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Remote sensing and advanced plant phenotyping handbook

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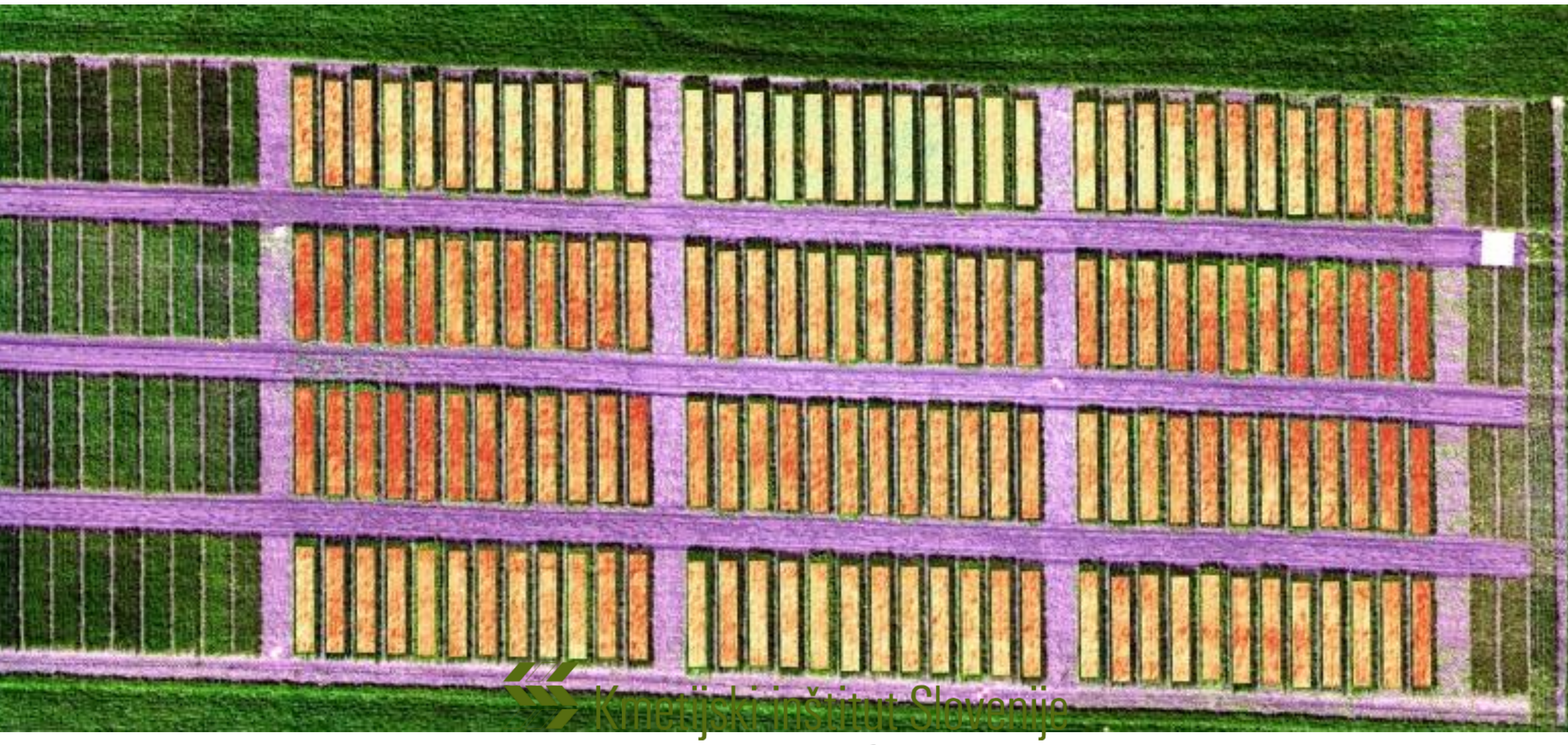

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1.1 What are precision agriculture, remote sensing, and phenotyping?

Precision agriculture is an approach that seeks to optimize resource management and increase crop productivity by leveraging technology, data, and advanced analytics. It involves the precise application of inputs such as water, fertilizers, and pesticides, tailored to the specific needs of individual plants or sections of a field. This precision is achieved through the use of various technologies, including remote sensing, geographic information systems (GIS), global positioning systems (GPS), robotics (including unmanned aerial vehicles), and data analytics. By utilizing these tools, precision agriculture enables farmers to make informed decisions tailored to the specific needs of their crops, and carry out more precise, targeted interventions. Ultimately leading to increased efficiency, reduced costs, and minimized environmental impact, thus increasing sustainability of modern agriculture. Precision agriculture helps conserve resources, reduce environmental impact, and mitigate the risks associated with unpredictable weather patterns and other uncertainties.

At the core of precision agriculture lies remote sensing. This is the science and technology of acquiring information about Earth's surface and atmosphere from a distance, without direct physical contact. It involves the use of sensors, such as cameras and specialized instruments, to detect and measure electromagnetic radiation reflected, emitted, or scattered by the target objects or the Earth's surface. This acquired data can provide valuable insights into various aspects of the environment, including land cover, vegetation health, weather patterns, and natural resources. In the context of agriculture, remote sensing enables us to capture valuable data about crops, soil conditions, and environmental factors without physical contact. It offers a non-invasive, efficient, and cost-effective means of monitoring agricultural systems at various scales, ranging from individual plants to large field areas. Remote sensing has emerged as a powerful tool in precision agriculture and plant phenotyping, revolutionizing our ability to monitor, analyse, and manage agricultural systems with unprecedented precision. Understanding the fundamentals of remote sensing in the context of precision agriculture and plant phenotyping is crucial for driving innovation and addressing the challenges faced by modern agriculture. The integration of remote sensing with other precision agriculture technologies, such as GPS and GIS, further enhances its capabilities. Georeferenced remote sensing data can be combined with field observations, weather data, soil information, and historical records to generate accurate and spatially explicit maps of crop health, nutrient status, yield potential, and pest infestation. These maps enable farmers to make informed decisions regarding variable rate applications of fertilizers, pesticides, and water, tailoring inputs to the specific needs of different areas within a field.

One of the key applications of remote sensing in precision agriculture is plant phenotyping, which involves the measurement and analysis of plant traits, such as growth rate, canopy structure, and physiological responses. Traditional methods of phenotyping are often labour-intensive, time-consuming, and limited in their spatial coverage. Remote sensing provides a solution to these challenges by enabling high-throughput phenotyping, allowing for rapid and comprehensive data collection over large areas.

Plant phenotyping is a critical aspect of crop breeding programs, enabling breeders to identify and select plants with desirable traits such as disease resistance, drought tolerance, and higher yields. Phenotyping involves the measurement and analysis of various plant characteristics, including growth rate, canopy structure, and physiological responses. However, traditional phenotyping methods can be time-consuming, labour-intensive, and limited in their scope. This is where remote sensing technology can revolutionize plant phenotyping by enabling high-throughput and non-invasive data collection over large areas. In the context of plant phenotyping, remote sensing can be used to measure various plant traits, such as leaf area, chlorophyll content, and canopy architecture, by analysing the reflectance or fluorescence of light emitted by plants. This information can then be used to identify and characterize different cultivars in the field.

One of the key advantages of remote sensing for plant phenotyping is its ability to provide rapid and high-throughput data collection over large areas. This can significantly accelerate the process of cultivar identification and characterization, providing valuable information for plant breeding programs. For example, breeders can use remote sensing data to assess the performance of different cultivars under different environmental conditions, allowing them to select the most promising candidates for further testing and development. Another benefit of remote sensing for plant phenotyping is its non-invasive nature. Traditional phenotyping methods often require destructive sampling or manual measurements, which can damage or disrupt plant growth and development. Remote sensing, on the other hand, can capture data without touching the plants, minimizing the impact on their growth and ensuring that the data is representative of their true characteristics. In addition to facilitating cultivar identification and selection, remote sensing can also assist in monitoring plant growth and development over time. This can provide valuable insights into the effects of different environmental conditions on plant traits and inform breeding strategies aimed at improving crop performance and resilience.

1.2 Basics of Electromagnetic Radiation

Remote sensing relies on the **interaction between electromagnetic radiation and objects of interest**. Understanding the basics of electromagnetic radiation and its characteristics is essential for comprehending the principles underlying remote sensing. Electromagnetic radiation is a form of energy that propagates through space in the form of waves. The electromagnetic spectrum spans from long-wavelength, low-frequency radio waves to short-wavelength, high-frequency gamma rays. Within this spectrum, we encounter familiar forms of radiation, such as microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays (Figure 1). Each segment of the spectrum carries distinct properties and interactions with matter. Electromagnetic radiation plays a crucial role in various natural processes, technology, and communication systems, shaping our understanding of the universe and enabling an array of applications in fields like medicine, telecommunications, and astronomy.

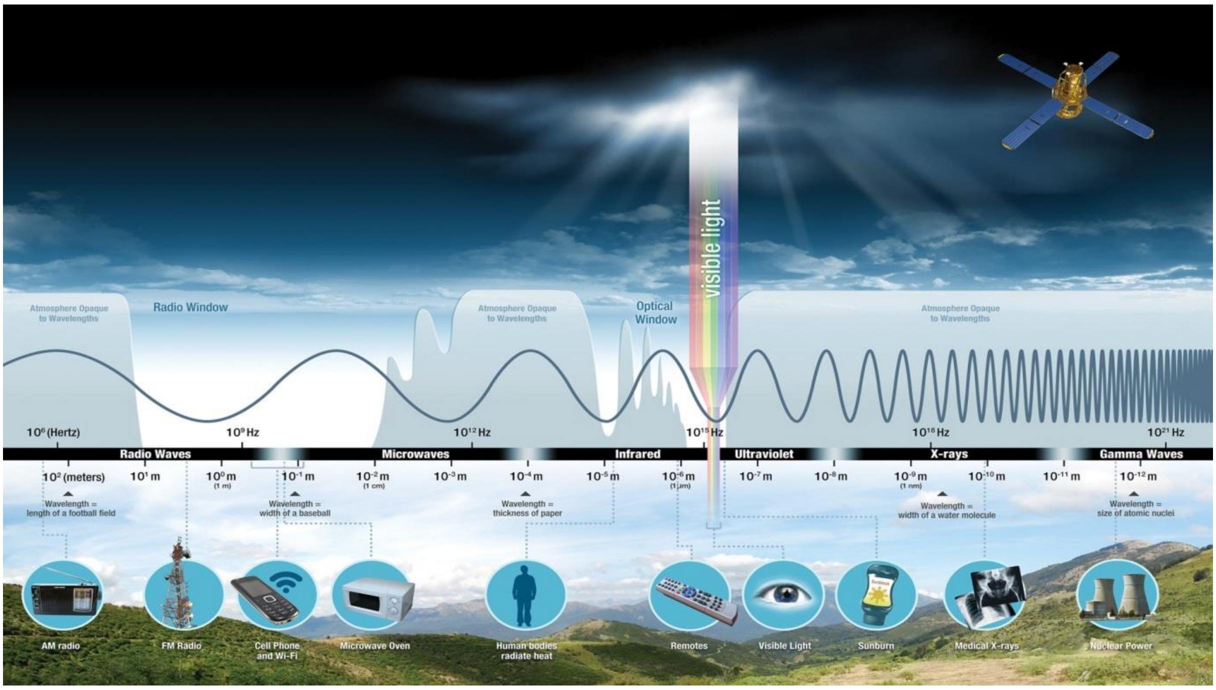
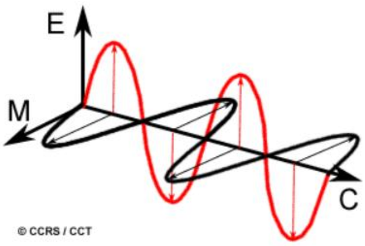
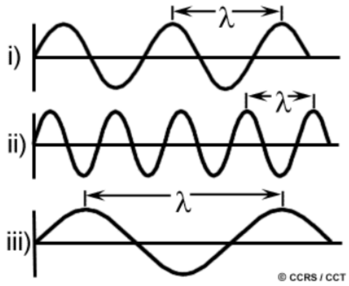


Figure 1: The electromagnetic spectrum. Source: NASA



Electromagnetic radiation, encompassing a wide spectrum of energy waves, follows the principles of wave theory, exhibiting fundamental properties and predictable behaviours. It consists of an electric field (E) perpendicular to the direction of travel and a magnetic field (M) orthogonal to the electric field. Both fields propagate at the speed of light (c).



Understanding remote sensing relies on two essential characteristics of electromagnetic radiation: **wavelength** and **frequency**. Wavelength (λ) represents the distance between consecutive wave crests, measured in meters (m) or related units such as nanometres (nm), micrometres (µm), or centimetres (cm). Frequency denotes the number of wave cycles passing a fixed point per unit of time and is measured in hertz (Hz), with multiples of hertz representing various frequencies.

Most of the electromagnetic radiation from space is absorbed or reflected in the atmosphere and doesn't reach the planet's surface. Only radio waves and light can penetrate the atmosphere in so-called atmospheric windows. This is why we can place radio and optical telescopes on the planet, but for more accurate measurements for astronomy, without atmospheric interactions, satellite-based sensors are required. One such atmospheric window is in the range between 400 and 1000 nm; this spectral range is also called the near to infrared spectrum (VNIR), and contains the visible light spectrum (400 to 700 nm), so called as it can be detected by the human eye. Everything you will ever see unaided is in this narrow spectral range. Moving to longer wavelengths, between 1000 and 2500 nm, we reach the short-wave infrared part of the spectrum. This part of the spectrum is sensitive to atmospheric conditions, as the atmospheric window doesn't reach all the way through the atmosphere equally for all wavelengths, anymore.

1.2.1 Characterizing radiation sources and surfaces: radiance, irradiance, and reflectance

Radiance, irradiance, and reflectance are fundamental concepts in remote sensing and the study of electromagnetic radiation. Sensors store image data as digital numbers, which refers to the numerical value assigned to each pixel in the image. It represents the brightness or intensity level of that pixel. The range and precision of the digital numbers depend on the bit depth, or the number of bits used to represent each pixel. For example, an 8-bit image can have digital numbers ranging from 0 to 255 (typically used in RGB sensors), while a 16-bit image can have values between 0 and 65,535. A wider range means a more precise representation of brightness values. The digital numbers allow for the quantification and storage of image data, enabling image processing, analysis, and display on digital devices. But digital numbers do not have physical meaning; they are simply numerical representations of brightness assigned to pixels. The conversion from digital numbers to physically meaningful values (usually to radiance) requires additional information and calibration specific to the imaging system and the type of data being captured. This process is called radiometric calibration, where we establish a relationship between the digital numbers recorded by a remote sensing instrument and the corresponding physical measurements or properties of the observed objects.

