

Review

Enhancing agricultural research within West Africa using sensor-based technologies

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The global population of the world is on an exponential increase, expected to surpass 9 billion by the year 2050. This places a huge demand on food securities and the need to address the challenges facing food security. Sensor-based techniques [e.g., Fourier-transform infrared spectroscopy] have enhanced the understanding and diagnosis of several disease conditions, including cancers and are increasingly being applied to answer research questions in other areas including agriculture. Methods employed are relatively non-destructive, rendering samples reusable to be analyzed by more conventional approaches as well as allow the fingerprinting of biological samples based on the vibrational modes of the molecules within the sample. Spectra are derived consisting of wavenumber-absorbance intensities within a typical biological experiment and a complex dataset is quickly generated. Biological samples ranging from biofluids to cytology to tissue sections derived from human or sentinel organism sources including plants can easily be observed using this technique. Using a reference range of a designated normal state, anything lying outside this is judged as potentially atypical. Discriminating chemical entities can be identified using computational approaches, which allow one to minimize within-category confounding factors. Technologies involving sensor-based approaches provide a sensitive, cost effective technique for biological and agricultural research. Sensor-based techniques allow the characterization of biological material based on its biochemical-cell fingerprint and could enhance the study of plant species in agricultural research.

Key words: Biochemical-cell fingerprint, biospectroscopy, computational analysis, Fourier-transform infrared, infrared spectra, fluted pumpkin, *Telfairia occidentalis*.

INTRODUCTION

Technologies incorporating sensor-based techniques (SBTs) have been around for many years. They have more recently gained attention due to their applications to understand occurrences in biological and environmental science such as cancer aetiology, biomonitoring and more recently, Butler et al. (2017) document a possible method of observing specific nutrient deficiencies in plant

using SBT. The sensitivity and resolution of most SBTs as well as the rapid generation of datasets representative of samples including the least variance detectable between samples make SBTs quite desirable.

Sensor-based techniques [e.g., Fourier-transform infrared (FTIR) spectroscopy, Raman spectroscopy] have been applied to understand several biological and

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environmental phenomena (Llabjani et al., 2011, 2014, 2012; Obinaju et al., 2014; Patel et al., 2011; Patel and Martin, 2010; Walsh et al., 2008, 2007b) and have significantly enhanced the study of molecular (bio-) chemistry of organisms including plants (Ivanova and Singh, 2003). They have been shown to differentiate between normal and diseased tissue types (Gajjar et al., 2013) as well as between nucleus and cytoplasm of cells (Holton et al., 2011). The possibility of studying a wide variety of sample types (Baker et al., 2014) using SBTs, is a significant advantage.

Environmental change is greatly modified by pollution and contamination, increased as a result of human and industrial activities (Callender and Rice, 1999). Increased contaminant concentrations have been shown in many urban and industrialized environments (Li et al., 2001; Zhang et al., 2005). Satterthwaite (1993) documents Waller (1991) as having identified urban air contaminants to include smoke/suspended particulates, sulphur dioxide, sulphuric acid, polycyclic aromatic hydrocarbons (PAHs) nitric oxide, carbon monoxide and some heavy metals. According to Obinaju and Martin (2013), a variety of these listed contaminants are found to be present in soil (Morillo et al., 2007) and water with increased contaminant concentration in most industrial areas (Li et al., 2001; Zhang et al., 2005). There is a vast amount of research on the effects of human exposure to environmental contaminants (Afroz et al., 2003; Brunekreef and Holgate, 2002; Dockery and Pope, 1994; Lin et al., 2007) and an equal amount of research on the effects of environmental contamination on agriculture, e.g., plant disease and plant growth (Das et al., 1997; Heagle et al., 1973). The effects of environmental contamination and climate change are huge threats to agriculture and crop production e.g. nutrient deficiencies and low yield. This in turn impacts hugely on food security.

Understanding change in biological molecules

Disease processes are most commonly identified using biochemical pathways within organisms and the evaluation of the unique chemical “fingerprints” (metabolite formation or gene/protein alterations) that specific cellular processes leave behind (Kenny et al., 2005; Mollerup et al., 1999; Welthagen et al., 2005). These fingerprints are considered cellular biomarkers in biological cells and tissue samples, and may indicate exposure to factors capable of initiating or altering cellular processes (Obinaju, 2012; Obinaju and Martin, 2013). Analytical techniques such as chromatography, microscopy or spectroscopy/spectrometry are able to detect these biomarkers within cells or tissue samples (Bi et al., 2007; Zhang et al., 2004), using them as indications of biochemical toxicity, initiation and progression of disease conditions or the presence of

certain chemicals within given samples.

