Section1: Introduction to Hyperspectral imaging

The information provided here is a short overview of NIR spectroscopy and hyperspectral imaging for a novice to this field. Please consult literature in the Reference section for more informative explanation and further reading.

What is NIR hyperspectral imaging?

Hyperspectral imaging is a combination of *imaging* and *spectroscopy*.

What does spectroscopy entail?

Spectroscopy studies the interaction between matter and electromagnetic radiation and records the amount of radiation absorbed or emitted as a spectrum over a specific wavelength range.

What is electromagnetic radiation?

In physics, electromagnetic radiation is defined as the waves of the electromagnetic field. The waves propagates through space and time and carries electromagnetic energy. Waves are characterized by their frequency of oscillation (number of times that a wave passes by a certain point per unit time) or by the wavelength (units expressed as nm or cm^{-1} for wavenumber). Waves of different frequencies make up the electromagnetic spectrum.

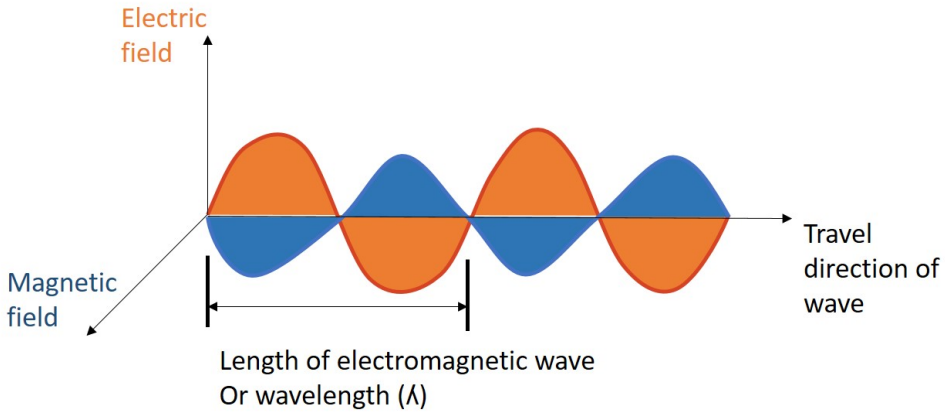

Figure 1: An electromagnetic wave can be defined by the length from one point to the identical point on the next wave and is indicated as wavelength (nm) or wavenumber (cm^{-1}).

What is the electromagnetic spectrum?

The electromagnetic spectrum covers the range of frequencies from the wavelengths with shorter frequencies linked to nuclear radiation (Gamma rays), which carries high energy,

followed by X-rays, ultraviolet (UV)-, visible-, infrared (IR) to the radio waves, which carry low energy (Fig 2).

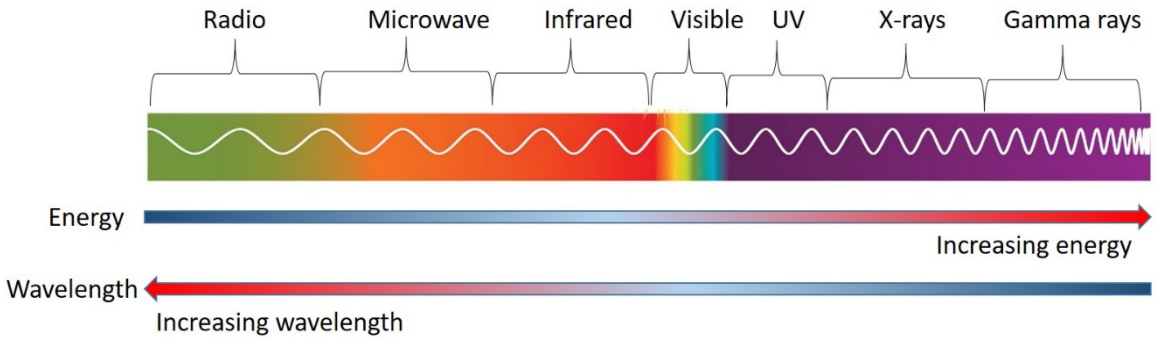

Figure 2: Schematic for the electromagnetic spectrum. Gamma rays have short wavelengths and this increase in the direction of the Radio waves. As wavelengths decrease the energy levels increase.

Where is the visible and NIR region?

Visible light covers the spectral range 400 – 780 nm and human and various other organisms can sense radiation belonging to this spectrum. Near infrared (NIR) and Short wave infrared (SWIR) light covers the range 780 – 2500 nm (Fig 3).

