methods, additional and complementary insights into materials can be gained. For example, combining µ-XRF with SEM-EDS broadens the range of detectable elements, including lighter elements [23]. This approach has proven valuable for examining different parts of a sample while optimizing the use of the same sample chamber, especially in situations where significant effort is required, such as high vacuum conditions. Several well-known companies have developed and offer XRF-capable experimental electron microscope setups.

It is worth noting that the volumes analyzed by the two techniques are very different. Electron beam and µ-XRF techniques analyze volumes of a few µm3 for the former and thousands of µm3 for the latter (information depth and excitation area greater than 20 µm). Despite this difference, both methods provide qualitative and quantitative information on chemical composition. However, their sensitivity varies with energy range. SEM-EDS has higher sensitivity for lighter elements, while µ-XRF has better sensitivity for heavier elements [16,23].

In addition, because EDS and μ-XRF have different excitation probabilities, using the results of one method may improve the accuracy of the results of the other method. Quantitative evaluation of elements in μ-XRF is mainly based on the fundamental parameter (FP) model normalized to 100%.

The accuracy of X-Ray fluorescence (XRF) analysis can be greatly improved by using energy dispersive spectroscopy (EDS) results of lower atomic number elements [25]. The combination of micro-XRF (μ-XRF) and energy dispersive scanning electron microscopy (SEM-EDS) has proven extremely beneficial for the comprehensive characterization of organic and oxide material samples. These elements cannot be measured directly using μ-XRF.

3.8 XRF vs XRD

X-Ray Diffraction -XRD- is used to determine the crystalline structure of material. It operates on the principle that the X-Rays are directed at a crystalline sample, they are then scattered in a specific direction(angle) based on the arrangement of atoms within the crystal lattice. This scattering produces a diffraction pattern that can be analyzed to provide information about the material's phase composition, crystallinity and other structural properties. This technique is mainly qualitative and is capable of detecting small amounts of crystalline materials. It provides detailed information about the lattice matrix parameters and symmetry. XRD method is less effective for analyzing amorphous materials since they do not produce distinct diffraction patterns and also require careful sample preparation to ensure that the sample is flat and properly oriented.

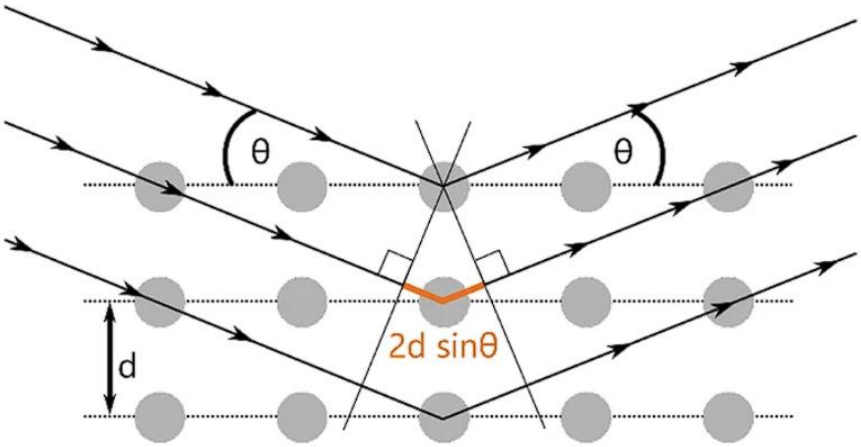


Figure 24. Bragg's Equation (Figure source: George F. Harrington· José Santiso. (2021)) [27]

When combined the analysis of crystalline materials with the knowledge of the chemical composition of the materials, the use of phase analysis in material testing becomes more accurate and efficient. Reducing the number of similar steps can make the analysis process faster. Many instrument concepts have been developed combining XRD and XRF techniques with resolution ranges less than 100 mm [16,23]. In addition, XRD analysis can be improved by using position-sensitive X-ray optics, such as capillary optics combined with monochromatic or synthetic multilayer optics [16].

By combining these two techniques, a more complete and accurate characterization of crystal samples can be achieved. In order to simplify the process of merging these two technologies, we created a pioneering XRD system that allows the seamless integration of both XRF and XRD techniques in one device [26]. These include analyzing the elemental composition of different materials, quantifying the amount of free lime in clinker and slag, conducting comprehensive analyzes of clinker phases, measuring Fe2+ levels by fusion, determining the iron phase during direct ferric reduction, the characterization of phases related to aluminum electrolysis and evaluation of thin films, among others [26].

3.9 Raman Spectroscopy and the XRF method

Raman spectroscopy provides valuable information regarding the chemical structure, phase, polymorphism, crystallinity, and molecular interactions of a sample. This data can be effectively combined with X-ray fluorescence (XRF) to enhance the elemental analysis of the samples.

Raman spectroscopy uses a concentrated monochromatic beam to induce inelastic scattering of molecules within a sample. The amount of energy lost in scattering is determined by the energy levels of the scattering molecules. This analytic method examines the energy levels of electrons in the outer shell [23]. These energy levels offer valuable insights into the atomic and molecular composition of materials.

Similar to μ-XRF, Raman spectroscopy, specifically μ-Raman spectroscopy, uses laser radiation sources with varying energy ranges to focus light on smaller areas of the sample, allowing the examination of different materials [23].

In order to investigate the possibility of analyzing the material composition of samples, a model integrating XRF and Raman spectroscopy was created [16,23]. The setup for μ-XRF includes a microfocus tube with Rh objective, multibacillary optics and a silicon drift detector (SDD). The sample is mounted on a three-dimensional motorized stage, allowing for simultaneous observation laser light onto the sample surface, particularly at wavelengths of 633nm and 787nm. The laser light and Raman spectrometer are connected to the microscope via optical fibers. This model is suitable for pigment investigation and geological sample analysis. [16,23,28,29]

4 Energy dispersive X-Ray Fluorescence Analysis of Biological Materials

4.1 Introduction

Substances of biological origin are derived from once-living organisms. This includes a wide variety of samples taken from both plants and animals, such as roots, leaves, fruits, flowers, honey, nectar, blood, meat, and grain. These materials are composed of energy-rich molecules, providing a vital source of nutrition for humans and animals alike. In addition to these nutritionally important elements, plants and animals can accumulate elements that appear to have no function in the basic biological processes of life. Such elements include cadmium, mercury, and, to a smaller extent, lead and arsenic. [30,31]

Because some of these elements can reach high levels in the body may have a toxic effect, it is obvious that physiological alterations at the cellular, tissue, and organ levels may be induced in a pathological sense leading to illness or death. Thus, with respect to this potential harm that can result from the toxic effects of these materials, monitoring the elemental composition of biological materials is of prime importance but is usually performed only on raw food ingredients or products.

Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF) is an X-ray-based multi-elemental analytical technique that allows for the measurement and analysis of elemental profiles in various samples. One of the primary advantages of EDXRF over commonly used alternatives like Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is its capability to analyze solid samples directly. The technique enables simultaneous multi-elemental profiling, which can also detect elements that may not be anticipated in the sample.

The EDXRF analytical equipment are suitable as they are small-sized and portable to be used for both field and laboratory investigations. The EQS is thus a valuable tool for those areas where analyses need to be run quite quickly on a large number of samples every hour.

