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Review Article

**NEAR- SPECTROSCOPY IN BIO-APPLICATION**<sup>1</sup>Raipuram Leekshitha, <sup>2</sup>.Dr. B. Poornima, <sup>3</sup> Veluru Jyoshna, <sup>4</sup> Murathoti Shireesha,  
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Renigunta Road, Tirupati, Andhra pradesh, 517506.**Abstract:**

*Near-Infrared spectroscopy (NIR) has emerged as a powerful tool in bio applications, offering non-invasive and rapid analysis of biological materials. This review explores the recent advancements and opportunities in the utilization of NIR spectroscopy in various bio-related fields. Analysis of biological tissues for disease diagnosis and monitoring. Non-invasive blood glucose monitoring, a holy grail in diabetes care, has been a focus of research, promising painless alternatives to traditional fingerstick methods. Its application has been significantly impacted by recent developments in instrumentation (such as miniaturized spectrometers) and spectrum analysis techniques (such as spectral image processing and analysis, quantum chemical calculations of NIR spectra). This review attempts to highlight NIR spectroscopy as a method that has reached maturity but has significant room for advancement in a number of dimensions across widely recognized bio-applications. Its practical value is evaluated critically and contrasted with alternative methods. The relationship between the fundamental properties and key elements of NIR spectra and the bio-application potential of NIR spectroscopy is emphasized.*

**Keywords:** NIR; NIR: analytical; bioscience; bio-spectroscopy; near-infrared spectroscopy;

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**INTRODUCTION:**

Near-Infrared spectroscopy (NIR) is a versatile and non-invasive analytical technique that has found wide-ranging applications in the field of biosciences and healthcare. It operates by measuring the interactions between near-infrared light and matter, providing valuable insights into the molecular composition and properties of biological samples. This introductory overview explores the fundamental principles and applications of NIR spectroscopy in the realm of biosciences.

Near-infrared (NIR; 1000-2500 nm; 10,000-4000 cm<sup>-1</sup>) spectroscopy can be summed up as a vibrational spectroscopy method that falls into a peculiar niche in the bioscience area. On the one hand, it has become the go-to tool for many applications involving the qualitative and quantitative evaluation of bio-related samples, such as in the analysis of medicinal plants or problems pertaining to the quality control of natural products. On the other hand, it competes with more well-known methods in a number of other fields, including Raman spectroscopy and infrared (IR, i.e., mid-infrared, MIR; 4000-400 cm<sup>-1</sup>; 2500-25,000 nm). The typical core fields include, for example, bioanalytical research and biomedical diagnosis, where NIR spectroscopy has traditionally been overshadowed by IR and Raman techniques and where there is still opportunity for advancement. The traditional focus areas include bioanalytical research and biomedical diagnosis, where NIR spectroscopy has typically lagged behind IR and Raman techniques but still has room to grow in acceptance. The entire potential of NIR spectroscopy in bio-applications hasn't been realized yet due to a number of factors, with some exceptions. Nevertheless, NIR spectroscopy is becoming more and more significant, and several recent developments have accelerated this trend. Recent studies have shown that NIR spectroscopy can be employed in novel ways to offer information that is challenging to obtain by competing approaches.

The goal of the current review is to offer a critical overview of NIR spectroscopy in a variety of bio-applications and to contrast its advantages and disadvantages. Comprehensive reviews that would try to depict the present state and promise of NIR spectroscopy in this field of study are lacking in recent publications. Due to this, it is helpful to briefly list the key parallels and divergences between these competing strategies. The physical foundation and consequences of NIR spectroscopy's primary benefits—such as the need for little sample preparation, the ability to examine damp samples, the

possibility of precise quantitative analysis—are explained. Nevertheless, a closer examination of the present restrictions won't be avoided.

The limitations of NIR spectroscopy are discussed, including its lower chemical specificity when compared to IR or Raman spectroscopy, its more difficult interpretation, which makes it harder to draw conclusions about the complex molecular makeup of biological samples, and its enhancement of certain vibrations (X-H groups) in the spectra. Depending on the application, certain qualities may be viewed as favourable or disadvantageous; in this case, deep sample penetration by NIR radiation and the evaluation of the sample in a larger volume should be noted. This makes NIR spectroscopy relatively better suited for in vivo examinations, a feature particularly important in biomedical applications. Special attention is given to evaluate the recent accomplishments in mitigating the method's limitations and improving its applicability, and its future development directions. The talk is based on a comprehensive examination of numerous NIR spectroscopy in biology applications found in published literature. The suggested classification solely serves the purpose of maintaining presentation clarity because NIR spectroscopy research frequently crosses boundaries. Review of studies on the characteristics of biological samples ranging from cells to tissues to complete organisms. Separately, body fluid analyses are examined because they are useful in many different domains, such as biological or forensic science. Due to the fact that NIR spectroscopy is a widely used tool for the examination of plant-related substances, including plant medicines, special attention will be paid to this field of applications. It is important to note the increasingly active clinical use of functional NIR spectroscopy (fNIRS) for functional neuroimaging in the field of medical diagnostics. Additionally, NIR imaging spectroscopy is a powerful method that may reveal the spatial distribution of the sample's chemical composition. Spectral imaging is very helpful for studying biological samples, and new methods are practical for keeping track of in vivo dynamical events taking place in the sample. The fundamental ideas for the very varied field of bio-applications of NIR imaging techniques cannot be fully presented in the current overview. Instead, a succinct overview and a few pertinent examples will be offered with the goal of giving a fundamental knowledge of the possibilities that these techniques offer in the field of biology. We will direct interested readers to specialized literature if they want a more thorough understanding of NIR spectrum imaging. For similar reasons, there is no introduction to data-analytical techniques, spectra pre-

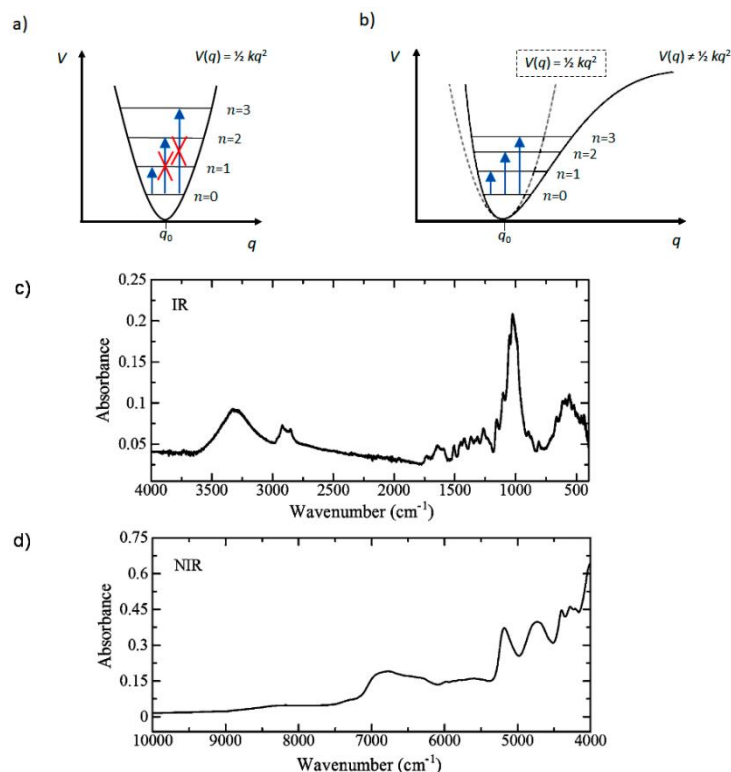
processing, or chemometrics in this paper. These tools are crucial to NIR spectroscopy, and interested readers are directed to a wealth of literature that covers these subjects, such as basic text books that explain chemometrics. A recent tutorial by Pasquini on NIR spectroscopy is noteworthy; it covers instrumentation, experimental and data-analytical tools, as well as imaging methods.