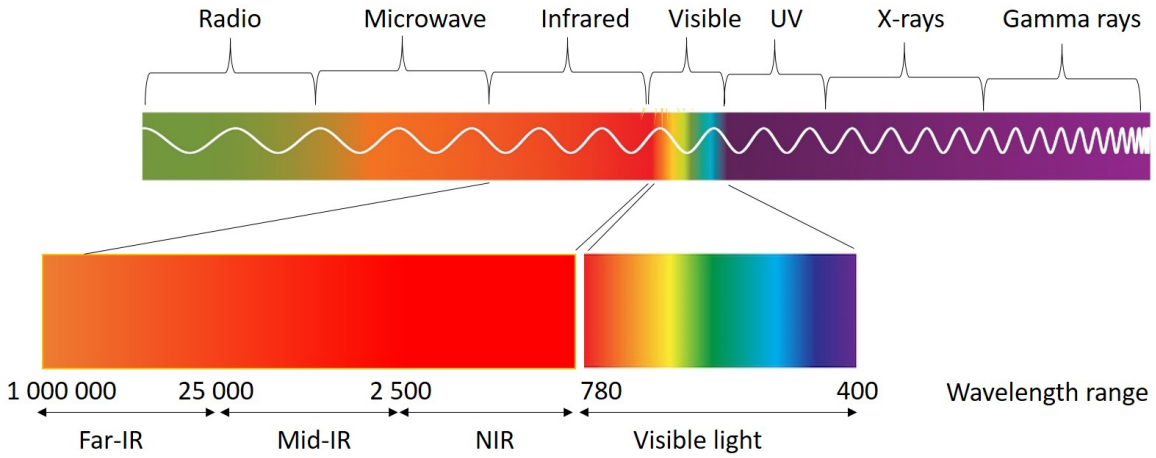

Figure 3: Visible and near infrared light spans the region 400 – 2500 nm. Visible light spans 400 – 780 nm and is followed by the infrared region. The NIR light spans 780 – 2500 nm, followed by the Mid-IR and the Far-IR region.

What is the response when radiation interacts with molecules?

Wavebands of different frequency carry different levels of energy and when radiation interacts with matter, the energy can be absorbed and result in different molecular responses (Fig 4). The wavebands with shorter frequency (UV, X-rays and Gamma Rays) result in ionization (loss

of electrons) when they interact with matter. Different types of instruments can measure the various types of molecular responses.

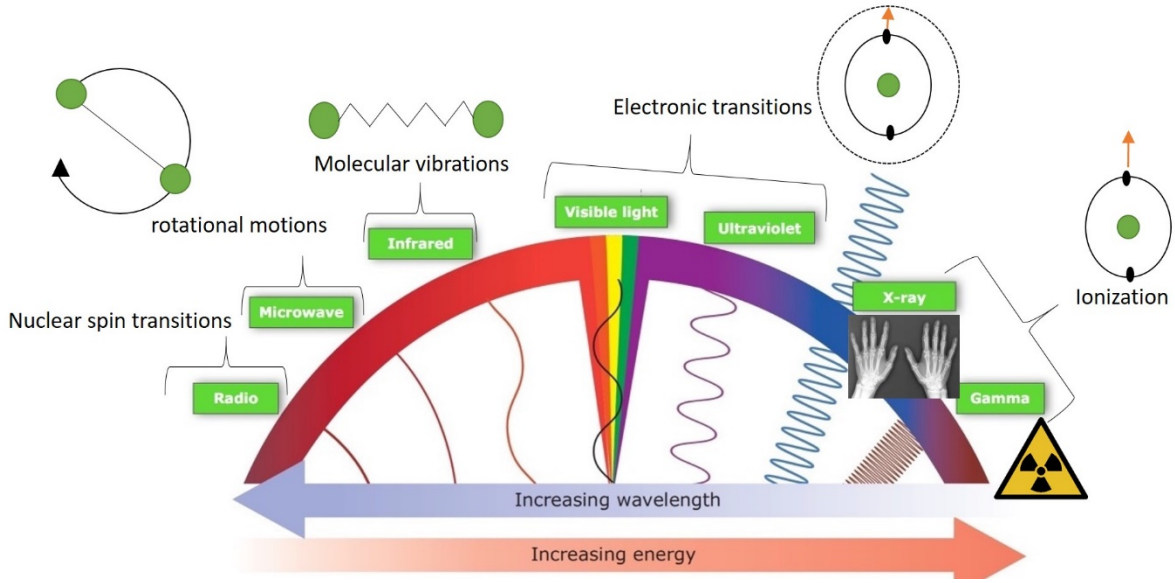

Figure 4: Different types of molecular responses in reaction to the absorption of energy from radiation.

