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Aquaphotomics
Origin, concept, applications and future perspectives

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Abstract. Aquaphotomics is a novel scientific discipline which has made rapid progress in just 14 years since its establishment in 2005. The main novelty of this field using spectroscopy is placing the focus on water, as a complex molecular matrix and an integral part of any aqueous system. Water is sensitive to any change the system experiences – external or internal. As such, the molecular structure of water revealed through its interaction with light of all frequencies becomes a source of information about the state of the system, an integrative marker of system dynamics. This novel field shifts the paradigm of seeing water in a system as a passive, inert molecule to one which can build various structures with various functionalities, giving water an active role in biological and aqueous systems. Owing to the high sensitivity of hydrogen bonds, the water molecules are incredibly adaptive to their surroundings, reshaping and adjusting in response to changes of the aqueous or biological systems, and this property in aquaphotomics is utilized as a key principle for various purposes of bio-measurements, bio-diagnostics and biomonitoring. This paper will present the origin and concept of aquaphotomics and will, through a series of examples of applications, illustrate many opportunities and directions opened for novel scientific and technological developments.

Keywords. Aquaphotomics, spectroscopy, water, light, bio-measurements.

1. ORIGIN OF THE NEW SCIENCE

The concept of aquaphotomics grew during years of experience with near-infrared (NIR) spectroscopy and mammary gland disease in cows (mastitis). First realizations about the role of water determining the state of biological systems were made by Roumiana Tsenkova, professor of bio engineering at Kobe University, Japan, during her early works at Hokkaido University while studying biological systems in-vivo with near infrared spectroscopy.

In 1996, when Roumiana Tsenkova moved from Hokkaido University to Kobe University in Japan with a five-year grant in Animal Husbandry, further investigations started at Kobe University and four other teams in Japan. The findings of this project opened the avenue for investigating water molecular systems in the biological world. She discovered that the water molecular matrix of milk and body fluids changes with inflammation of the mammary gland (mastitis) in dairy cows. In 2001, the seed of aquaphotomics was planted with a paper where R. Tsenkova and colleagues had applied NIR spectroscopy to measure milk proteins of healthy and mastitic cows.[1] They applied chemometrics and found that the two groups had different regression models for protein measurement and somatic cell count and that their average spectra differed in the area of water absorbance bands, which meant that the water structures, shaped by proteins and cells, changed with the disease. This was the first publication showing that water, acting as a biological/chemical matrix, can tell if a system is healthy or diseased. That was the time when the term water matrix started to be in use to denote water as a molecular network consisting of different water molecular structures that cause different behavior inside a system.

During those years, aquaphotomics basic spectroscopic experiments on water were performed as well by adding molecules or changing temperature or humidity or measuring water repeatedly and observing how the water spectrum changed at certain specific wavelength ranges.

One of the most spectacular observations from these early years was that the absorbance spectrum of water changed with consecutive measurements (in aquaphotomics called illumination perturbations). This result was first presented at the International NIRS conference in the year 2000 in Korea. Reactions varied from “I knew somebody would do it” and being interested to explore further, to “If this is true I will quit my job” because in conventional physical chemistry it is expected that good instruments will acquire the same spectra repeatedly. The fact that light changes the water seemed extraordinary and the exploration into the water spectrum as a source of information continued.

Further investigations were performed on cells, plants, and animals. Similar spectroscopic patterns, meaning similar absorbance bands changed when perturbations were introduced on other investigated systems. The realization emerged that the water absorbance bands (WABs) in the found patterns were related to the same wavelength ranges that were influenced in the basic water experiments performed earlier. These wavelength ranges were presented as water matrix coordinates (WAMACs), a new term introduced to denote the ranges in the electromagnetic (EM) spectrum where measured absorbance of biological and aqueous systems changed due to perturbations, providing information about particular water structures and water functionality.[2] Conventionally, only a few symmetric and asymmetric stretching vibration assignments of water molecules are known in the first overtone of water OH stretching vibrations for pure water systems.[3] However, the experimentally found patterns presented 12 ranges, see Figure 1 for a schematic depiction of those wavelength ranges (WAMACs).

It must be noted that these ranges are not completely fixed and new bands or extensions of the bands have been and will be found in further explorations with other systems and perturbations. It is tempting to try to assign the WAMACs to particular water structures, as has been done in various aquaphotomics publications.[2] In the NIR range, for such endeavors the found bands in wavelengths (*x* nm) are multiplied by an integer *o*, representing the expected overtone of the fundamental band of water structures (usually *o* is two for the first overtone), and converted into wavenumbers (y cm^{-1}) through the following formula: y cm^{-1} = 10,000,000 / $(o \cdot x)$ nm (adapted from $\tilde{\nu} = 1/\lambda$) and subsequently searched in literature for known assignments. However, since water vibrational modes and water inter- and intra-molecular bonding is complex and one system of molecules will entertain numerous types of water vibrations and inter and intramolecular bonding, it is usual that overlapping and shifting occurs and more than one band will respond to a change in the water matrix. Consequently,

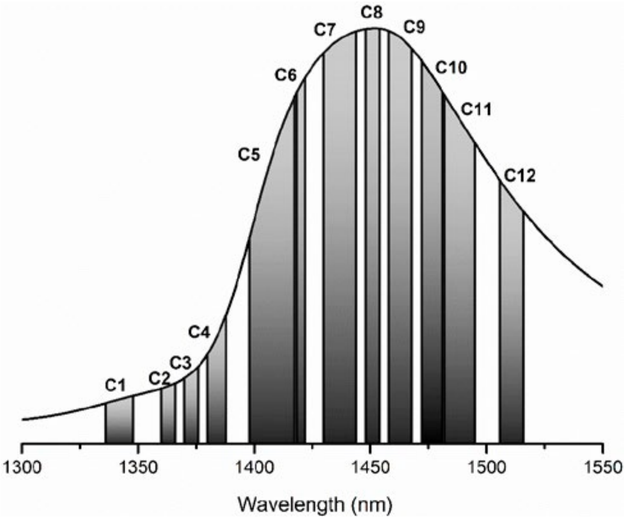

Figure 1. Schematic depiction of the water matrix coordinates (WAMACS), which are the wavelength ranges where the spectral absorption changes most in bio-aqueous systems due to pertubations.[4]

it is suggested not only to find assignments based on water molecular structures, but to work with the 'activated' water bands as 'letters' creating 'words' that can be assigned to a functionality per system–perturbation combination.[5]

'Activated' water bands are found through aquaphotomics analysis where the steps of the analysis, such as raw data inspection, conventional and chemometric analyses, provide certain quantitative outputs such as derivatives, subtracted spectra, regression vectors or loading vectors, discriminating power and others, which all unravel the water absorbance bands most affected by the perturbation of interest.[6] The spectra of aqueous systems are very complex, and changes caused by any perturbation will usually be subtle, but nonetheless persistent and consistent. Acquiring spectra under a certain perturbation without stabilizing the influence of other factors provides more spectral variations and therefore a robust model for water functionality related to examined perturbation. Averaging towards the main perturbation and subtracting of the average spectra is the first source of information about the specific bands that relate to the highest absorbance variations induced by the perturbation of interest. From all the water absorbance bands discovered during the multiple steps of aquaphotomics analysis, common absorbance bands will emerge to reveal perturbation-induced light absorbance pattern at specific water absorbance bands. In this way, aquaphotomics analysis provides a link between the observed water absorbance bands and the water functionality in the respective system.