### The principle of NIR spectroscopy in the context of Applications in Bioscience

NIR spectroscopy, along with IR and Raman, is a subfield of molecular vibrational spectroscopy, which uses electromagnetic radiation to examine the vibrational (internal) degrees of freedom (DOFs) of molecules. These DOFs, which are also known as stretching and deformation vibrations (modes), respectively, correlate to oscillatory changes in the bond lengths and angles between these bonds. The characteristic frequencies of molecular oscillations match to the wavelengths of incident electromagnetic radiation, where absorption phenomena may take place, giving rise to the appearance of an absorption

band in a molecule's spectrum. One must focus on the basic distinction between NIR spectroscopy and the other approaches, though. The model of the classical harmonic oscillator, whose distinguishing feature is the approximation of the genuine vibrational potential (i.e., vibrational energy) by a quadratic function (parabola; Figure 1a), provides the most approximate description of molecular vibration. The fundamental frequencies ( $\omega$ ), or the frequencies responsible for the bands visible in IR or Raman spectra (the wavenumber area of fundamental transitions is typically given as ca. 4000–400  $\text{cm}^{-1}$ ), are best described using the harmonic approximation. Notably, fundamental torsional and skeletal vibrations can be seen in the far-infrared (FIR) region below 400  $\text{cm}^{-1}$ . When compared, NIR region (12,500–4000  $\text{cm}^{-1}$ ) comprises non-fundamental bands, overtones (e.g., first overtones  $2\omega$ , second overtones  $3\omega$ , etc.) and combination bands (e.g., binary sum combinations  $\omega_a + \omega_b$ , ternary sum combinations  $\omega_a + \omega_b + \omega_c$ , etc.). This cornerstone difference dictates distinct disparity in the applicability of NIR spectroscopy to various problems encountered in the fields of bioscience.

Figure 1. (a,b) Vibrational potential, vibrational levels and transitions of diatomic (i.e., one-dimensional) oscillator in



(a) harmonic approximation, and (b) its real (anharmonic) nature, (c,d) comparison of infrared (IR) (c) and near-infrared (NIR) (d) spectra of the same sample (wood of Douglas fir species). The symbols denote:  $V$ —the potential energy;  $q$ —vibrational coordinate;  $k$ —force constant;  $n$ —vibrational quantum number. Panels (c,d) reproduced with permission from Springe.

**Merits of NIR Spectroscopy:**

- **Non-Destructive Analysis:**
- NIR spectroscopy is non-destructive, allowing for the analysis of samples without altering their composition or structure. This is particularly advantageous when studying biological samples or precious materials.
- **Wide Applicability:**
- NIR spectroscopy can be applied to a diverse range of sample types, including solids, liquids, and gases. It finds applications across industries such as pharmaceuticals, food and beverages, agriculture, chemicals, polymers, and more.
- **Speed and Efficiency:**
- NIR spectroscopy provides rapid results, often in seconds or minutes. This speed is especially beneficial in industrial settings where real-time monitoring and quick decision-making are essential.
- **Minimal Sample Preparation:**
- Unlike some other analytical techniques, NIR spectroscopy often requires minimal sample preparation. This reduces the time, effort, and costs associated with extensive sample pre-treatment.
- **Quantitative Analysis:**
- NIR spectroscopy allows for quantitative analysis, enabling the determination of the concentration of specific compounds in a sample. This makes it highly valuable for applications such as quality control and process optimization.
- **Reduced Chemical Waste:**
- Due to its non-destructive nature and minimal sample preparation requirements, NIR spectroscopy contributes to a reduction in chemical waste generated during the analysis process.
- **Cost-Effectiveness:**
- While the initial cost of NIR spectrometers can be relatively high, its cost-effectiveness comes from the rapid analysis, minimal sample preparation, and potential for real-time monitoring, which can lead to savings in the long run.

**Pitfalls of NIR Spectroscopy:**

1. **Complex Spectra:** NIR spectra can be complex, with overlapping bands, making it challenging to deconvolute and interpret the data accurately. Advanced data analysis techniques, such as chemometrics, are often required to extract meaningful information.

2. **Limited for Specific Compounds:** NIR spectroscopy is most effective for compounds with strong and characteristic NIR absorption bands. It may not be suitable for compounds with weak or indistinct NIR features.
3. **Calibration Required:** Calibration models are needed to correlate the NIR spectra to the properties of interest (e.g., concentration of analytes). Developing and maintaining accurate calibration models can be time-consuming and requires a set of representative reference samples.
4. **Light Penetration Depth:** NIR light has limited penetration depth, typically a few millimeters, which restricts its use for samples with significant opacity or thickness. For homogeneous analysis, the sample should be optically transparent in the NIR region.
5. **Sensitivity to Environmental Conditions:** NIR spectroscopy can be sensitive to environmental conditions like temperature, humidity, and vibrations. Strict control or compensation for these factors may be necessary for accurate and reproducible results.

**Comparison with Competing Techniques:**

- **Complementary Nature:** NIR spectroscopy is often used in combination with other analytical techniques, such as mass spectrometry, chromatography, or FTIR spectroscopy, to provide complementary information and improve the overall analytical capabilities.
- **Cost and Throughput:** Compared to high-resolution techniques like mass spectrometry, NIR spectroscopy often offers higher throughput and cost-effectiveness, making it suitable for rapid analysis of a large number of samples.
- **Sample Preparation:** NIR spectroscopy generally requires less sample preparation compared to some other techniques like chromatography, which can involve complex and time-consuming sample extraction, derivatization, or pre-concentration steps.
- **Sensitivity and Specificity:** Techniques like chromatography coupled with mass spectrometry (e.g., GC-MS, LC-MS) can offer higher sensitivity and specificity for trace-level analysis of specific compounds compared to NIR spectroscopy.
- **Qualitative and Quantitative Information:** While NIR spectroscopy is well-suited for

quantitative analysis, techniques like mass spectrometry can provide both qualitative and quantitative information, making them powerful tools for comprehensive analysis.

Approximate wavenumber regions, in which NIR absorption bands may appear for biological samples, are summarized in Table 1

Wavenumber in cm <sup>-1</sup>	Weve length in nm	Vibrational mode assignment and the associated most characteristic compound
8250	1210	3 C–H str. (C–H rich compounds, e.g., carbohydrates, lipids)
7375-7150	1355-1400	2 C–H str. + C–H def. (carbohydrates, lipids)
6980	1435	2 N–H str. (proteins)
6750	1480	2 O–H str. (carbohydrates, alcohols, polyphenols)
6660	1500	2 N–H str. (proteins)
6500	1540	2 O–H str. (carbohydrates, alcohols, polyphenols upon matrix effects, e.g., hydrogen bonded OH groups)
6400	1565	2 N–H str. (proteins)
6200-5800	1610-1725	2 C–H str. (carbohydrates, lipids)
5625	1780	2 C–H str. (C–H rich compounds, e.g., carbohydrates, lipids)
5500	1820	O–H str. + 2 C–O str. (carbohydrates)
5120	1955	3 C–O str. (carbohydrates)
4880	2050	N–H sym. str. + amide II (proteins)
4825	2075	O–H str. + O–H def. (alcohols, polyphenols)
4645	2155	Amide I + amide III (proteins)
4440	2255	O–H str. + O–H def. (carbohydrates, alcohols, polyphenols)
4360	2295	N–H str. + CO str. (proteins)

**Table 1.** Approximate positions of NIR bands that are meaningful for samples of biological origin (excluding water). In brackets provided are the exemplary chemical compounds for which the transitions are specific.

## Overview of Applications

### Analysis of Body Fluids

#### Blood

The examination of blood has been one of the earliest applications of NIR spectroscopy in medical diagnosis. Early adoptions include the investigation into oxygenation level of metabolites in blood pioneered by Jobsis in 1977 [20]. The development of NIR spectroscopy for the purpose of blood analysis has accelerated in the nineties with numerous trend-setting studies. The available instrumentation and proposed methods enabled more quantitative and direct measurements. For example, as demonstrated by Ozaki et al. in 1992, NIR absorption provides accurate information for the determination of deoxy hemoglobin concentration in venal blood. As the result of these early advances, NIR spectroscopy is nowadays a well-established tool for the analysis of blood and body fluids. Nonetheless, it remains an active and continuously progressing area of research.

#### Glucose in Blood

Although fairly matured, the analysis of blood glucose by NIR spectroscopy continues to be an intensively

studies topic with several recent literature reports reflecting the attention given to this field and the dynamics of the research. Recently, Uwadaira et al. re-evaluated in detail the suitability of the absorption bands in LW-NIR (long wavelength) vs. SW-NIR (short wavelength) for blood glucose examination. Typically, the LW-NIR region (ca. 7700–4000 cm<sup>-1</sup>; 1300–2500 nm) is used in blood glucose analysis. LW-NIR consist of the combinations or the first overtones of the OH, CH, and NH modes (Table 1). These bands are sharper than those in SW-NIR, which arise from higher overtones and combinations. However, the absorption index of LW-NIR bands is notably higher, and therefore, only shallow penetration depths is effectively achieved. Often this limitation makes mostly interstitial fluid in the epidermis layer of the skin being measured instead of deeper located tissues and blood vessels. In SW-NIR, however, there appears an absorption gap between visible absorption of hemoglobin and NIR absorption of water, creating an optical window for sensing the deeper layers of the tissue. The studies by Uwadaira et al. demonstrated that the correlation between SW-NIR spectra and blood glucose content is sufficient to successfully



construct calibration models based on partial least squares (PLS) regression. However, this could reliably be done only for individual subjects. It was observed that instead of directly using the change of blood constituents, a more effective for the development of a successful calibration model was body mass index (BMI).

