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Analytical Techniques for Biomedical Nanotechnology

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Chapter 4

FTIR spectroscopy and microscopy in biomedical nanotechnology

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Fourier transform infrared (FTIR) spectroscopy is one of the promising functional characterization tools used to confirm the available functional groups, drug functional characterization in pharmaceutical science, materials' characterization, tissues analysis including functional and morphological assessment, and confirmation of bindings between two active moieties. This approach is one of the potential methods to develop efficient optical sensors to detect a target analytic selectively. This chapter will cover the fundamental understanding of FTIR along with advanced biomedical applications.

4.1 Introduction

Human life has always been lived on the edge of a precipice. Thus the supervision of human health has been of major importance through the ages. It is one of the most crucial factors influencing the overall growth of a human being. Technological developments have led the way in the astonishing changes in the health sector, saving numerous patients and also ceaselessly enriching the quality of life. It is now known that as we move ahead into the era of technology, the number of benefits would rise. This is exactly where the concept of nanotechnology comes into the picture.

Nanotechnology is being referred as the most useful experimental technology in the 21st century providing immense opportunities in each discipline (Singh 2014). Indeed, figure 4.1 highlights the applications of nanotechnology in various fields. Nanotechnology has several definitions however, all the definitions focus on the development and design of bottom-up nanostructured materials that provide specific responses when they are exposed to definite stimuli. Nanotechnologies range from 10^{-9} m to 10^{-7} m in linear dimension (1–100 nm). For a perception of the size they are 80 million times smaller than an apple and 5 million times smaller than an ant. The idiosyncratic properties which are difficult for the bulk material to manifest are

doi:10.1088/978-0-7503-3379-5ch4

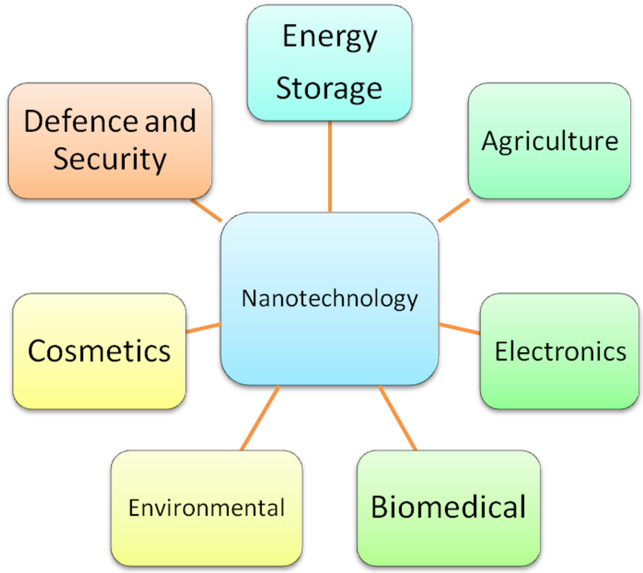

Figure 4.1. Application of nanotechnology in various fields.

the ability to achieve multiple functions from a single platform. It allows biologic barriers to be overcome that in the past restricted medical efforts for therapy and diagnosis (Pope-Harman *et al* 2007). Nanotechnology can permit the localization of therapeutic interruption to diseased areas inside the body and better control over temporal variables. Nanotechnology is thus a way to looking for transformation in biomedical sectors providing more sensitive and specific imaging techniques, nano-robotics (Gorjikhah *et al* 2016) and nano-devices for advanced detection of targeted and less toxic drug treatments, biochemical changes, tissue implantation as well as enhanced support for regenerative medicine thereby offering valuable and rewarding advantages to healthcare workers, patients, and society (Nalwa 2014). Thus biomedical nanotechnology is devoted to the exploration of nanotechnology for healthcare eventually attaining the goal of personalized health management thereby providing doctors and scientists with abilities they might never have dreamt of.

Despite the tremendous boons biomedical nanotechnology holds for healthcare, there is concern about its safety and the possibility of harmful effects (Polizu *et al* 2006). The apprehension that nanotechnology may have serious health and environmental risks has disrupted the field for many years (Ramos *et al* 2017). There was a huge need to develop methods to characterize nanomaterials in order to monitor the health and environmental hazard possessed by the nanotechnology since there is a lack of consensus about the morphology, size of the particle, surface charge after their interaction with the tissues and correlations with toxicity (Kaushik 2019). Thus, sophisticated instruments such as Fourier transform infrared (FTIR), scanning electron microscope (SEM), transmission electron microscope (TEM), x-ray diffraction (XRD) etc came into the limelight, which allowed evaluation of the nano-biomaterials and their interfaces (Saji *et al* 2010).

Thus this chapter will specifically focus on the FTIR characterization technique in detail along with its use and applications in various biomedical nanotechnology fields.

4.2 Nanotechnology in biomedical science

Researchers across the globe have discovered the significance of nanotechnology in the field of biological science. Thus with the evolution in nanotechnology, numerous research is being carried out in various areas especially in the biomedical field and many novel applications are being discovered day by day (Leso *et al* 2019). Figure 4.2 highlights the divergence of nanotechnology from its origin.

It has also been found that nanomachines and nanomaterials can be used for detecting disease, its grade and state and also the bio-molecular reactions. Nanosensors can also be employed to acquire biochemical reactions and processes taking place in the cell at any instant in time with the help of an image or video. Nanoparticles are also adopted as drug delivery agents targeting specific tissues being more selective in nature. Thus the breakthroughs of nanotechnology have paved the way from the clinical field with improvements in nanocoating, nano-fabrication and many more molecular nanotechnologies which helps in the