When molecules absorb the energy in IR radiation it results in molecular vibrations. Molecules display different types of vibrations for example stretching, bending, rocking, wagging and twisting (Fig 5).

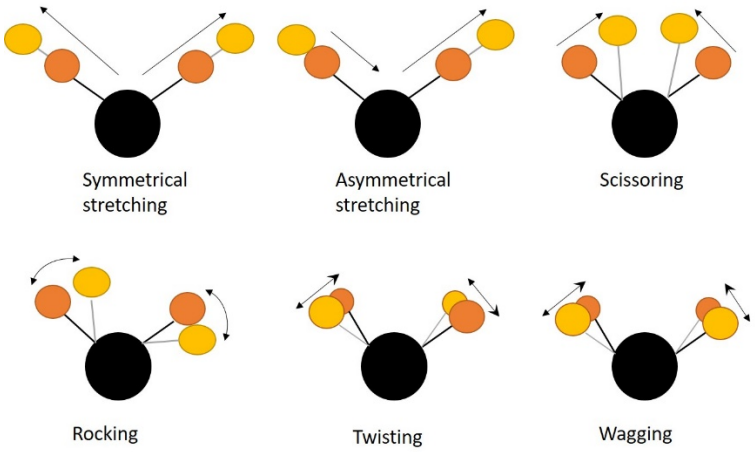

Figure 5: Diagram illustrating different vibrational modes of molecules, which include stretching, scissoring, rocking, twisting and wagging.

What type of molecules respond to NIR radiation and what does this response involve?

Molecules with covalent bonds (sharing electrons) such as N-H, S-H, O-H, C-H, C-O, C=C are continuously vibrating. When they absorb the energy in radiation at a specific wavelength, there is a change in the electric dipole moment (change in the positive-negative charge separation) of the molecule and the molecules transition to different vibrational levels (Fig 6).

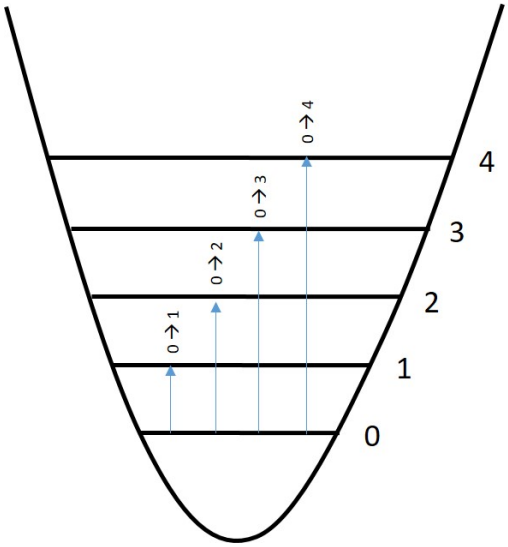

Figure 6: Molecules have different vibrational levels. The transition from ground state (v0) to the first vibrational level (v1) is known as the fundamental transition. When a molecule's vibrational level transitions to the higher vibrational levels, the response is known as an overtone.

Transitioning from the ground (v0) to the first vibrational state (v1) is termed the fundamental transition. Transitioning to the 2nd, 3rd or 4th excited state is known as overtones. Therefore, for a fundamental vibration, there is a series of overtones with decreasing intensity as the transition (overtone) increases (Workman and Weyer, 2012). **NIR spectroscopy measures these overtones**, which are generally very weak. The intensity of the transition from v0 to v2 is stronger than for the transition from v0 to v3. As a result, the response band for a molecule transitioning from v0 to v2, would appear at twice the energy (wavenumber) of the fundamental band (Fig 7). When more than one chemical bond in a molecule is excited simultaneously, the response appears as combination bands on the spectrum.