Interestingly, BMI seemingly affects the physical measurement conditions for blood glucose level. However, for certain subjects the individual calibration models were unsuccessful, leaving room for improvement towards robustness of this method. With aim to address this issue, the authors continued their research and proposed a simple approach in which a large data set containing ca. 400 carbohydrate tolerance tests (CTTs) was used. It suits systematic evaluation of every wavelength in NIR spectrum, if a direct correlation between blood glucose level and NIR absorbance can be determined. This approach successfully established NIR wavelengths at which absorption is strongly correlated to the blood glucose level. However, daily fluctuations even for the same person were observed at these wavelengths. As reported, the method is reliable for a 2-h analysis period. Therefore, further advances are needed to develop a more robust and universal prediction model. Further studies from other groups aimed at determining the informative wavelengths in the NIR region for non-invasive blood glucose prediction should be noted, e.g., Yang et al, or Suryakala and Prince who used PLS regression and principal component regression (PCR) models for this purpose. Non-linear regression by means of artificial neural network (ANN) has also been evaluated and compared with PLS regression by Jintao et al. This demonstrates well the room for improvement that still exists in this seemingly well-established application field of NIR spectroscopy. Much attention is paid to refining the chemometric analysis in this application. Improvements in accuracy and robustness are proposed, e.g., towards reducing the number of independent variables in calibration models for glucose concentration and correcting the individual physiology-related differences and dynamics of glucose, as reported by Dai et al. A set of two ANNs combined with particle swarm optimization (PSO), was proposed as a nonlinear calibration approach to this problem. The weight coefficients of the two ANNs, which represented the differences between individual and daily physiological rules, were optimized by PSO. This strategy successfully overcame individual differences and physiological glucose dynamics and thus, enhanced the robustness of predicting glucose concentration in blood. It is

worth to highlight the progressing applicability of NIR spectroscopy as a competing technique in the medical applications typically dominated by other tools. For example, blood-oxygen-level-dependent contrast functional magnetic resonance imaging (BOLD-fMRI) is a favoured technique for detection of brain cancer. Nonetheless, this method faces some limitations. In some cases of brain disorder in previous studies, e.g., stroke and brain tumour, BOLD-fMRI diagnosis had been demonstrated to produce incorrect image of activation areas. Sakatani et al. adopted NIR spectroscopy for improving the diagnostic reliability in such cases. The study compared NIR spectroscopy and BOLD-fMRI results of functional brain activation in patients. The characteristics of the cerebral blood oxygenation (CBO) changes corresponding to stroke and brain tumors were monitored by both techniques. Essentially, NIR spectroscopy offered a major improvement and delivered good diagnostic performances in the cases, where BOLD-fMRI performed unsatisfactorily. The study evidenced that application of NIR spectroscopy leads to superior accuracy and reliability in the functional imaging of diseased brains, in the cases where established techniques face limitations.

#### **Blood Oxygen Level**

In spite of this field being one of the earliest adoptions of NIR spectroscopy in medical diagnosis, recent literature indicates that it remains an active area of research with novel concepts being proposed. The specificity and sensitivity of NIR absorption to hemoglobin creates rich opportunities for non-invasive diagnosis. Recently explored directions include NIR imaging techniques for real-time in vivo visualization of the chemical distribution of Hemoglobin with differing properties. For instance, Mehnati et al. [29] developed a method of NIR multispectral imaging for optical differentiation of vessels according to hemoglobin concentrations. Successful application of this methodology for locating targeted vessels for mammography was limited, as the discrimination between vessels with various Hemoglobin concentrations needs to be further enhanced. Nonetheless, good image contrast was obtained, with promising prospects for early diagnosis and pre-screening breast cancer.

Nioka et al. developed an approach based on NIR spectroscopy for breast tumor diagnosis in patients undergoing biopsy. Continuous SW-NIR spectroscopy was employed to measure blood volume and blood hemoglobin concentration. The study aimed to verify whether angiogenesis and hypoxia are meaningful factors for cancer detection. This would be possible, if

the correlated parameters, the total hemoglobin content and oxygen saturation, can be used as biomarkers for those clinical conditions. The study revealed, that by monitoring high total hemoglobin and hypoxia scores, the sensitivity and specificity of cancer detection could be maintained at 60.3% and 85.3% levels, respectively. It was concluded that smaller-size tumors are more challenging for detection by NIR spectroscopy, whereas ductal carcinoma in situ (DCIS) can be detected using configurations presented in the study. It was noted that for larger-size tumour, a significantly higher deoxygenation occurs in DCIS than in benign tumour.

Interestingly, skeletal muscles can also be analysed to obtain useful information on the oxygen level in blood. This direction was recently discussed in detail by Chatel et al

Medical applications do not solely require the analytical tools for determination of blood oxygenation in vivo. A good example is the non-destructive measurement of haemoglobin in blood bags, as reported by Zhang et al. where this was accomplished using multipath-length VIS-NIR spectroscopy. A major difficulty in such application is the complex spectral and optical properties of blood bag material which require sophisticated approaches to yield accurate information on the contained blood. Noteworthy is the method based on refractometry for the analysis of blood oxygen level. Interestingly, not only blood absorption but also refraction can be used for this purpose. The refractive index of the haemoglobin solution is indicative for its oxygenation. Lazareva et al. accomplished good results through measurements of the refractive index values at 480, 486, 546, 589, 644, 656, 680, 930, 1100, 1300, and 1550 nm wavelengths covering the visible/near-infrared (NIR) region. Laser emission lines and multi-wavelength Abbe refractometer were employed for measuring hemoglobin aqueous solutions of different concentrations. Hemoglobin was obtained from human whole blood. The study reported specific increments of refractive index correlated with hemoglobin concentration from which the Sellmeier coefficients were calculated.

### Blood Stain

Blood stain analysis, in forensics, largely benefits from on-site capable methods. The miniaturized, affordable NIR spectrometers available nowadays, suit this role perfectly. Therefore, major focus is currently given to the adoption of the portable devices for this role. For instance, Pereira et al. developed a non-invasive, non-destructive method based on a hand-held NIR sensor

for confirmatory and in situ identification of dry blood stains on different substrates. The samples included human and animal blood stains. Additionally, for simulating potential false positives, stains from different commercial products that may resemble blood stains were used. The study involved a sophisticated suite of data-analytical methods. Pre-processing methods (standard normal variate, SNV; and normalization by range) preceded application of several supervised pattern recognition methods. The highest accuracy for discriminating human blood stain was achieved with the soft independent modelling of class analogy (SIMCA) algorithm, with resulting 100% correct classification for porcelain and glass, 80% for metal, and 90% for ceramic as substrates. Comparative methods involved PLS discriminant analysis (PLS-DA), genetic algorithm-linear discriminant analysis (GA-LDA), and successive projection algorithm-linear discriminant analysis (SPA-LDA). This demonstrated the suitability of on-site NIR spectroscopic detection of human blood stains on various substrates.