SBTs, particularly infrared (IR) spectroscopy, generate a chemical signature based on the structure and function of different biological systems (Martin et al., 2010). This technique may be applied to study conformation in biomolecules because various chemical bonds absorb light at different wavelengths within the mid-IR region, generating a vibrational spectrum consisting of wavenumber-absorbance intensities, referred to as a “biochemical-cell fingerprint” (Walsh et al., 2007a). These wavenumber-absorbance intensities (peaks) are representative of the biochemical composition of the sample interrogated, where peak areas can indicate the concentration of the molecule (Cakmak et al., 2006; Obinaju et al., 2014; Severcan et al., 2005) and the position of peak centroids, a measure of structural integrity within the sample (Palaniappan and Pramod, 2011).

Techniques incorporating sensors undergo constant protocol modifications to increase precision, minimize/eliminate bias, achieve certain sensitivity/selectivity levels and expand resolution. These modifications are mostly made possible by the application and use. Thus, this review focuses on the prospects of the application of SBTs particularly IR spectroscopy to enhance agricultural research by understanding the various changes occurring within plant samples as a result of interaction with environmental contaminants, including observing nutrient deficiencies in real-time.

PRINCIPLES AND METHODS

The interrogation of biological specimens using spectrochemical methods of analysis with the aim of acquiring information pertaining to an analyte of interest based on its inherent ability to absorb, reflect, bend, or scatter radiation as a consequence of its chemical bond structure could be defined as biospectroscopy and this method involves focusing the IR beam on a sample (cells or tissue sections), which absorbs the energy detected using methods which measure transmission, reflection or reflectance (Obinaju and Martin, 2013).

The analytical value of spectroscopy including IR spectroscopy is based on spectral bands occurring at more or less localized positions in the spectrum, which can be correlated to the presence/absence of characteristic structural features of the sample under study (Stuart, 2000). Plant cells or tissue samples could be interrogated using SBTs combined with suitable computational data processing approaches which allow the detection and measurement of biomarkers in the case of disease or a general response to environmental change (Obinaju et al., 2014). FTIR spectroscopy has been effectively been applied to study senescence in plant leaves (Ivanova and Singh, 2003).

It is generally understood that constituents of biological

cells absorb in the mid-IR region ($\lambda = 2\text{-}20\ \mu\text{m}$) based on the chemical bonds present, with specific absorption regions and spectral bands attributed to lipids ($\approx 1,750\ \text{cm}^{-1}$), carbohydrate ($\approx 1,155\ \text{cm}^{-1}$), secondary structure of proteins (Amide I, $\approx 1,650\ \text{cm}^{-1}$; Amide II, $\approx 1,550\ \text{cm}^{-1}$), and DNA/RNA ($\approx 1,225\ \text{cm}^{-1}$; $\approx 1,080\ \text{cm}^{-1}$) (Kelly et al., 2011). Using alterations in the spectral “biochemical-cell fingerprint” and peak assignments (Movasaghi et al., 2008) which typify the architectural structure of the cell or biomolecule under probe, the analyst can best understand mechanisms by which effects observed are possibly induced.

SAMPLE PREPARATION AND DATA HANDLING APPROACHES

IR spectroscopy, particularly attenuated total reflection FTIR (ATR-FTIR) spectroscopy techniques are applicable to a wide variety of sample types (Baker et al., 2014). However, each sample type requires adequate preparation prior to interrogation. For most spectroscopy, tissue cultures do not necessarily require elaborate preparation prior to interrogation aside from critical steps such as alcohol fixation and deposition on appropriate support matrix depending on technique (Obinaju and Martin, 2013). It is also important that sufficient amounts of material be deposited onto the support matrix to allow an absorbance reading of sufficient intensity (Martin et al., 2010). Generally, biospectroscopy techniques require the processing of the biological specimen with a view towards the capabilities of the technology to be employed (that is, a $10\text{-}\mu\text{m}$ -thick tissue section floated onto an IR-transparent substrate for transmission measurements) (Obinaju and Martin, 2013).

With excised tissue samples, considering the time lapse between excision and interrogation, it is critical to preserve tissue samples either by freezing in optimal cutting temperature (OCT) compound medium (Shim and Wilson, 1996) or embed tissues using paraffin wax in order to retain native biochemical states. The quality of spectral data obtained is dependent on sample handling and preparation techniques used prior to interrogation (Martin et al., 2010; Schwartz et al., 2003).