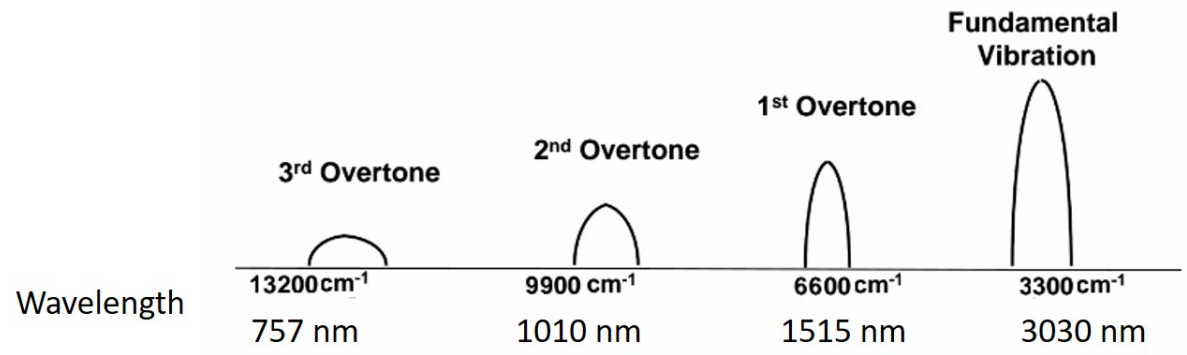

Figure 7: Fundamental and overtone transitions. A compound with the fundamental transition at 3300 cm^{-1} has overtones at multiples of 3300 cm^{-1}. For example, the 1st overtone occurs at 6600 cm^{-1} (2x 3300 cm^{-1}), the 2nd overtone occurs at 9900 cm-1 (3x 3300 cm^{-1}). (Figure adapted from https://alliedscientificpro.com/blog/welcome-to-our-blog-1/post/comparing-the-nir-spectroscopic-method-withftir-ft-nir-37).

What type of samples can be studied using NIR spectroscopy?

NIR spectroscopy can therefore be used to study organic samples, which contain the chemical bonds described above (C-H, O-H, N-H) because these functional groups absorb the energy from radiation in this region. **Therefore, instead of individual compounds, major functional groups were assigned to specific near-infrared regions** (Fig 8). From the diagram it can be seen that in a given wavelength range, a chemical bond will absorb the energy at a specific frequency when the energy matches the energy required to induce a vibrational response (Elmasry and Sun, 2010).

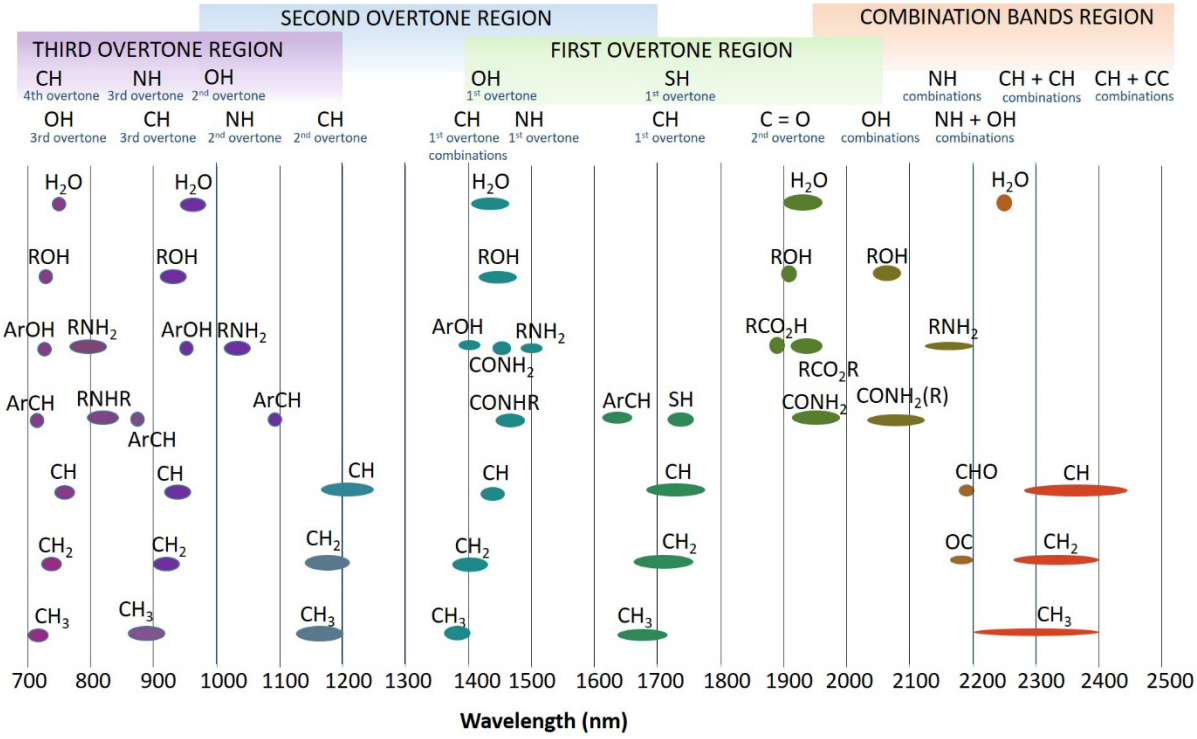

Figure 8: Diagram indicating the overtone and combination bands for different chemical bonds. (Figure adapted from Genot et al., 2014)