Radiance refers to the amount of electromagnetic energy emitted, transmitted, or reflected by a surface in a specific direction. It represents the radiant power per unit solid angle and unit projected area and is typically measured in watts per steradian per square meter ($W \cdot sr^{-1} \cdot m^{-2}$). Radiance provides information about the intrinsic properties of the surface and the distribution of energy in different wavelengths.

Irradiance, on the other hand, refers to the amount of electromagnetic energy incident on a surface per unit area. It quantifies the total power received by a surface and is typically measured in watts per square meter ($W \cdot m^{-2}$). Irradiance is important for understanding the energy available to interact with a surface and influences various processes such as photosynthesis, heating, and evaporation. Both radiance and irradiance are affected by the source of radiation, scattering, and sensor characteristics.

Reflectance is a dimensionless quantity that describes the ratio of the reflected radiation to the incident radiation (irradiation) on a surface. It represents the efficiency of a surface in reflecting different wavelengths of light and is often expressed as a percentage or a decimal fraction between 0 and 1. Reflectance is a normalized value that eliminates the influence of illumination and sensor characteristics. It is a crucial parameter in remote sensing as it provides insights into the optical properties and composition of objects or surfaces. By analysing the reflectance properties across different wavelengths, we can derive valuable information about the characteristics of plants, such as vegetation health, water content, or nutrient and micronutrient composition (Figure 2).

Together, radiance, irradiance, and reflectance play a vital role in the analysis of remotely sensed data. They enable us to quantify and interpret the interaction of electromagnetic radiation with various surfaces and provide valuable insights into the physical and chemical properties of objects and environments under study.

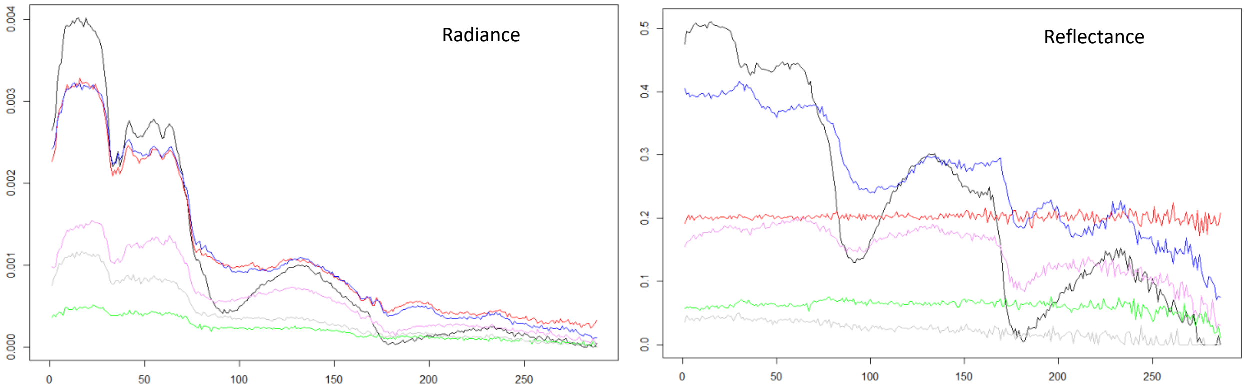


Figure 2: Comparing radiance and reflectance of several objects in the SWIR region. In radiance different objects and materials show similar trends, as they are affected by atmospheric effects, illumination, and sensor characteristics. Additionally, humans have the benefit of millions of years of evolution, so we have learned to distinguish between various materials based only on their radiance (because that is what we see). Computers do not have that benefit; using only radiance for object or material identification can lead to lower classification accuracy. Reflectance reveals the true spectral response of objects, allowing for accurate analysis of physical properties and characteristics. For example, only the black and blue lines are the spectral responses of plants. In radiance units we see that the blue and red lines are almost identical, but reflectance reveals that the red line is not a plant, but in fact soil. Similarly, grey (water) shows a similar trend as plants in radiance, but has a reflectance of close to zero. This is typical of water in the SWIR region, as most of the incident light is absorbed. Source: Archive KIS.

1.2.2 Interactions between electromagnetic radiation and objects

When electromagnetic radiation interacts with objects, several processes occur: **transmission**, **absorption**, **reflection**, and **scattering** (Figure 3). Understanding these interactions between electromagnetic radiation and objects is crucial for remote sensing applications. By analysing the amount and characteristics of radiation transmitted, absorbed, reflected, and scattered by objects, we can infer valuable information about their composition, structure, and condition.

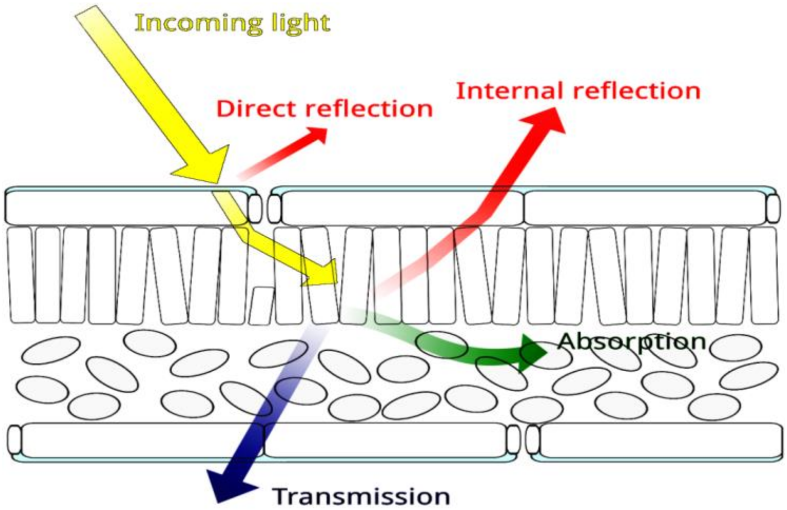


Figure 3: Reflection, absorption and transmission in plant leaf tissue. Source: Archive KIS.

Transmission refers to the passage of electromagnetic waves through an object without significant interaction or attenuation. Transparent materials, such as glass or water, allow most of the incident radiation to pass through them, making them ideal for transmitting light. Plant leaves can also transmit some amount of light, although the level of transmission varies depending on several factors, such as leaf structure, thickness, and pigmentation. The transmitted light can interact with other leaves, objects, or surfaces beyond the leaf, leading to further scattering, absorption, or transmission.

Absorption occurs when an object absorbs some or all of the incident radiation, converting it into other forms of energy, typically heat. The absorbed energy can excite molecules or cause vibrations within the object, leading to changes in its temperature or chemical state. Plants utilize this effect in photosynthesis. Different materials have unique absorption patterns, meaning they absorb specific wavelengths more strongly than others. This characteristic is exploited in remote sensing to identify and characterize objects based on their absorption properties.

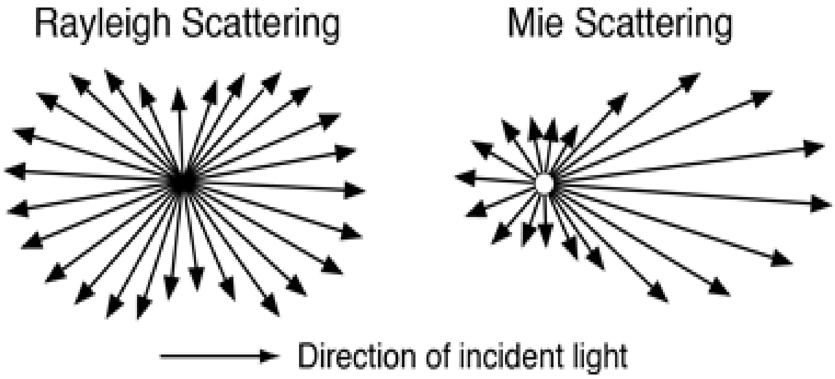
Reflection is the process by which electromagnetic waves bounce off the surface of an object. When light encounters an object, some of it is absorbed, while the remainder is reflected back towards the observer. The reflected light can provide valuable information about the object's surface properties, including its colour, texture, and reflectance characteristics. Reflectance is a fundamental property of objects that determines the proportion of incident radiation that is reflected. It varies across different wavelengths and materials, creating distinct spectral signatures. These signatures enable remote sensing techniques to differentiate between different objects or materials based on their unique reflectance patterns.

Scattering is a special type of interaction, which occurs most often in the atmosphere. It occurs when electromagnetic waves interact with particles or irregularities within an object or medium. The incident radiation is deflected in various directions, resulting in a scattering pattern. The scattering process is influenced by the size, shape, and composition of the scattering particles or irregularities.

Different types of scattering, such as Rayleigh scattering, Mie scattering, and non-selective scattering, can occur depending on the characteristics of the object and the wavelength of the incident radiation.

Rayleigh scattering dominates when particle size is smaller than the wavelength. These can be nitrogen and oxygen molecules, and atmospheric particulate matter (PM2.5 and PM10). This type of scattering is wavelength-dependent, short wavelengths (the colour blue) are scattered more strongly than longer wavelengths (colour red), but scattering occurs in all directions equally. This is why we see a blue sky, and red sunsets and sunrises. The more we pollute the atmosphere, the more blue light will be scattered, leading to beautiful sunsets.

Mie scattering, on the other hand, is wavelength independent, i.e. all wavelengths are scattered equally, and it dominates in one direction. This type of scattering occurs when particle size is similar in size to wavelength, for example water molecules. Clouds consist of water droplets, and due to Mie scattering, we see them as white. From space, all clouds are white, even the most severe storm. On the planet surface, we see storm clouds as white unless we are beneath them. Once they are above us, we see them as grey, due to shadowing effects from above-lying clouds.



Non-selective scattering occurs when particle size is much larger than wavelength, and has similar characteristics as Mie scattering. All wavelengths are equally affected, causing the scattered light to appear white or colourless. Non-selective scattering is responsible for phenomena such as the Tyndall effect, where light passing through a colloidal or particulate medium gets dispersed in all directions, making the medium appear milky or hazy.

In remote sensing, the reflectance of objects that we measure or observe is a comprehensive summary of all the interactions between electromagnetic radiation and the objects being studied. It encompasses processes such as transmission, absorption, reflection, and scattering.

1.2.3. Photosynthetically active radiation

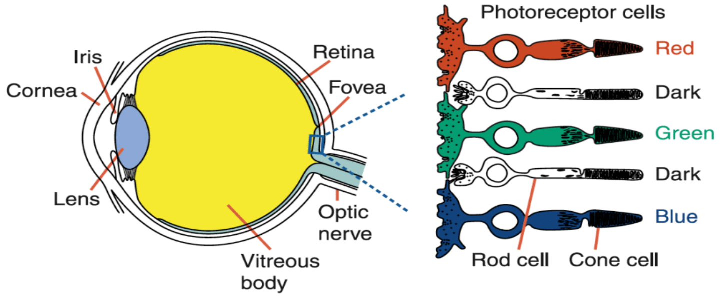
PAR, or **Photosynthetically Active Radiation**, refers to the portion of the electromagnetic spectrum that is essential for photosynthesis in plants. It encompasses the range of wavelengths between approximately 400 to 700 nm, which corresponds to the visible light spectrum. Plants utilize PAR for photosynthesis because it is within this specific wavelength range that chlorophyll, the primary pigment responsible for capturing light energy, is most effective. Chlorophyll molecules absorb light most efficiently in the red (around 660 nm) and blue (around 430 nm) regions of the spectrum, and reflecting light in green (around 550 nm). These absorbed photons provide the energy necessary for photosynthesis, the process by which plants convert light energy into chemical energy in the form of carbohydrates. The utilization of specific wavelengths by plants for photosynthesis is a result of their evolutionary adaptation to efficiently capture the available light energy. The absorption characteristics of chlorophyll pigments determine which wavelengths are most effective for photosynthesis, as they are responsible for capturing photons and initiating the chemical reactions within the plant cells.

Light outside the PAR range, such as ultraviolet (UV) or infrared (IR) radiation is less effective for photosynthesis because chlorophyll molecules have limited absorption capacity in these regions. UV light, with wavelengths shorter than 400 nm, carries higher energy but can be harmful to plants, causing damage to DNA and other cellular components. On the other hand, IR light, with wavelengths longer than 700 nm, carries lower energy and is less efficient in driving photosynthesis. The preference for PAR in photosynthesis is also influenced by the availability and abundance of light in the environment. Solar radiation consists mainly of visible light, with a significant proportion falling within the PAR range. Therefore, plants have evolved to optimize their photosynthetic apparatus to utilize the abundant and accessible light energy in this range. It is important to note that while PAR is crucial for photosynthesis, the efficiency of photosynthesis can vary among different plant species. Some plant species may have specific adaptations to thrive in low light conditions or in environments with different light spectra. This variation is reflected in the specific absorption characteristics of their chlorophyll pigments.

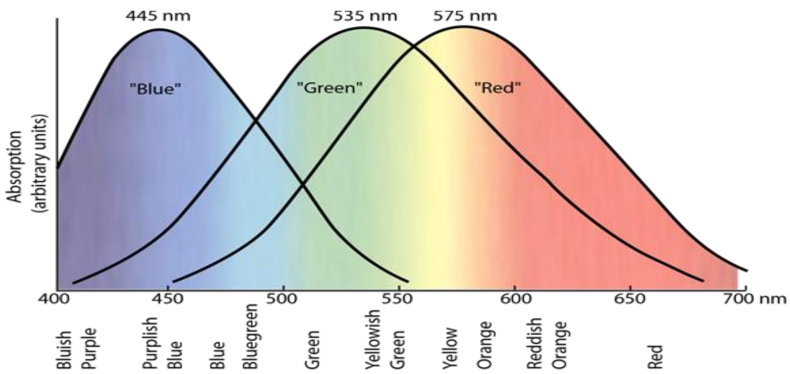
In agricultural applications and controlled environments, such as greenhouses or growth chambers, artificial lighting systems can be tailored to provide light within the PAR range, thereby maximizing photosynthetic efficiency and crop productivity. By providing light in the specific wavelengths that plants can readily absorb and utilize, growers can optimize the growth and development of plants under controlled conditions. A fact which is well-known and utilized by cannabis growers.