With the EDXRF method, a lot of important research opportunities have opened up on the planetary scale. For example, the opportunity to study and evaluate through elemental analysis both current and past influences of human activity on the environment: mining, industrial emissions, traffic, and intensive agriculture.

The EDXRF can be used for estimating the pollution of soil and plants by potentially hazardous elements [32, 33]. It can also be used to study how efficient pollutants are circulated and distributed along food chains [34, 35]. In turn, this may also explain the reasons for the imbalance provided in plant, animal, and human diets with micronutrients relative to major elements composing the crops. Finally, the application of EDXRF is due to the provision of important information on food safety and authenticity in terms of measures intended toward prevention and fighting food fraud. The actions carried out must not lead to a reduction in safety compared to what has already been achieved.[24]

4.2 Instrumentation of the EDXRF

As shown in the following Fig. 25, an EDXRF system can be divided into two essential parts: the excitation source and the spectrometer itself. Output pulses from a detector are next processed in an analog tail-pulse shaper for pileup rejection and then sent to an ADC (analog-to-digital converter) and multichannel analyzer for display or alternatively to a DPP.

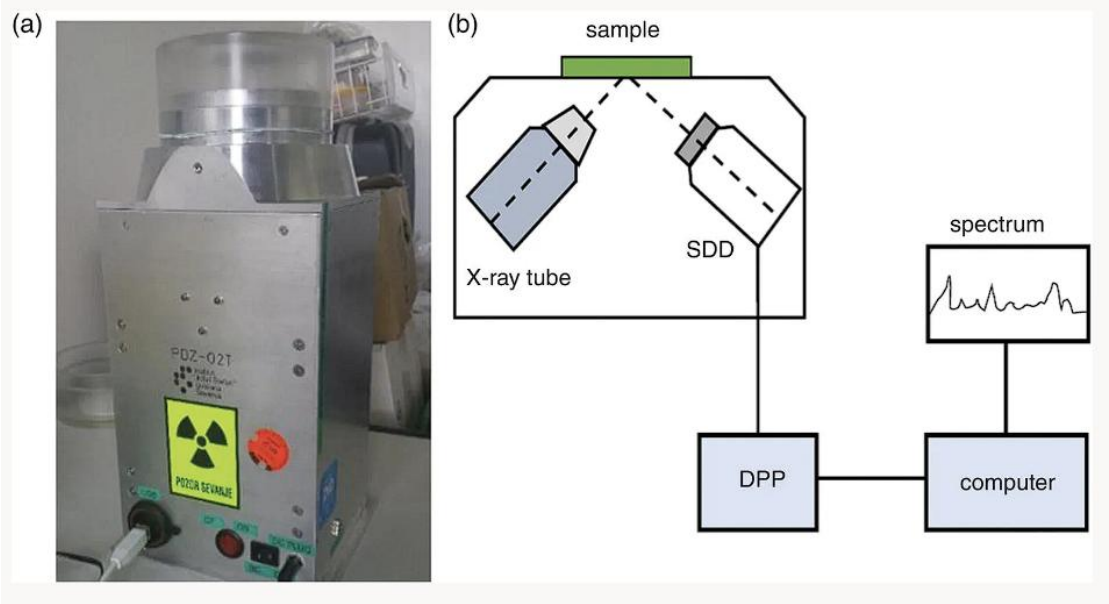


Figure 25. (a) Peduzo 02T portable X-ray spectrometer designed and built at the Jozef Stefan Institute, and (b) diagram of the XRF system consisting of an X-ray tube source, a silicon drift diode (SDD), a digital pulse processor (DPP), and a computer.

This can be used to excite elements in samples using radiation that is either polychromatic -produced from an X-ray tube- or monochromatic - produced by radioisotope sources. Examples of sources frequently employed for EDXRF analysis are listed in Figure 20.

Each of these sources emits X-rays at well-defined energies, thus exciting elements within a specific range of atomic numbers. Two or three sources are used in many systems because no single radioisotope source can cover all elements needed for proper geological or biological analysis.

Isotope	Half-life	Useful radiation	Energy (keV)	Elements excited efficiently
Fe-55	2.7 years	MnK-lineX-rays	5.9	Al-Cr
Co-57	270 days	FeK-line X-rays	6.4	<Cr
		γ-rays	14.4;122;136	
Cd-109	1.3 years	AgK-line X-rays	21.1	K-Tc
Am-241	470 years	NpL-line X-rays	14, 21	Sn-Tm
		γ-rays	59	

Figure 26.Commonly used radioisotope sources in EDXRF analysis. [15][16]

The source's half-life also plays a role and is more critical for the sources of Fe-55, Co-57, and Cd-109. The radioisotope Cd-109 has a half-life of 1.3 years; this is very short, and it will further demand constant replacing since its intensity will fall under the required excitation threshold.

Conversely, sample excitation is achieved through the use of X-Ray tubes for general characteristic and continuum X-Rays. There are two general types of these X-Ray tubes: air-cooled (ranging from 3 to 50 W) with low power and water-cooled (2 kW) with high power.

When using continuous radiation from an X-Ray tube on thick geological or biological samples, detection limits are poor in terms of element fluorescence because of a high level of background counts due to scattered radiation. The aspired current in the counter is previously mentioned by 29 Si Kα / 27 Al Kα ~50000; it means a current in A (29 Si Kα) that should be around 250 times higher than for 27 Al Kα.

It is the detector's ability to resolve neighboring lines in the fluorescence spectrum. This is important in avoidance of spectral interferences and overlap. Detector efficiency is a measure of how well the detector can absorb X-rays into its sensitive volume. Semiconductor detectors generally have good resolution and thus are found in most EDXRF instruments. Such detectors require liquid nitrogen cooling. Others are cooled electrically, with built-in Peltier elements.

For elements lower than Na in biomaterials, they either have very weak signals or are not detected in F limits because of their low yield for fluorescence and the absorption of weak energy XRF photon both by the sample and the surrounding air.

Fortunately highly sensitive units being fitted with zirconium filters (manufactured recently Japan) now allow determination down 0.1 p.p.m Cd rice grains. Additionally, accurately determining the presence of As in samples that contain Pb is difficult due to the close association between the As Kα-line and the Pb L3-line, the latter of which has an energy of 10.551 keV. [24]

4.3 EDXRF Spectra Quantification

X-ray spectrometers measure the intensity of radiative transitions of atoms in a sample, thus enabling elemental (qualitative and quantitative) information to be obtained regarding the samples.

The measured characteristic X-ray intensity depends not only on the type of excitation but also on some basic physical parameters that determine it: the probability of photoexcitation of the atoms. The radiation that hits the sample triggers the atoms to be excited; before any fluorescence radiation is emitted from the atoms, it is not detected by a detector.[14,36]

4.4 Sampling and Sample Preparation Limitations

For the analysis of biological materials, sampling, and sample preparation. The collected samples must represent with a high degree of accuracy the particular area, population of plants or animals, batch of food or food-related product to be analyzed. It is achievable by representative sampling to ensure that the concentration pertaining to those elements that are under interest is reflective in an accurate manner of a specific time and place. The analytical results obtained from representative samples will indicate how much of a typical person is representative of the population as a whole. This includes variations due to such factors as biological material, concentration of the element itself, sample collection and preparation, and technique of analysis. In a typical case like that of AAS or ICP-AES, sample preparation is usually through what is known as total matrix chemical digestion.