Experience and studying the patterns in this 'vocabulary' of all observed system-perturbation combinations can result in assignment of certain WAMACs to water structural behavior, see the often seen 'assignments' based on R. Tsenkova's experimental data in Table 1.

Table 1. The 12 WAMACS, their corresponding wavelength ranges, and the general 'assignments' often seen in R. Tsenkova's experimental data. They are in good agreement with the respective published assigned bands in IR range.

WAMAC	Wavelength range	General 'assignment'	
C1	1336-1348 nm	vapor like	H_2O asymmetric stretching vibration Protonated water clusters
C2	1360-1366 nm		Hydroxylated water clusters Water solvation shell
C3	1370-1379 nm		H_2O symmetrical stretching vibration and H_2O asymmetric stretching vibration
C4	1380-1388 nm		Water solvation shell Hydrated superoxide clusters
C5	1392-1412 nm		S_0: Trapped and Free water
C6	1421-1430 nm		Water hydration
C7	1432-1444 nm	bulk water	H_3O (Hydronium) S1: Water molecules with 1 hydrogen bond (dimer)
C8	1448-1458 nm		Protein transfer mode in acidic aqueous solutions Water solvation shell
C9	1460-1464 nm		S_2: Water molecules with 2 hydrogen bonds (trimer)
C10	1472-1482 nm		S_3: Water molecules with 3 hydrogen bonds (tetramer) H_3O_2 H_5O_2
C11	1492-1494 nm	ice like	S_4: Water molecules with 4 hydrogen bonds (pentamer)
C12	1506-1516 nm		Strongly bound water

Water is such a common element that the same 'bands' can be found in spectra of crystals as well as liquids and other (bio) systems. In this way, the building of the aquaphotome, which consists of the water absorbance bands and water spectral patterns (WASPs) related to specific states or dynamics of various systems, started.[2]

Every system–perturbation combination has its unique aquaphotome, i.e. the spectral pattern produced by the respective system under the respective perturbation. Aquaphotomes build up the aquaphotome database that contains all the respective WASPs of various systems under various chemical, physical, mechanical, biological, etc. perturbations.

To illustrate this, two entries in the aquaphotome database, for the wavelength range 1300-1600 nm, representing two system–perturbation combinations, in this case cow's milk – degree of increasing mastitis and water – increasing temperature, are provided in Table 2[2].

Finally, in 2005, R. Tsenkova proposed the establishment of aquaphotomics[2,9–11] as a new scientific discipline complementary to other "omics" disciplines, with the aim to study water in aqueous and biological systems, using light as a probe and a spectrum, which results from their interaction, as a source of information about the system.

Systematization of knowledge about water from the aquaphotomics research studies and experience in working with various aqueous and biological systems showed that water-light interaction over the entire EM spectrum can significantly contribute to the field of water science and provide better understanding of water molecular systems, and most importantly that it leads to the development of new technologies and applications.[2]

Table 2. Example of two entries in the aquaphotome database in the NIR region (1st overtone of water OH stretching vibrations, 1300-1600 nm) showing how development of mastitis influences the water matrix of milk and how increasing temperature influences the water matrix of ultrapure water.

WAMACS													
C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**Perturbation**	**System**
				1416nm down		1436nm down						Degree of mastitis increasing	Cow's milk[7]
				1410nm up						1492nmdown		Temperature increasing (6-80 °C)	Ultra-pure water[8]

2. SETTING THE STAGE

2.1. Aquaphotomics: Water – From passive to active component

The early aquaphotomics works were based on near-infrared spectroscopy. The analysis of water in this field was present since its inception,[12–14] but at that time water was still not considered as a molecular network system or a biologically relevant matrix.

In fact, for years, water has been described as the 'greatest enemy' of infrared (IR) and NIR spectroscopy on account of its dominant absorption. This attitude was a result of, at that time general, dominant opinion in biological sciences that water is an inert, passive medium. Living processes were described in terms of genes, DNA, proteins, metabolites or other single biomolecules acting as entities isolated from water. Research methods were focused on extracting information related to the structure of these biomolecules, and in the near infrared region dominant absorbance of water was seen as an obstacle to observing their absorbance bands. However, the field of water science has seen significant progress in the last decades which has changed the general opinion about water and its role in the living systems.[15–19]

Today, we are witnessing a paradigm shift – water is recognized as an active solvent, adapting its structure to the solutes that it accommodates, and in biological systems, water is seen as a biomolecule in its own right with an active role in the dynamics of biomolecular processes.[2,7,17]

2.2. Aquaphotomics: From a segmented to a global water-mirror approach

The main aim of the aquaphotomics field is to understand the role of the water molecular network in biological and aqueous systems by monitoring the interaction with light in the broadest sense, and investigating the whole EM spectrum of those systems under various perturbations. The name of this discipline is composed of three words: aqua – water, photo – light, and *omics*-all about something.[2]

Aquaphotomics presents the water spectral pattern as a multidimensional, integrative marker related directly to the respective system functionality. The foundation stone of aquaphotomics is a discovery that water in biological and aqueous systems works as a 'mirror' of the components and environment (matter and energy) and therefore its spectral pattern can be used to characterize the system as a whole. This is also called the WAter Mirror Approach (WAMA) (Figure 2).[2]

Water is an invaluable resource for human health, food security, sustainable development, and the environment. Understanding the role of water in aqueous and biological systems is of crucial importance. Historically, water properties have been extensively studied using a variety of methods from X-ray to THz spectroscopy. However, even if these techniques provide valuable information on water molecules, they generally focus on water as an isolated/separate chemical and physical subject only, in contrast to focusing on water as an interdependent connected active and 'functional' system, interrelated to its environment. There are no isolated systems in nature.

Likewise, from biological sciences point of view, up to recently, biologists have been focusing on pinpointing single biomolecules related to natural phenomena, without considering the contribution of all components of the system, and especially without considering water.

However, the function of single biomolecules is highly related to their molecular structure, which in turn is influenced by all components of the system, therefore biomolecules are not to be seen as separate from surrounding components. Moreover, their molecular structure is highly related to the creation of hydrogen bonds with the surrounding water molecules.

Therefore, in aquaphotomics, the water molecular structure of a bio-aqueous system is considered as a global 'mirror' reflecting the state, dynamics, behavior and 'functionality' of the respective system.

Figure 2. Water Mirror Approach: Just like the surface of the lake reflects its surroundings, the water on a molecular level is behaving like a mirror – its spectrum reflecting all the molecular components and influences of surrounding energies.