When applying NIR radiation to samples what response is measured by spectroscopy?

With this technique, a continuous-wave light beam emitting light in the spectral range 400 – 2500 nm is incident on the sample. When the light falls on an object it can be **reflected** without interacting with the sample (mirror like reflectance), this is known as specular reflectance (Fig 9). The incident light can also be scattered when interacting with the sample. **Scattering is influenced by the density, tissue composition, particle size and cellular structures (physical properties) in the sample.** The energy in the light can also be absorbed and this is more closely associated with the chemical composition of the sample. The light, which is not absorbed, can be reflected (diffuse). The **diffuse reflected** light contains information about the compounds near the surface of the object which absorb the light (Elmasry and Sun, 2010). The light can travel through the sample and this is measured as the **transmitted light**.

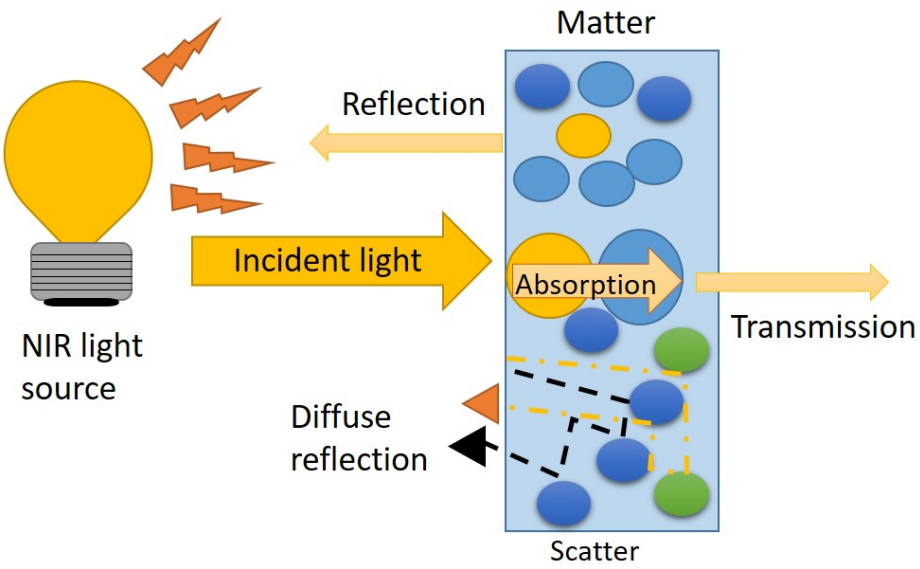

Figure 9: Diagram illustrating the various ways that light can interact with organic samples.

During spectroscopy, the light which is re-emitted from the sample is measured as reflectance, interactance or transmittance, and **the acquired spectra are then used to investigate the composition and quality of the samples**. The challenge in studying organic samples is that they contain mixtures of different compounds. The energy absorbed by the organic sample matrix is dependent on the chemical composition (type and concentration of molecules) of the sample (Workman and Weyer, 2012). Since all chemical bonds can absorb NIR radiation, the **resulting spectrum can be complex to interpret and contains both the chemical and physical information for the sample** (Elmasry and Sun, 2010).

How can NIR spectroscopy be used to investigate samples?

NIR is a **secondary analysis** method and data analysis requires multivariate statistical tools and calibration models.

Spectral data obtained by NIR spectroscopy can be used for:

1) Exploratory analysis - to determine similarities or differences between samples based on their chemical or physical make-up.

2) Models/calibrations can also be constructed to predict specific properties of samples

(A) **Categorical** models can be used to classify samples as belonging to specific groups based on their chemical and physical properties

(B) **Quantitative** models can be used to measure specific compounds in samples, but requires the availability of good chemical reference methods.

What does 'building calibration or models' involve?