### Haemodialysis

Hemodialysis can successfully be monitored in detail by IR and NIR spectroscopy as reported recently by Henn et al. The authors evaluated the suitability of both spectral techniques and compared their performances when used in hyphenation with PLS regression. The aim was to analyse quantitatively the blood constituents such as urea, glucose, lactate, phosphate and creatinine. These are important markers for the process of detoxification, particularly in ambulant dialysis treatment. The study aimed to compare IR and NIR techniques to determine the targeted molecules quantitatively in artificial dialysate solutions. These methods were directly assessed in accuracy by means of statistical errors determined in PLS regression analysis based on the same sample set. Noteworthy, the study included a detailed analysis of the wavenumbers meaningful for this purpose in both IR and NIR regions. The authors dissected the structure of the PLS regression coefficients vector developed for quantification of the target analytes in the sample on the basis of IR and NIR spectra (Figure 2). This detailed analysis unveiled that there are relatively few NIR meaningful wavenumbers in the regression models of glucose and urea concentration in artificial dialysate solutions. Interestingly, these wavenumbers are located in the regions free from strong absorption bands of water (Figure 2I). In order to take account the variations in the concentration levels during dialysis, a multilevel/multifactor design was employed. The conclusions from those results were that IR spectroscopy is better suited to analyse

the blood constituents; urea, glucose, lactate, phosphate and creatinine. This technique employed in a multi-reflection attenuated total reflection (ATR) mode enables a reliable prediction of all five target analytes under investigation. At the same time, NIR spectroscopy was successful only in the determination of urea and glucose. However, NIR spectroscopy provides considerable practical advantages such as easy sampling, or potential use of miniaturized sensors. Nonetheless, for both IR and NIR analyses,

the coefficients of determination  $R^2$  in PLS regression of at least 0.86 were achieved, as determined in the test-set validation (TSV) procedure for urea and glucose. The method applied to the analysis of lactate, phosphate and creatinine performed well in the IR region with  $R^2 \geq 0.95$  using TSV (Table 2) This study indicates that ATR-IR and NIR techniques are readily available for glucose and urea analysis. Yet, there exists room for improvement in the performance levels.

Model	Factor	$R^2$	RMSECV in mg/dL	RMSEP in mg/dL	LOD <sub>min</sub> in mg/dL	LOD <sub>max</sub> in mg/dL	LOQ <sub>min</sub> in mg/dL	LOQ <sub>max</sub> in mg/dL	
Urea	CV NIR	4	0.97	12	-	10	24	29	72
	IR	4	0.99	7.9	-	10	18	31	55
	TV NIR	4	0.98	-	19	-	-	-	-
	IR	5	0.99	-	6.6	-	-	-	-
Glucose	CV NIR	4	0.89	37	-	36	73	108	218
	IR	3	0.96	22	-	47	142	140	428
	TV NIR	4	0.86	-	54	-	-	-	-
	IR	2	0.99	-	11	-	-	-	-
Lactate	CV NIR	-	-	-	-	-	-	-	-
	IR	5	0.95	8.2	-	28	90	84	271
	TV NIR	-	-	-	-	-	-	-	-
	IR	8	0.99	3.0	-	-	-	-	-
Phosphate	CV NIR	-	-	-	-	-	-	-	-
	IR	8	0.99	1.1	-	1.0	2.6	3.0	7.9
	TV NIR	-	-	-	-	-	-	-	-
	IR	8	0.95	-	2.0	-	-	-	-
Creatinine	CV NIR	-	-	-	-	-	-	-	-
	IR	5	0.98	1.8	-	2.6	4.5	7.9	13
	TV NIR	-	-	-	-	-	-	-	-
	IR	4	0.96	-	2.1	-	-	-	-

Abbreviations: RMSECV—root mean square error of cross-validation; RMSEP—root mean square error of prediction; LOD—limit of detection; LOQ—limit of quantification; CV—cross validation; TV—test-set validation;  $R^2$ —coefficient of determination.

Table:2 Performance parameters of prediction of blood constituents least squares (PLS) regression models developed for IR and NIR spectra of a 5-component model mixture in artificial dialysate solutions

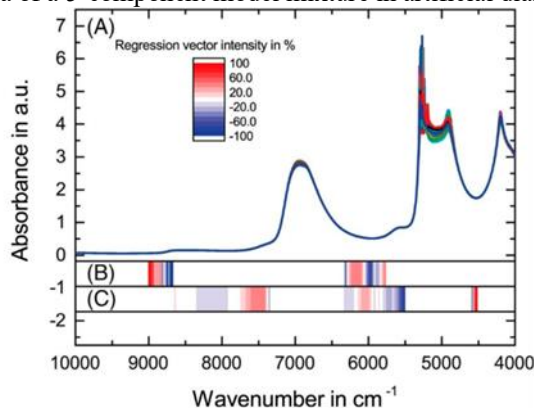


Figure 2. Dissection of the PLS regression vectors developed by Henn et al. [33] for prediction of blood constituents from NIR absorbance (I.A) and IR difference (II.A) spectra of a 5-component model mixture in artificial dialysate solutions. Relative (to the maximum value) intensity of the regression vector for glucose (B) and urea (C), lactate (D), phosphate (E) and creatinine (F).

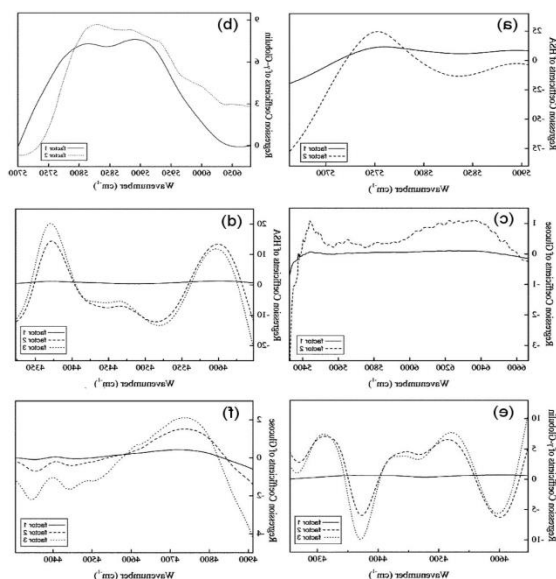
### Serum

Protein composition and glucose content in serum were frequent objects of NIR spectral studies. For example, Kasemsumran et al. conducted systematic examination of the analytical capability of NIR

spectroscopy in the analysis of human serum albumin (HSA),  $\gamma$ -globulin, and glucose for the needs of biomedical purposes. The aim of these studies was to evaluate the potential of NIR spectroscopy for simultaneous determination of HSA,  $\gamma$ -globulin, and



glucose in matrices of different complexity. The obtained results evidence the capacity of NIR spectroscopy supported by SCMW-PLS to simultaneously determine the concentrations of HSA,  $\gamma$ -globulin, and glucose in a complex biological fluid. Recently, a screening method for serum albumin based on NIR spectroscopy was proposed by Yao et al. That study focused on the wavelength selection for an efficient and rapid NIR analysis of human serum albumin, also achieved by a simpler RRPC-PLS model with just 15 wavelengths selected. Comparing the performances of the more complex and simpler RRPC-PLS models, the root-mean-square errors (RMSE) and correlation coefficients for validation were 0.505 g L<sup>-1</sup> and 0.997 for the optimal model (N = 24), and 0.530 g L<sup>-1</sup> and 0.997 for the simpler model (N = 15). The validation indicated better performance of RRPC-PLS compared with CARS-



PLS and EC-PLS, in regard to the model complexity and prediction accuracy, with predicted values nearly matching the reference values. Therefore, the conclusion was that RRPC-PLS method is a notable improvement over EC-PLS in application to NIR spectral determination of human serum albumin.

Figure 3. The structure of PLS regression coefficients vectors for simultaneous determination of HSA (a,d),  $\gamma$ -globulin (b,e), and glucose (c,f) concentrations from NIR spectra of model solutions developed by Kasemsumran et al.. Reproduced with permission from Royal Chemical Society.

### Saliva

Instead of blood or serum, saliva is an alternative source of diagnostic information useful in examining various conditions, e.g., cancer, diabetes, or oral

leukoplakia. Compared with blood or serum, saliva is a less complicated matrix with less variable chemical composition. Yet, it contains proteins, nucleic acids, mucins, amino acids, enzymes, and primary metabolites, which are highly informative biomarkers for various physiological conditions of the body. The ease and convenient acquisition of a sample from patients, makes it particularly suited for application of rapid screening method by means of spectroscopy. For those reasons, saliva carries a notable diagnostic potential useful and very practical for biomedical applications of NIR spectroscopy. Consequently, the technique was adopted relatively early for salivary biomarker profiling. Oral cancer cells appear in saliva at early stages of cancer, while oral epithelial cells are transmitted into it continuously during the cancer development. This makes saliva analysis sensitive subject for analysis in oral cancer detection at early stages. For instance, Murayama et al. proposed a diagnostic method based on NIR spectroscopy for detection of oral cancer from one drop of saliva without any specific diagnosis marker. In that study, the NIR spectra of one drop of saliva were measured using a capillary tube method. Principal component analysis (PCA) with the second and third factors calculated with the second derivative NIR spectra clearly discriminated between the two groups. It is noteworthy that the absorption profile of saliva in the NIR region is highly essential for in vivo studies of oral cavity. Methods involving saliva are also in the scope of functional NIR spectroscopy studies. Nonetheless, it may be noted that in the field of analysis of body fluids, IR and Raman techniques found great usefulness, while the full potential of NIR spectroscopy has not yet been entirely uncovered.