2.3. Aquaphotomics: From single disciplinary to multidisciplinary approach

Water has been studied by different disciplines in many different ways and all of them use their own particular terminology. It is quite difficult to translate scientific findings from one area into another. Aquaphotomics provides an opportunity to start building up a “water vocabulary”[5] where the water vibrational frequencies, i.e. water absorbance bands (WABs) are the “letters”, and the water absorbance spectral patterns (WASPs) are the “words” identifying different phenomena in order to translate findings of water between different disciplines. These letters and words create fingerprints that are stored in the aquaphotome database, per bio aqueous system and ‘perturbation’, which can be physical (temperature, humidity, pressure, electromagnetic radiation, and so on), biological (disease, certain enzymes, DNA, and so on), or chemical (concentration of salts, and so on) (as presented in Table 2).

2.4. Aquaphotomics: From a reductionist to a global and integrative approach

Genomics, proteomics, metabolomics, and other “omics” disciplines have revolutionized life science. However, these disciplines study isolated elements, therefore reducing the system to its parts.

Systems biology and other functional “omics” disciplines integrate proteomics, transcriptomics and metabolomics information to provide a better understanding of cellular biology, thereby taking a more integrative approach, but still integrating isolated elements.

Aquaphotomics is introduced as a global and integrative approach (Figure 3). All elements investigated in other "omics" disciplines can also be investigated through measurements of the water that surrounds them, an 'indirect' type of measurements where water serves as a sensor and an amplifier.[20] The status, dynamics, and functionality of an intact system can also be measured directly with aquaphotomics, without reducing the system to its parts, by e.g. measuring the skin, the leaf and so on.

2.5. Aquaphotomics: From a static, destructive/invasive to a dynamic, non-destructive/non-invasive approach

In "omics" disciplines such as genomics or proteomics, creating databases and further using them for understanding biological processes, requires isolation of individual elements (genes, proteins) one at a time, making such analyses extremely time-consuming and laborious. It requires the destruction of the analyzed object and thus provides only a single time-point (static) picture of the processes. Considering the speed, plasticity and multifactor-dependence of biological processes it is clear that such static one-at-a-time approach should be complemented with more dynamic and real-time methods.

The aspect of dynamics has been partly addressed by metabolomic profiling, where a snapshot of the physiology of the cell at a specific moment can be acquired. Even though metabolomics and aquaphotomics are taking completely different approaches, they try to solve the same problem – to provide a systematic view of the processes with time-dependent information of their interconnections. In contrast to metabolomics, aquaphotomics does not destroy the sample with the measurement, therefore it can study the same object **fast, non-destructively, non-invasively and continuously**, thereby it is able to **monitor ongoing processes dynamically**.

Through an understanding of water–light interaction dynamics and its relation to biological functions, aquaphotomics brings together the knowledge acquired by other "omics" disciplines describing single elements of biological systems and upgrades it to a systemic, integrated level as water does in biological and aqueous systems.

2.6. Aquaphotomics: Relationship to conventional spectroscopy

The aquaphotomics approach is complimentary to the conventional spectroscopy approaches, too. In most of the VIS-NIR-IR spectroscopy studies, the water absorption bands are considered as masking the real information. For example, in order to measure bio molecules like proteins and glucose, water is evaporated in order to "see" better the absorbance bands of glucose.

In contrast, in aquaphotomics, the **water spectral pattern** is considered as **the main source of information. Water is the matrix, the "envelope", the "scaffold" of the system**.[21]

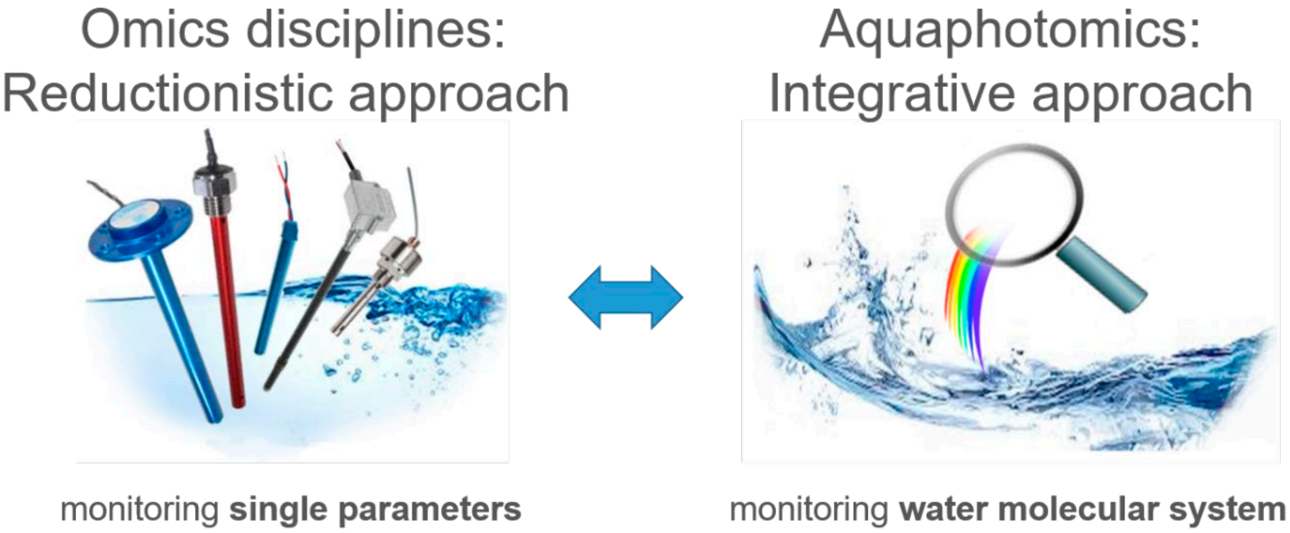

Figure 3. Aquaphotomics encompasses all other "omics" disciplines, providing an integrative approach to studying aqueous and biological systems.

In aquaphotomics, the 'functionality', the biological state, the biological reaction to a change (dynamics) of the bio-aqueous system is the key, instead of the presence of individual molecules.

In most conventional spectroscopy studies, quantitative models are made for each separate component to be used to diagnose a system, where combining the models multiplies the errors thereby producing inaccurate results. In aquaphotomics, instead of looking for the individual components, the water spectral pattern is used as a global marker, and monitoring this marker can provide information about changes in the system.

In aquaphotomics analysis, specific water molecular structures (presented as water spectral patterns) are related to the status, dynamics and 'function' of the bio aqueous systems studied, thereby building an aquaphotome – a database of water spectral patterns correlating water molecular structures to specific 'perturbations' (disease state, contamination state, reaction to light, change in temperature, and so on). The process of extracting information from water spectra in aquaphotomics requires a field-specific approach.[6]

3. APPLICATIONS

Aquaphotomics is well developed in the visible and near-infrared range of the spectrum. Aquaphotomics is used for fundamental studies as well as many field applications, where various spectroscopic techniques and measurements setups and devices can be applied, such as transmission and transflection spectroscopy, using handheld devices or benchtop systems.