1.3 How sensors "see"

Understanding how human eyesight functions is essential for grasping the concepts of multispectral and hyperspectral systems used in remote sensing. Human eyesight is based on the perception of visible light, between 400 and 700 nm. The eye consists of several components, including the cornea, lens, and retina. When light enters the eye, it passes through the cornea and lens, which help focus the incoming light onto the retina. The retina contains specialized cells called photoreceptors, specifically rods and cones. Rods are responsible for vision in low-light conditions and detecting shades of grey, while cones are responsible for colour vision in brighter conditions. Cones are further divided into three types, each sensitive to a particular range of wavelengths associated with red, green, and blue colours. These are the three primary colours, resulting in trichromatic vision. This means that the human eye can differentiate and perceive a wide range of colours, all the colours of the rainbow, within the visible spectrum based on the combination of these primary colours. The concept of colour is a perceptual phenomenon that arises from the way our visual system interprets different wavelengths of light. Colour perception is subjective and can vary among individuals, but it plays a crucial role in our understanding and interpretation of the world around us.



While our eyes can detect specific ranges of wavelengths of light, the information captured by the photoreceptors is then transmitted to the brain via the optic nerve. The brain processes this information, combining signals from the different types of cones to create the perception of colour and enabling us to see the world around us. And this process enables us to see so-called non-spectral or imaginary colours. These are colours don't exist in nature, they don't have their own wavelengths. Our perception of these colours arises from the way our visual system processes light information. The colour brown results from a mixture of red and green and is interpreted by our brains as a subdued and dark colour. Brown is basically a very dark orange. On the other hand, magenta, a vibrant purplish-pink hue, is a combination of red and blue light wavelengths that our eyes perceive as a distinct colour. These non-spectral colours add a fascinating dimension to our perception of the world, expanding the range of hues beyond what occurs naturally in the visible light spectrum.

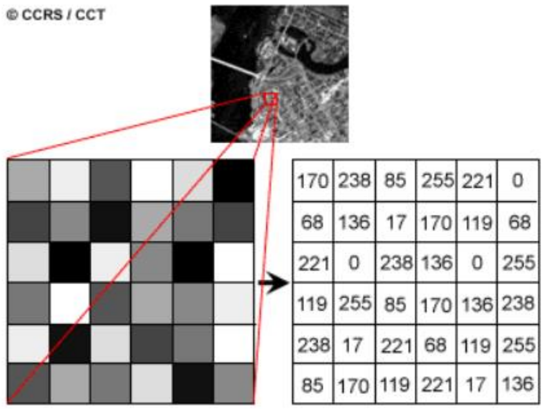


We utilize the same approach, combining three spectral bands, to produce colour images. This is the RGB (red-green-blue) colour system, utilized for computer screens, smartphones, digital cameras… And when we expand this approach to parts of the light spectrum invisible to us, such as near infrared and short-wave infrared, be get multispectral sensors, with up to 20 bands spread throughout the spectrum between 400 and 2500 nm. And when we increase the number of bands, to several hundred or thousands of narrow and contiguous spectral bands, we get a hyperspectral sensor. These systems are designed to detect and capture electromagnetic radiation across a broader range of wavelengths, beyond the visible spectrum, and in much more detail. This way they can provide information about objects or materials that may not be visible to the human eye. This level of spectral detail enables us to identify and differentiate between various materials with similar visual appearances, such as different types of vegetation and early (i.e. pre-symptomatic) detection of plant health.

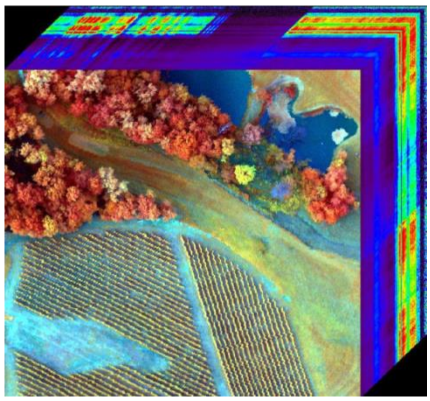
1.4 Characteristics of images

It's important to differentiate between the terms "images" and "photographs" in remote sensing. An image refers to any pictorial representation regardless of the wavelengths or remote sensing device used to detect and record electromagnetic energy. On the other hand, a photograph specifically refers to images that have been detected and recorded on photographic film, i.e. a light-sensitive film undergoes chemical reactions. While all photographs are images, not all images are photographs. In remote sensing we therefore use the term “images” talk about “imagery data”. Please note, this is different from “imaginary data”, such as the impact of invisible pink unicorns on crop growth.

Photographs can be described digitally through a process that involves converting the visual information captured in the image into numerical data. This transformation enables the representation, storage, and manipulation of the photograph using computers and digital systems. By dividing the photograph into small discrete units called pixels, each pixel is assigned a numeric value or digital number that corresponds to its brightness level. This digital representation allows for precise recording and reproduction of the photograph's visual content. Of course, sensors which record digital images directly capture electromagnetic energy as a numerical array. Even though these two ways of capturing remote sensing data are interchangeable, some details can be lost in the conversion process. Images have several benefits over photographs. Metadata (data about the images, such as sensor type, acquisition date and settings, sensor characteristics, GPS data) can be automatically included in the generated images. Additionally, digital photographs can be further enhanced, edited, and analyzed using various image processing techniques and software tools, offering flexibility and creative possibilities for manipulating and interpreting visual information. By combining images with GPS data (a process called georeferencing) we obtain spatially and spectrally accurate data for each pixel of the image.



As previously mentioned, multispectral and hyperspectral images are recorded by capturing electromagnetic radiation across different bands or wavelengths. Each band can be considered as a separate image capturing specific portions of the electromagnetic spectrum. The sensor is equipped with filters or detectors that are sensitive to specific wavelength ranges. As the sensor scans the scene, it simultaneously records the intensity or radiance of the reflected or emitted energy within each band. These separate band images are then combined or overlayed to generate a spectral image. In a multispectral image, typically a few discrete bands (such as red, green, and blue) are combined to create a colour composite image, allowing human visual interpretation. On the other hand, hyperspectral images consist of numerous contiguous narrow bands covering a wider range of wavelengths. The hyperspectral data can be visualized as a spectral cube (also called a hyperspectral data cube), where each pixel represents a spectrum across the entire range of bands, providing detailed information about the material composition and characteristics of the observed objects or features. The overlaying or combination of these band images enables the generation of a comprehensive spectral image, offering insights into the unique spectral signatures and properties of the captured scene.



Digitally, we can combine and display channels of information using the primary colours: blue, green, and red. Each channel's data is represented as one of the primary colours, and the relative brightness (digital value) of each pixel determines the proportions in which the primary colours combine to represent different colours. When displaying a single channel or wavelength range using this method, all three primary colours are actually employed. As the brightness level is the same for each primary colour, they combine to form a black and white image with various shades of grey. However, when displaying multiple channels, each assigned to a different primary colour, the brightness levels may vary for each channel/primary colour combination, resulting in a colour image (Figure 4).

Humans see colour as a combination of three bands, red, green, and blue. This is called the RGB colour space. Colour spaces are mathematical models that define and organize colours in a structured manner. They provide a standardized way to represent and describe colours, facilitating communication and analysis across various applications. Popular colour spaces, such as RGB (Red, Green, Blue) and CMYK (Cyan, Magenta, Yellow, Black), define colours based on combinations of primary colour components. Other colour spaces, such as CIE L*a*b* and HSL/HSV, offer alternative ways to represent colours using perceptually meaningful attributes like brightness, hue, saturation, and lightness. Different colour spaces can be more meaningful for computers than RGB because they offer specific advantages in various computational tasks related to colour processing and analysis. Each colour space has its own strengths and limitations, and their appropriate usage depends on the specific requirements of the task at hand, such as accurate colour reproduction, colour matching, or colour manipulation. While RGB is a widely used colour space, it has certain limitations that make other colour spaces more suitable for certain applications.

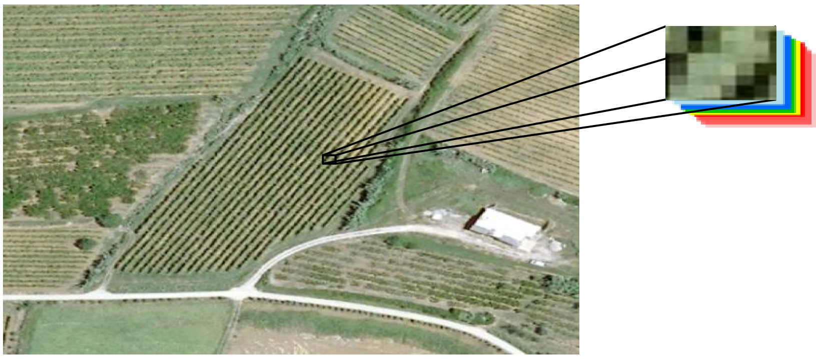


Figure 4: Multispectral image of a vineyard, presented in RGB colour space. Individual multispectral bands are colour-coded (a different colour for each band) in the zoomed-in part on the right. Source: Archive KIS.

One key advantage of alternative colour spaces is their ability to separate colour information into perceptually meaningful components. For example, in the CIE L*a*b* colour space, the L component represents lightness, while the a and b components represent the red-green and blue-yellow colour opponency. This separation allows for more intuitive and precise colour manipulation, such as adjusting brightness or altering colour balance, as these operations can be performed independently on specific components. Different colour spaces may offer enhanced capabilities for specific applications. For instance, the HSV (Hue, Saturation, Value) colour space is often preferred in computer graphics and image processing for tasks such as colour selection, manipulation, and image segmentation (a common process in remote sensing, where an image is separated into different subsets, such as plants and soil, and each subset is extracted separately for analysis), as it provides a more intuitive representation of hue and saturation.

Alternative colour spaces can be more meaningful for computers because they offer better perceptual separation, improved perceptual uniformity, device independence, and specialized features tailored to specific computational tasks. They expand the computational possibilities for handling and analysing colours, allowing for more accurate, efficient, and visually pleasing outcomes in various applications.

1.5 Spectral signatures

A spectral signature can be defined as the unique pattern or curve that represents the reflectance or emission characteristics of a material or object across the electromagnetic spectrum. It illustrates how the intensity of electromagnetic energy changes as a function of wavelength. The spectral signature is typically visualized as a graph or curve, with the x-axis representing the wavelength and the y-axis representing the reflectance or radiance values (Figure 5).

Spectral signatures play a vital role in remote sensing and are fundamental to understanding and interpreting the properties of materials and objects based on their interaction with electromagnetic energy across different wavelengths. These signatures provide valuable information about the composition, physical properties, and behaviour of observed targets, enabling us to identify, differentiate, and study various features of objects under study. The structure of a spectral signature is determined by the specific properties and composition of the target material or object. Each material has its own distinct spectral response, resulting in a unique signature. The shape, position, and amplitude of the curve provide valuable information about the target's optical properties, such as absorption, transmission, and reflection characteristics. Spectral signatures exhibit various features, including peaks, valleys, and inflection points, which correspond to specific wavelengths where the target material interacts strongly with electromagnetic energy.

The use of spectral signatures is widespread in remote sensing applications. One of the primary applications is material identification and classification. By comparing the spectral signature of an unknown target with a library of known signatures, scientists can identify the material or object in images. This technique is commonly employed in mineral exploration, vegetation analysis, land cover mapping, and geological surveys. Spectral signatures are also utilized for characterizing and monitoring environmental parameters. For example, in the field of agriculture, spectral signatures of crops can provide insights into their health, growth stages, and nutrient status. By analysing changes in spectral signatures over time, farmers and researchers can assess crop conditions, detect stress or disease, and optimize resource allocation. Furthermore, spectral signatures are employed in change detection studies, in time series analysis. By comparing the spectral response of an area or object captured at different times, we can identify and quantify changes that have occurred. This is particularly useful in monitoring land use changes, early detection of plant health, and plant breeding.

The spectral signature of plants is characterized by distinct patterns across various spectral regions, including the visible, red edge, near-infrared (NIR), and shortwave infrared (SWIR). In the visible region, plants typically exhibit relatively low reflectance due to strong absorption by chlorophyll. The rededge region is particularly important for vegetation analysis, as it captures the transition from low reflectance (and high absorbance) in the red region (around 600 nm) to higher reflectance in the NIR. This transition is influenced by chlorophyll (and other accessory pigments) absorption and the structural properties of leaves, providing valuable information about vegetation health and photosynthetic activity. The NIR region (800 to 1000 nm) is characterized by high reflectance in healthy vegetation. This response is attributed to the strong scattering of light within leaf tissues and the presence of internal cellular structures. NIR reflectance is sensitive to vegetation density, leaf area, and water content, making it useful for assessing vegetation vigour and biomass estimation. The SWIR region (1000 to 2500 nm) is influenced by various factors, including leaf internal structure, water content, and, importantly, plant biochemistry. Plants adapt to stress events by changing their biochemistry, which we can measure non-invasively using remote sensing. These changes occur before any visible symptoms develop, since the later require a change in pigment structure and ratios. Pigments, such as chlorophyll, carotenoids, and anthocyanins, are crucial for photosynthesis, the process by which plants convert sunlight into chemical energy for growth and development. Plants try to preserve their pigments as long as possible during severe stress events as a survival mechanism and an adaptive response to unfavourable conditions. Utilizing remote sensing in the SWIR region, early detection of plant stress is enabled. Spectral signatures are also affected by atmospheric effects, such as water absorption, which reduces the signal received on the Earth's surface. Therefore, atmospheric correction techniques are applied to mitigate these effects, and retrieve accurate spectral information.

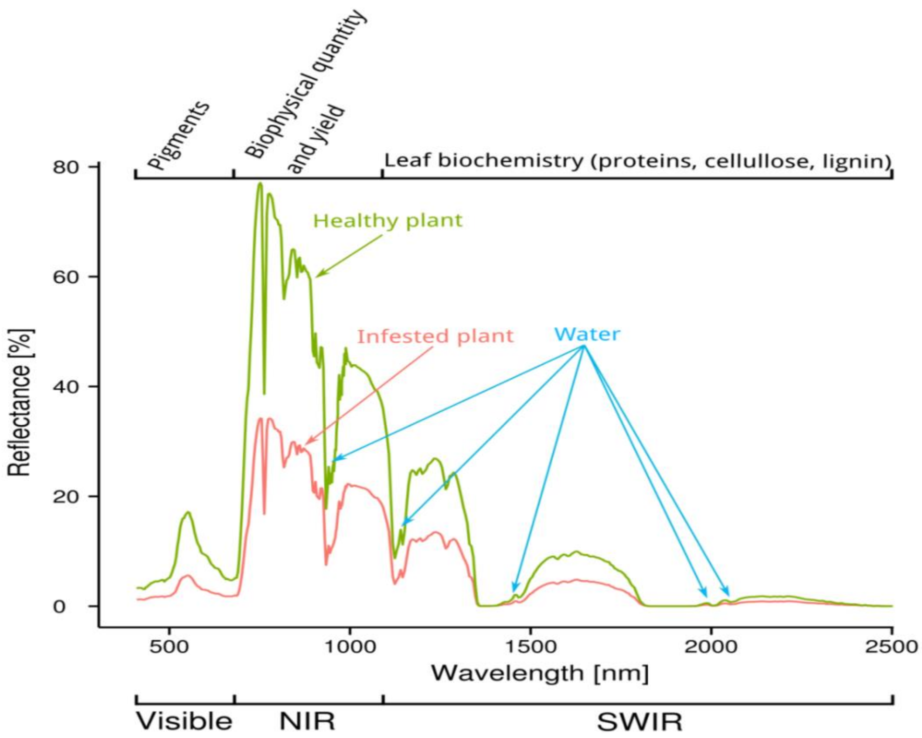


Figure 5: Typical spectral signatures of plants. Note the four spectral regions, visible, rededge, NIR, and SWIR. This hyperspectral signature also shows atmospheric water absorption bands. These signatures were captured using artificial lighting at small distances, up to 3 m. This is why not all of the light in these bands was absorbed and we get at least some information. Using aerial and satellite imaging (distances of more than 10 m and using only sunlight for illumination) all of the light in these bands would have been absorbed, reflectance would be 0 %.

