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Aquaphotomics tenth anniversary

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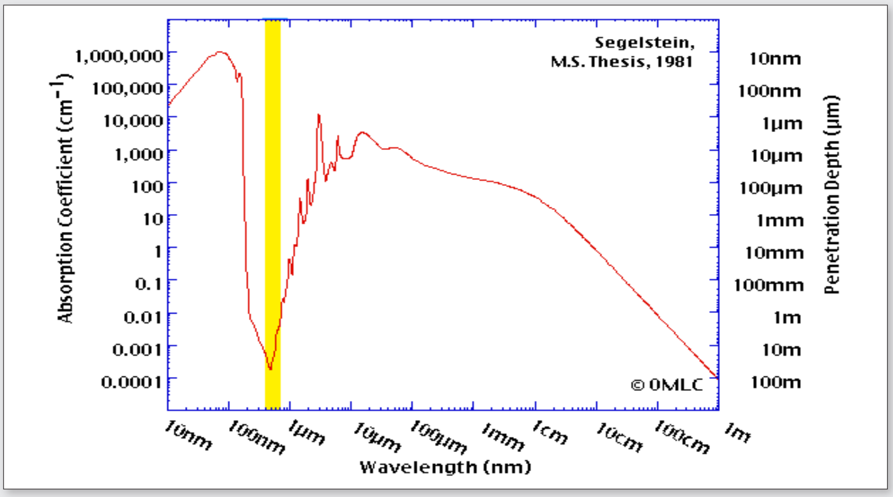


Figure 2. Water electromagnetic spectrum.

Celebrating anniversaries is a good reason to review achievements. Aquaphotomics (Figure 1) emerged from near infrared (NIR) spectroscopy as a new scientific field covering the "avoided" part of the NIR spectrum where water has a strong absorption. This defines the role of aquaphotomics as complementary to conventional NIR spectroscopy to explore the role of water in biological and aqueous systems. As water is the only unique substance that absorbs any kind of electromagnetic radiation, Figure 2, aquaphotomics could be defined as water spectroscopy covering the whole electromagnetic spectrum. Water is a matrix and a continuous, dynamic network of water molecules, the fabric of living and aqueous systems. Quantitative information about its spectral changes at various energy levels opens a new scientific window for direct observation of system functionality. The NIR is the most informative and suitable range for *in vivo* water measurements and non-invasive monitoring aiming at diagnosis, identification and understanding of system functionality. Aquaphotomics is the field where spectroscopy, time series and multivariate analysis meet to discover new phenomena in biology, chemistry and physics.

Water is perhaps the most measured substance by NIR spectroscopy due to its strong signal compared to other molecules and its strong effect on properties and chemical activity of materials. Water (moisture) is commonly analysed using a single wavelength and appropriate path length. Tomas Hirschfeld, Phil Williams, Roberto Giangacomo, Mutsuo Iwamoto, Yukihiro Ozaki and other scientists in the field of NIR spectroscopy have explored water in their studies. They showed that water can be used to indirectly measure other molecules such as salts, sugars, proteins etc. that either do not have absorbance in the NIR range or because their molecular structure can be studied through investigating the surrounding water. Karl Norris used to give the "famous" example that the spectrum of protein in water minus the spectrum of water does not give the spectrum of the respective protein and it has become a strong motivation for me to answer the question "why?". In the beginning, our group has learned from these studies trying to understand and to explain spectral changes caused by disease in biological systems. In 2001, at the 10th International NIR Spectroscopy Conference in Kyongju, Korea, for the first time, we reported that in the wavelength range of the water first overtone we found the most prominent changes