The NIR range is especially suitable, the perfect window for non-invasive measurements of aqueous systems and living biological systems, as NIR light can penetrate deep (1-10 mm)[22] into the aqueous systems, and does not get fully absorbed, making it possible to measure the transflectance spectrum of the light that comes back out of the bio-aqueous system after interacting with the water and other components of the system.

In the next sections, a brief overview of the various areas of aquaphotomics applications will be given.

3.1. Basic studies and solute measurements and analysis

It is well established that different water species, for example, water dimers, trimers, hydration and solvation shells, contribute very specifically to the spectrum.[23–25] The changes in water spectrum accurately and sensitively reflect the changes of water molecular species, hydrogen bonding and charges of the solvated and solvent molecules or clusters. Big data sets of water spectra acquired under various perturbations reveal immense information about the water molecular system dynamics and the role of water in bio-aqueous systems ('functionality').

One of the proof-of-concept studies showing that water behaves as a mirror on a molecular level had an objective of measuring concentrations of different salts ranging from 0.002 to 0.1 mol/L.[26] Salts were used to demonstrate the water mirror approach since they do not have absorption bands in the NIR range, therefore any result would be entirely due to changes of water matrix in response to perturbations of salts at different concentrations. This was also the first work using NIRS to examine the effect of salts in such low concentrations. In a multi-center study, with three different locations and three different spectrometer systems, it was demonstrated that the water mirror approach of aquaphotomics enabled predictions of concentrations with a limit of detection at 1000 ppm level, which indicates that under specified conditions, aquaphotomics approach improved the detection limit for NIRS around five times.[27]

A recent paper on the essentials of aquaphotomics explains the details of the experimental methodology, chemometric tools aquaphotomics uses and how the information on changes of the water matrix in response to perturbation of interest can be extracted from the complex water spectra.[6] This paper shows how simple tools, such as spectra subtraction can reveal that salts (potassium-chloride in the concentration range of 10-100 mM) do change the water molecular system and that change is reflected in the absorbance spectra of salt solutions (Figure 4).[6]

Figure 5 shows the aquagrams corresponding to those solutions. Aquagrams are a visual representation of a WASP – this type of graphs displays the normalized absorbance values at the selected WABs. Aquagrams are very convenient visual tools to explore the differences between different perturbation steps, or different groups of interest.

Using the same concept, other molecules – single or in mixtures, in minute concentrations were measured such as mono- and di-saccharides.[28] This was the first research confirming the applicability of NIR spectroscopy for qualitative and quantitative analysis of mono- and di-saccharides at millimolar concentrations with the detection limit of 0.1-1 mM. Figure 6 shows the aquagrams for lactose in 0.02-100 mM concentration range depicting that higher concentrations of lactose increase strongly hydrogen-bonded water, i.e. act as structure makers, and decrease weakly hydrogen-bonded water.

Similar findings have been reported for proteins in solution,[29] metals in solution,[30] etc.

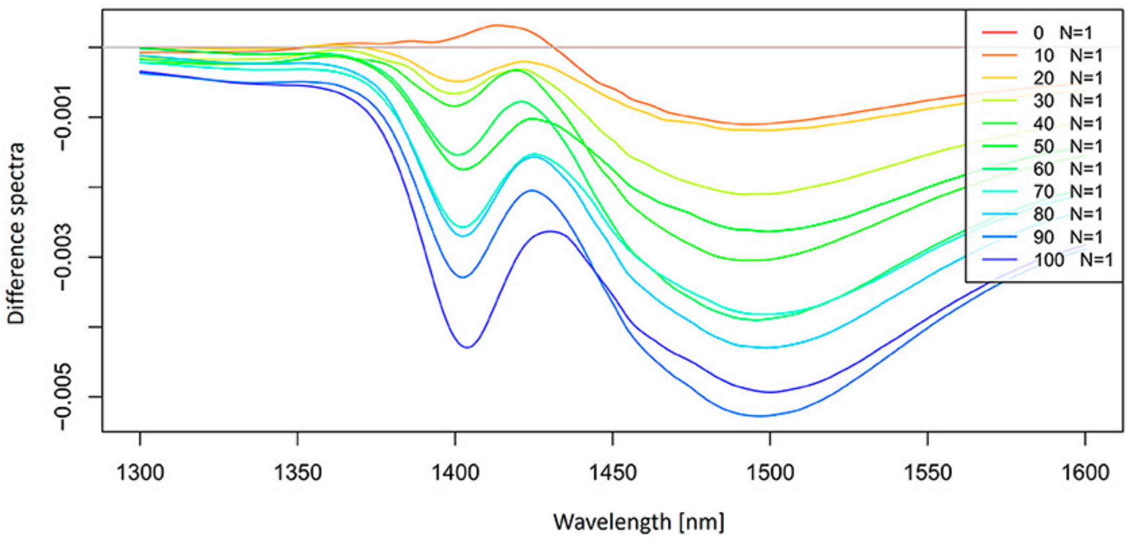

Figure 4. Difference absorbance spectra in the spectral range of 1300–1600 nm (OH first overtone) of pure water and aqueous solutions of potassium-chloride in the concentration range of 10–100 mM. The average spectrum of pure water was subtracted from the spectra of potassium-chloride solutions.[6]