### Cell-Related Studies

IR and Raman spectroscopy are matured tools for carcinoma diagnosis. In this field, NIR spectroscopy is advancing and feasible methods are becoming well established. Early developments of NIR spectroscopy at that direction include approaches to detect prostate cancer cells. NIR calibration models for the analysis of glucose, lactate, glutamine, and ammonia as the prostate cancer markers were established by Rhiel et al. For the calibration, an adaptive procedure was developed with aim to selectively remove interfering metabolism-induced covariance between glucose, lactate, glutamine, and ammonia that arose in the cultivar of PC3-human prostate cancer cells. PLS regression models were generated from single-beam NIR spectra measured in the region of 4800 and 4200 cm<sup>-1</sup>. In the first attempt, the calibration models were developed on the basis of full spectral range; however, in the next steps an optimization of spectral windows

was carried out and used for fine-tuned calibrations. This enabled lowering the standard error of prediction (SEP) to 0.82, 0.94, 0.55 and 0.76 mM, respectively for glucose, lactate, glutamine and ammonia. Successful validation of NIR spectroscopy for off-line determination of the concentration levels of nutrients and metabolites in a serum-based cell culture medium formed an important step. Further, benefits from performing cell separation prior the spectral analysis became apparent as well. Such treatment was developed for on-line monitoring of human prostate cancer cells. A polypropylene filter was used for retaining the cells upon centrifugal filtration in that case.

Applicability of electronic absorption spectroscopy extending to NIR region to characterize breast cancer cells was studied by Zhang et al. The investigation focused on the behaviour of gold nanorods (AUNRs) in metastatic breast cancer cells. Of particular note, this has been done in an extended ultraviolet-visible-NIR (UV-Vis-NIR) region (25,000–10,000  $\text{cm}^{-1}$ ; 400–1000 nm). It is an interesting example of how

electronic absorption bands, typically studied in UV-Visible region (400–800 nm), that extend to NIR region can be used for practical bio-applications (Figure 4). UV-Vis-NIR absorption spectra of AUNRs in the living cells were used together with the information gained from other techniques (inductively coupled plasma mass spectrometry, ICP-MS; transmission electron microscopy, TEM) to monitor the properties of AUNRs in a highly metastatic tumour cell line. It was observed that the characteristic surface plasmon resonance (SPR) peaks of AUNRs can be detected with living cells that have taken up the nanorods. Further, the peak area of transverse SPR band was determined to be proportionally related to the amount of AUNRs in the cells, giving the developed method quantitative character of analysis. The established easy-to-use UV-vis-NIR absorption spectroscopic method can be used to monitor the behaviour of AUNR. Zhang et al. have demonstrated how this capacity can be successfully applied to monitor the appearance of metastatic breast cancer cells.

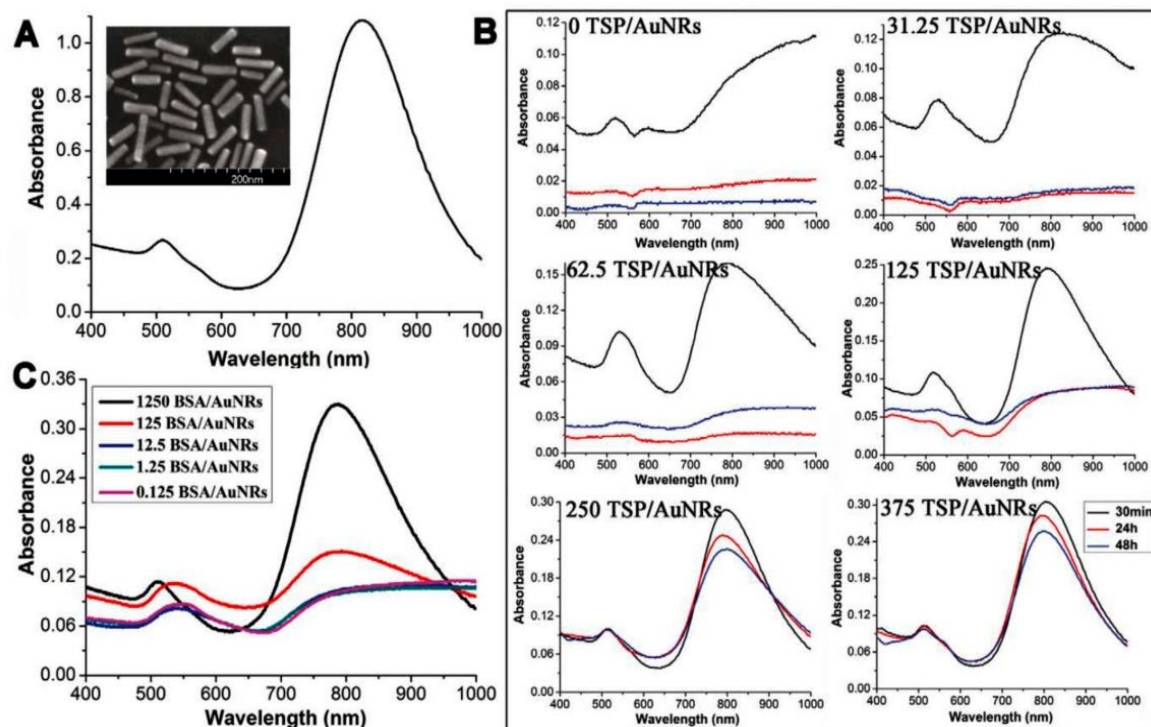


Figure 4. UV-vis-NIR spectrum of AUNRs suspension in water (A). Spectra of gold nanorods (AUNRs) dispersed in serum containing media (SCM) with 0–30% of fetal bovine serum (FBS) and different incubation times (B). Spectra of bovine serum albumin (BSA)/AUNRs samples (C).

An interesting problem of the appearance of biological cells in milk was examined as a factor potentially influencing the performance of NIR spectroscopy in

food analytical applications. Tsenkova et al. investigated the effect of high somatic cell count (SCC) index on the accuracy of NIR spectroscopic

determination of fat, protein and lactose content in non-homogenized cow milk. In that study, transmittance spectra of 258 milk samples were analysed in SW-NIR region (14,285–9090  $\text{cm}^{-1}$  ; 700–1100 nm). The most accurate calibrations, as evaluated through analysing SEP values and the correlation coefficient, were obtained for the samples with low SCC index. In contrast, the accuracy decreased notably in the scenario, where calibration models constructed on the basis of low SCC milk were subsequently used to predict the target analytes in samples with high SCC, and vice versa. Therefore, high cell content influences the accuracy of determination of fat, protein and lactose content in milk. These observations demonstrated the influence of cell content on the robustness of analysis of the chemical composition in milk by NIR spectroscopy; a factor that needs to be considered in similar applications.

### Analysis of Tissue

Vibrational spectroscopy, in particular imaging instrumentation, is a remarkably powerful tool for examination of tissues. Noteworthy are the applications in medical diagnosis, where IR and Raman spectroscopy are nowadays matured techniques, with prime importance for carcinoma diagnosis. Much alike the latter, a major attention in NIR bio-spectroscopy has been given to tissue analysis. From the point-of-view of tissue analysis in biomedical sciences, NIR spectroscopy is conveniently partitioned according to short-wave (SW; 13,333–9090  $\text{cm}^{-1}$ ; 750–1100 nm) and long-wave (LW; 9090–4000  $\text{cm}^{-1}$ ; 1100–2500 nm) NIR wavelength intervals. At short NIR wavelengths, the absorption of hem proteins (hemoglobin, myoglobin, and oxy-derivatives) and cytochrome of the tissue dominate the spectra and provide information concerning tissue blood flow, oxygen saturation and consumption, and the redox status of the enzymes. In the LW-NIR region, the observed absorptions are more complex and indicative for different biomolecules that may appear in tissues (Table 1). Valuable information concerning the chemical composition of the tissue with its main components of lipids, proteins, carbohydrates, and water can be gathered from LW-NIR region.

NIR spectroscopy, for instance, delivers quantitative chemical information from breast tissue based on oxy-hemoglobin and deoxy hemoglobin, water, and lipids. From these parameters, total hemoglobin concentration and tissue hemoglobin oxygen saturation were determined and provided information on tumour angiogenesis and hyper-metabolism. As an

illustrative example, distribution of total hemoglobin (tHb) and tissue oxygen saturation (stO<sub>2</sub>) levels in breast tissue can be monitored non-invasively in NIR spectral images (Figure 5). In addition, expert groups in this area have used NIRS (in conjunction with a tracer) to directly assess absolute and relative values of skeletal muscle perfusion in healthy and patients with chronic diseases.