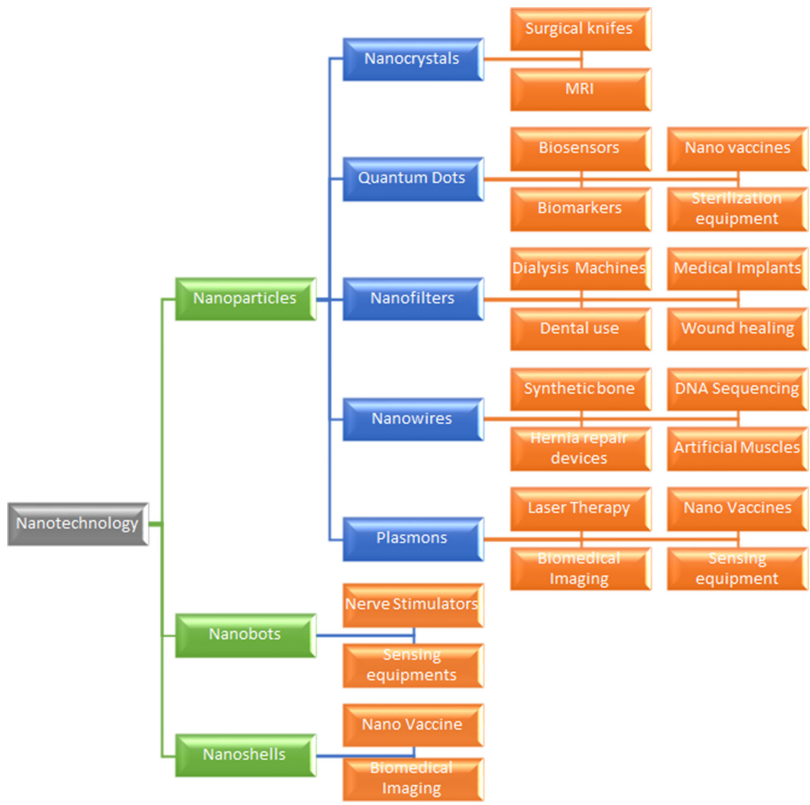

Figure 4.2. Divergence of nanotechnology from its origin.

management, detection, study, and treatment of numerous diseases (Whitman *et al* 2008). Treatment of these diseases incorporates the modification of the cellular activities, repair of the biological tissues and the substitution of diseased tissues with healthy tissue. Nanotechnology can also be used in the field of corrective surgery, termed nanosurgery, and also has potential in the field of regenerative medicines.

4.3 Methods of FTIR spectroscopy and microscopy

Depending on the type or amount of the sample to be examined, various methods of analysis by FTIR microscopy and spectroscopy is achieved and this is shown in figure 4.3.

4.3.1 FTIR spectroscopy

When the sample is irradiated with infrared radiation, FTIR scrutiny of the sample is carried out and results in more sensitive and accurate results. The samples to be

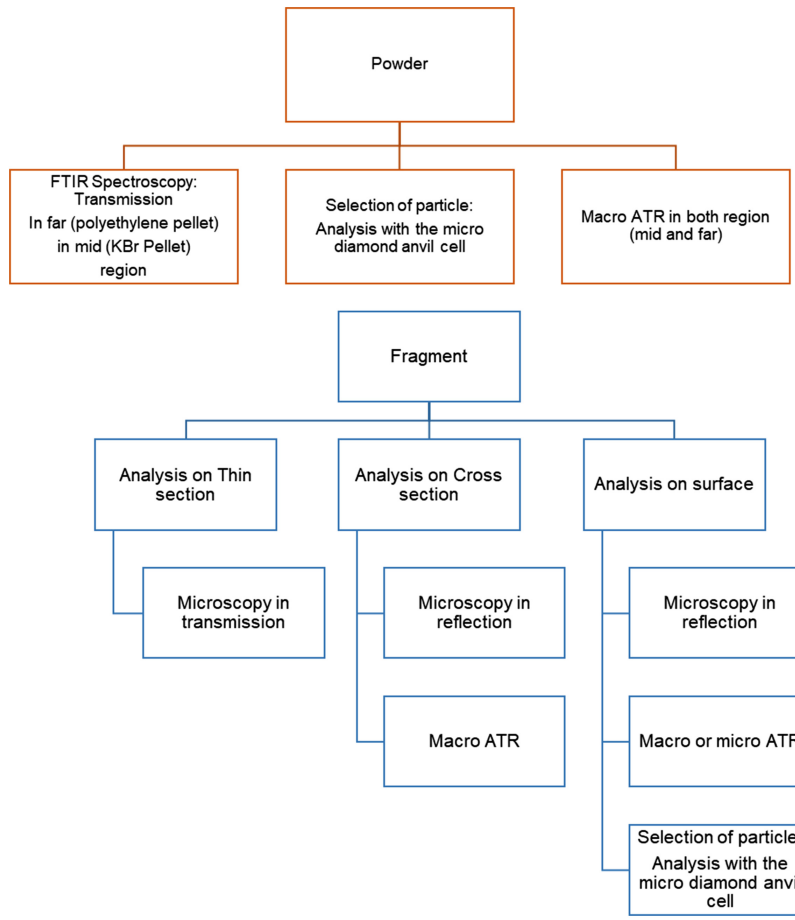

Figure 4.3. Several ways of analysis by FTIR spectroscopy and microscopy.

inspected are formulated as very fine and narrow films in between the infrared crystal clear aperture and are disseminated in the inactive infrared powdered substance whereas the non-transparent samples are just inspected in reflectance without any formulation (Prati *et al* 2010).

The principle for this technique is that whenever the radiation is passed through two separate media, the radiation is split into dissimilar fractions in transmitted and reflected beams based on the ratio of refractive index of two different materials. When the inspected surface of the sample is not reflecting completely there is the existence of scattering, refraction and dispersed reflection which results in the difficulty of elucidating the spectra thus macro attenuated total reflection (ATR) is used to acquire a spectra similar to that of the spectra acquired in the transmission. The sample to be examined is placed in proximity with a crystal also known as internal reflection element (IRE) having more refractive index than that of the sample, so when the radiation irradiates the crystal and passes to the sample at critical angle, the total reflection is generated but then the angle of incidence is more than that of the critical angle, the result is attenuated in which a disappearing wave is produced on the surface of the crystal which is capable of penetrating the sample.

4.3.2 FTIR microscopy

The instrument being used for the FTIR microscopy is a FTIR microscope which is a combination of an optical microscope and a FTIR spectrometer. The most critical component of this method is the spatial resolution of the microscope in order to characterize the tiny and nanoparticles or the fine and narrow layers (Prati *et al* 2010). In order to obtain good signal-to-noise ratio (S/N ratio) a minimum of a 20×20 μm infrared beam opening is required in the case of a single element mercury cadmium telluride (MCT) detector. In this technique the diamond anvil cell, an easy device which is mounted directly on the FTIR microscope stage is used for the analysis of micro or nanoparticles. The micro ATR technique is diversely utilized in this microscopy since it permits the scrutiny of narrow areas keeping the aperture constant due to the magnification element present in the internal reflection element.