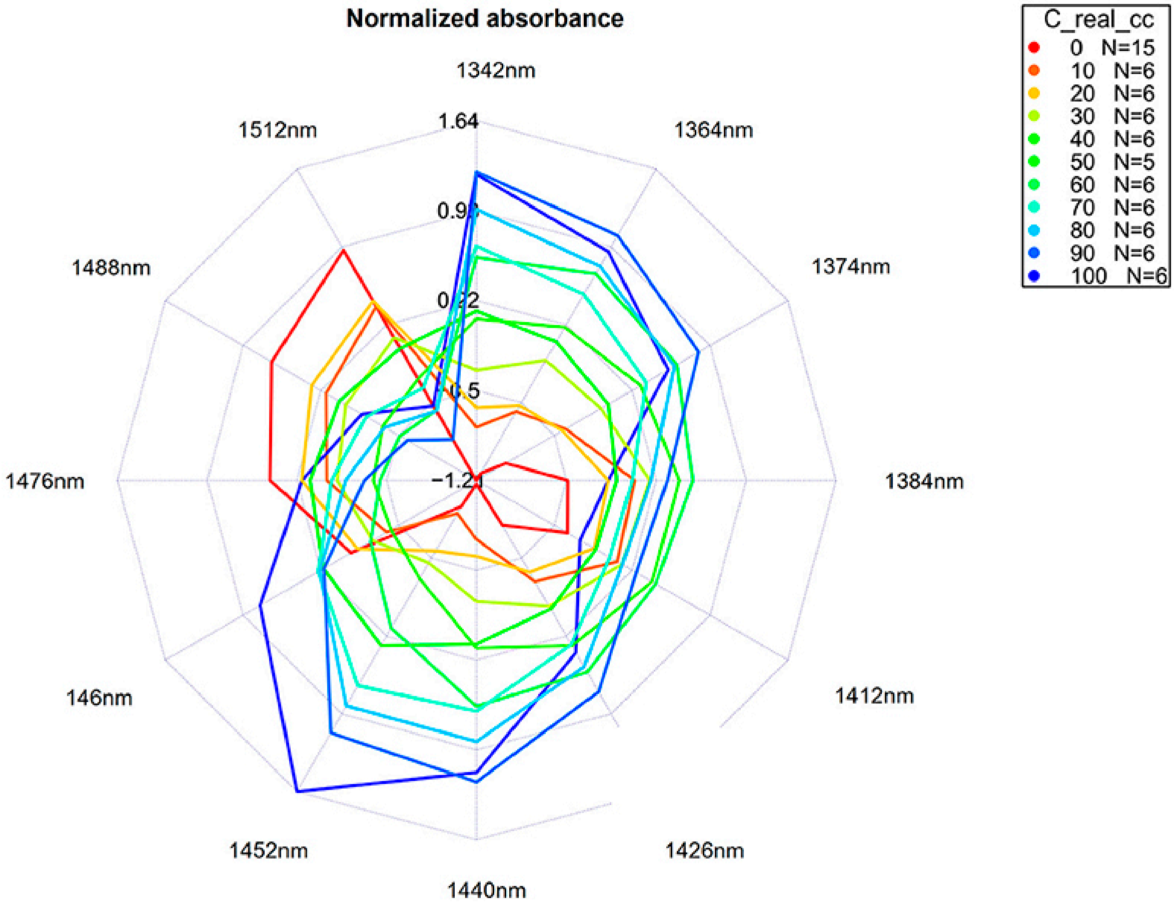

Figure 5. Aquagrams of aqueous solutions of potassium-chloride in the concentration range of 10–100 mM in the spectral range of 1300–1600 nm (OH first overtone).[6]

Contrary to the common understanding of overtone spectroscopy (100 to 1000 times lower absorbance than in the mid-IR range), it has been shown that even very small concentrations[31] of the solutes could be measured with NIR. The water-mirror approach provides measurements of solute concentrations previously thought impossible at ppm,[30–32] even ppb levels under certain experimental conditions.[30,33–35]

Apart from the concentration of analytes, this approach also was successfully applied to the measurement of physical parameters of water systems, such as pH and acidity,[36] and the effects of mechanical filtration on water.[37]

Thus, aquaphotomics contributed to basic knowledge about water-light interaction under perturbations and showed potential for fundamental applications.

3.2 Protein studies

Through work in the field of protein-water interactions, aquaphotomics provided insight into their dynamics and the significant role water plays in their functionality. One of the first studies analyzed prion protein isoforms[38] – the proteins which are the cause of neurodegenerative diseases. One of the possible mechanisms of converting the protein into the misfolded form was thought to be binding of Manganese (Mn) instead of Copper (Cu). The aquaphotomics analysis of Mn and Cu prion isoforms in water solutions revealed that while binding of copper resulted in increased protein stability in water, the binding of manganese resulted in protein instability and the subsequent changes led to fibril formation.

Subsequently, another study investigated the formation of amyloid fibrils[39] – another type of protein linked to neurodegenerative diseases. Changes during fibrillation of insulin were monitored in 2050–2350 nm and 1300–1600 nm spectral regions, covering the bands related to protein and related to water absorption. The results showed that all the steps of conformational changes of the protein, confirmed by the results in 2050-2350 nm region were reflected also in the changes of the water molecular network in the 1300-1600 nm region (Figure 7).

These studies unquestionably demonstrated one fundamental fact – proteins and water act together – they

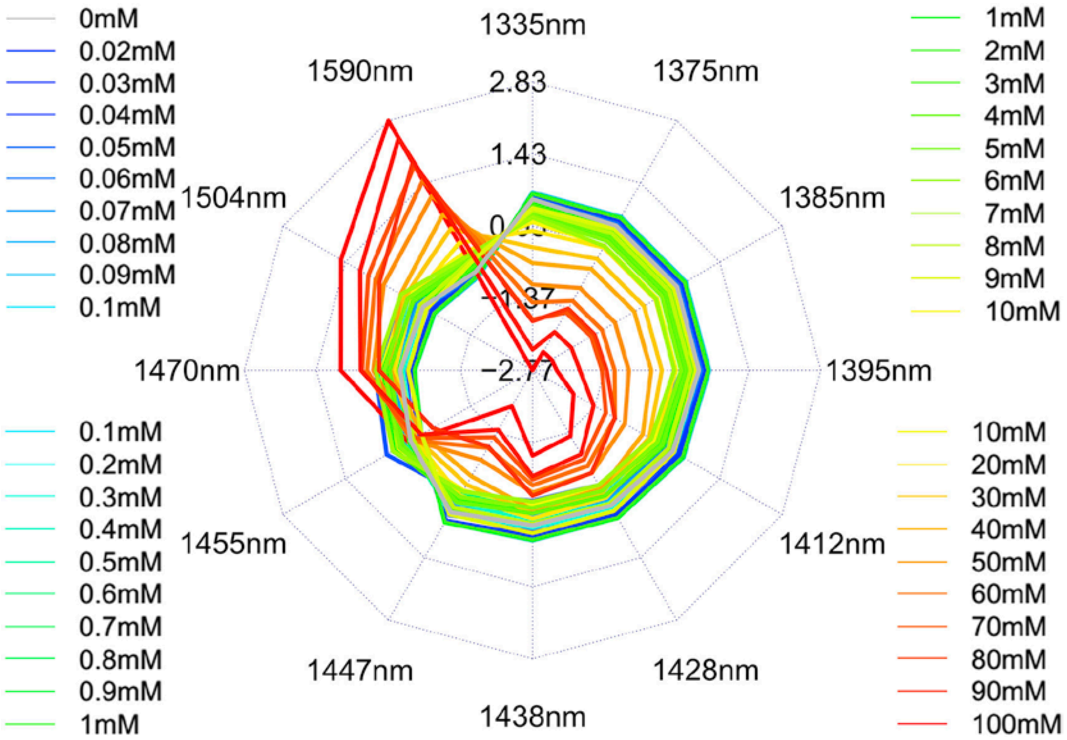

Figure 6. Aquagrams of lactose solutions in 0.02–100 mM concentration range. The radial axes are the 12 water matrix coordinates (WAMACs) and the dynamic changes for low to high lactose concentration from structure maker to structure breaker properties – the water spectral pattern (WASP) changes gradually and the dominance of highly hydrogen-bonded water structures increases with increasing lactose concentration. Figure is adapted from original.[28]

are a system. Proteins are not isolated entities in an inert medium, and all the complexity of their function in living systems can only be understood if the water is recognized as an active part of it.