Sample dissolution can be quite challenging and time-consuming and may even, in some cases, limit the application of analytical methods for both environmental monitoring and regular quality control in production processes. Typically, organic matter digestion and dissolving analytes in soil and plant samples have been performed with dry ashing (combustion of the sample) or wet digestion (digestion with strong acids). It is extremely difficult to digest samples containing silicon, such as soils and plants, by wetting because it

would be necessary to use HF acid to totally break the matrix if the determination of the total concentration of the analytes in the sample were desired. Sand baths/hot plates digestion systems are slowly being replaced by microwaves/closed vessels to avoid the loss of volatile components (Hg, Se, As). These procedures are based on microwaves and cut down time for digestion with reduced chances of contamination or loss of analytes. However, it is more difficult to perform the hydration of samples containing silicon, such as soils and plants, using acid HF in the case he acid should be completely dissolved if we should determine the concentrations of analytes.

Some of the main disadvantages of INAA are as follows: high cost; not being able to apply nuclear reactors for irradiation; rather long analysis time because of the cooling phase of decay active short-lived radionuclides that interfere. Additionally, INAA is unable to recognize certain environmental components that are significant (like Pb); this conclusion is based on the fact. High costs and the cooling down of short-lived radioactive decays (which last for just a few seconds) for later NAA after several other analytical steps are time consuming are the major drawbacks. The other one is that, in many countries, this method cannot be performed in nuclear power plants due to radiation exposure. However, it is valued for its high sensitivity and accuracy.

Biological samples are dried in an oven or freeze-dried at a temperature from 60 to 100°C. This will dry moisture before the grinding process. In the grinding or crushing stages, it is very easy to get contaminated by the tools. Special importance has to be attached to the use of suitable materials especially when analyzing trace elements. Agate mortars and pestles may leave biological materials contaminated, introducing Ti, V, Cr, Mn, Fe and Pb at trace levels [15]. For EDXRF analysis, approximately 100–200 mg of homogeneous solid material in powder form is needed; but any inhomogeneities make the measurements highly error-prone, particularly for light elements [3]. The thickness must be kept constant while forming the pellets to avoid any deformation due to inhomogeneity.

Sample moisture content can significantly affect the accuracy of solid biological measurements by EDXRF. An increase in moisture content lowers

the apparent concentration of the sample because of dilution. This phenomenon is usually noticeable in the case of analytes that have X-ray lines of low energy, under 5 keV. Whereas elements presenting X-ray lines of higher energy, such as Pb, Cd, Ba, and La, may show just a little difference. The distortion's degree and nature depend on the energy of the X-ray emission of the analyte, as well as the sample composition. In practice, however, when the moisture content is relatively low (i.e., between 5 and 20%), the net error is overall probably not that significant.

It can be analyzed directly using EDXRF in transparent disposable spectro-cups which have bottom polymeric that are 2.5 micrometers thick or thinner. One other alternative is to take measurements indirectly via precipitation. Small-element concentrations are low in biological fluids like juice, urine, and sap. Precipitation generators come in several varieties for this specific task.

After all, consider cobalt, iron, nickel, and zinc, which may be precipitated using ammonium pyrrolidine dithiocarbonate. Such precipitants are each selective since they precipitate only one type of element.

5. Total Reflection X-Ray Fluorescence and it's suitability for analyzing Biological Samples

In very small amounts various elements present in biological systems play an important role and have significant implications. These elements may be essential— that is to say they are required in certain amounts for normal biological functions. But, on the other hand, alternatively, they may also be non-essential and if present within the body can give rise to some specific types of diseases.

Inorganic nutrients are incorporated into proteins that are vital to life and they influence the chemical reactions within living systems [3]. Such elements as cadmium, nickel, arsenic, beryllium, and chromium are elements that have the potential to be cancer-causing, also toxic to human and animal health by effecting an increase of some trace elements such as zinc, strontium, and lead in the cartilage which could trigger arthritis [30,37]. Besides, some toxic

elements can enter biological systems through contaminated food, air, water causing harmful effects on humans or animals. The concentrations of these elements in the materials above play a biological role and thus are very important.

The concentrations of these elements in the above materials play a biological role. And, therefore, are of immense importance Destructive classical methods for such determinations destroy the samples of cell tissue. They also have a demand for relatively large amounts of sample. Additionally, ICP-OES and ICP-MS have a consumable sample that is specifically designed to be utilized in the analysis. If there is any question about the results, the composition can only be re-calculated.

As a consequence, there is a need for fast non-destructive/non-consumable techniques for analysis of the composition of biological samples. X-ray fluorescence analysis (XRF) is able to give important information about the sample that is easy to prepare without negative impact on the sample. Using XRF, the desired components can be identified in biological systems without having to analyze them adversely.

5.1 Advantages and Limitations of conventional XRF for Elemental Determinations in Biological Systems

The theory of X-ray fluorescence has been expounded in many works [38, 39], therefore, coherence has limited the account to just a few lines in this section. When high-energy x-rays (energy > 1 keV) impinge on a sample and produce core-shell electrons based on the bond energy, it results in electron hole induction and the atoms are no longer sustainable. The atoms will be sustained by filling the gap with the electrons donated by the outer shells.

The energy of photons coming out as a result of this transition falls within the regime of energies for X-ray and is well defined. This energy level of X-rays so generated is one of the definite values for individual elements concerned and therefore is spoken of as characteristic X-rays. Moseley's Law [38] states that this energy of characteristic X-rays is connected with some atomic number from

an element. The linear relationship ideally between the x-Ray intensity and the concentration of the element indicates how much of the element is in the sample because they do not get destructed themselves while exciting the sample. The characteristic x-Ray lines are sharp.

XRF analysis is non-destructive and non-consumable, although it requires some additional sample preparation steps in terms of pelleting, dissolution, or bead preparation. After the XRF analysis, the sample is available for further investigation; it can be reused or reprocessed with other techniques if doubt creeps into the analysis. In addition to elemental analysis, with the help of XRF, one can study elements' distribution in samples of bones, hair, nails, and cancer as well as normal tissues at the μm scale using very small X-ray spots of few microns in size. μ-XRF can be used to study medical treatme nts by analyzing the distribution of elements of body parts (e.g. bones/hair/nails/etc.)

The above description proves how simple XRF analysis is and how much it can tell about a biological sample. It has some drawbacks, of course. The first of which is that it cannot detect lower concentration elements in the ppb range. This is because of the high background that is generated by scattering of X-rays penetrating deep into the sample and also because when the emitted electrons lose energy in the form of X-rays, they appear as spectral background. An effect resulting from this limitation is that XRF will need a considerably large amount of sample during analysis, more especially with very valuable, toxic, or rare samples, like forensic and biological samples. Which may not be practical at all times. Additionally, matrix effects are so severe in XRF analysis without a matrix-matched standard. It results in grossly inaccurate values.