The capabilities of NIR spectroscopy, e.g., non-invasive deep tissue sampling, make it potent tool particularly useful in the case of breast cancer. Therefore, considerable attention has been given to this application in the literature. A comprehensive review article discussing in detail early diagnosis and monitoring of breast cancer by NIR imaging is available from Sari et al. NIR spectroscopy as a diagnostic tool for cancer has been employed for various other cases, e.g., carcinoma of colorectal tissues, pancreas, or skin. On the other hand, several other relevant chemical features of tissues may be determined by NIR spectroscopy. NIR studies on human tissues include cervix, brain, skin, prostate, lung, head and neck, pancreas, and colorectal tissues, in which the absorption bands characteristic for one or more of the abovementioned biomarkers.

TABLE 1. CONT.

Wavenumber Region [ $\text{cm}^{-1}$ ]	Wavelength Region [nm]	Parameters Measured	Ref.
6798, 5233	1471, 1911	DNA	[72]
4866, 4604, 4261	2055, 2172, 2347	proteins	[72]
9000–7905 6000–5500	1111–1265 1666–1818	lipids	[72]
15,385–25,000	650–400	tissue scattering profile	[73]

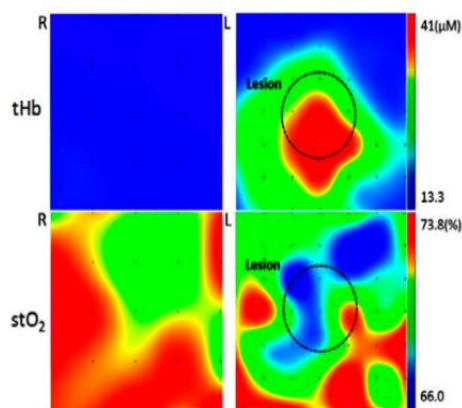


Figure 5. NIR image presenting the distribution of total hemoglobin (tHb) and tissue oxygen saturation (stO<sub>2</sub>) concentrations on both left and right breast acquired from a 56-year-old subject.

### Analysis of Medicinal Plants and Phytopharmaceutical Applications

The therapeutic and medicinal properties of herbs and plants are most frequently connected with existence of

individual bio-active compounds. Hence, their content affects the practical value and general usefulness of any given natural product. The chemical composition and the concentration levels of these bio-active compounds can successfully be analysed by NIR spectroscopy. One can notice a worldwide increasing trend in using natural drugs derived from medicinal plants. High demand to ensure quality of the natural medicine calls for analytical techniques capable of high-throughput, rapid, non-invasive, simultaneous in situ analysis of chemical and physical parameters of fresh plants, and intermediate or final products. Quality control plays a critical role for medicines derived from plants, as the chemical composition of natural drugs is prone to a much greater variation than conventional pharmaceutical products. Methods based on portable, miniaturized NIR instrumentation are greatly favoured in this role, as can be concluded from recent literature. Portable instrumentation enables direct quality assessment and optimization of the cultivation conditions, greatly enhancing the content of the active chemical in the final product, e.g., by selecting the harvest time. However, novel handheld NIR devices differ in the design principles and their applicability and performance profiles of handheld remain continuously investigated in various scenarios.

For example, characterization of the performance of portable NIR spectrometers in analysing medicinal plant extracts formed one of the main goals of the study by Kirchler et al. In their systematic examination, the authors described the capability of miniaturized NIR spectroscopy supported with various tools in determining the antioxidative potential and related properties of natural drugs. The study dissected performances of one benchtop (NIR Flex N-500 FT-NIR, Büchi, Flawil, Switzerland) and two different types of miniaturized NIR spectrometers (Micro NIR 2200, VIAVI Solutions, Milpitas, USA; and micro PHAZIR, Thermo Fisher Scientific, Waltham, USA) in the determination of the rosmarinic acid (RA) content of dried and powdered *Rosmarinus officinalis*, folium (i.e., *Rosmarini folium*). Noteworthy, the underlying technology and, thus, the operational parameters (e.g., spectral range, resolution, etc.) and the resulting analytical performance of the various miniaturized NIR spectrometers differ. Therefore, it is an active field of research in NIR spectroscopy to perform systematic evaluation studies of the applicability of certain instruments to various analytical problems. An interested reader is pointed to a focused literature covering the details of the

technological aspects and issues related to the performance levels of these instruments. The performance profiles were assessed with a number of different data-analytical methods. NIR spectra measured with three spectrometers (Figure 6) were calibrated through PLS regression models against the reference measurements by high performance liquid chromatography (HPLC) (Table 4). Prediction accuracy as determined by cross validation (CV) revealed that the benchtop spectrometer achieved the best result with a  $R^2$  value of 0.91 and a RPD of 3.27. A miniaturized NIR spectrometer, the Micro NIR 2200 achieved satisfying prediction performance with  $R^2$  of 0.84 and a RPD of 2.46. The analysis performed using the handheld micro PHAZIR, with a  $R^2$  of 0.73 and a RPD of 1.88, was less accurate and demonstrated room for improvement. In addition to inspecting the PLS regression models, the spectrometers were further evaluated in their sensitivity at different wavelengths by two-dimensional correlation spectroscopy (2D-COS) analysis (Figure 7). The relative differences between the sensitivity of the spectrometers were visualized by 2D hetero-correlation plots. This step allowed identification of discrepancies between the micro PHAZIR and the Micro NIR 2200 compared to the benchtop instrument. To gain better understanding of the factors which determine the constructed PLS regression vectors, quantum chemical calculation of the NIR spectrum of RA was carried out. This approach yielded independent information on the NIR absorption features of the target molecule (RA) and enabled interpretation of the main influences in the regression coefficients plots.

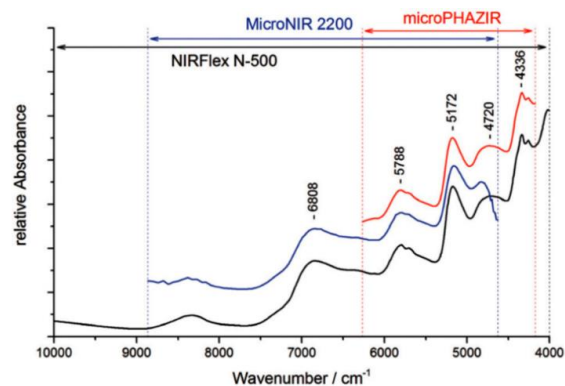


Figure 6. NIR spectra of *Rosmarini folium* samples measured on benchtop (NIR Flex N-500) and handheld (micro PHAZIR and Micro NIR 2200) spectrometers