3.3. Water quality monitoring

Water monitoring is one of the most natural applications of aquaphotomics. Since measurements of very small concentrations of solutes was proven to be achievable using salts as model systems,[40] the next direction of research was concerned with detection of pesticides (Alachlor and Atrazine, concentration from 1.25 – 100 ppm) which were measured with high accuracy by applying aquaphotomics principles achieving the detection limit of 12.6 ppm for Alachlor and 46.4 ppm for Atrazine.[40]

A great step forward in water quality monitoring was made by moving on from the detection of individual contaminants to monitoring the water by utilizing the water spectral pattern as a holistic, integrative marker.[41] The proposed concept has significant advantages – it allows cost-effective, reagent-free, continuous screening of water quality where even small disturbances are reflected in the water spectral pattern which serve as a signal for possible contamination, reducing the possible need for conventional solute analysis.[41] The concept is radically novel, because it shifts the perspective of water quality defined by a set of physico-chemical and microbiological parameters to the definition of water quality as a water spectrum within some defined limits – i.e. the spectrum integrates the influence of all single markers into one integrative, holistic marker which can be easily monitored in real time. The applicability of the proposed concept was evaluated on different types of water solutions (acid, sugars, and salt served as model contaminants) as well as in real life groundwater system.[41]

In addition, the same principles of using the water spectrum as an integrated marker characteristic for each water was applied for discrimination of commercial mineral waters[42] and for discrimination of water before and after filtering as mentioned earlier.[37]

3.4. Food quality monitoring

Most fresh foods contain more than 70% water, while fresh fruits and vegetables can contain up to 95% water.[43] Thus the quality is deeply related to their water status. NIR spectroscopy (780 – 2500 nm wavelength region of the electromagnetic spectrum) has been used as a non-destructive tool for food quality monitoring for a long time.[44] It has been found that in many foods the NIR signal is dominated by the absorbance of water and multivariate analysis of NIR spectra frequently demonstrates that the water absorbance band, located around 1450 nm, is the main contributor to quality prediction.[2] This confirms that water status is a key indicator of food quality.

Aquaphotomics has been applied to understanding the role of water in food quality, for instance in the detection of surface damage in mushrooms,[45,46] quality monitoring of milk,[47,48] detection of honey adulteration,[49] monitoring of the cheese ripening,[50] investigating sugars in dehydration of apples[51] and apple sensory texture,[52] influence of packaging materials on cheese and winter melon[53] and many more.[43]

3.5. Materials and nanomaterials studies

Aquaphotomics studies on water-material interaction hold great promise in understanding some of the

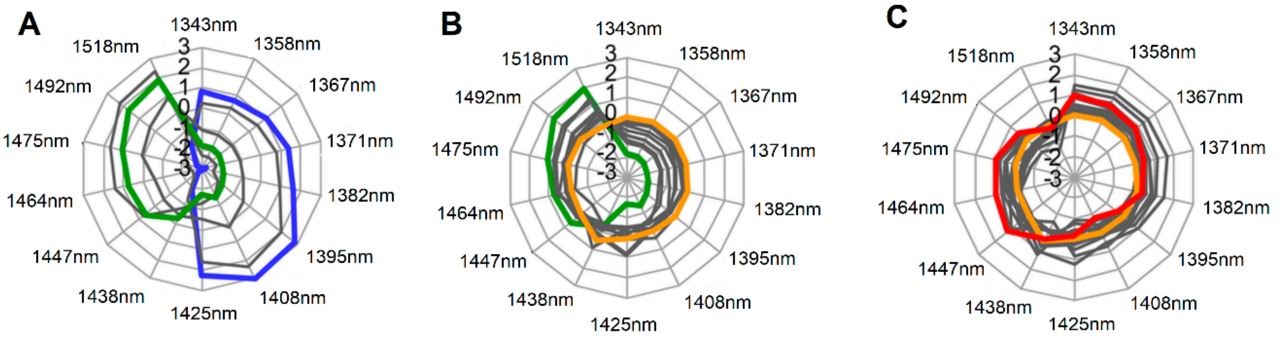

Figure 7. Time dependency of water spectral changes along the fibril formation process depicted by aquagrams. (A) 6 to 10 min for nucleation. (B) 10 to 18 min for elongation. (C) 18 to 30 min for the equilibrium phase. The WASP is plotted every 1 min, starting from the 6th minute, and those at 6 min, 10, 18 min and 30 min are colored by blue, green, orange, and red, respectively, the rest are colored grey. Figure adapted from original.[44]

very complex properties that are of interest for many applications such as wettability or biocompatibility.

Recent aquaphotomics studies utilizing time-resolved IR spectroscopy are excellent examples of other regions in EM spectra that contribute to our understanding of water-light interaction and the functionality of different water species in the biocompatibility of polymers.[54–56] Other studies revealed crucial importance of ratios of different water species that contribute to excellent wettability of titanium dioxide surfaces.[57] More recent studies explored the state of water in hydrogel materials of soft contact lenses[58,59] – something that was in the past exclusively done using destructive calorimetric methods, and now for the first time performed on lenses in hydrated conditions similar to physiological conditions – these studies revealed that the water spectral pattern holds information about degree of damage of polymer networks and of protein deposits on the surfaces of worn contact lenses. They show the potential of using aquaphotomics in the exploration of water and hydrophilic materials at the same time in a completely non-destructive manner.

Other aquaphotomics studies showed how nanomaterials shape the water matrix. For example, studies showed that fullerene-based nanomaterials in very low concentrations act as water structuring elements.[60–62] This finding may actually provide the explanation for the peculiar findings of their excellent antioxidant and radioprotective properties which far exceed theoretical calculations based solely on fullerene structure.[63] Similar to the findings on biomolecules and water interaction, nanomaterials, or materials in general should not be viewed as systems functioning in an isolated manner – they form a system when they interact with water, and this results in the functionality and the properties as we know them.

3.6. Microbiology studies

Aquaphotomics made a significant contribution to the field of microbiology by not only providing a fast and nondestructive analysis, but by contributing to better understanding of the mechanism of action of some microorganisms.[64–66] An example of such an application was in growth monitoring of probiotic, non-probiotic and moderate bacteria strains.[65] The three groups could be classified according to their probiotic strength with high accuracy – and each bacteria strain influenced the water in a specific way producing unique spectral pattern (Figure 8). The absorbance bands that contributed most to the classification were in the first overtone of water OH stretching vibrations (1300-1600 nm). Aquagrams of the three groups are shown in Figure 7, probiotic bacteria strains (in red) were characterized by a higher number of small protonated water clusters, free water molecules and water clusters with weak hydrogen bonds.[65] The discovery that strong probiotic bacteria shape water by producing more free water and less hydrogen-bonded water species, i.e. they break water structures in a way comparable to an increase in temperature, provides novel insight on their mode of action in biological organisms.

Another study showed that even in the first overtone of the combination bands of water OH stretching vibrations (1100-1300 nm) the aquaphotomics approach allows successful, rapid selection of probiotic bacteria strains.[64] It was shown that the differences in the water spectral pattern of different bacteria strains were related to the presence of extracellular metabolites, which have a different influence on water molecular structure.[66]

3.7. Plant biology studies

NIR spectroscopy coupled with suitable discrimination analysis provides an opportunity to gain information on the health status of plants in real time and non-destructively, even allowing a biological specimen to remain alive for continuous *in vivo* monitoring during biotic stress such as a viral infection or abiotic stresses such as cold and drought.