5.2 Factors Limiting the Application of XRF for Biological Sample Analysis

However, it does have some drawbacks. The first limitation is that it cannot detect lower concentration elements in the ppb range. An effect resulting from this limitation is that XRF will need a considerably large amount of sample during analysis, which may not be practical at all times; more especially very valuable, toxic or rare samples like forensic and biological samples. Moreover, in XRF analysis matrix effects are so severe without a matrix-matched standard. It results in grossly inaccurate values because of the comparison of deep penetration of x-rays into the sample.

Another phenomenon is that the elemental X-ray lines of matrix elements with energies higher than those of analyte lines at the absorption edge of the analyte are absorbed by the analyte, and in this way, they excite the analyte elements to give off their characteristic X-ray lines. Hence, these lines have higher intensity values. This results in analyte X-rays producing lines of higher intensity than could be produced when excited by the excitation X-rays alone. In Wavelength Dispersive X-Ray Fluorescence angles between excitation X-rays & sample and detector & sample keep changing, and hence background keeps changing as well. In WDXRF, the distance between the sample and the detector is a few centimeters, higher than in EDXRF, where the distance is a few millimeters. This restricts its applicability to forensic, precious, and biological samples, etc.

5.3 Improving XRF for trace element determination: Total reflection X-ray fluorescence spectrometry (TXRF)

The question now is how to make XRF applicable to the determination of trace elements. The answer is the complete elimination of the above-discussed limiting factors. This can be done by: (i) reducing spectral background to a minimum, (ii) making matrix effects equal to zero, and (iii) decreasing the sample-to-detector distance to the minimum possible value. They based their proposal on the concept that when chromium thin films are deposited on it and

excited by an X-ray beam under TXRF conditions, the background would be much reduced due to the total reflection of the excitation beam on an optically flat support. It is classified as a separate method among X-ray spectrometries TXRF [14, 40].

5.3.1 Principles of TXRF

Yoneda and Horiuchi were the ones to first propose the idea for trace element analysis with TXRF. It has since been used in more recent and advanced areas of materials characterization. The basic principles based on which TXRF analysis operates arise from instrumental changes in three key aspects compared to conventional EDXRF to achieve orders-of-magnitude improved sensitivity and excellent performance in terms of precision and accuracy:

- In the case of TXRF, the angle of incidence of the primary beam is small with respect to the sample surface and support, smaller than the critical angle (typically ~1 degree for most materials) depending on the energy of the X-rays and the density and atomic number Z of the reflector material. This feature of TXRF avoids deep-seated incidence of the X-rays in the sample support, decreasing the background to a detectable level more compared to conventional XRF, which in turn results in higher than standard detection limit ratio.
- If a primary-incident X-ray beam is tilted by less than the critical angle, the sample support has to have a flat polished surface, maintaining a consistent angle below the critical angle, to reflect the whole incident beam from it. In this case, at the point of intersection of the incident and reflected rays, if there is a sample movie a few nanometers thick, then it will be illuminated by the incident light and the light will be entirely reflected from the support. This configuration causes the sample to be excited two times more intensely than EDXRF, leading to a twofold increase in the fluorescent X-ray intensity, or about a factor of two.

Since the specular and reflectance angles are typically less than 1 degree, the detector can be placed very near the sample on the holder. This will ensure that the angle between the X-ray beam and the detector is approximately 90 degrees— a beam-detector configuration of 0-90°, which is necessary to reduce the background level in XRF (here TXRF).

5.4 Suitability of TXRF for Elemental Analysis in Biological Samples

The biological samples such as blood, serum, urine, plasma and tissues are over 80% water. Removal of water leaves residues that are in nature mostly low Z elements (e.g., C, H, N, O). Therefore matrix effects are not a big issue. An example of trace element analysis in these samples using TXRF is a very simple sample processing technique where the sample is just mixed with an appropriate internal standard. Dental plaque and also hair plus nails and bones among other biological materials can be directly analyzed using small quantities of sample so that the matrix effects become negligible; alternatively these samples can be subjected sample processing before analysis. It should be mentioned that trace elements from biological samples have a basic view of human health whereby their levels of concentration can act as indicators to good health or disease involvement and risk chances of diseases. Processes with animals and plants at the cellular level trace element have elicited a large number of research studies reported in various literature sources.

It could also be a consequence of pollution, or the local environment of an individual at that moment introduces some extra elements into the biological system. Besides, the level of some metals in different body compartments may reflect the presence of serious diseases or disorders. Then, during cancer treatment carrier agents such as platinum are administered into the body of a person and as such one must have knowledge on the mode of action of platinum-based drugs. For interference elimination, generally, sample digestion and matrix separation are included in these procedures.

For each excitation energy, stepwise TXRF emission spectrum at each energy is measured for a short time. Emission intensities were also quite low except

samples burned twice (namely in 10 and 1% oxygen). The obtained TXRF emission spectra at each excitation energy are in turn fitted using a least-squares fitting program to determine XANES such as the FEFF6 generated XANES shown. The standard used (gray) was tabulated data on TiO2. This yielded the degree of oxidation as an output for analysis from measurement of XANES. In this way, the spectrum can be taken and instantly compared to a standard without any long-term stability of the system. Such applications are very valuable but impossible with regard to the average laboratory using SR sources as for instance Bruker ATSARA that are especially designed to accommodate synchrotron radiation for XRF analysis and fast mapping, but with different optics than the laboratory TXRF spectrometers [31].

6. X-Ray Fluorescence Spectrometry to Study Gallstones, Kidney Stones, Hair, Nails, Bones, Teeth and Cancerous Tissues

6.1 Trace Mineral Elements in Biomedical Samples

It is critical to be able to measure major and minor elements in biological samples if we are going to assess the medical importance of these minerals. Minerals are of value in sustaining good health and well-being: some essential components are already present in the human body in high concentration. On the other hand, very minimal exposure to all these elements initiates the development of signs of toxicity, especially in the renal tubule, before proteinuria marks their presence. As a priority, this kind of element in biological samples must be quantified with sufficient acuity [3, 40].

Elemental analysis of samples can be done by several analytical techniques, for instance [3, 40]. For the assessment of trace and major elements, colorimetric methods or ion specific electrodes are commonly used. Whereas atomic absorption and emission spectrometric methods are more frequently used when determining the levels of trace elements to assess deficiencies or toxicity of essential and nonessential elements.

6.2 Applications of XRF for Biological Specimens

XRF is one of the most common methods when studying metals, minerals, environmental samples, food components, body fluids, and biopsies [3,41]. In 1971 the in vivo determination of lead (Pb) was first reported; completed by a series of studies which are being continued to improve this phenomenon. These developments have led to numerous alternative methods of XRF.

6.2.1 XRF Applications to Calcified Tissues (Teeth and Bones)

Oral tissues, dental calculus, human teeth, and bones are some examples of biological samples that could be evaluated using XRF in the analysis process. Enamel, dentine, and cementum are the three components that make up teeth. There is a similarity between them and bones in the sense that they are made up of various salts, such as hydroxyapatite, and carbonate, which absorbs other cations to form a crystalline solid compound. The inorganic composition of teeth is 85 percent. This is necessary for bone to retain these elements during the remodeling process carried out by osteoblast and osteoclast cells, and for teeth to maintain these elements through the simple replacement of old adsorbed salts with new ones. Despite the fact that a great deal of research has been conducted over a long period of time, dentine mineralization is still not fully understood.