1.6 Remote sensing sensors and platforms

1.6.1 Sensors

Remote sensing utilizes different types of sensors, which can be placed on different platforms. A platform refers to the vehicle or system that carries the sensors and instruments used to collect data and imagery. The choice of sensor(s) and platform(s) always depends on our requirements, what we are interested in. In this regard we have to answer a few simple questions:

1. *What kind of information we need*? If we require only to know which plants are in stress, but not what kind of stress, we might use only thermal imaging.
2. *How much detail do we need?* Should data be acquired for individual plants, or are entire stands enough? Are spectroscopic measurements enough or do we need images?
3. *How frequently should data be acquired?* Is one measurement enough or do we need time series data, for example for early detection.
4. *What is our budget*? Remote sensing isn't cheap (although it's improving), and our choices are always limited by what we can afford.

There are two primary types of sensors used in remote sensing, active and passive. Active sensors emit their own energy source, such as pulses of light or microwaves, and measure the response or reflection from the target object. Some examples of active sensors include lidar (light detection and ranging) and radar systems. On the other hand, passive sensors detect and measure the natural radiation emitted or reflected by the target object without emitting their own energy. Examples of passive sensors include optical cameras, multispectral and hyperspectral sensors, and thermal infrared sensors. Both types of sensors have their advantages and disadvantages, see Table 1 for details.

Table 1: Advantages and disadvantages of active and passive sensors.

	Advantage	Disadvantage
Active	Direct measurements of the target object's properties, such as its distance, elevation, or surface roughness.	Limited coverage area due to their narrow field of view or scanning limitations.
	Penetration through vegetation, clouds, and other obstructions, allowing for data collection even under challenging environmental conditions.	Require specialized equipment and sophisticated data processing algorithms to interpret and analyse the acquired data.
	High vertical resolution, enabling the measurement of complex 3D structures and vegetation profiles.	Depend on their own energy source, making them susceptible to limitations in energy availability and potential interference from external sources.
Passive	Can cover large areas, making them suitable for broad-scale analysis and monitoring.	Sensitive to environmental factors such as cloud cover, atmospheric interference, and shadows, which can affect the quality and availability of data.
	Lower operational costs as they rely on natural sources of energy, such as sunlight.	Rely on sunlight, making nighttime data collection challenging or limited to specific sensors, such as thermal infrared sensors.
	Can capture a wide range of information, from visible light to thermal radiation, enabling various applications in different domains.	Lower vertical resolution compared to active sensors, making them less suitable for detailed analysis of vertical structures or complex 3D objects.

In agriculture, both active and passive sensors find applications, but passive sensors are more commonly used due to their wider availability and cost-effectiveness (Table 2). Passive sensors, such as multispectral and hyperspectral sensors, provide valuable information about vegetation health, nutrient content, and stress detection, aiding in crop monitoring and precision agriculture practices. For plant phenotyping, passive sensors are particularly useful due to their ability to capture detailed spectral information. Hyperspectral sensors, with their high spectral resolution, enable precise identification and characterization of plant traits, such as chlorophyll content, water stress, and disease detection. These sensors provide valuable data for breeders and researchers to understand plant responses and improve breeding practices. On the other hand, active sensors have some applications in plant phenotyping, they are more commonly used for measuring canopy height, biomass estimation, and vegetation structure in forestry and ecosystem studies. Combining passive and active sensors can provide a comprehensive understanding of both spectral and structural characteristics of plants. This integration allows for the assessment of not only the physiological properties of plants but also their 3D structure, canopy architecture, and biomass distribution.

Table 2: Application of various sensors in agriculture.

Sensor Type	Applications
RGB Cameras	Canopy cover, Height Canopy Architecture and canopy colour (e.g. chlorophyll concentration using greenness indices).
Spectral sensors	Canopy Architecture, LAI, Vegetative indices, Biochemical composition of the leaf/canopy. Pigment concentration, water content, indirect measurement of biotic/abiotic stress.
LiDAR and time of flight sensors	Canopy height and canopy architecture, Estimation of LAI, volume, Biomass
Thermal sensors	Stomatal conductance. Water stress induced by biotic or abiotic factors.
Soil Sensors: electromagnetic induction (EMI), ground penetrating radar (GPR) and electrical resistance tomography (ERT)	Mapping of soil physical properties, such as water content, electric conductivity or root mapping.

Another important aspect of sensors is their resolution. In remote sensing, resolution refers to the level of detail and clarity with which an object or feature can be observed and represented in the acquired data. Different types of resolution are commonly considered in remote sensing. **Spatial resolution** pertains to the smallest discernible unit on the ground, determining the size of individual pixels or picture elements in an image. High spatial resolution provides fine details and can distinguish smaller objects, while lower spatial resolution may blend objects together. **Spectral resolution** refers to the number and width of spectral bands or wavelengths that sensors can capture. Sensors with higher spectral resolution can distinguish and analyse a broader range of electromagnetic radiation, enabling identification of subtle differences in the reflected or emitted energy. **Temporal resolution** relates to the frequency and regularity of data acquisitions over time. Frequent data capture with high temporal resolution enables monitoring changes and dynamic processes. **Radiometric resolution** refers to the sensor's sensitivity to detect and differentiate variations in the intensity or brightness of reflected or emitted energy. Higher radiometric resolution provides more levels of intensity, resulting in greater data accuracy and subtle distinctions. The consideration of these various resolutions is crucial in selecting appropriate remote sensing data for specific applications and analysis requirements (Table 3).

Table 3: Comparison of selected characteristics of some satellite, airborne and UAV sensors.

Platform	Spatial Resolution	Spectral Resolution	Revisit Time	Number of Bands	Spectral Range
Sentinel-2	10-20 m	10-20 nm	5 days	13	VNIR SWIR
WorldView-3	0.31-1.24 m	2-4 nm	1-4 days	8	VNIR SWIR
GeoEye	0.41-2.0 m	0.5-0.9 nm	1-3 days	4	VNIR
Planet	3-5 m	10-20 nm	1 day	4-7	VNIR
Hyperspectral Airborne	0.3-3 m	1-10 nm	Varies	>100	VNIR SWIR
Multispectral UAV	5-30 cm	5-10 nm	Varies	3-20	VNIR SWIR
Hyperspectral UAV	< 10 cm	1-3 nm	Varies	>50	VNIR SWIR

Please note that the values provided in Table 3 are approximate and can vary depending on specific sensor models, configurations, and acquisition conditions. Spatial resolution may vary depending on the altitude or flight height of airborne or UAV platforms. Similarly, spectral and radiometric resolutions can differ among sensors and instrument setups. Revisit time for satellites refers to the frequency at which the same location can be imaged, while for airborne and UAV imaging, it depends on flight plans and project requirements. Spectral ranges mentioned represent the general regions of the electromagnetic spectrum covered by the respective platforms.

1.6.2 Platforms

Remote sensing platforms encompass a diverse array of vehicles and systems designed to collect data and imagery. These platforms serve as carriers for sensors and instruments, enabling the acquisition of valuable information about our planet. Satellites, one of the most widely recognized remote sensing platforms, orbit the Earth and provide a global perspective, capturing data on a regular and systematic basis, allowing for long-term monitoring. Aircraft, including airplanes and drones (unmanned aerial vehicle – UAV), offer flexibility in terms of sensors, spatial, spectral, and temporal resolution, and targeted observations, allowing for detailed and customized data collection. Ground-based platforms, such as hand-held, stationary sensors or vehicles, provide close-range monitoring and specialized measurements. Each platform brings its own unique advantages, capabilities, and limitations, making the choice of platform dependent on factors like the desired spatial coverage, temporal resolution, data accuracy requirements, and cost considerations (Table 4). Often, a combination of different platforms and sensors is employed to optimize data collection and analysis. Remote sensing platforms play a vital role in various fields, including environmental monitoring, agriculture, disaster management, and urban planning, aiding in our understanding of Earth's processes and informing decision-making processes.

Table 4: Characteristics of remote sensing platforms.

	Advantage	Disadvantage
Ground-based	Highly accurate and precise measurements, particularly when positioned in close proximity to the target area or object.	Collect data from specific locations, limiting their coverage area and making them less suitable for large-scale analysis.
	Data can be collected and analyzed in real-time, allowing for immediate decision-making and response.	Localized measurements, which may not capture variations across larger areas.
	Often have lower initial costs and operational expenses.	Require proper installation, calibration, and regular maintenance, adding logistical considerations and costs.
Airborne and UAV	Very high spatial resolution, allowing for detailed analysis of small areas.	Due to their limited flight range and endurance, drones are better suited for smaller study areas compared to satellites.
	Can be deployed at specific locations and altitudes, providing targeted data collection and the ability to access challenging or inaccessible areas.	Subject to airspace regulations, flight restrictions, and permits; this can limit their use in certain locations or scenarios.
	Collect data quickly, enabling near-real-time monitoring and analysis.	Adverse weather conditions, such as strong winds or precipitation, can impact operations and data collection.
Satellite	Capture images of large regions, making it suitable for broad-scale analysis and monitoring.	Lower spatial resolution compared to other platforms, making it less suitable for detailed analysis at smaller scales.
	Consistent and continuous data over extended periods, enabling the study of temporal changes and trends.	Depending on the satellite and orbit, revisit times can range from several days to weeks, resulting in potential gaps in data availability.
	Sensors can capture data in multiple bands, allowing for detailed analysis and identification of different objects and materials.	May involve significant costs, and data access can be restricted due to licensing or proprietary restrictions.

2. Advanced plant phenotyping[1]

2.1 Introduction

Conventional phenotypic approaches, which compare a large number of lines/genotypes for a wide range of traits over a range of contrasting environments is extremely time consuming and expensive and is therefore difficult to fully exploit especially for the smaller organic and low-input market sectors. Advanced plant phenotyping is a cutting-edge approach that involves the comprehensive measurement and analysis of plant traits to gain a deeper understanding of plant growth, development, and responses to environmental factors. It has become increasingly crucial in agricultural research and breeding programs to address the challenges of food security, climate change, and sustainable agriculture. By utilizing advanced technologies such as high-resolution imaging, remote sensing, robotics, and sensor networks, researchers can capture detailed data on various plant characteristics including growth patterns, leaf traits, root architecture, physiological processes, and stress responses. This wealth of information helps breeders and scientists identify key traits associated with yield, stress tolerance, and disease resistance, enabling the development of improved plant varieties. Advanced plant phenotyping requires an interdisciplinary approach, bringing together experts from fields such as biology, genetics, computer science, engineering, and data analytics. Collaboration among these disciplines facilitates the development of sophisticated phenotyping platforms, automated data collection, and analysis pipelines.

The biggest challenge for massive phenotyping is to design the tools that can allow researchers to collect, access, organize, integrate, analyse, and manage phenotypic databases across the population. New imaging technologies and bioinformatics software are now available, in combination with computational tools are helping for high throughput phenotyping. Recently, phenotypic analysis has become a major limiting factor in genetic and physiological analysis, in plant sciences as well as in plant breeding. With rapid developments in plant molecular biology e.g. Next Generation Sequencing (NGS) and in molecular-based breeding techniques, an increasing number of species have been sequenced. Nowadays high-definition genotyping can be carried out on a large numbers of plants in an automated way, thereby allowing association genetics studies. However, the link between genotype and phenotype has progressed more slowly due to the time/labour constraints in phenotyping a large number of genetic lines across multiple environments. Many breeders/research institutions carry out extensive phenotyping activities often under time and resource constraints, which may lead to results that are difficult to interpret especially for quantitative resistance to key diseases.

Despite the remarkable advancements, phenotyping still poses challenges. The sheer volume and complexity of data generated by advanced phenotyping methods have created a bottleneck in data analysis and interpretation. Researchers are working to develop efficient data management strategies, analytical tools, and standardized phenotyping protocols to maximize the potential of advanced plant phenotyping and accelerate crop improvement efforts. By addressing these challenges, advanced plant phenotyping holds great promise for enhancing our understanding of plant biology and supporting the development of resilient and high-yielding crop varieties to meet the growing global demand for food. There is therefore an urgent need to develop and train researchers and plant breeders in the use and application of Advanced Phenotyping capabilities in terms of digital, thermal and multi/hyper spectral imaging in terms of when, where and how these can be efficiently utilised.

[1] Based on ECOBREED deliverable **D 7.2 Production of materials for improved phenotyping training** (autors: Pavol Hauptvogel, Ankush Prashar, Uroš Žibrat, Paul Bilsborrow, Matej Knapič, Leonidas Rempelos, 2021)

2.2 Application of high-throughput phenotyping in crop studies

In recent years, many approaches have been discussed and incorporated into the constantly improving highly automated, non-destructive phenotyping of plants. Plant phenotyping is the extensive evaluation of multiplex plant features like architecture, phenology, pest/disease resistance, and yield and quality traits. The detailed and specific phenotyping strategies are required for genome-wide association and permit high-resolution linkage mapping investigations, and also to practice models of genomic selection for plant advancement.

The improvement of correctness and throughput of phenotypic assessment at all biological levels - phenomics, metabolomics and genetics - are the main objectives of modern phenotyping. The phenotyping system allows precision and replicative datasets because of non-destructive data collection and minimizes labour and costs due to its:

- automation,
- improved data integration,
- remote sensing capabilities.

Advanced phenotyping platforms produce significantly more data than conventional phenotyping approaches and they need special systems for data management, access and storage. New statistical tools are needed for enlarging the experimental design and for making greater use of integration of data together with deriving biologically significant signals from experimental and environmental noise. The phenotyping field covers two major challenges:

1. Analysis of a large quantity of genetic lines and,
2. Replication of measurement of dynamic traits (i.e. traits whose phenotype changes during the growth period).