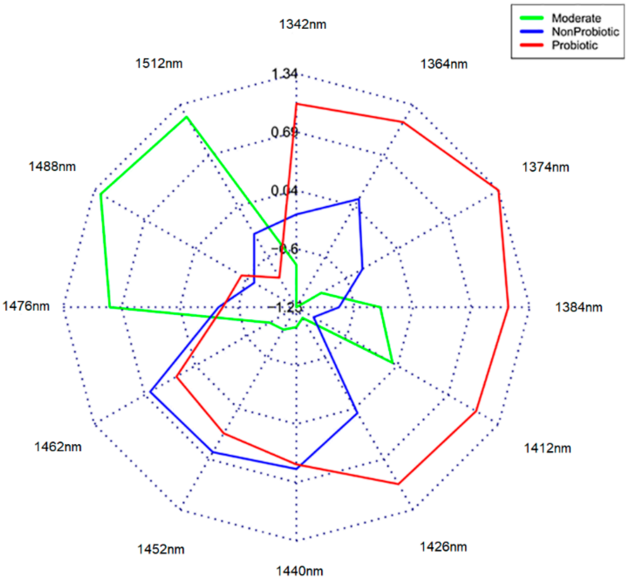

Figure 8. Aquagrams of culture media of groups of probiotic, moderate and non-probiotic strains. Average values of normalized absorbance values of the water matrix coordinates for each group are plotted on each wavelength axis. Figure adapted from original.[65]

Aquaphotomics provided a methodology to follow the impact of a virus infection based on tracking changes in water absorbance spectral patterns of leaves in soybean plants during the progression of the disease.[67] Compared to currently used methods such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and Western blotting, aquaphotomics was unsurpassable in terms of cost-effectiveness, speed, and accuracy of detection of a viral infection. The diagnosis of soybean plants infected with soybean mosaic virus was done at the latent, symptomless stage of the disease based on the discovery of changes in the water solvation shell and weakly hydrogen-bonded water which resulted from a cumulative effect of virus-induced changes in leaf tissues. Tracking the cumulative effect of various, probably even unknown biomarkers of viral infection in leaves provided grounds for successful, early diagnosis based on aquaphotomics principles.

Similarly, different water spectral patterns were found in leaves of genetically modified soybean with different cold stress abilities.[68] This research on discrimination of soybean cultivars with different cold resistance abilities has proven that resistance to cold can be characterized by different water absorbance patterns of the leaves of genetically modified soybean. Again, different genetic modifications resulted in a multitude of bio-molecular events in response to cold stress, whose cumulative effect was detected as a specific water spectral pattern of leaves – i.e. the higher the cold resistance, the higher was the ability of cultivar to keep the water structure in less-hydrogen bonded state, providing a supply of "working water" in the conditions of decreased temperature.

In another study, aquaphotomics was applied for exploration of the remarkable property of extreme desiccation tolerance i.e. the ability of some plants, called resurrection plants, to survive extremely long periods in the absence of water and then to quickly and fully recover upon rewatering.[69] Application of aquaphotomics to study one such plant *Haberlea rhodopensis* during dehydration and rehydration processes, revealed that in comparison to its biological relative, but a non-resurrection plant species *Deinostigma eberhardtii*, *H. rhodopensis* performs fine restructuring of water in its leaves, preparing itself for the dry period. In the dry state, this plant drastically diminished free water, and accumulated water molecular dimers and water molecules with 4 bonds (Figure 9). The decrease of free water and increase of bonded water, together with regulation during drying which is directed at preservation of constant ratios of water species during rapid loss of water, was thought to be the underlying mechanism that allows preservation of tissues against the dehydration-induced damages and ultimately the survival in the dry state, as well as resurrection to its fully functional state upon rewatering.

3.8. Bio-measurements, bio-diagnostics, and bio-monitoring

As briefly mentioned in the introduction, aquaphotomics was founded as a novel discipline on the applications of NIR spectroscopy for milk quality analysis and cow mastitis (mammary gland infection) diagnosis.[1,33,47,70] These works showed that as the various milk components change during the different stages of infection, they influence the water matrix of milk differently, therefore water spectral patterns found by the aquaphotomics analysis are suitable as a biomarker for diagnosis of mastitis.[71] Furthermore, for blood, and urine, it was shown that water spectral patterns were able to function as a biomarker. The water spectral patterns of blood, milk, and urine of mastitic cows, revealed that the same water absorbance bands are activated in different body fluids in response to the presence of disease.[33]

Not only the presence of disease can be detected using aquaphotomics principles, but also basic physi-

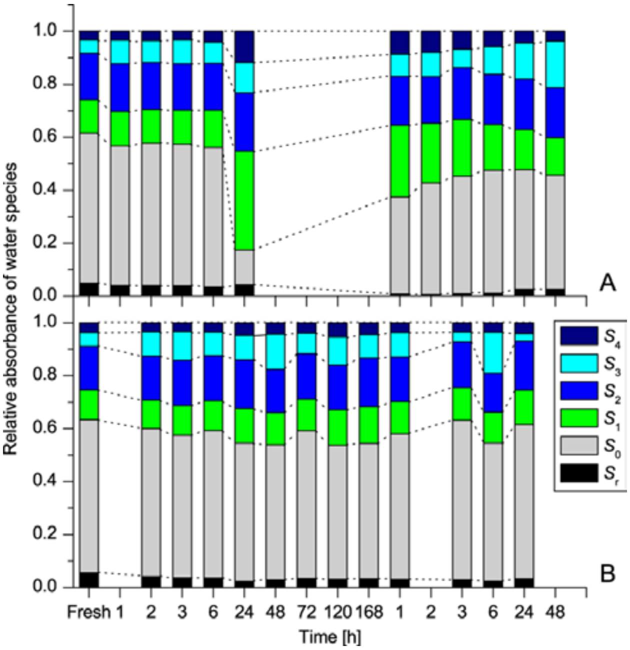

Figure 9. Dynamics of different water species (S_i = water molecules with *i* hydrogen bonds, S_r = protonated water clusters) during dehydration and rehydration of *Haberlea rhodopensis* and *Deinostigma eberhardtii*. Relative absorbance of water species in *Haberlea rhodopensis* (A) and *Deinostigma eberhardtii* (B) during desiccation and subsequent rehydration.[69]

ological changes can be tracked. For example, the water spectral pattern of urine was used to detect the ovulation period in the giant panda,[72,73] as well as in the Bornean orangutan,[74] and the water spectral pattern of milk was used to detect the ovulation period of dairy cows.[75] The method proved to be able to detect hormonal changes in a very low range (0.80 ng/ml to 127.88 n/ml)[73] more rapidly and therefore can be practically done more often than conventional analysis and without using reagents.