Due to the enhanced potential of FTIR microscopy with the development of imaging techniques, enormous FTIR spectra are being accumulated and it also enables the collection of the patterns that shows the distribution of various compounds. A FTIR microscope incorporated with the combination of FTIR spectrometer and the microscope is the advance in this technique which is capable of enhancing the spatial resolution of the system thereby maintaining an adequate standard of spectra. This technique also provides more energy in comparison to that of the traditional techniques being used.

4.4 FTIR spectroscopy in biomedical applications

4.4.1 Cancer study, diagnosis, and treatment

Cancer is one of the primary diseases responsible for many deaths and occurs extensively across the globe. Worldwide nearly one in three individuals are diagnosed with cancer in their lifetime and one in six die as a result of cancer.

The primary objective in cancer research is to innovate a rapid, accurate, and economical technique for the detection of cancer in order to increase the likelihood of survival. Cancer is a stepwise disease that counts in the existence of epigenetic disparity in susceptible cells and addition of absolute as well as transmittable genetic irregularities as the first event and second event being the modification of the biomolecules in both molecular structure aspects and composition. It also takes into account an array of mutations and also alters the response to the microenvironment of the cell, seizure of the responses of the hosts immune and metastasis cancer cell phenotypes that eventually add to chemotherapy. Methods of vibrational spectroscopy that are sensitive to the chemical alteration at molecular scale are employed to detect the presence of cancer (Yano *et al* 2003).

FTIR has attracted huge interest in cancer research because of its ability to explicate quantitative as well as qualitative data of biochemical content. FTIR spectroscopy, as an efficient technique for the investigation of chemical alteration and the composition at the molecular level, has been adopted to detect carcinomas (Su and Lee 2020). The detection of a disease thus depends on biochemical changes instead of morphological changes in tissues. On the basis of the vibrational transitions obtained from the interaction with the IR light of the chemical bond in the sample, the molecular fingerprint is obtained for the biological samples. This method is indeed capable of early detection of the cancer before the abnormalities are obtained from the morphological studies by detecting biomolecular changes (Kumari *et al* 2018). FTIR microscopy and the spectroscopy techniques are capable of detecting the alteration of the chemical content during carcinogenesis progression. Somewhere in the middle of the 20th century these techniques were marked as highly sensitive, label-free, specific, and non-invasive for the tissues of various types of cancer such as colon, breast, skin, stomach, prostate, ovary, cervix etc. The spectrum of the FTIR for the biological sample is the combination of the absorption bands of all lipids, carbohydrates, nucleic acid and proteins, see figure 4.4 which depicts the general bands of FTIR for the biomolecules but specifically for oral epithelium (Wang and Wang 2021). Recent advances in modifications in the chips of integrators, waveguide, detectors, infrared sources, and software development boost the clinical translation of FTIR technique as a rapid, fruitful, cost effective, and automated diagnostic technique.

Biomarkers are defined as molecular changes that are related to the disease present in the body tissues or fluids that are necessary for diagnosing and screening in order to start clinical interventions rather than studying the morphological changes as in present histopathology methods. FTIR can be used directly for tissue analysis, which not only allows early detection but also enhances the accuracy thereby minimizing the discrepancies in the pathologist's interpretation (Levin and Bhargava 2005).

The leading areas where FTIR spectroscopy can be used:

- Differentiation of mormal and infected tissues in organs (liver, breast, cervix, colon).
- Keeping track of unusual growth of cells in tissues (body fluids and thyroid)
- Contrast between cancer and other pathologic conditions which have similar clinical manifestation (colon cancer and IBD)
- Supervision of the effect of the chemotherapy and the grading of the tumor.

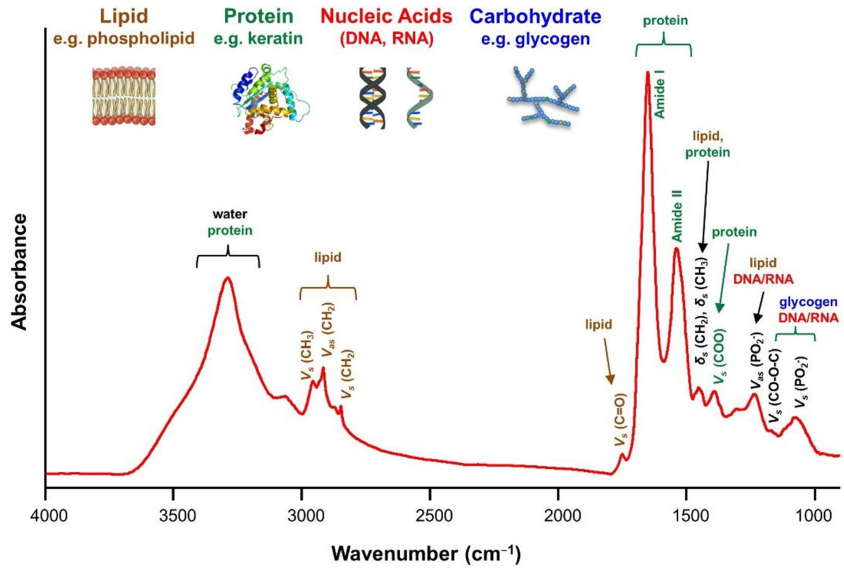

Figure 4.4. Common FTIR bands for biomolecules in oral epithelium. Reproduced from Wang and Wang (2021). CC BY 4.0.

Steps in cancer diagnosis using FTIR spectroscopy:

- Preparation of the sample.
- Collection of the data.
- Analysis of the data.
- Computational methods.
- Prediction or diagnosis.