To briefly explain, during NIR spectroscopy, the NIR light is directed onto the sample and a detector measures the amount of light reflected or transmitted as a spectrum over a specific wavelength range. The spectrum contains information both regarding physical properties and about the type and concentration of the compounds. By imaging a large number of samples with specific properties of interest (bruised apples vs undamaged apples) or fruit with different sugar concentrations, the spectra are analysed to generate a calibration model.

For **categorical models classes are assigned to specific spectra** (bruised or undamaged) collected from samples with these properties. A model can be constructed based on these spectra, which can identify these properties (classes) in the future even when they are not visible to the eye. Examples of these applications include constructing models to class pharmaceutical tablets, which look similar but have different chemical contents (see application note 1 on the website) or to identify bruising in fruit.

Quantitative models are constructed by imaging samples and applying chemical reference methods to quantify the property (protein, fat, sugar, cellulose, water etc) of interested. The reference values are assigned to the spectra of the samples and used to construct a calibration model, which can be used to quantify the property of interest in the future without having to repeat chemical reference methods. To construct these types of models, one has to obtain samples with varying levels of the property to be measured. Additionally, these samples should be representative of all samples to be analysed in the future. For example to construct a calibration to measure fruit sugar contents, fruit with different maturity levels, originating from different locations, seasons etc should be included.

How does quantitative analysis work?

Molecules absorb the energy and the response is measured as a spectrum. The absorptivity of the matrix and the number of molecules present in the sample surface will influence the spectral response. The Lambert – Bouguer-Beer law (Beer's law*)* can be used for the quantification of compounds. The law states that the absorbance of a compound as measured by a spectrophotometer is equivalent to the absorptivity of the compound (ε), the concentration of the compound (C) and the path length (L) of the sample container (F1).

A = εCL **or** **C =** $\frac{A}{\varepsilon L}$ **(F1)**

In NIR spectroscopy of 'solid' samples it is generally, the amount of light reflected (R) that is measured. The absorbance can be determined by using the formula (F2) to convert reflectance to absorbance.

$$\mathbf{A = log10\ (\frac{1}{R})} \qquad \mathbf{(F2)}$$

Why is it necessary to apply multivariate data analysis to construct models?

As mentioned earlier, the challenge of NIR spectroscopy is that the spectra have broad bands due to overlapping of combination and overtone bands. As a result, the bands for the individual compounds are not well separated (Workman and Weyer, 2012). Additionally, several compounds absorb NIR energy at all wavelengths, which makes it difficult to determine the baseline and this complicates quantitative methods, which assess the peak height or peak area to determine concentration. This necessitates the application of multivariate data analysis tools to interpret the bands and compensate for the background interference (Workman and Weyer, 2012).

What is the difference between conventional and NIR hyperspectral imaging?

Conventional NIR instruments

Handheld NIR instruments operate in a staring mode, also called single-point spectroscopy. The light re-emitted from a specific area or point on the sample is being measured. To obtain a spectrum, which is representative of the entire sample, several measurements in different locations have to be collected and the average spectrum determined. There are different types of hand held or desktop instruments available on the market with various applications. These instruments also offer different options for sample presentation.

Hyperspectral imaging cameras

In contrast, during hyperspectral imaging the sample is placed on a translation stage, which moves the sample past the camera. The camera collects the spectra in a push broom/or line scanning mode (sample is scanned line by line) and the data is stored in the Band Interleaved by Line (BIL) format. This spectral data is a three dimensional (3-D) data set as it contains the pixel location in x and y (2-D) for each pixel and the spectral information for each pixel. Collectively, this 3-D dataset is termed a hypercube (Geladi, Grahn and Manley, 2010) (Fig 10).