Thermogravimetric mass spectrometry, C/N analysis, and X-ray fluorescence of the dentin and enamel of healthy human, bovine, pig, and ovine teeth were analyzed. In the intra-enamel comparison, dentin had a higher content than enamel for C and N, respectively. Human enamel is characterized as the most mineralized tissue, with TG-MS analysis indicating a lower carbon content and the presence of additional components in enamel compared to dentin. It was determined that Mg, S, Sr, and Zn are more abundant in dentin compared to enamel, as assessed by the wavelength dispersive X-ray fluorescence method.

The wavelength dispersive X-ray fluorescence (WDXRF) method revealed that Mg, S, Sr, and Zn concentrations were elevated in dentin relative to enamel. The study revealed higher concentrations of Mg, S, Sr, and Zn in dentin compared to enamel. The concentrations of P, Ca, Cl, Cu, and K, along with the Ca/P ratio, were elevated in enamel compared to dentin. The study revealed that human enamel and dentin contained significantly higher levels of calcium and chloride, while exhibiting lower concentrations of magnesium, sulfur, and zinc in comparison to animal tissues. The authors concluded [36] that while other species exhibited significant differences, human and bovine enamel and dentin showed notable similarities.

Tooth decay is one of the most prevalent dental problems today. Several tests are essential in order to correctly surveying the carious areas. Toothache diagnosis is usually based simply on an X-ray transparency of the tooth and a physical checkup. Demineralization of tooth minerals results in low calcium content, the earliest symptoms of caries. Kitasako et al. [36] and his team recently used a scanning XRF microscope to measure the calcium content on the surface of demineralized tooth samples. They also used microradiography in contact to measure the degree of mineralization. The technique has been applied in the direct measurement of calcium using X-ray fluorescence microscopy in demineralized teeth [36, 42].

Analysis of trace and heavy elements in teeth can reveal the cause of dental diseases that can be initiated from heavy elements in the environment [23, 25]. Several Test have been conducted to investigate the trace elements' contents in teeth from heavily and lightly polluted areas using XRF spectroscopy.

This was done with XRF spectroscopy. According to the authors, the presence of zinc, sulfur, and lead is related to environmental pollution sources [23]. They also state that the content of zinc and lead in the teeth of smokers is higher than that in non-smokers. Oprea et al. have studied several tooth samples for trace, minor and microelement content by XRF analysis [25].

Various tooth tissues were analyzed for several measurements (e.g., human enamel, dentin, and cementum). Different ages and professions exhibit slight

variations in elemental composition. This also pertains to gender differences in dietary habits, nutritional status, and lifestyles.

Nicotine, aside from being one of the potential toxicants in tobacco products and a major driver of tobacco addiction, appears to mediate the effects of smoking on hemodynamics. One other thing, it must be said, nicotine is related to a lot of diseases as well [19]. The adverse effect of smoking on oral health was also stated by Albandar et al. In their findings, they found that smokers had few teeth than nonsmokers. They also found that tobacco was r\Harmed only the person who smoked it but inhaled into their lungs and then into their bloodstream with effects at bone metabolism. Johnson et al. reported that exposure peri-implant tissue to tobacco was reasons enough over high rate implant failures among smokers as general condition effect by tobacco result raised higher chances factors for failure situations.

Quantitative analysis of the samples showed the presence of a number of elements such as calcium, potassium, phosphorus, magnesium, carbon, and sodium in deciduous teeth. The same elements along with iron and lead were also quantified in permanent teeth. As theirs was that the concentration of these toxic elements is more into the group that smokes than into nonsmoking group Higher Pb, Cd, and Co concentrations with lowest Ca, P, and Na concentrations of potentially toxic elements were noted in the older group (over 60 years old). Those aged 20 to 40 who were non-smokers had higher Ca levels than other groups.

Apart from deciduous teeth and permanent teeth samples showing high content of calcium, potassium, phosphorous, and magnesium, the same elements were quantified in a sample for deciduous teeth. The presence of these same elements plus iron and lead indicates that the concentration of toxic elements is more into the group that smokes than into nonsmoking group. Higher concentrations of Pb, Cd, and Co with lowest concentrations of potentially toxic elements such as Ca, P, and Na were found in the older group (over 60 years old). Those non-smokers who were aged 20 to 40 had higher levels of calcium than the rest of the compared groups.

In the study by Spector et al., bone Sr was measured in 238 pediatric samples using portable XRF. It was found that in most subjects bone Sr could be measured with a portable XRF analyzer in 2 min, indicating the feasibility of this approach for widespread application and explaining possible gender differences in bone Sr storage capacity and Sr-related metabolism. The work was published at 9:00 a.m. in the Journal of Bone and Mineral Research. It, therefore, needs to be highlighted that there is no problem with running without breakfast. Morning exercise on an empty stomach will not shut down your metabolism.

Aaron James Specht et al. [47] described the in vivo measurement of Pb in human bone using an optimized commercial XRF and used a previous version of the instrument for bird bone measurements in the field. The authors measured bone lead in resected exposed bald eagle bones by ICP-MS, a Cd-109 KXRF system, and portable XRF system. Regression of bone lead measurements obtained with both KXRF and portable XRF systems against data from ICP-MS yielded high coefficients ($r = 0.93$ and $r = 0.92$, respectively).

7. The use of Energy Dispersive X-Ray Fluorescence for Clinical Diagnosis

7.1 Introduction

As previously discussed, Energy Dispersive X-Ray Fluorescence (EDXRF) is a sophisticated analytical method progressively utilized in clinical diagnostics for non-destructive elemental analysis of biological specimens. EDXRF offers significant insights into metabolic disorders, toxicity, and disease pathogenesis by detecting and quantifying trace elements in tissues, blood, urine, and other body fluids.

EDXRF has proven highly valuable in identifying heavy metal toxicity (e.g., lead, mercury, and arsenic exposure) and evaluating imbalances of key elements such as iron, zinc, and calcium, which are crucial in situations such as anemia, osteoporosis, and neurodegenerative disorders. The advantages encompass

minimal sample preparation, expedited analysis, and the capability to evaluate both solid and liquid samples in situ.

7.2 Determination of arsenic concentrations in human scalp hair for diagnosis of arsenic poisoning.

Consumption of arsenic (As) contaminated water is the primary cause of arsenicosis, which ultimately results in severe suffering and death for the affected individual. Poisoning or arsenicosis is a medical condition resulting from elevated levels of arsenic in the body. The World Health Organization establishes a safe limit for arsenic in drinking water at 10 µg/l, while the government of Bangladesh sets its standard at 50 µg/l [1, 2]. Various studies indicate that consumption of water containing arsenic at concentrations exceeding 150 µg/l can increase mortality rates in patients with chronic diseases such as cancer, heart disease, and diabetes by approximately 64%. However, it may pose risks for patients with normal, non-chronic diseases. The manifestation of arsenic disease (arsenicosis) is contingent upon the intake of arsenic compounds and their subsequent excretion from the body. As typically enters the human body through three primary routes: (i) inhalation via air, (ii) ingestion through water and food, and (iii) absorption through the skin. Reports indicate that 40–60% of arsenic can be retained by the human body following its entry into the system[3]. The development of symptoms associated with arsenicosis is influenced by several factors, including exposure duration, the body's defense mechanisms, dietary habits, individual arsenic uptake concentration, and overall duration of exposure. It is generally accepted that a period of 2 to 20 years is necessary for the manifestation of the disease's symptoms [40].