Diagnosis using FTIR spectroscopy

The dried homogeneous samples (blood components, plasma, body fluids, and whole blood), which are mounted on transparent window materials are used for the collection of the biological sample which is collected by simple IR spectrometers. For frozen, fresh, or fixed tissues, FTIR microscopy is utilized where sections of microtome tissue are present. Colon or cervix-like tissues is easier to estimate since they have zones of proliferation which are defined. Usually, identical tissue sections which are of continuous nature are stained histochemically in order to get a direct comparison between the two methods so as to confirm the diagnosis. Imaging techniques use the data output, thereby allowing huge arrays of spectral quantification with the use of focal plane area detectors (Khanmohammadi *et al* 2011). The details of the spectra obtained for every point is then subjected to numerous mathematical analyses and the information is utilized for the generation of a color code for every point. The intensities of the colors are then reassembled to acquire a pseudo-colored image. In such cases, the malignant tissues have different colors to the normal tissue. Even though this type of examination takes a longer time, it maps the complete area simultaneously, identifying malignancies at each point if any. In the case of microscopic study there are chances of missing a few areas during the

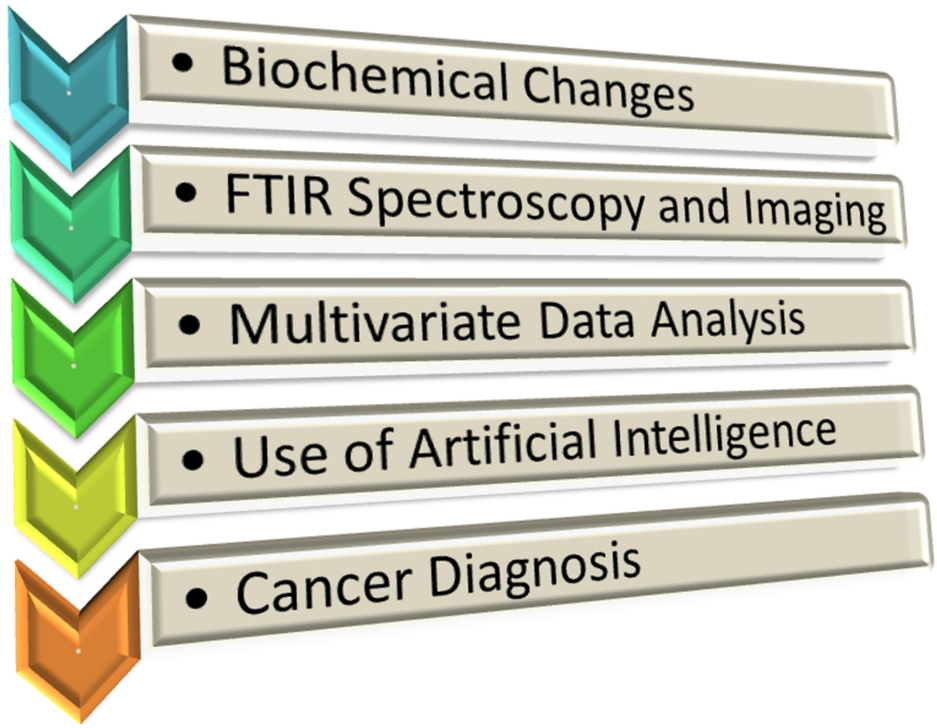

Figure 4.5. The general process for cancer diagnosis.

arbitrary sampling for the cancer. The general process for cancer diagnosis can be viewed in figure 4.5.

Different types of body materials which include tissue, bladder wash, blood, extracellular vesicles septum, urine etc are studied using FTIR spectroscopy in order to develop efficient alternatives for the management and diagnosis of cancer. Since every molecule has its own, unique spectrum which depends on the amount of IR and the wavelength absorbed, FTIR results in the formation of a unique fingerprint of the absorbance peaks of the various parameters that are present in the sample to be examined (Sahu and Mordechai 2005). These biochemical fingerprints are distinguished and are unique for the alteration of molecules in every disease thus provide the exact status for the patient's health.

In order achieve accurate results and distinguish between healthy people and those suffering from cancer, the technique utilized must have high sensitivity, accuracy, and specificity. FTIR spectroscopy does possess all the above characteristics. It has also been discovered that FTIR has the ability to distinguish between cancer and other diseases like hepatitis, heart disease, and many others. It also has applications in the rapid diagnosis of tumors which helps in to assist surgery. It is also found that coupling the ATR-FTIR with optical fiber helps in the evaluation of the surgical resection margin in the colorectal cancer surgery(Li *et al* 2005). FTIR with PCA-LDA helps to detect the difference in the spectrum of a healthy colon and a diseased colon, and cancer in a post- and pre-chemotherapy colon, which means

that it is easier to monitor the efficacy of chemotherapy. Additionally, it also defines the tumor margin even of a mono cell of cancer before resectioning which helps to increase the chances of survival of patients.

When attenuated total reflectance Fourier transform infrared (ATR-FTIR) microspectroscopy was utilized for colon cancer detection as per the spectral data using the linear discriminate analysis (LDA) technique classification 100% sensitivity, 95.8% accuracy, and 93.1% specificity was achieved. Ovarian cancer is diagnosed by using ATR-FTIR spectroscopy in combination with (GA-LDA) genetic algorithm with linear discriminant analysis and when combined with (PC-DFA) principal component discriminant function analysis, formalin-fixed archival prostate cancer tissues were analyzed (Khanmohammadi *et al* 2007). When FTIR was utilized for the oral, pharyngeal, and laryngeal cancer, biochemical changes in the sputum was detected which was used as a non-invasive way to monitor the progression of the disease. In the case of skin cancer, FTIR spectroscopy is used to differentiate normal samples from those of the melanoma and non-melanoma samples with better accuracy (Menzies *et al* 2014, Shakya *et al* 2020). It has the ability to forecast the drug effectiveness on the tumor cells and also recognize and measure different subpopulations of tumor cells, which a prime factor that can aid fruitful immunotherapy, either with the addition of an antibody or without it. It is also found that high accuracy, up to 97%, is achieved for lung cancer annotation. Accuracy of 95% has also been achieved in the identification of subtypes of the prognostic adenocarcinoma using automated FTIR by reduction of manual errors during spectrum processing and the sample preparation. In general approximately 40 min are required for the pathological examination whereas FTIR only takes up to three minutes for the same evaluation. FTIR spectroscopy also has application in the grading, classification, and staging of cancer wherein up to 100% of specificity and sensitivity is achieved (Andrus and Strickland 1998).