The interrogation of cells or tissue samples generates complex spectral data sets and necessitates the application of suitable data handling tools in order to extract important discriminating information (Obinaju and Martin, 2013) and several approaches exist for processing spectral data including peak picking (Garrett et al., 1991), regional integration of spectra (Chen et al., 2003), principal component analysis (PCA) (Jolliffe, 2005), linear discriminant analysis (LDA) (Martin, 2007) and machine learning algorithms, which allow for classification according to exposure and effect (Llabjani et al., 2012; Trevisan et al., 2012).

Interpretation of IR spectral datasets is based on a

multivariate approach [PCA with or without LDA] (Martin et al., 2010) which allows the identification of wavenumber-related biomarkers of effect by reducing the initial number of variables present in each IR spectrum to a small number of factors. This approach has been extensively used in spectra data processing (Obinaju et al., 2014; Ukpebor et al., 2011; Walsh et al., 2008, 2007b).

TECHNIQUES AND APPLICATIONS OF SBT FOR AGRICULTURAL RESEARCH

These various SBTs, including FTIR spectroscopy, monitor the vibrational modes of functional groups within biomolecules and enable a correlation between chemical information and histological structures where shifts in peak positions, changes in bandwidth intensities and band area values of the IR bands are used to obtain valuable structural and functional information about the system of interest (Stuart, 2000). The sensitivity of IR spectroscopy has been greatly enhanced over time, enabling its application to answer various biological questions (Ahmad et al., 2008; Barber et al., 2006; Llabjani et al., 2012, 2010; Obinaju et al., 2014; Ukpebor et al., 2011).

Continuous environment change is most likely to impact agriculture as well as food security (Obinaju, 2010). The components of environmental change, that is, environmental contamination by chemical compounds, possess the potential to alter structural components as well as biochemical processes of plant and animal cells (Lewtas et al., 1993). These alterations particularly to plant cells could mean reduced growth/yield or the onset of disease in the affected plant species. Several biological techniques rely on the subjectivity of pathologists and simple statistical tests to determine or predict these effects and complex procedures/staining to enable section architectural visualization is adjudged time consuming, subjective and ultimately renders samples non-transferable to other techniques (Obinaju et al., 2014). However, this can be overcome using SBTs (Walsh et al., 2008). A major advantage of using SBT is that analyses can be conducted in the absence of any need for complex labelling or enzymatic requirements, as often needed by more conventional approaches (Obinaju and Martin, 2013).

Hypothetically, using the fluted pumpkin (*Telfairia occidentalis*) species as a potential case study with sample preparation and data acquisition processes as illustrated in Figure 1 and in Obinaju and Martin (2013), an example of the potential application of biospectroscopy in agricultural research is explained. Determining crop nutrient status particularly by foliar and soil analyses, relies currently on analytical techniques such as flame photometry and flame atomic absorption spectroscopy. These methods however efficient and sensitive, require