Arsenate and arsenite are two carcinogenic arsenic compounds that tend to accumulate in human hair, nails, bones, and skin. The toxic effects of arsenic are typically observed in specific regions of the body. The analysis of those organs can effectively quantify the level of arsenic exposure in an individual. The collection of bone, skin, and nail samples from

the human body is complex and may occasionally be unfeasible when compared to scalp hair. Furthermore, it was observed that arsenic has a strong tendency to deposit in human scalp hair, which is composed of keratin. Consequently, hair tissue contains the highest concentration of arsenic compared to other tissues in a suspected patient. At the time of exposure, arsenic combines with hair keratin and becomes trapped in the hair, making it the optimal sample for analysis. Hair tissue serves as an effective biological indicator for chronic arsenic exposure due to the slow excretion of arsenic through hair.
Measuring the concentration of arsenic in scalp hair provides an accurate indication of arsenic exposure levels in the body. Numerous analytical methods have been proposed for the determination of arsenic in scalp hair. Among these, Energy Dispersive X-ray Fluorescence (EDXRF) is the safest and simplest technique, providing more accurate results as it does not require digestion or chemical treatment for sample preparation.

7.3 Determination of Lead Concentrations

7.3.1 Background

Autism spectrum disorder is on the rise. Autism spectrum disorder comprises a group of profound pervasive developmental conditions with etiological factors of environmental, genetic, and epigenetic origins. Some features appear to run in families. Autism spectrum disorder is a neurodevelopmental disease which invariably presents as impeded social functioning with associated lack of relatedness to others, poor communication and repetitive behavioral symptoms. Autism is considered as a result of disturbance in prenatal and postnatal normal neurobiological processes with the disease's significant genetic etiology, but minor other factors play a role and are also much more common.

Johnson and Myers [43] postulated that, in general, teratogenic effects on the central nervous system of the developing embryo can result from early

pregnancy environmental exposure to essential minerals and heavy metals. Lead is included among the toxic heavy metals because of great concern as an undesired impurity that can be emitted from numerous common sources into the environment in polluted air or water after the combustion of gasoline and industrial activities. Pb can enter the body by inhalation, ingestion of food or drink, and by various other pathways. For a developing fetus or child, Pb is more sensitive than it is for an adult because their blood-brain barrier is relatively immature; there is high gastrointestinal absorption due to common hand-to-mouth behavior as well as increased ventilation rates per body weight that results in more being inhaled while also being more likely to ingest soil or dust.

Exposure to lead has been related to autism in studies throughout the world. Nonetheless, there is not full agreement on this issue. These are ‘toxic metals’ that can have adverse effects on brain development and function of other organs and systems in the body. Nercotizing lead-induced neurological, physical, and behavioral ailments afflict kids more often. The Centers for Disease Control and Prevention define levels of blood lead at 10 µg or greater as “elevated” or “worried.” However, research has indicated that even lower concentrations in the blood are associated with lower levels of intelligence in children between 3 and 5 years of age. In view of this information, the CDC has proposed lowering the threshold for an elevated blood lead level to 5 µg/dL.

7.3.2 Role of EDXRF in Diagnosis of Blood Lead Level

Lead is absorbed primarily through the walls of the intestine or by inhalation. An estimated 10-15% of lead in adults and 25-45% in children may be absorbed through the gastrointestinal (GI) tract. Once it enters the body, it follows different paths to reach different organs. When in the human body for only 20 hours, the concentration in bone tissue increases from 8% to 90%. Blood is the fluid that carries Pb throughout the body. Serum and plasma do not contain all of the information necessary regarding the content of Pb in human blood. The

measurement of lead content in whole blood is, therefore, more accurate than S-Pb or P-Pb.

Another major study reported that mean plasma lead levels represented only about 0.29% of whole blood lead levels. Techniques based on AAS, ICP-AES, polarography, and ICP-MS have been applied for decades in the analysis of lead in blood, either in situ in the sample chamber (S-Pb) or following precipitation of the matrix components (mainly proteins) and subsequent analysis of the purified Pb in solution (P-Pb). In addition to this, whole blood samples may not be viable owing to the presence of fat bodies in the samples. It's very difficult to measure large biological samples at once for fat-rich whole blood samples.

Up-to-date, bodily tissue (urine, blood, plasma, nails or hair) analysis has turned out to be the most widely chosen and efficient method used for disease recognition and monitoring. While there is an understanding of the presence of various toxic substances (Pb, Cd and As), there can be an application for the evaluation of these substances using EDXRF. Further, EDXRF has the ability to detect larger amounts of certain specific essential elements in the body's tissues; for instance, Fe in urine or Mn in blood, which can initiate a disease or functional decline if not detected properly. Furthermore, it can be said that the XRF analysis method using EDXRF spectrometer for identification and measurement of components in all kinds of substances including body tissues is a 100% unique approach. It is efficient and accurate and can identify components in small to medium sample sizes. It is efficient and accurate.

8. Synchronous Radiation X-Ray Fluorescence Analysis

8.1 Introduction

V.A. Trunova [48] presents a brief review of the application of synchrotron radiation X-ray fluorescence with the VEPP-3 storage ring of BINP SB RAS for the determination of the element composition of various types of samples. Among them are biological and geological samples, samples of the

environment, and archaeological samples. The principal advantage of SR-XRF over other X-RFAs is in the special properties of synchrotron radiation that allow analysis of low-mass samples in the milligram range at several orders of magnitude lower mass than was possible with older analytical methods.

More specifically, a strategy for determining the chemical composition of low-mass samples (from fractions of a milligram) had been developed for special samples, e.g. lunar soil (basalt), fragments of grass moth bodies, and fragments of human myocardial tissue biopsy material. It is also an analytical technique, which is used in elemental analysis of biological tissues. One major advantage of the method is that sample dissolution, decomposition and concentration are not necessary. This way, risk for contamination and loss of the analyzed components are reduced, and the analysis time is cut by about half. [3,48]

X-ray optics developed on new synchrotron radiation sources can greatly enhance the older X-ray optics by selectively exciting components in samples of complex composition and enabling optimal detection conditions to be chosen; (ii) Since SR is naturally polarized, it allows excitation radiation scattered by a sample as a background to be reduced at the detection angle; ideally, this reduction may reach one or even two orders of magnitude (iii) High intensity of the SR beam allows area analysis with high resolution when investigating very thin samples, hence increasing s/b in some cases as well [30]. The advantages of SR include the possibility to work with relatively small cross sections and high coherence of X-rays, while this can also be obtained by conventional methods if focused, which has been used successfully in microanalysis [30].