Taken all together, FTIR spectroscopy carries potential to be used as the pioneer clinical technique for the study, diagnosis, and treatment of cancer. Moreover from the many examples and applications discussed in this section, it is proved that this techniques can be used extensively on multiple types of cancer. This indeed offers extreme results for the accuracy, specificity, and sensitivity for cancer detection when compared to the conventional methods being used. In addition, this technique validates the accurate grading, classification, and staging of cancer. When an automated process is used, the need for complex and time consuming processes is also removed. This technique is also employed for the follow up and monitoring of cancer patients. Healthy people can easily be differentiated from those with disease with greater accuracy. Therefore, FTIR spectroscopy is a boon for the transformation in the diagnosis and treatment of cancer.

4.4.2 Nanoscale imaging

Fourier transform infrared spectroscopic imaging and Fourier transform infrared microscopic imaging are comparatively modern techniques which have attracted considerable attention. The leading advantages of these techniques are their high molecular responsiveness in combination with a computative resolution at molecular scale. Another benefit of these techniques is their potential to examine samples

under indigenous conditions, due to which it is easy to have insight of the sample without the requirement of stains, additional marker or fixation (Steiner and Koch 2009). This imaging technique is chemical distinct, readily apparent, non-destructive, and label-free with a vast scope for application.

Advances in the field of instrumentation has made FTIR imaging a versatile technique for a wide range of applications. The morphology of various samples can be examined using Fourier transform infrared microscopy imaging. This technique enables us to track down the high resolution spectral images having applications in medical and biochemical fields. High resolution and quantitative data is obtained from the FTIR microscopy which highlights the chemical distribution in the sample. In comparison with conventional histology, FTIR microscopy is particularly quantitative following the principle of Beer–Lambert's law which states that absorbance is directly proportional to the molecular absorbance (Andrew Chan and Kazarian 2016, Kazarian and Chan 2009). Infrared nano-spectroscopy permits innovative potentiality for chemical and structural evaluation of nano-composites, biomaterials, optoelectronic devices and many more. With the evaluation of far FTIR absorbance spectra, the organic material's FTIR spectrum at nanoscale is analyzed thereby permitting the identification and characterization in reference with standard IR data available (Amenabar *et al* 2017).

It is important to follow the proper workflow to obtain the image which helps to proceed further towards diagnosis and treatment, a typical workflow is shown in figure 4.6. Using traditional methods it was not possible to observe the local alteration of biochemical content past discectomy which is now possible with the use of FTIR microscopy. The collagen and proteoglycan content in the IVD is

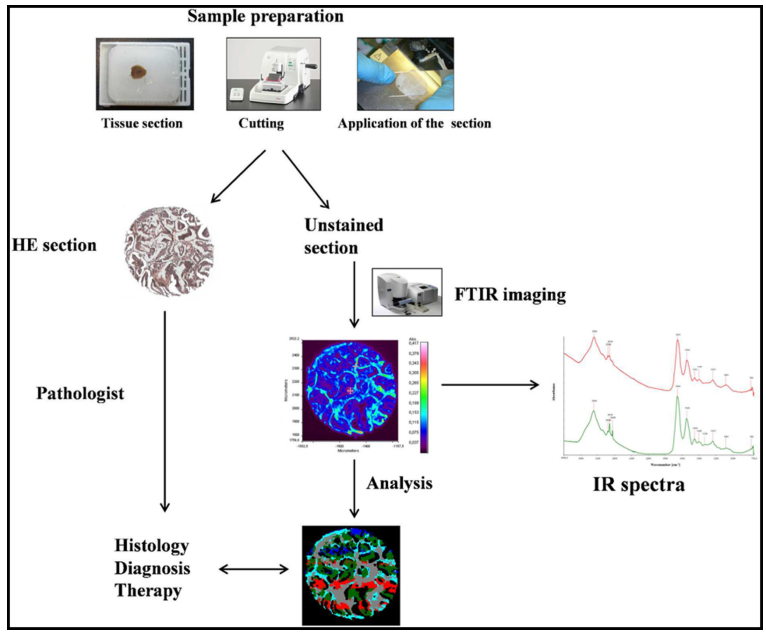

Figure 4.6. Typical workflow of sample preparation, infrared spectroscopic analysis, spectral diagnosis and image building. Reproduced from Pezzei *et al* (2013) with permission of The Royal Society of Chemistry].

quantified and it was found that there is some robust correlation with the Pfirrmann category of degradation (Sloan *et al* 2020). FTIR, a combination of the Fisher discriminant analysis (FDA) and principal component analysis (PCA), was utilized for sample identification of healthy and deteriorated articular cartilage. Imaging of the cartilage and the infrared spectrum of the sample is achieved as the result of FTIR which depicts the concentration of the sample where every pixel is illustrated with false color corresponding to the IR spectrum. Following the strict procedure for sample collection of articular cartilage, the sample was examined thoroughly and the report was generated (Mao *et al* 2015). Results have shown that this technique has an accuracy of approximately 95% and on comparison it was found that the prediction groups were identified accurately.

Tissues of the central nervous system are analyzed in order to understand the structural and biochemical changes linked with the neural degradation diseases including brain tumors, Alzheimer's, transmissible spongiform encephalopathy, Parkinson's, and multiple sclerosis by using this technique in combination with x-ray fluorescence microscopy (XRF) which is indeed a powerful approach for the identification of the chemical composition of a tissue along its trace element distribution. The results obtained are specific, highly accurate, and rapid. Various sections of the central nervous system (CNS) are also comfortably differentiated on the basis of existence or non-existence of myelin as healthy CNS tissue stores a greater amount of myelin in white matter than in gray matter (Caine *et al* 2012).

This practice was extended for the comparison of coronal brain tissue sections of a C6 glioblastoma multiform tumor-bearing rat to sections from a healthy animal by adapting the multiple least square (MLS) fitting procedure for the evaluation of the relative absorbance percentages of pure brain protein, lipid and nucleic acid compounds in FTIR images. The results obtained display the distribution of protein and lipids in the spectroscopic image extracted. Due to the intrinsic packaging property of tissues, augmented dissimilarity was noticed in the IR bright field in comparison with that obtained from the visible microscopic images. The graph of vibrational peak ratio of lipid-protein shows the gradients in the concentration (Levin and Bhargava 2005). Thus it was concluded that this technique is of importance in terms of its adaptability as an insight into the formation of tumors and extending it to the favorable results of numerous therapy processes.