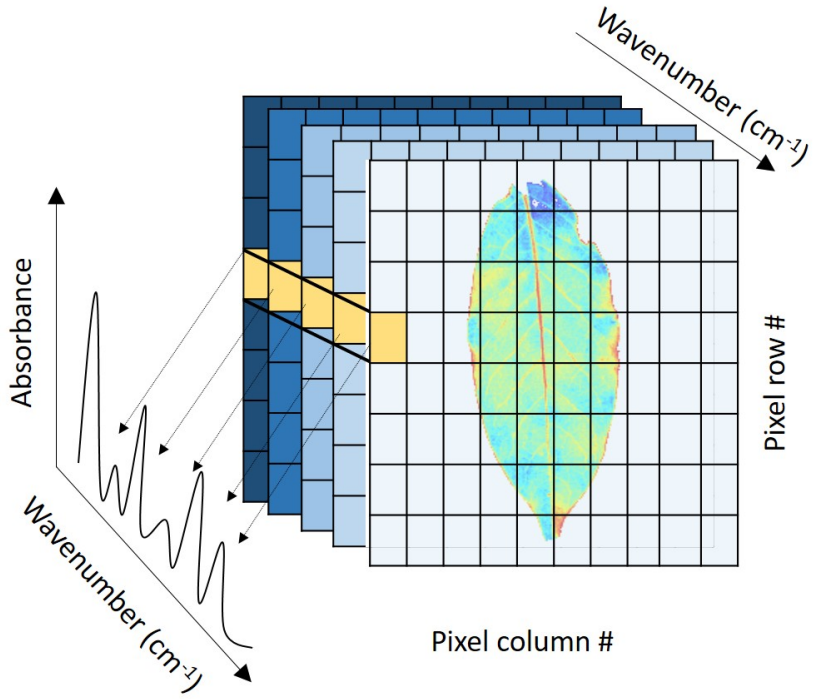

Figure 10: Schematic representation of a hyperspectral image (hypercube) for a leaf. The spectra to the left corresponds to the orange pixel in the diagram and may contain information about the chemical composition of the pixel.

Since the spectral information is collected for each pixel, this technology can be used to generate chemical maps or images, which facilitate visualization of the **spatial distribution of chemical compounds associated to bands** of interest (Fig 11). This is especially useful for analysis of samples of heterogeneous nature.

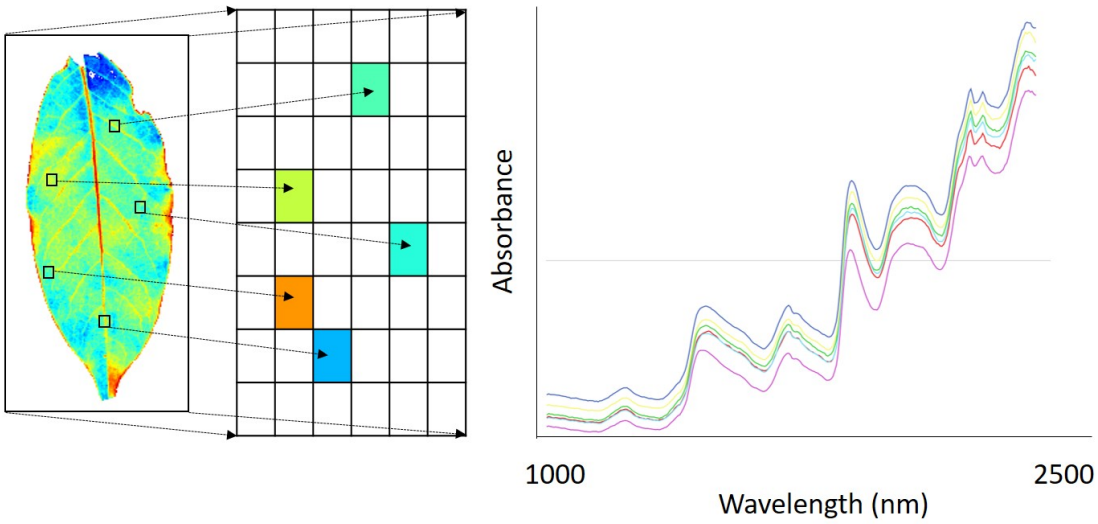

Figure 11: An example of a chemical image representing the intensity at a single wavenumber in each of the pixels within the image. The chemical image is shown as a cross section of the data cube at wavelength (λ = 1928). To the right, the representative spectra for some of the pixels in the cube were plotted. The band at 1928 nm is the H_2O band and the chemical image can be seen as a representation of the water distribution in the sample.

Hyperspectral imaging cameras in the Vibrational Spectroscopy unit

The unit has VNIR and SWIR hyperspectral imaging cameras (Fig 12). The cameras records the absorption spectrum in the spectral range 400 – 1000 nm (VNIR) or 950 – 2500 nm (SWIR) for each pixel. The spectral resolution for the VNIR is 3.26 nm (182 bands) and for the SWIR it is 5.45 nm (288 bands). There are different lenses available to adjust the field of view (FOV) of the cameras to accommodate samples of different sizes. These lenses not only determine the FOV, but also determines the pixel size. The spatial resolution for the VNIR is 1800 pixels per line and for the SWIR it is 384 pixels per line.