SR sources are highly specialized; usually, there are dozens of analytical stations in high-tech laboratories which, as a rule, and dedicated sources. There are nearly 50 SR sources in the world today. The Siberian synchrotron radiation source and Terahertz at the synchrotron radiation center (SSTRC) built-in BINP SB RAS since the end of the 1950s is VEPP-3 storage ring (2 GeV). In operation also hosts Sibir-2 (2.5GeV) at the Kurchatov Institute research installations.

Specific features of SR lepton storage: high luminosity, low angular divergence, continuous spectrum of radiation with linear polarization realized at SSRC, etc. let us conduct the research. The technique has greatest spread in study of complex biological objects. Multi-element non-destructive analysis will be provided for low mass samples (detection limit 0.01 μg/g) with unique capabilities of SRXRF. NIST-1 has all the necessary equipment for determination of elements over a wide range.

Visible X-ray elemental analysis stations have, however, been used for studies on biological samples of various types [30,44]. Such methods were developed for chemical composition determination of low-mass (a few milligrams) biological samples like biopsy material fragments of human myocardial tissue [39, 45], lunar regolith (basalt) and fragments of the lunar corpse of the moth [41]. The described sample preparation method is the only technique suitable for quantitative analysis of rare and low-mass (<several milligrams) biological tissue samples, most notably biopsy samples.

8.2 Obesity and interaction of elements in lungs and liver of humans

Obesity leads to the development of a pathological system characterized by a stable metabolic disorder. The liver serves as the primary organ for chemical homeostasis, participating in the metabolic processes of proteins, fats, and carbohydrates. The lungs execute numerous non-respiratory functions and, in conjunction with the liver, participate in fat metabolism [49–55]. Considering the significant role of BEs in metabolic processes, it is pertinent to investigate the levels of BEs in the liver and lungs during the early stages of alimentary obesity (AO) development. The primary objective of this study was to determine the concentrations of chemical components and inter-element correlations (IECs) in the liver and lungs of rats by simulating metabolic changes influenced by food rationing.SRXRF has been utilized to quantify the concentrations of K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr in liver and lung samples of Wistar rats. The subjects of this experiment comprised (i) healthy rats, (ii) rats with AO, and (iii) rats with AO that received zinc sulphate. ZnS reacts with water over an

extended period (AO+Zn). Each group was partitioned into two subgroups. The experiment involving the initial subgroup concluded with the animals in a state of physiological hunger, followed by the retrieval of liver and lung tissue. In contrast, the animals in the second subgroup were sacrificed two hours post-ingestion of lard. The rats exhibiting physiological hunger showed no intergroup differences in the bioelement (BE) content in either the liver or the lungs. In comparison to healthy animals, the rats exhibiting AO showed physiological hunger redistribution of IECs, indicating a sustained metabolic disorder. Supplementation of zinc (Zn) in the diet of rats did not influence body weight or the concentration of BEs (including Zn) in the liver and lungs. Nonetheless, the IECs in the tissue samples of rats exhibiting physiological hunger also underwent alterations. This redistribution was distinct from that observed in the rats with AO. The IECs altered shortly after the ingestion of lard, indicating persistent changes in the animals' metabolism.

The study established that epithelial tissues, such as nails, can facilitate the elimination of chemical elements from the body. Consequently, the elemental composition of the nail plate holds significant potential for non-invasive health diagnostics. The analysis revealed a dynamic relationship between the metabolic functions of the liver and lungs in AO, as indicated by the elemental composition. Elements such as Br, Rb, and Sr, which are not well-studied, play a significant role in forming correlations, highlighting their importance in metabolism. Furthermore, the degree of deviations in IECs within cells and tissues can facilitate early diagnosis of disease progression and assessment of treatment efficacy.

9. Conclusions

Synchrotron X-ray fluorescence microscopy is currently the only microanalytical imaging technique that can be used with hydrated biological materials and still provide submicron spatial resolution. In addition to being able to obtain micro-XANES spectrum, the method is specially suited for investigating transition

metal subcellular speciation. However, Fluorescent small-molecule probes are still very useful. instruments to examine the thermodynamic and kinetic availability SXRF microscopy of transition metal cations in living cells enhances this strategy in a complementary manner by providing numerical microanalytical information about fixed or frozen samples. Combined with additional imaging techniques, such as microradiography, electron microscopy, Such synergy is further expanded by SIMS, FTIRM, or MRI to investigate the physiology of transition metals in cells.

References

[1] Kevles BH (1996). Naked to the Bone Medical Imaging in the Twentieth Century. Camden, New Jersey: Rutgers University Press. pp. 19–22. ISBN 978-0-8135-2358-3.

[2] Sample S (27 March 2007). "X-Rays". The Electromagnetic Spectrum. NASA. Retrieved 3 December 2007.

[3] Selvik G. Roentgen stereophotogrammetric analysis. Acta Radiologica. 1990 Jan 1;31(2):113-26.

[4] Dawood Tafti1; Christopher V. Maani. StatPearls

[5] Seibert JA. The AAPM/RSNA physics tutorial for residents. X-ray generators. Radiographics. 1997 Nov-Dec;17(6):1533-57.

[6] Seibert JA. X-ray imaging physics for nuclear medicine technologists. Part 1: Basic principles of x-ray production. J Nucl Med Technol. 2004 Sep;32(3):139-47

[7] X-ray Generator Contributed by D Tafti, MD. StatPearls

[8] Technical Note/ x-Ray detectors- Hamamatsu Photonics

[9] J. G. Brown, X-Rays and Their Applications (1966)

[10] Henke, B.L., Lee, P., Tanaka, T.J. et al. (1982). Low-energy X-ray interaction coefficients: Photoabsorption, scattering, and reflection: E= 100–2000 eV Z= 1–94. At. Data Nucl. Data Tables 27 (1): 1–44.

[10] Compton, A.H. (1923). XCV. Absorption measurements of the change of wave-length accompanying the scattering of X-rays. London Edinburgh Dublin Philos. Mag. J. Sci. 46 (275): 897–911.

[11] Peter Brouwer. THEORY OF XRF. Getting acquainted with the principles. 5th Edition, PANalytical BV, The Netherlands. (2018)

[12] Geochemical Instrumentation and Analysis. Karl Wirth, Macalester College and Andy Barth, Indiana University~Purdue University, Indianapolis

[13] Dr. Pradeep Patil. D.Y Patil hospital and medical College. (2021)

[14] Suvorova, D., Khudonogova, E., and Revenko, A. (2017 May). X-ray fluorescence determination of Cs, Ba, La, Ce, Nd, and Ta concentrations in rocks of various composition. X-Ray Spectrom. 46 (3): 200–208.

[15] Micro X-ray Fluorescence (μXRF) - XOS

[16] Our Techniques [internet]. San Diego: Eurofins EAG Laboratories; [2015–2021] [cited Apr 2021]. https://www.eag.com/techniques (accessed November 2021).

[17] Basics of Inductively Coupled Plasma Mass Spectrometry. SHIMADZU

[18] Montaser, A. and Golightly, D.W. (1992). *Inductively Coupled Plasmas in Analytical Atomic Spectrometry*. New York: *VCH Publishers*, Inc.

[19] Gholap, D.S., Izmer, A., Samber, B.D. et al. (2010). Comparison of laser ablation-inductively coupled plasma-mass spectrometry and micro-X-ray fluorescence spectrometry for elemental imaging in Daphnia magna. Anal. Chim. Acta 664 (2010): 19–26.