In most cases, in the field and in laboratory platforms, the following detection systems are being used:

- (semi)-automatic evaluation of morphometric parameters using RGB image analysis,
- chlorophyll a fluorescence kinetic imaging,
- hyperspectral or multispectral analysis of the light spectral reflectance
- thermal (IR) imaging,
- environmental monitoring systems,
- soil status monitoring systems.

2.2.1 Controlled environment platforms

In many indoor installations, systems are composed of regulated watering and nutrient regimes controlled by automatic weight systems and environmental controls in the imaging.

Control and programming of platform systems, as well as data analysis, are performed with sophisticated and user-friendly software packages. Such newly developed phenotyping systems have tools for estimating many photosynthetic parameters; RGB systems can estimate plant morphology, IR-thermal cameras can evaluate stomatal conductance, and hyperspectral imaging systems can evaluate metabolomics of experimental plants at different stages of growth.

The first major automated phenotyping platforms for a controlled environment were built in Australia (Australian Plant Phenotyping Facility in Adelaide and the CSIRO facility in Canberra). However, in the last decade, the development of phenotyping platforms has been concentrated mostly in Europe, including two main commercial developers of phenotyping systems - Lemnatec (Germany) and Photo System Instruments - PSI (Czech Republic).

2.2.2 Field based phenotyping platforms

Field phenotyping is a critical component in understanding plant development and improvement traits and it's the understanding of interaction between the genetic and environmental factors which help us understand the critical crop production and stress related traits. High throughput phenotyping methods in the field help us to increase selection efficiency for breeding traits by accounting for variability in management and environmental factors. There are diverse approaches available for field phenotyping ranging from point sensors which can be hand-held or in the form of field based fixed systems, vehicle mounted and driven platforms or aerial vehicle based systems which can be either unmanned or manned with potential for the use of satellite based systems for continuous monitoring. The most developed phenotyping platforms (up to 2018) are listed in Table 5.

Table 5: Automated and semi-automated high-throughput plant phenotyping platforms

Location / Producer	Platform	Features	URL
PSI	PlantScreen™	Conveyor phenotyping system in controlled environmental conditions with analysis of chlorophyll fluorescence, kinetic and thermal imaging, morphometric and RGB analysis, and hyperspectral and NIR imaging, uses an automated weighing and watering system	**http://www.psi.cz**
LemmaTec	Scanalyzer^{3D}	Comprehensive non-destructive 2D-3D assessment of plant physiological traits in controlled environmental conditions	**http://www.lemnatec.com**
INRA	Phenopsis	Specific platform for phenotyping *Arabidopsis* plant growth under controlled environmental conditions.	**http://bioweb.supagro.inra.fr**
INRA	Phenoscope	Automated phenotyping device to handle and monitor hundreds of individual's pots.	**http://www.phenoscope.versailles.inra.fr**
INRA	Phenodyn	Temporal of hundreds of monocot crop species.	**http://www.phenome-fppn.fr**
INRA	Phenoarch	Automated platform based on a LemmaTec system to analyze the genetic determinants of plant responses to environmental conditions.	**http://www.phenome-fppn.fr**
Phenospex	FieldScan	Phenotyping under field- or semi-field conditions that is designed to screen large populations.	**http://www.phenospex.com**
WPS	WSP	Fully automated digital phenotyping system using high-throughput RGB sensors.	**http://www.wps.eu**
Keygene	PhenoFab^{R}	Greenhouse service operation that combines phenotyping technology with trait interpretation to exploit phenotypic variation.	
Jülich Plant Phenotyping Centre	Growscreen	Non-invasive method designed to quantity shoot morphometrical and functional parameters and root architecture.	**http://www.fz-juelich.de**
Wageningen UR	PhenoBot	Autonomous mobile robot with camera promises to output direct registered depth and color image for morphometric analysis.	**http://www.wageningenur.nl**
Wiwam	Wiwam Conveyor	Integrated robotic system for phenotyping of larger plants with automated irrigation and measurement of a variety of plant growth parameters at regular time intervals.	**http://www.wiwam.be**
Australian Plant Phenomics Facility	PlantScan	Provides non-destructive analyses of plant morphology, structure and function by using high-resolution cameras with cutting-edge information technology.	**http://www.plantphenomics.org**

Ground-based field sensing platforms allow plot-level data capture both at organ and canopy analysis along with **global positioning systems** (GPS) enabled navigation and spatial analysis. There are some fixed systems available in the form of field scanners (for example systems at Rothamsted Research, Zurich field phenotyping platform, Arizona field scanner system etc). Similarly, mounted platform systems or technology (which can be in form of tractor mounted or adapted with another mobile system) is not a suitable fit for all systems and has to be adapted depending upon the crop and planting specifications along with accounting for inconsistencies and plant characteristic variabiliity. Table 6 provides some advantages and disadvantages of different systems (adapted from Deery et al., 2014).

Table 6: Advantages and disadvantages of different platforms (adapted from Deery et al., 2014)

Platform Type	Disadvantages	Advantages
Fixed systems	Generally expensive; can only monitor a very limited number of plots	Unmanned continuous operation; after-hours operation (e.g., night-time); good repeatability
Permanent platforms based on cranes, scaffolds or cable-guided cameras	Limited area of crop, so very small plots; expensive	Give precise, high resolution images from a fixed angle
Towers/cherry-pickers	Generally varying view angle; problems with distance (for thermal), bi-directional reflectance distribution function (BRDF), plot delineation, *etc.*; difficult to move, so limited areas covered	Good for the simultaneous view of the area; can be moved to view different areas
Mobile in-field systems	Generally take a long time to cover a field, so subject to varying environmental conditions	Very flexible deployment; good capacity for GPS/GIS tagging; very good spatial resolution
Tractor-boom	Long boom may not be stable	Easy operation; constant view angle; wide swath (if enough sensors are mounted as on a spraying bar); mounting readily available (needs modification)
Manned buggies	Requires a dedicated vehicle (expensive)	Flexibility with the design of the vehicle (e.g., tall crops, row spacing); Constant view angle; very adaptable
Autonomous robots	Expensive; no commercial solutions available; safety mechanisms required	Unmanned continuous operation; after-hours operation (e.g., night-time)
Airborne	Limitations on the weight of the payload depending on the platform; a lack of turnkey systems; spatial resolution depends on speed and altitude	Can cover the whole experiment in a very short time, getting a snapshot of all of the plots without changes in the environmental conditions
UAVs	Limited payload (weight and size); limited altitude (regulations) and total flight time (hence, total covered area); less wind-affected than blimps; regulatory issues depending on the country	Relatively low cost compared with manned aerial platforms; GPS navigation for accurate positioning
Manned aircraft	Cost of operation can be expensive and may prohibit repeated flights, thereby reducing temporal resolution; problems of availability	Flexibility with the payload (size and weight); Can cover large areas rapidly

2.3 Non-destructive analyses of growth and physiology by automated imaging

The system for automated non-invasive analysis of above-ground plant performance may be based on different technical solutions or measured signals. In the following sections, the most important/developed sensors that have been used for Advanced Phenotyping will be introduced. The systems highlighted below are systems which can be adapted and used for different imaging platforms depending upon the spectral and spatial resolutions of the sensors. The major discussion below is mainly done keeping in mind the basis for controlled environment imaging systems with highlights about changes for field-based systems.

2.3.1 Visible RGB imaging of plant above-ground biomass

In most cases, the main observed trait in plant biology is above-ground growth and biomass production. In addition to numerous secondary traits describing the morphology of shoots, the primary and universal trait is the size (volume, fresh mass, dry mass) of biomass and the rate of biomass formation. The standard way to assess biomass production is via destructive sampling, by a simple weighing of the fresh (FW) and dry (DW) mass. However, this can be done only at the end-point analyses. Similarly, leaf area and consequently the plant growth rate are usually determined by manual measurements of the dimensions of plant leaves, or by passing harvested leaves through a leaf area machine. Such measurements are highly time consuming and thus cannot be used for large scale experiments involving a large number of genotypes. Therefore, controlled system plant phenotyping facilities prefer to evaluate the growth rate using imaging methods which employ digital cameras with subsequent software image analysis.

This enables a faster and more precise determination of the leaf area and other parameters called the projected area. In general, non-invasive techniques of shoot growth determination have proven very reliable and high correlations between the digital area and the shoot fresh, or dry weights, respectively, have been reported in Arabidopsis, tobacco and different crop species. Similarly, other common growth parameters such as stem length, number of tillers and inflorescence architecture can be assessed non-destructively and manually, but again the time requirements, limit the number of plants analysed. High-throughput approaches for analyses of these rather species-specific traits would be very valuable however with the exception of Arabidopsis the range of accessible solutions has been rather limited.

Recent phenotyping platforms run the procedures to capture and automatically analyse the images of above-ground area. The images are taken from a top and/or side view, in which the several views are analysed taken when plants are rotating by a fixed angle. An example of top view and side view image is shown in Figure 6.

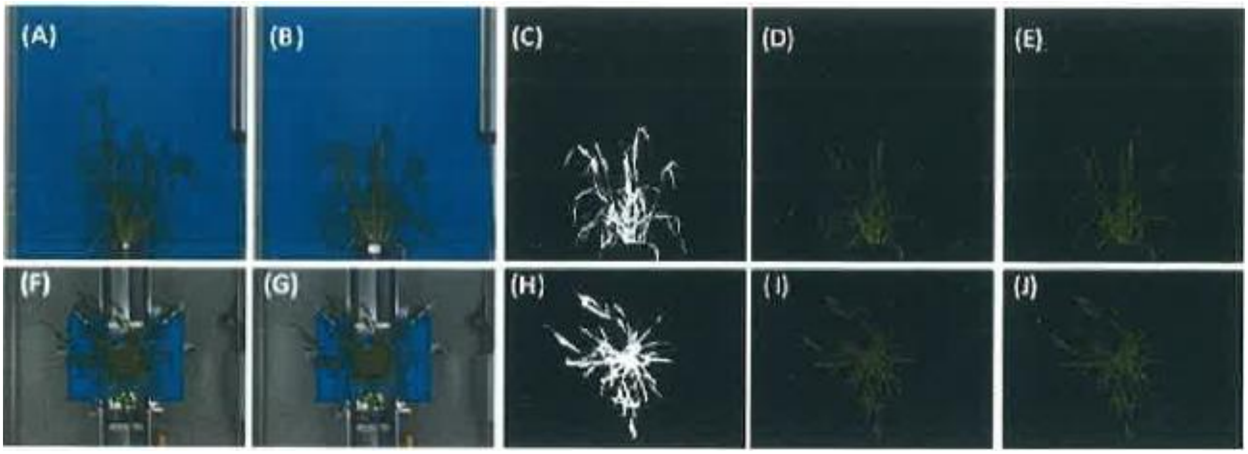


Figure 6: Top and side view of a wheat plant. The original figure from two different angles from the side (A, B) and top (F, G) was segmented and the background, including pot and substrate was automatically identified during image processing by the system (C, H), then removed, so the final image for the numerical analysis conists only from the plant parts on the black background (D, E, I, J). The image from commercial PlantScreen® phebnotyping system (PSI, cech republic), Slovak PlantScreen. SUA Nitra, Slovakia.

As an example, the RGB imaging system of PlantScreen® phenotyping system (PSI, Czech Republic) enables the researcher to calculate automatically this set of parameters using different modes of view. From the image captured from the top (top view), the following parameters can be calculated:

- Area (pixel count / mm^2)
- Perimeter (pixel count / mm)
- Roundness
- Compactness
- Eccentricity
- Rotation mass symmetry (RMS)
- Slenderness of leaves
- Color index
- Leaf tracking and leaf analysis

Using the series of side-view images, the following parameters can be calculated:

- Plant height (pixel count / mm)
- Growth width (pixel count / mm)
- Area (pixel count / mm^2)
- Perimeter (pixel count / mm)
- Compactness
- Number of leaves
- Leaf angle

A more complex procedure is the use of combination of top and side view, which enables estimation of the following growth traits:

- Total biomass volume or mass
- Leaf movement
- Relative growth rate

Similar to PlantScreen, studies are also evaluating the use of field, tractor or buggy mounted systems in the field for organ level and plant based traits. This includes the parameters mentioned above.

Similar object in motion combined with photogrammetry based approaches are being used from a UAV based systems using RGB cameras to understand different canopy traits (height, canopy cover etc). The benefit being that the UAV based approaches give us canopy trait characteristics for a large number of varieties under differing field/ environmental conditions.
Canopy cover estimates can be estimated automatically from images using simple threshold and segmentation based analysis available in many image processing programmes. This can help estimate the leaf area index (LAI) and light interception. Further approaches can be used or have been developed to extract traits like shape, compactness e*tc.*

2.3.2 Chlorophyll fluorescence imaging

The technical development and release of commercially available devices have led to a wide expansion of practical applications of chlorophyll fluorescence in plant biology, stimulating the progress in photosynthetic research and in crop science. The routines for distinguishing of different fluorescence quenching were developed, leading to important discoveries on the excess light energy dissipation and partitioning of the light energy between photochemical and non-photochemical processes. Although the different technical solutions enabled measurement of chlorophyll fluorescence operating efficiencies, most of the results have been obtained thanks to development of pulse amplitude modulation (PAM) fluorometers, using saturation pulse method.

Thus, since its introduction, now more than 30 years ago, the saturation pulse (SP) method employing the PAM technique has become a common way to assess the photosynthetic electron transport in plant tissues or other photosynthetically active samples. The parameters derived from chlorophyll a fluorescence measurements based on the PAM method provides information about the fluxes of energy originating from the de-excitation of chlorophyll molecules in photosystem II (PSII) in plant chloroplasts, by non-invasive assessment of almost any plant. The parameters of chlorophyll fluorescence analysis can be calculated from quite a few fluorescence intensities, obtained either in dark-adapted or light-exposed samples. The principle of the modulation technique lies in the measurements of the rise of total fluorescence in response to a measuring pulse. Thanks to using the increment of fluorescence instead of the total values, the fluorescence parameters can be determined even under conditions of actinic light. Different light environments lead to different states of PSII de-excitation fluxes, which is reflected in changes of fluorescence intensities.