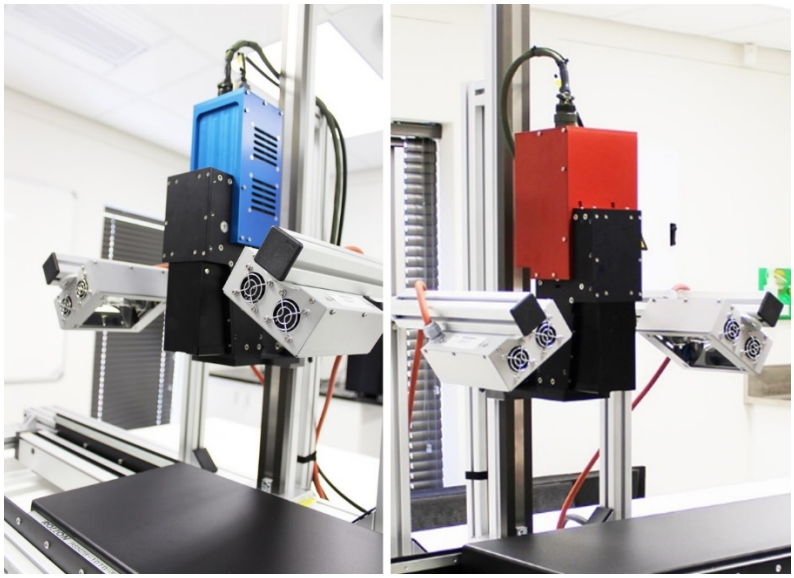

Figure 12: Hyperspectral imaging cameras in the vibrational spectroscopy unit include (L) the visible near infrared (VNIR) and (R) short wave infrared (SWIR) camera, which operate in the 400 – 1000 nm and 950 – 2500 nm spectral range respectively.

What are the advantages of using NIR spectroscopy?

1) The technique is non-destructive and easy.
2) It is fast and requires little or no sample preparation.
3) It does not generate any waste and does not use harsh chemicals. Therefore, NIR spectroscopy is environmentally friendly.
4) The method is versatile and can measure several properties and constituents in a sample simultaneously.
5) Hyperspectral imaging facilitates visualization of spatial distribution of chemicals within the sample.
6) It can save costs on expensive reference methods.
7) Calibrations can be reliable and precise.
8) It enables large amounts of samples to be analyzed in less time.

What are the limitations of NIR spectroscopy?

- This is not a primary analysis method, it requires calibrations and good reference methods for the quantification of analytes of interest.
- Broad bands are difficult to interpret or assign to specific functional groups.
- Requires separate calibrations for each commodity and analyte of interest.
- Each calibration requires setting up a spectral database, which represents the full range of samples that may be encountered under various conditions.
- Calibration models may be constructed using hundreds or thousands of representative samples to obtain robust prediction models.
- Methods have to be compared continuously to assess the precision and accuracy relative to a reference method.
- NIR is not suitable for trace analysis, the compound which you are interested in has to be a one of the main constituents.
- This method can't be used to determine molecular structures of compounds
- It is not suitable for analysis of very dark samples.
- To distinguish between samples, the objects have to have characteristic absorption features which should be present in a minimum concentration or should 'span' a pixel to allow to be detected (Elmasry and Sun, 2010).

Interested in using NIR spectroscopy to analyze your samples?

Contact the unit manager Janine Colling (jcolling@sun.ac.za) for more information.

USEFUL TERMINOLOGY

Spectral range – describes the wavelength regions covered by the hyperspectral imaging system.

Spectral resolution – the absolute limit of the hyperspectral imaging system to separate two adjacent monochromatic spectral features emitted by a point in the image.

Spatial resolution - indicates what is the size of the smallest object that can be seen on the surface of the sample as an object separate from its surroundings (Elmasry and Sun, 2010). This is determined by the field of view (FOV) which is dependent on the type of lens (1 m, 30 cm or microscope) being used. For details, please contact the unit manager.

References and useful books on Vibrational Spectroscopy and Hyperspectral imaging

Chan ECY, Griffiths PR, Chalmers JM (2010). Applications of Vibrational Spectroscopy in Food Science. John Wiley & Sons Ltd, UK

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Genot V, Bock L, Dardenne P, Colinet G (2014). Use of near-infrared reflectance spectroscopy in soil analysis. A review. Biotechnology, Agronomy, Society and Environment 18: 247 – 261.

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