FTIR imaging was further studied for pathological changes in bone by studying the healing process of fractured bones in availability and non-availability of therapy wherein the treated samples had more mineral content per matrix. Even the crystals had enhanced size for the same treated samples and also cellular activities were examined at the place of fracture with the help of fatty acids present in the cell membrane.

In breast implants, which are common for women in the western world, the leakage of silicone gel has become a primary concern. The shells of silicon elastomers being used for the enhancement of the breast are filled with silica gel which can leak from the implant either due to failure of the material or due to damage leading to tissue disorders. In order to evaluate the histopathological difference, the presence of the gel in the tissue is important and would easily be recognized by the FTIR imaging. Thus the chemical difference can be observed between the silicone gel and the tissue and the further treatment can proceed.

FTIR being a highly efficient technique is thus also adopted for application in the field of pharmaceuticals. This is well suited for kinetic study for the dissolution of polymers. Dissolution rate and polymer and solvent diffusion are monitored in this as a function of time with knowledge of the initial conditions. Thus the FTIR image is obtained as a result of the chemical change in the infrared spectra. Since the dissolution, structural properties, and drug release profiles are affected by the various component distributions, the FTIR imaging is also fruitful in this pharmaceutical area.

FTIR is also being applied for the diagnosis and treatment of various diseases in skin tissue, prostate tissue, esophageal tissue, etc. The results obtained have had high accuracy and sensitivity compared with the tradition methods and are rapid. When FTIR-guided LCM (laser capture microdissection) was used, an exemplary potential biomarker in bladder cancer was discovered, as shown in figure 4.7. Thus this

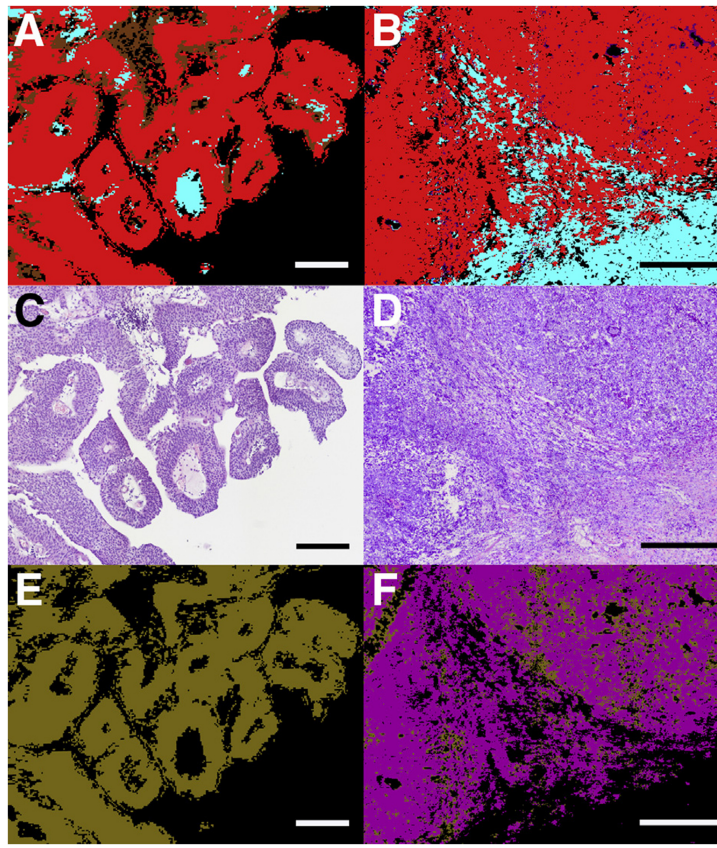

Figure 4.7. Fourier transform infrared (FTIR) imaging index color images obtained label-free before hematoxylin and eosin (H&E) staining of bladder cancer tissue. A and B: low-grade and invasive high-grade carcinoma (red, bladder cancer; cyan, connective tissue; brown, inflammation; purple, urothelial cells). C and D: corresponding H&E staining after FTIR imaging. The label-free classification matches the cancerous regions. E and F: results of the second random forest, which classified the cancer as invasive high-grade (purple) or low-grade (olive) carcinoma. E: no invasive high-grade carcinoma is observed. Scale bars Z 200 mm. Reproduced from Witzke *et al* (2019), with permission from Elsevier.

method enabled label-free spatial categorization with great accuracy and no interobserver variability, in addition to the molecular resolution of the proteomic analysis.

4.4.3 Drug release and drug delivery

Explication of the drug release mechanism and assessment of the effectiveness of the drugs in a biological specimen is essential in order to formulate the drug and control its quality. This is affected by numerous factors of synthesis like microstructure polymorphism and the drug delivery system. Proper development and the application of FTIR can be seen in figure 4.8. For the improvement of pharmaceutical tablets, proper knowledge of the tablet mechanism and the drug release process are prerequisites. Thus the application of FTIR imaging and nano-FTIR technique in biological specimens sets a base for proper understanding of the mechanism and process of drug release. Conventional methods of detection are slow, complex, and have less relevance globally, thus FTIR since it has more advantages over conventional techniques is applicable for a wide range of applications specifically in the field of pharmaceuticals for drug release and drug delivery applications (Song *et al* 2020).

In order to study and investigate the dissolution of tablets and the drug release, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) has been used with the mono reflection accessory of ATR with the crystal of diamond. This allowed *in situ* FTIR imaging of the dissolution of tablets that yield more specificity toward chemicals and also provide spatial resolution at rapid speed.