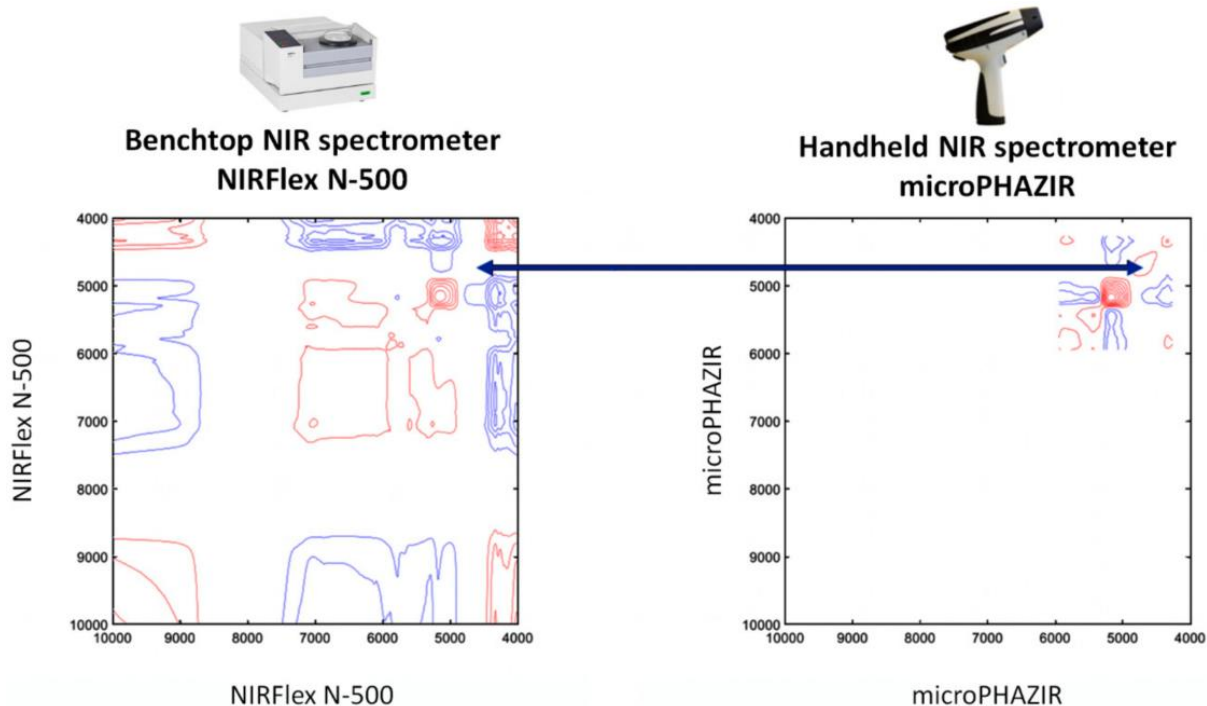


Figure 7. A visual assessment and evaluation of the chemical sensitivity profiles of NIR spectrometers (reference benchtop vs. handheld) by two-dimensional correlation spectroscopy (2D-COS)

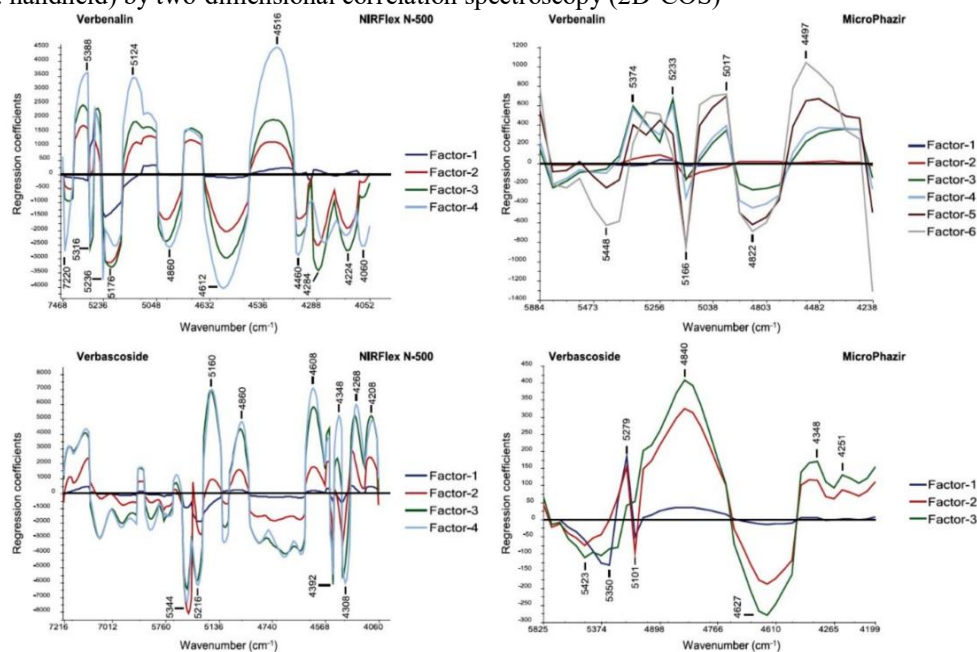


Figure 8. PLS regression coefficients plots for the best performing calibration models for verbenalin and verbascoside content in *Verbena officinalis* samples. The models were constructed for NIR spectra measured on NIRFlex N-500 (benchtop) and microPhazir (miniaturized) spectrometers

Table 5. Performance parameters of PLS regression models for the prediction of rosmarinic acid content CRA in *Rosmarinus officinalis*, folium



Spectrometer	NIRFlex N-500		microPHAZIR		MicroNIR 2200	
samples	60		60		60	
outliers	6		8		4	
$C_{RA}$ ( $w/w$ ) range/%	1.138–2.425		1.138–2.425		1.138–2.425	
validation method	CV	TSV	CV	TSV	CV	TSV
$R^2$	0.91	0.91	0.73	0.73	0.84	0.85
SECV/%	0.072	0.069	0.12	0.11	0.091	0.11
SEP/SEC	1.46	1.43	1.28	1.24	1.55	2.09
factors	8	8	5	5	11	12
RPD	3.27	3.41	1.88	2.06	2.46	2.14

Abbreviations: CV—cross validations; TSV—test-set validation; SECV—standard error of cross validation; SEP—standard errors of prediction; SEC—standard error of calibration;  $C_{RA}$ —rosmarinic acid content ( $w/w$ ).

The capability of analysing living plants brings essential benefits to several other fields of practical applications. Natural products often have complex and variable chemical composition. Thus, determination of their quality, origin, detection of adulteration and authenticity check have particular importance. In this area, NIR spectroscopy has become often used, e.g., for quality assessment and authentication of traditional Chinese medicines (TCMs). As an example, Huck-Pezzei et al. could successfully discern pharmaceutical formulations produced from either *Hypericum perforatum* or *H. hirsutum* that originate from China. The analysis done by NIR spectroscopy differentiated plant species, varieties and cultivars, and plants grown in different locations and under different growth conditions.

The potential of NIR spectral imaging for analysis of plant tissues. As they are micro-structured samples, the ability to simultaneously provide chemical and

topological information finds a particular usefulness here. As an example, NIR spectral imaging is powerful asset in quality control of drugs. This potential has been demonstrated by Sandasie t al. by their method developed for authentication of Echinacea based medicines appearing on the pharmaceutical market. Echinacea species are often included in various formulations to treat upper respiratory tract infections. The study involved three species, *E. angustifolia*, *E. pallida* and *E. purpurea*, acquired from local market in South Africa. By employing NIR hyperspectral imaging operating in the range 10,870–3978  $\text{cm}^{-1}$  (920–2514 nm), with aid of PCA it was possible to clearly discriminate between the three Echinacea species from the leaf and root material (Figure 9). The method accurately predicted the raw material content in several commercially available products and identified products that did not contain crude Echinacea material as well.

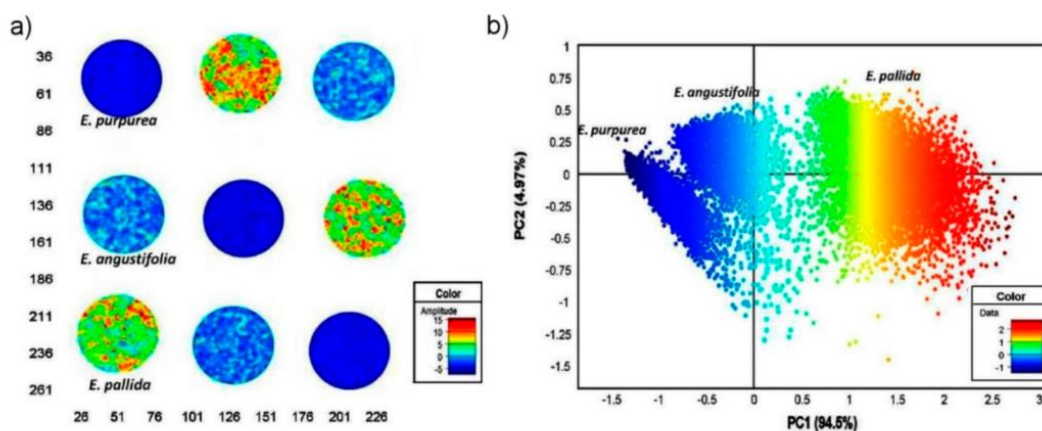


Figure 9. The analysis of NIR images of medicinal plants. PCA score image (t1) of Echinacea sp. leaf powders based on colour amplitudes (a). The corresponding score plot (PC1 vs. PC3) shows minimal separation of the pixel clusters (b). (EAL—*E. angustifolia* leaf, EPL—*E. purpurea* leaf *E. pallida* leaf)

### Entire Organisms

As explained in Section 2, the physical principles underlying NIR spectroscopy make this technique relatively more suitable for deep sample sensing and

interrogation of sample in-volume. These features give NIR spectroscopy the capacity for examining entire biological organisms. A good example serves here the recent study by Ishigaki et al. [83]. Therein, the properties of fish embryo have been comprehensively investigated in vivo at the molecular level. The development of fertilized eggs of Japanese medaka fish, *Oryzias latipes*, was monitored by NIR conventional spectroscopy and imaging techniques. The 6200–4000  $\text{cm}^{-1}$  region of the NIR spectrum contains useful information on the inner components of the egg, such as proteins, lipids and water. Furthermore the embryo growth time, oil droplets and yolk undergo changes in their chemical structure, which is accessible for NIR spectroscopy (Figure 10).