The measurement (Figure 7) was done on dark-adapted sample (kept for 20 minutes in darkness before measurements). After modulated measuring light was turned on (ML ON), the fluorescence signal increased, reaching the value of minimum fluorescence in the dark-adapted sample, Fo. Then, the short saturation pulse (SP, intensity 10,000 umol nr^2 $s\text{-}^1$ for i second) was applied, which led to the fluorescence intensity increase to the maximum value, Fm, followed by the decrease back, almost to the Fo level. In the next step, the non-saturating actinic light was turned on, leading to a steep increase of fluorescence signal (F'), reaching the maximum at given actinic light intensity (Fp) followed by the gradual decrease, reaching after few minutes the steady-state value of actinic-light influenced fluorescence signal (Fs'). The same saturation pulse applied in the sample exposed to actinic light led to an increase of fluorescence signal (peak), reaching the maximum fluorescence in light-adapted state, Fm', followed by the fast decrease back to the Fs' level. In order to obtain minimum fluorescence of light adapted sample (Fo'), a short period of far-red excitation was applied, leading to decrease of FS' signal to Fo' value. Individual fluorescence intensities (Fo, Fm, Fp, Fs', Fm', Fo') represent the variables used for calculation of all fluorescence parameters (quantum yields, quenching parameters, etc.) derived from modulated chlorophyll fluorescence measurements.

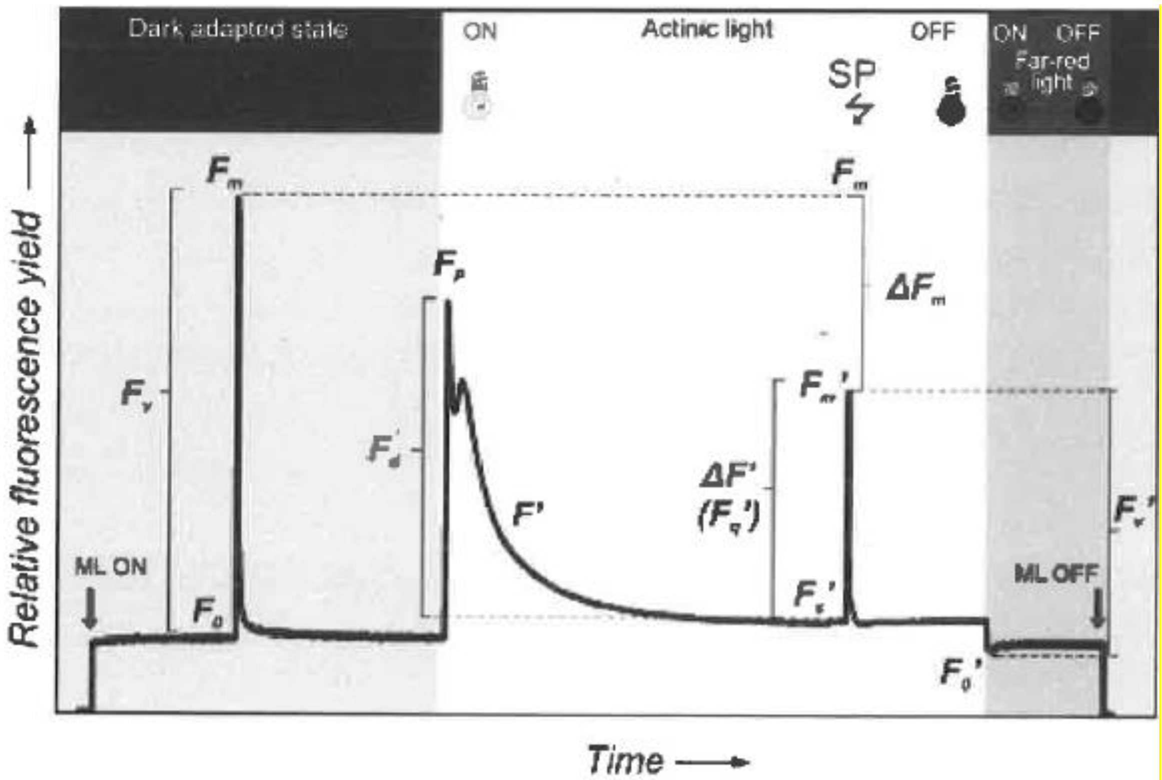


Figure 7: The principle of the fluorescence measurement using the saturation pulse method with quenching analysis by Kalaji et al 2017.

Most of the instruments for chlorophyll fluorescence analysis integrate the signal of the measured area. Anyway, advances in the technology of imaging detectors, LED light sources and processing of the data enable construction of systems for chlorophyll fluorescence imaging, which provides spatial resolution to the fluorescence records. These systems represent probably the most useful innovation of the technique of chlorophyll fluorescence, with universal applicability. Fluorescence imaging devices have been constructed for their use either at the microscopic, plant, leaf or organ level or for remote sensing of chlorophyll fluorescence. Using the sensitive camera to image the fluorescence signal can be useful to observe the photosynthetic responses at sub-cellular levels, in plant cells, tissues, leaves or other plant organs, as well as at the whole plant level. In addition to integrated data, the images provide precise visual information about photosynthetic performance at the different level of organization. The fluorescence imaging technique makes possible the observation of the spatial and temporal heterogeneities resulting from variation in internal and/or environmental factors on photosynthesis over the large observed area. The conventional point measurements can just barely (or not at all) detect the heterogeneities easily detectable using the chlorophyll fluorescence imaging.

Chlorophyll fluorescence imaging techniques can be used also for some special applications. The responses of stomata, especially the heterogeneity and dynamics of stomatal opening and distribution can be well identified. In addition to numerical assessment, the images can be created in false colour palates to encode the areas differing in stomatal opening by different colours in a sufficient pixel resolution of the images. Such images correspond to topological maps identifying the heterogeneity of the values of the measured parameters across the sample. Hence, imaging approaches may help to avoid the imperfection typical for the point measurements of chlorophyll fluorescence that are responsible for many incorrect or imprecise results. The imaging technique can be easily used for the analysis of different protocols, such an induction curve or light curve, including the calculation of quenching parameters and parameters of energy partitioning in the dark- or light-adapted state. The visible spectra of PAR is commonly used for excitation of chlorophyll fluorescence, but application of other spectra (mostly UV-radiation) is also possible, providing a specific type information, different from PAR excitation. Commonly, three types of light have to be used, i.e. pulse-modulated measuring light, actinic light for continuous light exposition and saturating light pulses. Similar to point measurements, the five key fluorescence levels are used to calculate fluorescence parameters for each measured pixel: Fo, F_0', Fm, Fm' and Fs'. Technical limitations in some of devices disable the direct Fo' measurement by far red light; therefore, Fo' has to be calculated using the formula. The commercially available fluorescence imaging devices provide the full operating possibilities, including programming of any common light curve, induction curve and recovery) or user-defined protocols. Typical sets of fluorescence parameters that can be achieved using automated chlorophyll fluorescence imaging are shown in Table 7. The sensitivity of the chlorophyll fluorescence imaging to the stress effects was previously documented by many studies. Drought stress led to the heterogeneous distribution of values of chlorophyll fluorescence parameters on the leaf surface.

Table 7: The fluorescence parameters provided automatically by the FlourCam® imaging system as a part of automated phenotyping facility of SAU Nitra.

Fluorescence parameter	Definition
F, F'	Steady state fluorescence emission from dark- or light- adapted leaf, respectively
Fo, Fo'	Minimal chlorophyll fluorescence intensity measured in the dark- or light-adapted state, respectively
F_m, F_m'	Maximal chlorophyll fluorescence intensity measured in the dark- or light-adapted state, respectively
F_v, F_v'	Variable chlorophyll fluorescence (F_m-Fo) measured in the dark- or light-adapted state, respectively
F_q'	Difference in fluorescence between Fm' and F'
Fv/Fm	Maximum quantum yield of PSII photochemistry measured in the dark-adapted state
F_p	Peak fluorescence during the initial phase of the Kautsky effect
Rfd	Fluorescence decline ratio in steady-state (F_p - F')/F'
Φ_{PSII}	PS II operating efficiency; effective quantum yield of photochemical energy conversion in PSII (Fq'/F_m')
NPQ	Non-photochemical quenching (F_m/F_m') -1
qL	Fraction of PSII centers that are 'open' based on the lake model of PSII (F_q'/F_v')(Fo'/F')
ETR	Electron transport rate

Plant breeding represents one of desired future applications of fluorescence imaging, such as high-throughput screening of varieties resistant to biotic and abiotic constraints. Up to now, the evaluation of disease or stress resistance in breeding programs is done mostly using visual scoring by skilled breeders; in addition to high time requirements, this approach can generate bias between experimental repeats and evaluations of different experts. To reduce the time requirements and to improve the objectivity, advanced high-throughput phenotyping tools are needed. A strong point of chlorophyll fluorescence imaging is the fact that it can be used to screen a large number of plants in a short time. Moreover, it can be integrated in robots for automatic analyses).

In addition to previously mentioned, the imaging was used to show the effects of herbicides or the herbicide induced accumulation of reactive oxygen species (ROS) in plant tissues. Chlorophyll fluorescence imaging also identified heterogeneities caused by chilling stress, induction of photosynthesis, wounding, fungal diseases, viral infections, nutrient stress, senescence, drought, and ozone stress. Chlorophyll fluorescence imaging enables a study of the interactions between leaf structural properties and environmental conditions, directly related to photosynthetic assimilation.

The challenge using fluorescence imaging is to process all the data collected in a scientifically meaningful way. As an example of a possible solution, the data can be analysed by frequency distribution parameters.

There are also several limitations of chlorophyll fluorescence imaging. For example, for reliable imaging measurements, it is critical that the whole sample area is illuminated homogeneously - this is, however, very difficult to achieve in larger plots. Moreover, the positions of leaves (leaf angle, distances of the leaf from the light sources) can cause large heterogeneity of illumination of the samples. Whereas the values of the maximum quantum yield of PSII photochemistry can be correct (except the parts of plants in which the incident light of saturation pulses will be below the saturating level), the values of efficient quantum yield of PSII photochemistry (<&PSII) and ETRpsn can be partially overestimated in the positions with lower incident actinic light intensities. Despite some risks, the chlorophyll fluorescence imaging represents an emerging technique with a high potential for practical use.

2.3.3 Spectral Sensing

Spectral remote sensing methods assess changes in plant spectral signatures, due to disease, abiotic stress, or ontogenetic development, within and outside the visible part of the electromagnetic spectrum. Plant optical properties are characterized by three processes: (1) transmission through the leaves and stems, (2) absorption by chemicals inside tissue (e.g. metabolites, water, pigments, proteins, cellulose, lignin), and (3) reflectance from inside tissue structures and from a leaf surface. Plant spectral signatures are therefore always a complex combination of these three processes. Most optical sensors do not measure plant physiological parameters, but instead measure the sum reflectance of various plant tissue and metabolic products. Spectral imaging sensors can obtain the spectral absorption and reflectance characteristics of crops, which can be used to monitor the crop planting area and crop growth, to evaluate the biological and physical characteristics of a crop, and to predict crop yield. Plants have different defense mechanisms for preventing entrance and colonization pests and pathogens, such as induction of hypersensitive reactions, production of antimicrobial metabolites and proteins, and plant tissue structure. These changes lead to highly specific changes in reflectance. A simple examplified form, Spectral reflectance from plant or crop tissue is inversely related to the chlorophyll content and relies on the interaction when light penetrates tissue, where it can be absorbed, reflected from the surface or transmitted through the leaf and dependent leaf pigment content. In a healthy plant, the maximum absorption spectrum is generally found in the blue spectral region (400-500 nm and the red spectral region of chlorophyll band (660-680 nm) and hence reflecting most of green and infrared light, making it appear green to human eyes. In case of stress scenario, for e.g nitrogen deficiency reduces leaf chlorophyll concentration leading to lower light absorption and higher reflectance in the visible or infrared range. Thus these spectral signatures can help us in understanding the reflectance mode for different crops, different varieties and different stress acenarios. The most commonly used sesning su=ystems include, Multispectral and hyperspectral imaging sensors which can be deployed under controlled, fixed and mobile field based and UAV based systems to obtain and characterise crops and varieties based on their spectral signatures.

2.3.3.1 Multispectral Imaging/sensing

Multispectral imaging sensors are defined as hardware that are capable of sensing and recording radiation from invisible as well as visible parts of the electromagnetic spectrum, which have been widely used for crop phenotyping due to the advantages of low cost, fast frame imaging and high work efficiency; however, they are limited by the low number of bands, low spectral resolution, and discontinuous spectrum

2.3.3.2 Multispectral and Hyperspectral imaging of light reflectance

The development of new, more accessible cameras and sensors has enabled the spread of use of hyperspectral imaging of light spectral reflectance from remote sensing applications into plant phenotyping. The absorption of light by endogenous plant compounds is used for calculations of many indices which reflect the composition and function of a plant. A typical example is the normalized difference vegetation index (NDVI), which was originally developed to estimate plant chlorophyll content. Another example is the photochemical reflectance index (PRI), which can be used to estimate the photosynthetic efficiency. The absorption of compounds (e.g., chlorophylls, carotenoids, anthocyanins, water, lignin, etc.) at a given wavelength can be used for direct estimation of their concentrations in the plant. For practical reasons, measurement of absorbance is replaced by measurements of reflectance.

The simple NDVI analysis as discussed in the first example can be carried out using low cost multispectral cameras. These are sensors that are capable of sensing and recording radiation from invisible as well as visible parts of the electromagnetic spectrum, which have been widely used for crop phenotyping due to the advantages of low cost, fast frame imaging and high work efficiency; however, they are limited by the low number of bands, low spectral resolution, and discontinuous spectrum.

A slightly more sophisticated range of these sensors include hyperspectral sensors which have more narrowly placed wavebands for higher spectral resolution. Depending on the measured wavelengths of reflected signal, various detectors are used. The most frequent are the VNIR (350-1200 nm) detectors, less abundant are the SWIR (short-mid wavelength infra-red region; 1000-2500 nm) detectors. Both wavebands are valuable for plant phenotyping. The reflectance signal can be detected at selected wavelengths or separated spectral bands (so-called multispectral detection). The whole spectral region can also be measured even for each pixel when cameras are applied and the hyperspectral imaging is carried out (Figure 4). Whereas the hyperspectral imaging in the VIS-NIR spectral region is used for evaluation of several indices as mentioned above, the SWIR spectral region is mainly used for the estimation of the plant's water or lignin content. Despite the many indices that have been defined so far, based on the reflectance measurements, it is difficult to assess them accurately. For this reason, critical revision of all reflectance indices is needed to evaluate which of them provide the required information in the best way.