Aquaphotomics has also been applied in the human medical field, one of the earliest works utilized NIR spectra (600-1100 nm) for detection of HIV-1 virus in plasma.[76] The results yielded a good correlation with those obtained by the reference ELISA method suggesting that this can be a rapid and accurate screening method for HIV-1 infection, and for other viral diseases too.

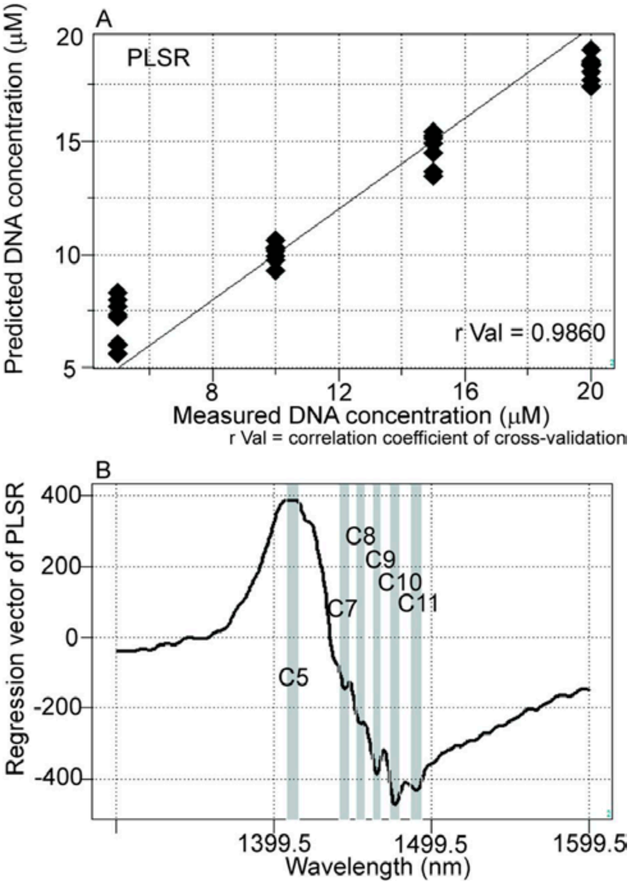

Figure 10. NIRS regression model for DNA concentration. (A) Y-fit for DNA concentration of partial least squares regression (PLSR) with pretreatment by mean centering, smoothing (21 points), orthogonal signal correction (OSC) (one component), and active class validation. N = 32, number of applied latent variables = 2, rCal = 0.9978, SEC = 0.3882, rVal = 0.9860, SECV = 1.5131. (B) Regression vector of the PLSR calibration model for DNA concentration showing characteristic water peaks at the 1400–1500 nm spectral interval.[79]

Feasibility of discriminating different organ tissues was shown in brain, liver, kidney and testes tissue of mice,[77] and detection of concentrations Cu, Mn, Fe in the same tissues was also reported.[78]

One of the groundbreaking works dealt with the detection of UV induced changes in DNA based on the changes in the water spectral pattern of DNA solutions.[79] Non-invasive identification and measurement of very low concentrations of 3D conformations of DNA were possible, see Figure 9. The formation of UV-induced cyclobutane pyrimidine dimers caused an increase of strongly hydrogen-bonded water, which has been found in previous studies to be typical for oxidative stress. Apart from the effect of UV radiation, even the dose of irradiation could be measured indirectly by the changes induced in the water spectral pattern, i.e. in the strength of water covalent bonds and other changes in the water matrix. Figure 10 shows the Y-fit and regression vector for DNA concentration.

Aquaphotomics was also proposed for *in vivo* monitoring of topical cream effects,[61,62] and in recent international conferences, other applications e.g. in the field of therapy monitoring have been proposed, such as dialysis efficacy, which uses a similar approach to what was used for water quality monitoring. It is expected that new publications about more aquaphotomics applications will follow. The next step with great potential is to bring these aquaphotomics applications to the market.

4. CONSIDERATIONS

As water is highly influenced by its environment, depending on the required limit of detection for the application, environmental changes have to be taken into account. In previous work it has been recommended that parameters of the environment, such as temperature, pressure, humidity, should always be recorded to match every measured spectrum.[6] Sensitive applications for subtler changes in the water matrix may require monitoring of high and low frequency measurements, magnetic fields, CO_2 levels, background radiation, and other factors such as periodical changes in the year, related to moon phases or sun spot activity, to interpret water spectral changes. All these measurements and controls are needed in order to confirm that the water spectral pattern related to each of these environmental perturbations is different from the water spectral pattern found for the perturbation of interest.

When it comes to unknown possible influences, not only environmental but also related to drift of instrument and more, it has been recommended to scan pure water samples (as environmental control) at regular intervals during the experiments.[6] In subsequent analysis the spectra of these pure water samples are used for correction, either by using EMSC (extended multiplicative scatter correction) using the first loading of PCA (principle component analysis) as an interferent spectrum,[26] or by applying a closest spectrum subtraction technique,[80] in order to remove environmental influences not concerning the perturbation of interest. It is not always possible to 'remove' an influence entirely, but knowing its spectral pattern will help separating it from the spectral pattern of the main perturbation. For that purpose, new data analysis methods have to be explored and developed.

5. FUTURE PERSPECTIVES

With the theoretical and technological advancements in spectroscopies in the entire EM range the development of aquaphotomics based applications has become more feasible. Analytical tools and data processing have improved significantly and the miniaturization of sensors on the technological side has opened up the potential for high accuracy field applications being more cost-effective at the same time.[81]

Also, the mentioned advantages of being non-destructive, fast and capable of comprehensive system (real-time) monitoring and diagnosis provide great potential to complement conventional technology used to perform similar tasks. As discussed in this paper, aquaphotomics can be applied in many fields, such as agriculture (plants and animals), biotechnology, life science, medicine, industry, and basic science. Further pilot studies in real-life settings, combined with market research and sensor design specific for each application will pave the way towards implementation of aquaphotomics in daily life.

In our opinion, cross-disciplinary research can also benefit from aquaphotomics by considering water as the matrix of life. Water is the bridge and provides a new common platform for science and technology,[4] a common 'mirror' for all disciplines. For example, the fractality and coherence of liquid water, as predicted by quantum electrodynamics, have been shown through NIR spectral analysis of the isosbestic behavior induced by temperature perturbation.[82] Applying the concept of aquaphotomics in this way opens up the potential for exploring systems on micro and macro level, from cells to space. In the future, the areas of biotechnology and life science, as well as basic science of water will benefit as aquaphotomics provides a complimentary way of exploring water – through its interaction with matter and energy in real time. These interactions can be observed in various systems, such as liquids, cells, whole organisms, space, using the available technology and its upcoming advancements. A new language around water spectral patterns per system and perturbation, such as being built with the aquaphotome database, will enable new discoveries and understanding of bio aqueous systems and the role that water plays. Next steps, such as the further building of the aquaphotome database, will stimulate cross-disciplinary science and will help understand the role of water as a functional 'biomolecule' in the dynamics of life.

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