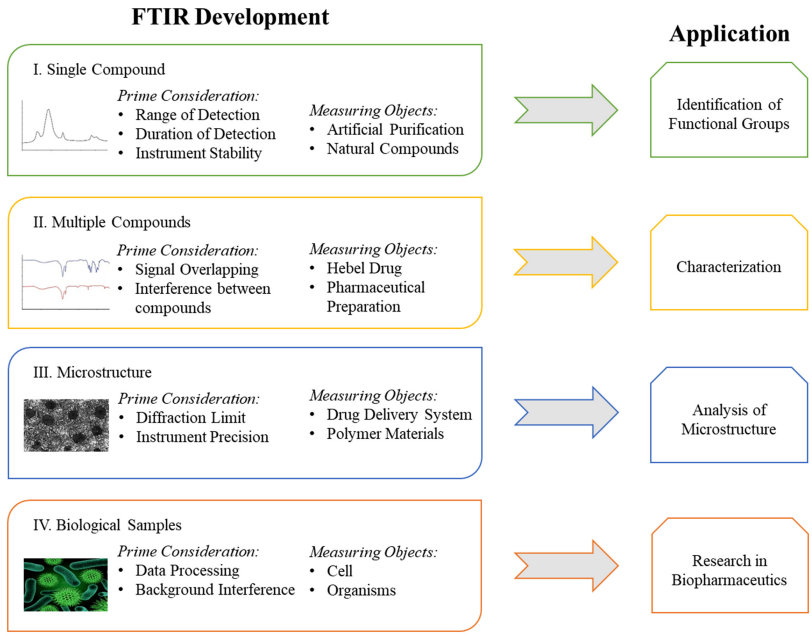

Figure 4.8. FTIR development and its application.

For example, the ATR-FTIR spectroscopic imaging technique was used to study the actions of drugs like ibuprofen and methylprednisolone on dissolution. The advantage of ATR mode is that it requires much less sample preparation whereas in the FTIR it provides information about the functional group present in the sample. Thus the chemical snapshots of the pharmaceutical tablet can be collected as a function of time in order to sort out the profiles of water, drug, and excipient to provide an understanding of drug release after the dissolution of the tablet (Kazarian and Ewing 2013).

Combining the mathematical models with the ATR-FTIR spectroscopic imaging enables a superior prognosis of the drug release and drug delivery thereby improving the particulars of novel systems. Combining ATR-FTIR and nano-FTIR techniques along with the chemometrics becomes a prospective tool for overcoming the inadequacy of traditional IR detection techniques. Nano-FTIR and ATR-FTIR enablesresolution far from the limit of optical diffraction to be acquired and to attain more effectiveness of the drug release mechanism. Structural details of drugs, their characterization and nano-structure scrutiny for can be analyzed in great detail using the FTIR technique. FTIR is used for the characterization of nanoparticles synthesized for anti-HIV drug delivery application. It shows well resolved peaks (Dev *et al* 2010). Peaks shown at various wavelength ranges represent the presence of various chemical bonds or linkage and some peaks are also the result of the interaction between the drug and the polymer.

The innovative approach of surface enhanced ATR-FTIR spectroscopy will permit recognition of the materials even at minute concentrations of the sample which would make it possible to study the nanoparticles for the drug delivery applications and will also feature the manifestation of drug particles which are nano-crystalline in nature. Further growth is based on ATR-FTIR (Tian *et al* 2016) imaging in microfluidic devices for the study of fluid flows. The primary benefit of using this combination is the speed of quantification, which provides a tremendous amount of chemical data which is crucial for the dynamic systems looking into the release of drugs in the pharmaceutical field (Chan and Kazarian 2005).

Contrasting the standard tests of dissolution, FTIR spectroscopic imaging provides insight and accurate data regarding the changes in the tablet during the course of dissolution. Experiments on real tablets can also be done with the help of ATR-FTIR spectroscopy imaging due to its very low sample preparation and thickness independency of the sample.

Various types of nanoparticle and modified nanoparticles have also been used for the enhancement of drug delivery by the improvement of the efficiency of the drug, reducing the dosage and also minimizing its side effects. Thus FTIR as a characterization technique is utilized.

To sum up, the ATR-FTIR imaging technique has tremendous benefits over traditional techniques of dissolution. Thus to differentiate the bio-molecular structures of the drugs, FTIR spectroscopy is a superior technique to adopt. The proper understanding of the mechanism and the process of drug release can be easily realized and studied with the use of FTIR spectroscopy.

4.4.4 Bioinformatics

Bioinformatics is an interesting field in the domain of life sciences. It is comprised of interdisciplinary work ranging from analysis of molecular sequence and genomics data, genome annotation, building biological networks, development of database along with data management systems. Apart from regular biological studies bioinformatics facilitates the development of methodologies and analysis of data using a wide range of tools; it includes storing large volumes of biological data, and organizing this data to make it user friendly. Bioinformatics helps to understand the complex data easily with the help of sophisticated computing facilities. Specially designed computer programmes consist of cloud computing along with a combination of statistics and mathematics. The well-known approaches are pattern recognition, reconstruction, machine learning, simulation, molecular modeling, etc (Ibrokhim 2016, Mount 2001). FTIR has showed significant use in pattern finding, biomarker identification, imaging, and diagnosis in extracting biological information. Figure 4.9 shows the possible contributions of FTIR in bioinformatics.

Computational molecular biology is also a most popular approach which is nevertheless similar to bioinformatics. In the several biomedical applications it is

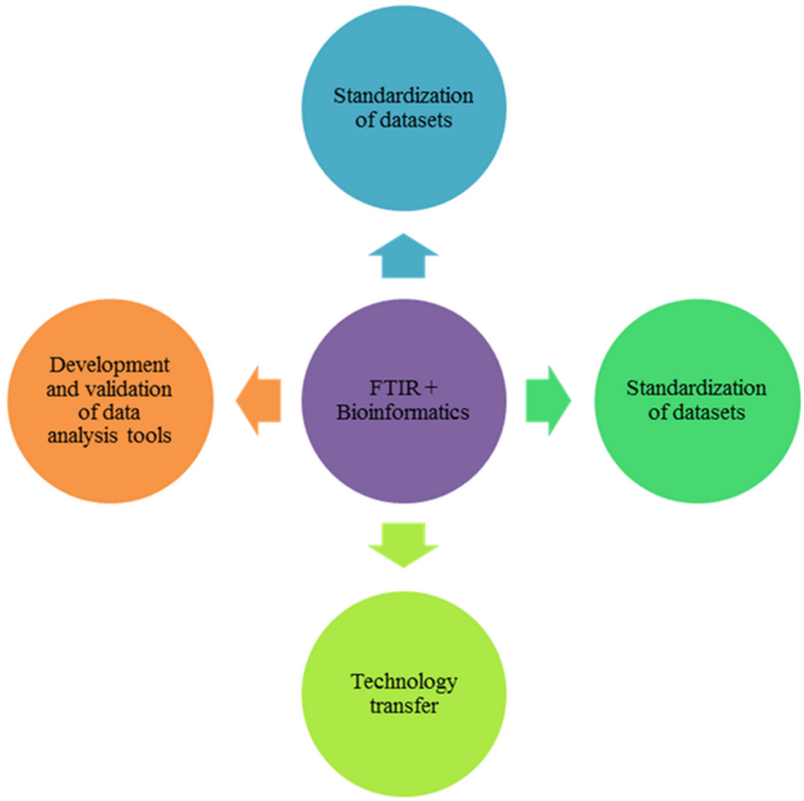

Figure 4.9. Combined approach highlighting the role of FTIR in bioinformatics.

very tedious task to store the data and identify the appropriate molecule within a stipulated time, FTIR has helped to overcome such restrictions.