[20] Garman, E.F. et al. (2005). Elemental analysis of proteins by micro-PIXE.

Progr. Biophys. Mol. Biol.,Elsevier 89 (2): 173–205.

[21] PIXE (Particle Induced X-ray Emission). Universidad Autonoma de Madrid

[22] Experiences from eight years of firing demolition wood using ammonium sulphate to mitigate superheater corrosion. Conference: 28th Int Conf on Impacts of Fuel Quality on Power Production and the EnvironmentAt: Åre, Sweden. (2022)

[23] Haschke, M. and Boehm, S. (2017). Micro-XRF in scanning electron microscopes. Adv. Imaging Electron Phys. 199: 1–60.

[24] X-Ray Fluorescence in Biological Sciences. Vivek Kumar Singh, Jun Kawai, Durgesh Kumar Tripathi (2022)

[25] Haschke, M., Eggert, F., and Elam, W.T. (2007). Micro-XRF excitation in an SEM. X-Ray Spectrom. 36: 254–259.

[26] Yearly, R. (2015). Better Together: XRF and XRD [internet]. United States: ThermoFisher Scientific https://www.thermofisher.com/blog/mining/better-together-xrf-and-xrd/.

[27] Back-to-Basics tutorial: X-ray diffraction of thin films. George F. Harrington· José Santiso. (2021)

[28] Aibéo, C.L., Goffin, S., Schalm, O. et al. (2008). Micro- Raman analysis for the identification of pigments from 19th and 20th century paintings. *J. Raman Spectrosc.* 8: 1091–1098.

[29] Presser, V., Keuper, M., Berthold, C., and Nickel, K.G. (2009). Experimental determination of the Raman sampling depth in zirconia ceramics. *Appl. Spectrosc.* 63–11: 1288–1292.

[30] Hussain S, Mubeen I, Ullah N, Shah SS, Khan BA, Zahoor M, Ullah R, Khan FA, Sultan MA. Modern diagnostic imaging technique applications and risk factors in the medical field: a review. BioMed research international. 2022;2022(1):5164970.

[31] Suvorova, D., Khudonogova, E., and Revenko, A. (2017 May). X-ray fluorescence determination of Cs, Ba, La, Ce, Nd, and Ta concentrations in rocks of various composition. X-Ray Spectrom. 46 (3): 200–208.

[32] Nečemer, M., Kump, P., Ščančar, J. et al. (2008). Application of X- ray fluorescence analytical techniques in phytoremediation and plant biology studies. *Spectrochim. Acta Part B* 63 (11): 1240–1247.

[33] Nečemer, M., Kump, P., and Vogel- Mikuš, K. (2011). Use of X- ray fluorescence- based analytical techniques in phytoremediation. In: Golubev I., editor. *Handbook of Phytoremediation*. Hauppage (NY): Nova Science Publishers: p. 331–358

[34] Kavčič, A., Mikuš, K., Debeljak, M. et al. (2019). Localization, ligand environment, bioavailabilityand toxicity of mercury in Boletus spp. and Scutiger pes-caprae mushrooms. Ecotoxicol. Environ. Saf. 184: 109623.

[35] Kavčič, A., Budič, B., and Vogel-Mikuš, K. (2020). The effects of selenium biofortification on mercury bioavailability and toxicity in the lettuce-slug food chain. Food Chem. Toxicol. 135: 110939.

[36] Balcaen, L., De Schamphelaere, K.A.C., Janssen, C.R. et al. (2008). Development of a method for assessing the relative contribution of waterborne and dietary exposure to zinc bioaccumulation in Daphnia magna by using isotopically enriched tracers and ICP-MS detection. Anal. Bioanal. Chem. 390: 555–569

[37] Ali A, Chiang YW, Santos RM. X-ray diffraction techniques for mineral characterization: A review for engineers of the fundamentals, applications, and research directions. Minerals. 2022 Feb 6;12(2):205.

[38] van der Laan G, Figueroa AI. X-ray magnetic circular dichroism—A versatile tool to study magnetism. Coordination Chemistry Reviews. 2014 Oct 1;277:95-129.

[39] Klockenkämper R, Von Bohlen A. Total-reflection X-ray fluorescence analysis and related methods. John Wiley & Sons; 2014 Dec 10.

[40] Potts PJ, Ellis AT, Kregsamer P, Marshall J, Streli C, West M, Wobrauschek P. Atomic spectrometry update. X-ray fluorescence spectrometry. Journal of Analytical Atomic Spectrometry. 2003;18(10):1297-316.

[41] Uo, M., Wada, T., and Sugiyama, T. (2015). Applications of X-ray fluorescence analysis (XRF) to dental and medical specimens. Jpn. Den. Sci. Rev. 51: 2–9

[42] Jaswal, B.B.S., Rai, P.K., Singh, T. et al. (2019). Detection and Quantification of heavy metal elements in gallstones using X-Ray fluorescence spectrometry. X-Ray Spectrom.: 1–10.

[43] Gholap, D.S., Izmer, A., Samber, B.D. et al. (2010). Comparison of laser ablation-inductively coupled plasma-mass spectrometry and micro-X-ray fluorescence spectrometry for elemental imaging in Daphnia magna. Anal. Chim. Acta 664 (2010): 19–26.

[44] van der Laan G, Figueroa AI. X-ray magnetic circular dichroism—A versatile tool to study magnetism. Coordination Chemistry Reviews. 2014 Oct 1;277:95-129.

[45] Achkar, A.J., Regier, T.Z., Monkman, E.J. et al. (2011 Dec 6). Determination of total x-ray absorption coefficient using non-resonant x-ray emission. Sci. Rep. 1: 182

[46] N. Hiraishi , S. Kobayashi , H. Yurimoto , J. Tagami. Ca doped remineralization study on dentin by isotope microscopy. Dental Materials

Volume 34, Issue 4, April 2018.

[47] Aaron James Specht, Marc Weisskopf, Linda Huiling Nie. Portable XRF Technology to Quantify Pb in Bone In Vivo.

[48] V.A. Trunova. Synchronous Radiation X-Ray Fluorescence Analysis of Microelements in Biopsy Tissues.

[49] Grippy, M.A. (1999). Pathophysiology of lungs (in Russian). M.: BINOM.

[50] Zilber, A.I. (2007). Studies in respiratory medicine (in Russian). M.: MEDpress-inform.

[51] Syromyatnikova, I.V., Goncharova, V.A., and Kotenko, T.V. (1987). Metabolic activity of lungs (in Russian). L.: Medicine

[52] Anghey, E., Grant, L., Furman, B.L., and Dryden, W.F. (1977). The effects of oral zinc supplementation in the mouse. J. Comp. Pathol. 87 (1): 1–14.

[53] Kryzhanovsky, G.N. (2011).Basics of general pathophysiology (in Russian). M.: Medical news agency.

[54] Lepekha, L.N. (2007). Surfactant system in healthy and sore respiratory systems. Respiratory Medicine. M.: GEOTAR - Media.

[55] Trinner, K.S. (1960). Thermogenesis function and alkalinity of the lung tissue reaction (in Russian). M.: Academy of Sciences of the USSR.