In recent years, hyperspectral imaging (HSI) has been widely accepted as a nondestructive, rapid and safe method of qualitative analysis of plants. Spectral data can present much information about the object state. Usually, the dataset is very large and needs to be analysed by appropriate multivariate and machine-learning methods. The investigated spectral parameters of leaf tissue are estimated non-destructively and interpreted with various methods, e.g. principal component analysis. The following ways of HSI applications are the most frequent:

- Analyses of leaf pigments (chlorophyll, carotenoids, and anthocyanins).
- Spectral reflectance is also known as a fast method for determining nitrogen levels in plants. The general principle of spectral analysis involves reflectance values measured at different wavelengths. Nitrogen content is predicted from linear dependence of reflectance and reference values of leaf nitrogen content.
- Hyperspectral and fluorescent imaging provides a means to directly and non-invasively detect and quantify secondary metabolites such as flavonoids and terpenoids.
- Hyperspectral imaging has shown high effectiveness for assessing fruit and vegetable quality and their safety regarding surface defects, contamination, starch index, bruising, sugar content, freeze damage, firmness and bitter pits. Defect detection with HSI analysis is based on identifying the spectral trait wavelengths for the defect using these spectral parameters.

- HSI was found to be useful for automatic detection of pest and disesae infections
- Classification models have been established to detect insect damage. Fruit analysis after hyperspectral imaging has shown better detection ability compared to the standard conventional visual investigation.
- The moisture content and colour of surface are also needed for estimation of fruit and vegetable quality.
- HSI has been used to classify crop seeds including maize, barley, rice, oat, soybean and wheat seed for the presence of weed seeds.
- HSI at the leaf level is proven to be relevant for the estimation and quantification of pest and fungal invasion. As a non-destructive diagnostic tool HSI has high potential.

2.4 Non-destructive analysis of growth and physiology by fast manually operated techniques

Although automated systems represent the best choice for phenotyping, an alternative, cheaper way to assess specific traits related to plant phenotype and physiological responses are the modern hand-held tools with low cost and low labour demand. We propose some of them, which are routinely performed at SUA Nitra.

2.4.1 Fluorescence excitation ratio method to assess flavonoid, anthocyanin, chlorophyll and nitrogen content of plants

Chlorophyll a fluorescence represents the re-emission of light absorbed by photosynthetic pigments with emission spectra in the red to far-red region (600-800 nm), with peaks at ~680 and -730 nm. Although the emission of chlorophyll fluorescence is directly related to the photochemical activity running on the thylakoid membranes in the chloroplast (see chapter on Fluorescence Imaging), the fluorescence signal is strongly influenced also by optical properties of plant tissues not directly related to photochemical processes. It was shown, however, that adjustment of leaf optical properties is not purposeless, but usually serves as protection of photosynthetic structures. Thus, in addition to others, an important defence mechanism against the deleterious effects of solar radiation involves synthesis of relatively stable compounds that serve as light screens and/or internal traps. Depending on concentration in cells and tissues, the protective compounds reduces the fraction of radiation absorbed by light-sensitive cell components, and thereby diminishes light-induced damage. Probably the most important position among compounds providing the passive photoprotection (screen) in plants are flavonoids and anthocyanins.

The phenolic compounds, (flavonoids, hydroxycinnamic acids) have absorption maxima in the UV part of the spectrum. The flavonoids are located either in the epidermal vacuoles, cell walls, or dissolved in epicuticular wax. They have absorption maxima around 260 nm (isoflavones, flavanones), 320 nm (hydroxycinnamic acids), 260 nm and 340 nm (flavones) or 360 nm (flavonols), although the relative importance of the different phenolic compounds as the UV-screen remains an open question.

Based on the strictly UV-absorbing properties, the effects of phenolic compounds on visible light-induced chlorophyll fluorescence is negligible, whereas their presence strongly suppress the chlorophyll fluorescence emission under UV excitation. This phenomenon has been successfully applied for estimation of transmittance of UV radiation by chlorophyll fluorescence. As experiments have confirmed that the phenolic compounds in the epidermis are responsible for most of the UV-absorption of the leaf, the ratio of visible light-excited to UV-excited chlorophyll fluorescence can serve as an indirect measure of content of the UV-absorbing phenolic compounds in leaves, as shown by the model presented (Figure 8).

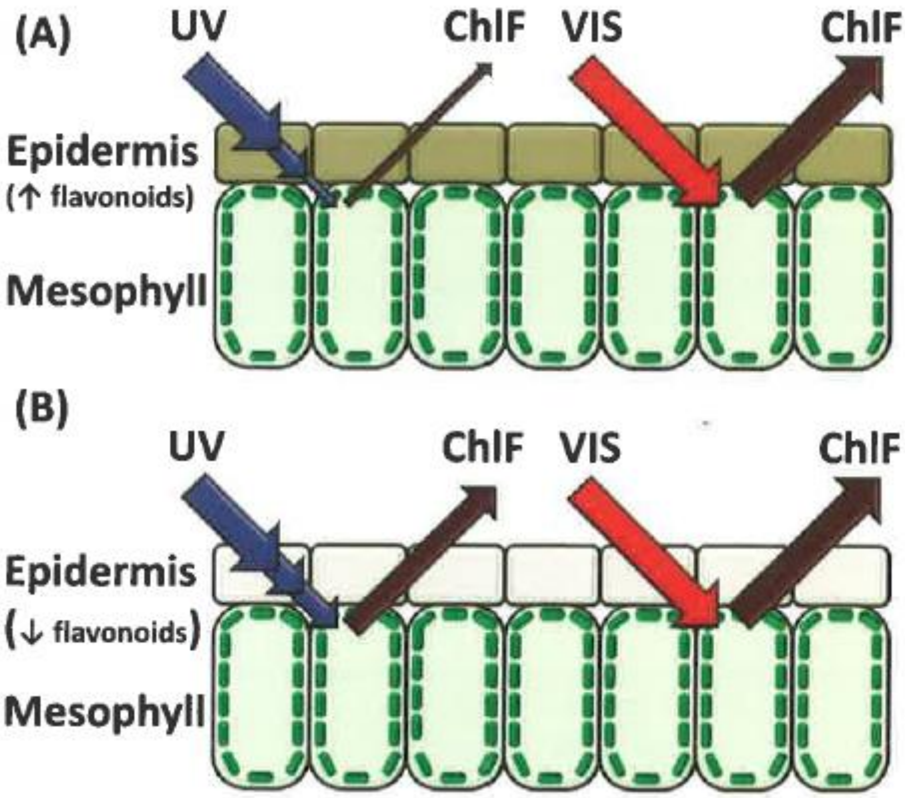


Figure 8: A schematic drawing of the adaxial part of a leaf cross-section illustrating the principle of the chlorofyll fluorescence (ChlF) method for assessement of content of UV-absorbing compounds in the sample with high (A) and low (B) flavonoid content. The thickness of the beams indicates relative intensity. The figure created by the authors is published in Sytar et al. (2016).

In parallel, anthocyanins are water-soluble vacuolar pigments of higher plants. They are responsible for the red coloration of plant tissues, especially in fruits but can also occur in plant leaves. In many cases, significant accumulation of anthocyanins is induced as a result of environmental stresses such as low temperature, nitrogen and phosphorus deficiencies, UV-B stress, drought, pathogen infections, or due to toxic effects. Anthocyanins absorb strongly in the green region of the spectrum. The spectral band around 550 nm (green) is sensitive to anthocyanin content. Thus, similarl to flavonoids, the ratio of red (or blue) light-excited to green light-excited chlorophyll fluorescence can serve as an indirect measure of anthocyanin content in plant samples, as shown in the model (Figure 9).

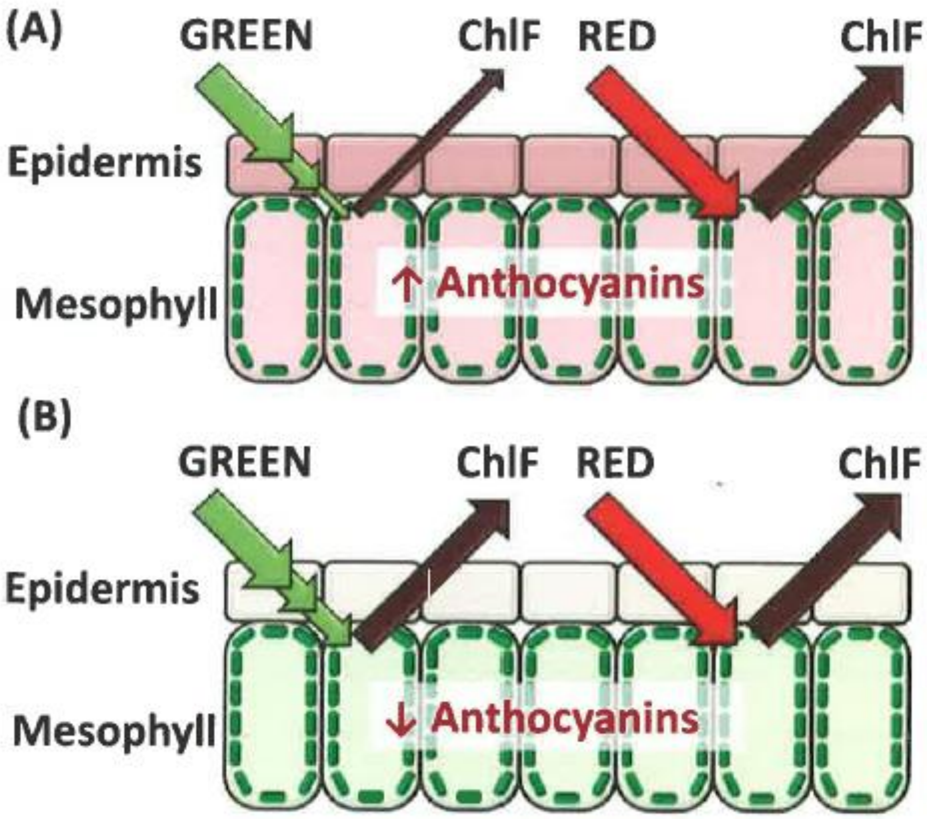


Figure 9: A schematic drawing of the adaxial part of a leaf cross-section illustrating the principle of the chlorofyll fluorescence (ChlF) method for assesement of content of anthocyanins using simultaneous green and red excitation in the sample with high (A) and low (B) anthocyanin content. The thickness of the beams indicates relative intensity. The figure created by the authors, is published in Sytar et al. (2016).

In previous decades, the numerous studies examined and confirmed the possibility of using the chlorophyll fluorescence signal in estimation of phenolic and anthocyanin contents. In addition to self-constructed devices or standard fluorometers combined with external light sources and filters, which have been used in the majority of studies, factory-made special devices for this purpose have also been introduced.

The research group of Z. Cerovic (France) developed several devices using the principle of multispectrally induced chlorophyll fluorescence described above. In principle, they introduced two types of devices: leaf clip-based instrument (commercially available under trademark Dualex, Force-A, France) as well as the non-contact type of instrument (under trademark Multiplex, Force-A, France) (Figure 10).

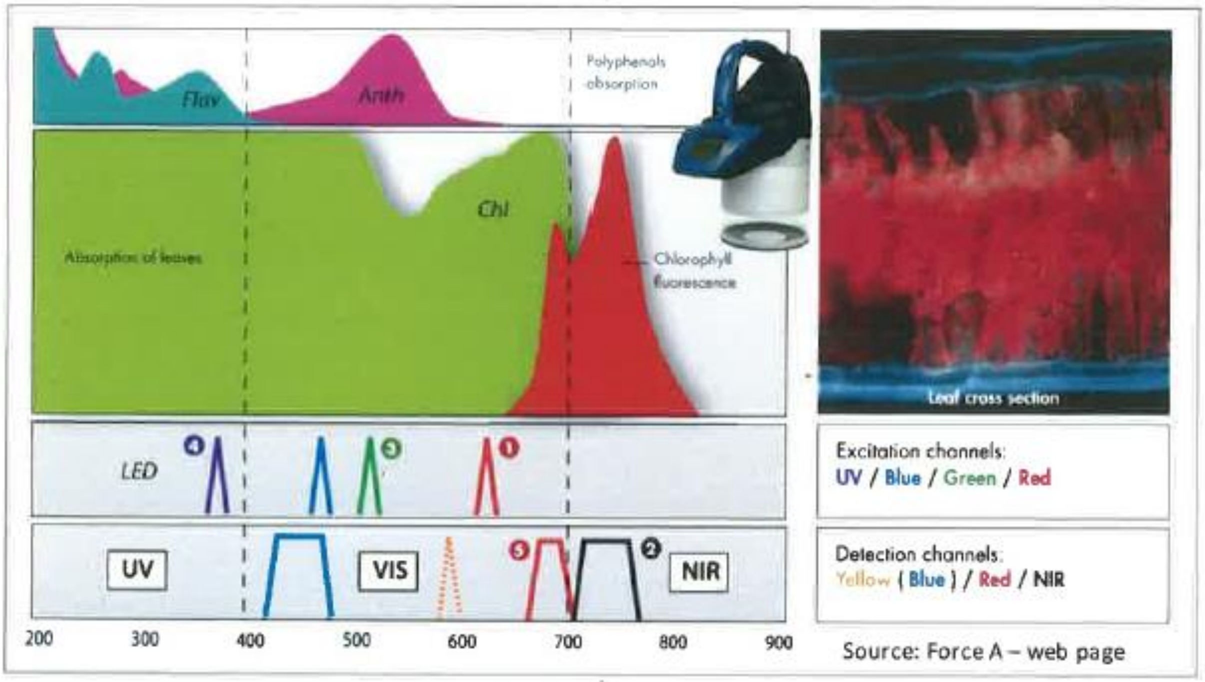


Figure 10: A scheme of the wavelengths emitted by the LED-units of Multiplex-3 device (Force-A, France), and detected by 4 detector units of the device, which serve to estimation of flavonoid, anthocyanin, chlorophyll and nitrogen contents in leaves and/or fruits.

While the Dualex system measures only two signals (UV and VIS-light induced chlorophyll fluorescence), several kinds of Dualex are produced, specialized for estimation of UV-absorbing compounds (flavonoids), anthocyanins or chlorophylls. In contrast, the Multiplex system measures simultaneously different fluorescence signals after excitation under several spectral regions of light (UV, blue, green, red excitation); thus, this system enables estimation of flavonoid, anthocyanin, chlorophyll and other information from a single measurement. Thanks to the fact that the Multiplex system does not need any leaf clip, it can be used for measurements even with objects other than flat leaves, e.g. fruits, stems, flowers, etc. This makes this system especially useful for special applications, potentially also in automated systems (as it needs no direct contact with plants).

Moreover, the ratio of fluorescence signal measures in parallel at different wavelengths can serve to estimate the chlorophyll content and, in combination to FLAV signal, provide an estimate of nitrogen content, as SFR decrease and FLAV increase in nitrogen deficient conditions, hence, the ratio NBI =SFR/FLAV represent a good and reliable indirect estimate of the level of nitrogen nutrition.