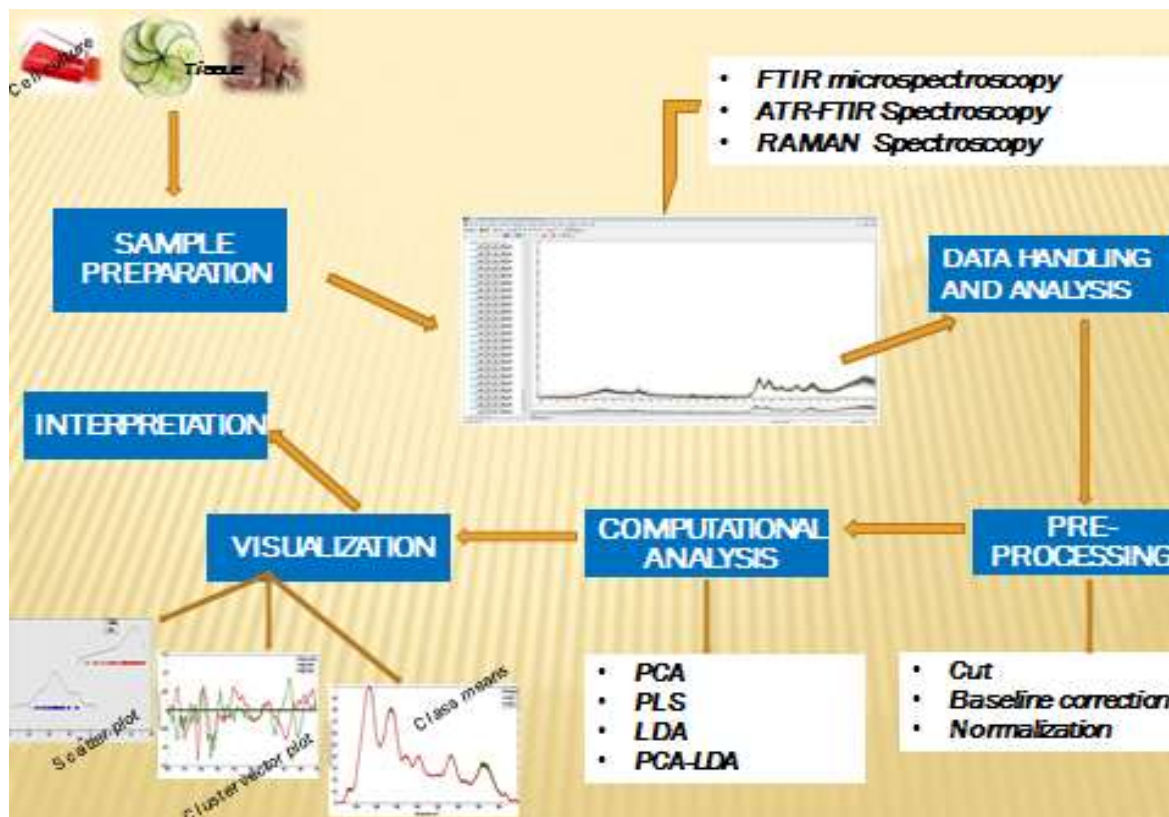


Figure 1. Schematic diagram showing the various processes involved in biospectroscopy analysis from sample preparation to data visualization and interpretation. Samples can either be (1) snap-frozen in liquid nitrogen or using optimal cutting temperature (OCT) compound medium; (2) embedded using paraffin wax; or (3) fixed in a mixture of alcohol and distilled water. Data interpretation can be done based on identified peaks and wavenumbers in plots/graphs.

a nutrient extraction step usually involving acid digestion which can often be time-limiting and may remove any information regarding spatial origin and distribution (Butler et al., 2017).

The increased awareness of the health protecting and promoting properties of consuming green vegetables has increased over the years. With increasing environmental change including environmental contamination, there is growing concern as to the possibility of exposure to contaminants *via* dietary consumption and the effects of soil contamination on the growth parameters of dietary crops especially fruits and vegetables.

T. occidentalis is a tropical vine and a member of the Curcubitaceae family grown in West Africa as a leaf vegetable, for its edible seeds and more importantly its rich nutritional and perhaps therapeutic value (Egba et al., 2014). There are genetic variants of the fluted pumpkin species particularly within Nigerian states (Fayeun et al., 2012) with certain levels of variation in their individual vegetative characteristics, e.g., vine length, foliage width and number of branches per plant (Aremu and Adewale, 2012; Cyril et al., 2014; Fayeun et al., 2012). The distinction between each variant could

easily and more rapidly be detected based on the mean spectra and cluster vector plots derived using IR spectroscopy coupled with multivariate computational analysis. The various intensities and peak areas could provide a measure of the concentration of each desirable molecule/nutrient within the parts of the interrogated plant. Furthermore, in cases where the interrogated plant is exposed to an element of environmental change, that is, chemical contaminant or UV radiation, a comparison of the spectra of exposed plant, to the spectra of a control or unexposed plant could provide an indication of the possible areas of the plant targeted by the said element, e.g., decreased absorption bands for cellulose (1030 cm^{-1}) indicating changes to leaf pigmentation (Obinaju et al., 2014), changes to the polysaccharide (Pectin 1055 cm^{-1}) region as an indication of calcium (Ca) deficiency in plant tissue (Butler et al., 2017).

Conclusions

Food security is a major threat and requires novel and innovative, not to mention rapid yet cost effective

methods of dealing with the challenge, especially through agricultural research. SBTs offer improved understanding and characterization of molecular alterations in both plant and animal species especially those occurring as a result of exposure to environmental contaminants (Ahmad et al., 2008; Obinaju et al., 2014; Ukpebor et al., 2011) because methods employed are specific and sensitive to changes within the biochemical constituents of cells and tissues at certain wavelengths (Baker et al., 2014) and while spectral analysis may require basic knowledge of statistical software packages such as MATLAB®, the various techniques provide a suitable avenue to sort for desirable traits, monitor plant nutrient stress in real-time before the manifestation of symptoms indicating deficiency and track the activity of environmental contaminants and pathogens in agricultural species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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