4.4.5 Microbial cell identification and differentiation

The biomedical samples do not show inherent characteristics such as absorbance, transmission or reflection that is considered a prerequisite for FTIR analysis. Microbial cells need appropriate analysis since they have structural and biological diversity. In order to obtain useful information of structure as well as composition of whole cells FTIR plays an important role. In many biomedical applications the microbial cells occupy a wide range of characteristics thus FTIR is suitable to identify them accordingly. In this analysis scheme pattern recognition is used as it consists of a number of vibrational bands. As FTIR provides adequate qualities of cell structures it is preferred over the available classical microbiological techniques. In this analysis FTIR provides valuable information about total composition, structure of all structures present; these parameters show significant variation in the properties along with time, temperature of growth, and nutrient conditions. FT-Raman spectra have been used to characterize a number of microorganisms to date. The prime advantages of this technique are to provide quick support for estimation of gross composition of microbial cell samples and its behavioral change with respect to time, temperature, as well as growth.

4.4.6 Microbial microcolonies

Figure 4.10 shows the essential steps to be taken in the primary analysis phase of any microbiological sample analysis. These steps may be carried out using different

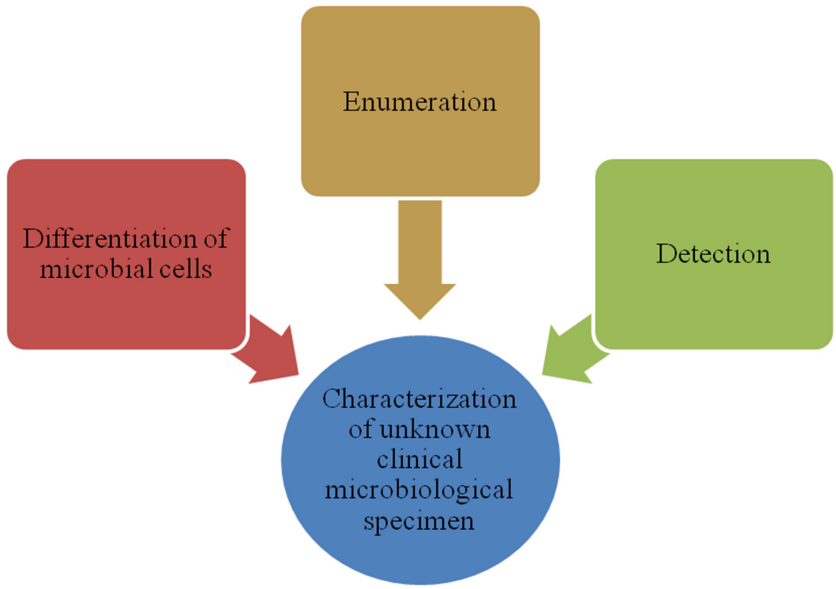

Figure 4.10. Essential steps in the characterization of unknown clinical microbiological specimen.

analysis techniques which may then be followed by the counting of colony-forming units, optical density analysis, etc. In view of these essential steps it would be beneficial to combine many of them to obtain faster and more efficient responses. FTIR is an important analysis tool in this scenario as well, before using FTIR for such systems the following prerequisites may be studied:

1. Is there any limitation in terms of real detection limit for microorganisms?
2. Do you have adequate sampling facilities?
3. Reproducibility of the sample.
4. Whether the sample which is to be analyzed will react in affirmative way in terms of absorbance, reflectance etc.

4.4.7 Identification and diagnosis of disease states

In addition to the identification of microbiological cells, FTIR is also applicable for analysis of human cells, tissues, biofluids, etc. The variation in the spectrum provides a wide range of data in terms of structural change along with compositional change due to any unwanted strain or disease. FTIR is used to analyze the biofluids (such as synovial fluids, to diagnose arthritic disorders in joints), blood and serum (clinical diagnosis), the characterization of disease states in tissues, such as a particular type of cancer (skin, breast or colon cancer); Alzheimer's disease—abnormalities in the central nervous system; analysis of normal leukemic or lymphocytes cells.

4.5 Conclusion

Nanotechnology in the field of biomedical science is a promising area of research having potential to improve the health of patients suffering from serious diseases. This chapter primarily highlights the applications of sophisticated characterization and diagnostic techniques like Fourier transformed infrared (FTIR) spectroscopy and microscopy in the diversified field of nanobiotechnology. Detailed applications in the field of pharmaceuticals like drug release and delivery along with cancer treatment and diagnosis, Nanoscale imaging, bioinformatics, microbial cell identification and differentiation, microbial microcolonies, identification and diagnosis of disease states are discussed. FTIR analysis signifies the presence of chemical bonds and functional groups in biological tissues. Moreover on the basis of absorption spectra obtained as a result of FTIR, the grade of cancer can be determined. FTIR imaging is proven to be fruitful since it is helpful in the easy identification of surface morphology. Microscopy enables the direct visualization of the biomolecules. It has potential for studying dynamic systems like drug delivery and release. From the unique absorption spectra obtained as a result of FTIR analysis, spatial distribution of the various bio-chemicals can be studied thoroughly.

FTIR thus can be referred as an advanced characterization technique for histopathological studies owing to its lower requirement with regard to sample preparation, low cost, high speed and less time consuming results, zero damage, high sensitivity, accuracy and specificity towards the discrimination and detection of complex biological tissues, cells and tissue sections.

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