In summary, the Multiplex-3 device (Force-A, France) measures:

- Total flavonoid content determined as the **FLAV index,** derived from UV absorption properties of flavonoids.
- Total anthocyanin content determined as the **ANTH index,** derived from green-light absorption properties of anthocyanins.
- Total chlorophyll content determined as **SFR index** derived from red/far-red fluorescence,
- Estimate of the level of nitrogen nutrition as **NBI index** (nitrogen balance index), Based on SFR and FLAV indices.

2.4.2 Analysis of fast fluorescence kinetics by the JIP-test

As the methods based on saturation pulse analysis are relatively time-consuming, a big effort has been applied to develop a more efficient way of measurement of photosynthetic performance and environmental effects. In recent decades, the exponential increase of the studies applying fast fluorescence kinetics has been observed. Chlorophyll fluorescence induction represents a plot of measured fluorescence intensity as a function of time of continuous illumination (Figure 11).

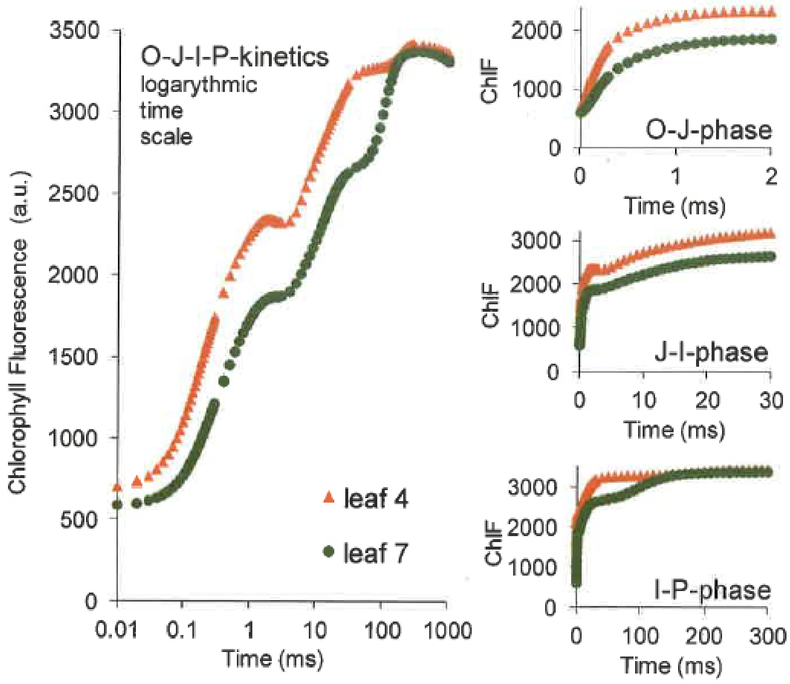


Figure 11: Examples of O-J-I-P-curves recorded in two different leaves of barley (*Hordeum vulgare* L. cv. Kompakt) in post-anthesis stage. The leaves are numbered in the order in which they appeared. Leaf 7 represents a penultimate leaf (the second leaf from the top, well exposed to sun), leaf 4 (an older leaf, below the others) was almost completely shaded inside the canopy. The main graph (left) shows the entire O-J-I-P-kinetics plotted on a logarithmic time scale. The small graphs (right) show individual phases plotted on a regular time scale: O-J phase in time o -2 ms, J-I phase in time 2-30 ms, and I-P phase in time 30-300 ms. Data of the authors published in Zivcak et al. (2017).

Such a curve recorded under continuous light has a fast (less than one second) exponential phase, and a slow decay phase (duration of a few minutes). The rise has a typical polyphasic shape, clearly evident when the curve is plotted on the logarithmic time scale, or if the individual steps are plotted separately, at different time resolution (Fig. 8). The shape of OJIP-transient is sometimes denoted as a 'fingerprint' of a sample of a given physiological status; any deviation of the curve indicates photochemical changes at the thylakoid membrane level. The analysis of OJIP curves taking the theoretical assumptions and probabilities derives different photosynthetic parameters for the dark adapted state of the photosynthetic systems. The nomenclature for 'OJIP' is as followed: O for origin or Fo level measured at 50 us (or less) after illumination, J and I represent intermediate states measured after 2 ms and 30 ms, respectively, and P is the peak or Fp = Fm (maximal fluorescence). This is valid only if sufficient light intensity is used. In heat-stressed samples, another peak occurs between Fo and Fj at app. 300 us, which is usually called K-step; therefore, some authors call the fast chlorophyll fluorescence induction the OKJIP-curve or transient curve. The OJIP curve from Fo to Fm is correlated with the primary photochemical reactions of PS II and the fluorescence yield is controlled by a PS II acceptor quencher (the primary quinone acceptor, QA). Thus, the OJIP transient can be used for estimation of the photochemical quantum yield of PS II photochemistry, and electron transport properties. The OJIP fluorescence curve analysis can be used to monitor the effect of various biotic and abiotic stresses, and photosynthetic mutations affecting the structure and function of the photosynthetic apparatus. There are several groups of parameters derived from the fluorescence rise. In addition to the basic fluorescence values and fundamental parameters, such as Fo, Fm, Fv/Fm (similar to the saturation pulse method), there is also a group of parameters derived from the JlP-test, introduced by Strasser and Govindjee (1992). We can divide it into the fluorescence parameters derived from the data extracted from OJIP transient and the biophysical parameters calculated using the previous group of fluorescence parameters. In plant stress research there are several possible ways of interpreting the data. A multiparametric approach is based on the visualization of data e.g. by spider plots or pipeline models. On the other hand, the model offers the integrative parameters enabling simple assessment of the status and vitality of the photosynthetic apparatus, which are sensitive and created mostly for possible practical applications in pre-screening or selection in research and breeding programs.

From numerous JlP-test parameters, for practical applications in the crop research **Performance Index (PI)** was also introduced. This complex parameter integrates several independent structural and functional properties of the photochemistry, reflecting the functionality of both photosystems II and I and providing quantitative information on the current state of plant performance under stress conditions.

However, the current level of knowledge does not entitle us to draw further conclusions about photosynthetic performance based on fast chlorophyll fluorescence only. Even usefulness of the fast chlorophyll fluorescence for leaf photosynthetic performance testing could be proven in the future, more probably, the method will remain mostly a tool for assessment of the stress effects on the photosynthetic functions. In this respect, the availability of user-friendly portable fluorometers for high-frequency record of OJIP-transient and the useful software for the analysis of experimental data, make the JIP test derived from the fast chlorophyll fluorescence attractive even for users without a deep knowledge of photochemical processes at the thylakoid membrane level. As we have mentioned above, the small and portable devices allow efficient data records even under field conditions. The chlorophyll fluorescence induction kinetics contains valuable information about the photochemical efficiency of primary conversion of incident light energy, electron transport events, and related regulatory processes. These issues can be deciphered using advanced mathematical models based on the analysis of fluorescence curves, providing a large number of fluorescence parameters. They can be divided into:

- Parameters directly derived from fluorescence data (Fo, Fm, Fv, Area),
- Specific quantum yields, i.e. energy fluxes per absorbed light spectra (TRo/ABS, DIo/ABS, ETo/ABS, ETo/ABS)
- Energy fluxes per active reaction centre (ABS/RC; TRo/RC; ETo/RC, DIo/RC)
- Energy fluxes per excited cross section: (ABS/CS; TRo/CS; ETo/CS, DIo/CS)
- Density of reaction centres (RC/ABS; RC/CSo; RC/CSm)
- Probabilities of electron transport between individual steps of the electron transport chain
- Performance indices and driving forces (PI_{ABS}, PI_{TOT}, df)

All parameters are precisely and simply defined and they can be used to characterize the status of PSII photochemistry, which reflects the effects of external factors and the status (vitality) of plants.

2.4.3 Analysis of leaf chlorophyll content using chlorophyll meters

Chlorophyll content represents an important indicator of plant health status and basic information of the limitations of photosynthetic capacity. The main methods for determination (HPLC, spectrophotometric) are destructive. An alternative way is the use of chlorophyll content meters, which have been used successfully in many species to estimate leaf chlorophyll, and allows measuring of chlorophyll content on the same leaf over time. The readings from chlorophyll content meters can be also used to predict the nitrogen status of leaves and hence the efficiency of fertiliser uptake. The meters/devices that calculate chlorophyll content indices (e.g. SPAD value, CCI index) are based on measuring the reflectance, absorbance or fluorescence at particular wavelengths. The most common hand-held chlorophyll absorbance meters, of which several are commercially available, measure absorbance by the leaf at two different wavelengths of light: red and near-infrared. The red light is strongly absorbed by chlorophyll. The second is a 'reference wavelength' necessary to adjust for differences in tissue structure.

It was shown that all the chlorophyll meters available on the market are useful, but precision is not always the same in all conditions; especially, the fluorescence-based instruments have some limits. The values of different types of devices should therefore not be combined and compared.

2.5 Thermal sensors

The sensors mentioned above mainly work on the phenomena of absorption, reflectance and fluorescence, but one of the other powerful tools for phenotyping, especially for traits related to stress responses, especially water is the use of thermal imaging. The approach works on the basis of simple physics phenomena of evaporation causes cooling and in case of plants, they interact with environment through interface of “stomata”, maintaining a carbon-water and energy exchange balance and adapt to ever-changing conditions. Thus stomata play an important role in plant adaptation and growth by balancing the need to minimise water loss while maintaining photosynthetic gains. Evaporative cooling through transpiration is a major component of the leaf energy balance and thus any stomatal closure in response to drought stress, therefore, will be manifest as a warmer temperature, so that thermal imaging can be used to quantify stomatal closure (Prashar and Jones 2016).

Similar to spectral reflectance sensing, thermal sensing suffers from difficulties of background interference such that techniques are necessary to obtain a pure signal from the canopy only. These can include the overlaying of spectral images or extraction of canopy variation or thresholding. Various automated or semi-automated methods have been proposed and are used for canopy temperature extraction. Examples of use of thermal sensing include from plant level to canopy level and also include crops in various categories from small grain cereals to broad leaf crops like maize and potatoes and even fruit trees (Prashar et al 2013; Prashar and Jones 2014). Infrared thermography can also be used for plant stress detection:

1. Abiotic stresses
    a. Drought
    b. Salinity
    c. Heat and frost stress
2. Biotic stress
    a. Pathogens affecting above-ground parts
    b. Soil-borne diseases
3. Screening for indirect yield and quality traits
4. Dynamic and spatial variation in stomatal conductance

2.6 Potential use of sensors for non-destructive crop phenotyping of wheat and potato traits

There are a range of different types of sensors atht are available for use in Advance Phenotyping and the information presented in Tables 8 and 9 examines the potential for use in the phenotyping of wheat and potato respectively.

Table 8: Potential use of sensors for non-destructive crop phenotyping of wheat traits

Winter wheat	Digital	Spectral	Thermal
Crop vigour			
Growth habit	Applicable	Applicable	Applicable
Germination %	Applicable	Applicable	Applicable
Tillering ability	Applicable	Not applicable	Not applicable
Leaf Area Index	Applicable	Applicable	Applicable
Green Area Index	Applicable	Applicable	Applicable
Plant height	Applicable	Applicable	Applicable
Crop phenology			
Days to maturity	Not applicable	Potential	Potential
Days to anthesis	Not applicable	Potential	Potential
Yield related			
Yield	Not applicable	Not applicable	Not applicable
Harvest Index (HI)	Not applicable	Not applicable	Not applicable
Ear number per m2	Potential	Not applicable	Not applicable
Number of grains per ear	Not applicable	Not applicable	Not applicable
Disease %	Applicable	Applicable	Applicable
Grain quality			
Protein %	Not applicable	Applicable	Not applicable
Hectolitre weight	Applicable	Not applicable	Not applicable
Hagberg Falling Number	Not applicable	Potential	Not applicable

Applicable   Potential   Not applicable

Table 9: Potential use of sensors for non-destructive crop phenotyping of potato traits

Potato	Digital	Spectral	Thermal
Crop vigour			
Growth habit	Applicable	Applicable	Applicable
Leaf area index	Applicable	Applicable	Applicable
Plant height	Applicable	Applicable	Applicable
Crop phenology			
Days to maturity	Applicable	Applicable	Potential
Yield related			
Yield	Potential	Potential	Potential
Harvest index (HI)	Potential	Potential	Potential
Tuber number	Applicable	Potential	Potential
Average tuber weight	Applicable	Potential	Potential
Disease %	Applicable	Applicable	Applicable
Tuber quality			
Dry matter %	Not applicable	Not applicable	Not applicable
Tuber size	Applicable	Applicable	Applicable
Skin colour	Applicable	Potential	Not applicable
Eye depth	Potential	Potential	Not applicable
Cooking/baking quality	Potential	Potential	Potential

Applicable (green) Potential (orange) Not applicable (red)

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Pop quiz!

1) If there were no particles and no scattering, what colour would the sky be?
2) What colour is the Sun?
3) Why aren't plants fashionably black?
4) Why do plants like to keep their pigments fashionable and trendy even during stressful times?
5) How can remote sensing help scientists study plants without actually being physically present with them?
6) Why is it important to analyze different spectral regions, such as visible, near-infrared, and shortwave infrared, when studying plants using remote sensing?
7) Can remote sensing use anything else than electromagnetic radiation?
8) What is the prevailing source of electromagnetic radiation on Earth? This remote sensing device do you personally use to detect this energy?
9) Why do remote sensing systems often neglect or ignore the blue and UV parts of the spectrum?
10) When Legolas says: "A red sun rises. Blood has been spilt this night" what does he really mean by that? Why does the rising sun appear red?
11) You want to characterize and classify several wheat varieties using airborne remote sensing. What would be the best way of doing this?
12) What is the advantage of displaying spectral bands in an RGB colour space, compared to using individual, grayscale images?
13) You're undergoing a lifestyle change and want to change your wardrobe. Could you simply change the colour space of your clothes? Why?
14) You plan on performing aerial multispectral imaging. At what time of day would you plan data acquisition, and why?
15) If you wanted to monitor crop growth over an entire growing season, for an entire country. What type of sensor characteristics would be best for this (spatial, spectral, temporal resolution), and why?
16) You want to phenotype several potato varieties, set up in an factorial design, with several replicates. Which sensing platform and sensor would you use, and why?
17) How can plant phenotyping contribute to improving crop yields and food security?
18) What are some of the advanced technologies used in plant phenotyping, and how do they help scientists gather data about plants?
19) How can plant phenotyping aid in the development of drought-resistant or disease-resistant plant varieties?
20) You live on an isolated island, without internet and electricity or any way of sending any kind of material, and wish to send an image to a friend on a neighbouring island. How would you do this?