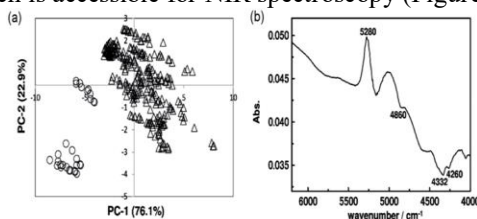


Figure 10. PCA scores plot of NIR spectra of yolk measured over the development time of *Oryzias latipes* embryo (a).  $\Delta$  indicates the data collected from the first to the tenth day and denotes data collected at the day before hatching. Loadings plot of PC-1 (b)

Through analysing NIR spectra and imaging data, one can non-invasively follow the chemical footprint corresponding to metabolic changes occurring in a developing embryo. Insights into subtle features of the chemical environment characteristic to the biological structures of an egg were obtained as well. For example, the study suggested that oil droplets contain relatively more strongly hydrogen-bonded

water, and the water environment typical for yolk seem to differ from those found in the other structures. Furthermore, secondary structure of proteins could have been assessed through the characteristic bands at 5756 and 4530  $\text{cm}^{-1}$ ; e.g., the appearance of membrane structures was proposed at certain locations within the egg.

### Introduction to Functional NIR Spectroscopy (fNIRS)

Functional NIR spectroscopy (fNIRS) is an optical topography (i.e., imaging) technique used for monitoring brain function in clinical applications. It is an area of medicine, in which NIR spectroscopy exemplified a unique potential and has been extensively adopted for research and diagnosis. One can find a remarkably rich scientific and medical literature devoted to fNIRS, which well reflects the vigorous discussion over its development and applications. For this reason, it falls beyond the capacity of this work to discuss in detail the applications of fNIRS. Instead, the discussion will focus on introducing the fundamental principles of this technique, while interested reader will be pointed to the most relevant exhaustive literature covering key discussed aspects of fNIRS. Previous sections mentioned the typical high permeability of organic materials to NIR radiation. The adoption of NIR spectroscopy for non-invasive sensing of brain function is possible as the transmissivity through biological tissue (so-called “Near-infrared window in biological tissue”) is high enough to enable percutaneous measurement of cerebral cortex located beneath the skull. Brain neuro-activity (i.e., firing of nerve cells) influences active energy metabolism in the cerebral cortex with an increase in blood volume and blood flow to supply glucose and oxygen to brain as the secondary effects. Hence, through neuro-vascular coupling the neural activity is correlated with the blood volume change, and the latter parameter is an accurate index of the brain activity (Figure 14).

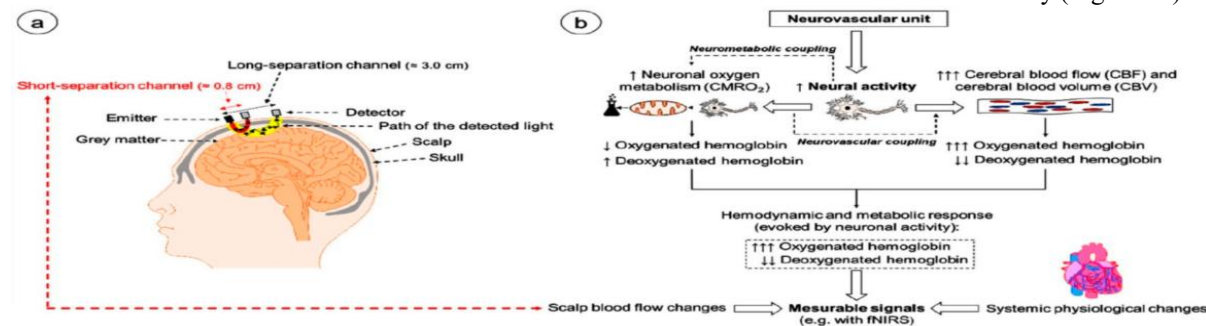


Figure 14. (a) Schematic illustration of the neurovascular unit and the changes in cerebral hemodynamics and oxygenation induced by neural activity. (b) Exemplary illustration of a possible NIRS montage on the human head and the assumed banana-shaped course of detected light of “short-separation channels” and of “long-separation channels”.

Nowadays, the instrumentation and methodology of fNIRS improved (e.g., in terms of portability, spatial resolution, time-to-result) and is highly suitable for imaging of brain activity in real-time with minimal inconvenience for patients. Autonomous fNIRS sensors configured as wireless wearables enable continuous monitoring of patient's brain activity throughout his normal daily life. The neural activity in cerebral cortex is associated with high level functions such as motion, sensation, perception, speaking, etc. This makes it suitable for fNIRS to be used as a substantial aid in diagnosing even complex problems, e.g., in psychiatry. Moreover, fNIRS provides valuable research data indispensable for advancing neuroscience and our basic understanding of the higher functions of brain and improving rehabilitation therapies.

It should be noted that, there are other techniques established in medical practice for non-invasive assessment of brain activity, e.g., functional magnetic resonance imaging (fMRI), positron emission tomography (PET), magnetoencephalography (MEG), electroencephalography (EEG), each with specific advantages and disadvantages. Brief comparison of the characteristics of fNIRS opposed to the competitive technique is provided beneath. The prime advantages of fNIRS are simplicity and safety of the instrumentation. Unlike MEG or PET, no high-energy photon radiation is used, meaning that no radiation exposure risk exists and an ordinary non-shielded room is suitable. For the same reason, repeated measurements can be performed on relatively more vulnerable subjects, e.g., infants or elders. Safety-related aspects of fNIRS are exhaustively discussed in the literature. Unlike fMRI, fNIRS is convenient for patient, as the diagnosis can be performed with the subject holding a natural posture (sitting, standing or lying). The sensor require. minimum wiring or can be wireless, hence the subject can be diagnosed under motion and activity. Finally, fNIRS is vastly superior to all other functional imaging modalities in its cost-effectiveness and portability. While the cost factor might be negligible in the developed world, it should be emphasized that this particular benefit is critical to wide-spread modern medical imaging diagnostic tool in the developing countries such as the African continent. For further information of fNIRS, an interested reader may consider review articles describing the history of this discipline by Ferrari and Quaresima or by Boas et al. Systematic discussion of the methodology, in a tutorial-resembling form is available from Herold et al. A solid review of the general principles and applications of fNIRS is available in a form of review article by Irani et al.

Comprehensive discussion of the recent advances and applications of fNIRS throughout different areas of medicine can be found in review articles, e.g., by Yang et al. (stroke diagnosis and rehabilitation), Mihara and Miyai (neurorehabilitation), or Ernst et al. (applications in psychiatry). Novel concepts of sensor miniaturization and autonomy in the form of wireless wearables were described in detail by Pinti et al, current state of knowledge in the area of preprocessing of fNIRS imaging data were dissected by Pinti et al., while prospects for future advances of fNIRS in the whole were summarized by Quaresima and Ferrari.

### CONCLUSION AND FUTURE PROSPECTS:

With its main benefits being quick analysis, broad applicability to varied samples, ability to examine moist samples, flexible instrumentation including small sensors permitting portability, NIR spectroscopy provides enormous promise in various bio-applications. However, compared to rival methods, it is still a discipline in development in some areas and has space for advancement. This calls for a mention of the field of bioanalytical research and medical diagnosis, where IR and Raman spectroscopy still pose a serious threat. However, recent research suggests that NIR spectroscopy is constantly advancing into new sectors of use. The interpretability of NIR spectra has improved recently, closing the gap with IR or Raman techniques, which are traditionally prized for their greater chemical specificity. When dealing with materials of biological origin that are chemically complicated, this becomes very important. Innovative handheld NIR spectrometers are essential for on-site analysis of bio samples, such as medicinal plants, in order to improve growing conditions and guarantee the greatest quality of natural medicines. Engineering remote airborne NIR sensors deployable on UAVs for environmental monitoring were recently made possible because to advancements in instrumentation. The development of data-analytical techniques, which is also encouraged by other fields, supplements this. With the continuance of present research strands and the emergence of completely new ones, it is anticipated that NIR spectroscopy will significantly expand its utility across the field of bio-applications in the upcoming decade.

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