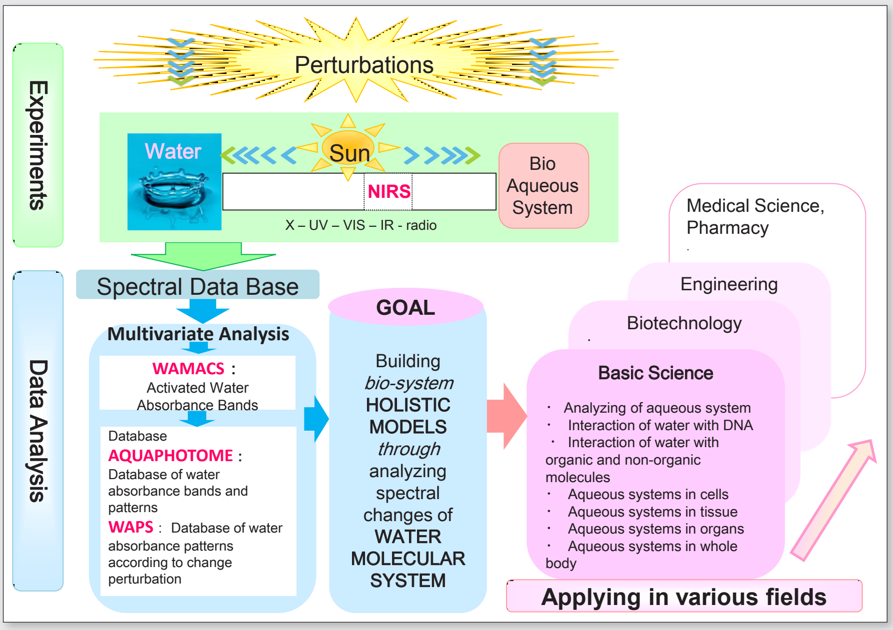
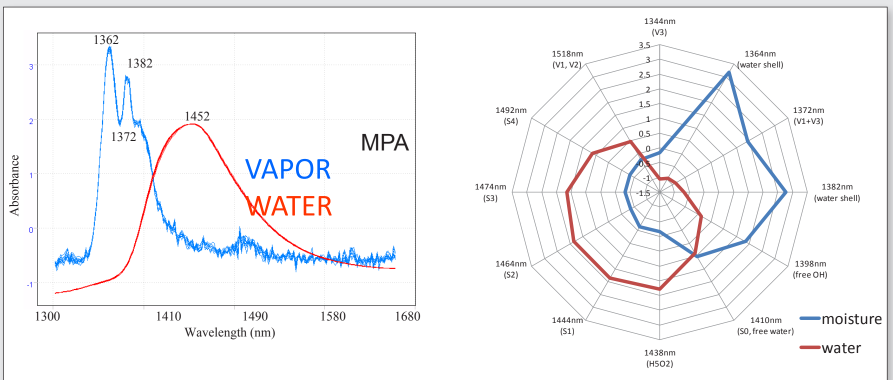


Figure 1. Aquaphotomics overview.

in milk from healthy cows versus milk from cows with mammary gland inflammation.[1] That was the first attempt ever to relate the water absorbance spectrum of a system to its health status. Karl Norris noticed this as an important achievement and we felt his strong encouragement. Our next work on prion protein, PrP, isoforms identification using real-time NIR spectral data acquisition played an important role in paving the way to aquaphotomics. It was a collaborative study with Dr David Brown and Dr Ilina Iordanova from the University of Cambridge, UK, and a continuation of our joint work with Professor Y. Ozaki's group on protein–water interaction studied by NIR spectroscopy. For the first time, we demonstrated the power of NIR spectroscopy for non-invasive monitoring and spectral data acquisition as a time series. We were able to identify three isomers of PrP [PrP, PrP(Cu) and PrP(Mn)] only using the first overtone of water spectral monitoring data and to describe water spectral changes in the process of PrP fibrillation caused by Mn in the protein's octapeptide region.[2] The results were published in *BBRC* and our paper became a milestone for using time-resolved NIR spectroscopy as a tool with which to investigate the role of water in understanding the mechanism of protein fibrillation. Later on, after continuing in the same direction and finding specific water spectral patterns for various systems and conditions, we proposed Aquaphotomics as a new scientific discipline at the 12th International NIR Spectroscopy Conference in Auckland, New Zealand. We presented a talk "Visible-near infrared perturbation spectroscopy: water in action seen as a source of information".

The first workshop on Aquaphotomics was held at the 13th International Conference in Sweden in 2007 followed by another one at the IDRC in Chambersburg in 2008. The members of the workshops came from different countries: Ireland, Italy, Bulgaria, Canada, Japan, Thailand, Hungary, New Zealand, The Netherlands etc. The First International Symposium on Aquaphotomics was held in Brussels in 2014. The Second is going to be in Kobe, Japan, 26–29 November 2016. Thanks to the research of different groups working on aquaphotomics, spectral data of various systems and their analysis with the aim of identifying the role of the water in each respective system has been very successful and has led to very interesting findings. In different systems, we were able to identify always the same water bands activated in different combinations and patterns following various perturbations. These water bands are called Water Matrix Coordinates, WAMACS, and the specific spectral patterns, Water Absorbance Patterns, WAPS. An overview of these results was written as an editorial in the Special Issue of *Journal of Near Infrared Spectroscopy* on Aquaphotomics published in 2009.[3] The Aquagram, Figures 3 and 4, is a star chart displaying normalised water absorbance at already known water bands represented on axes starting from the same point. Each axis represents relative values for all the compared observations at the same absorbance band. The chart depicts water absorbance patterns, WAPS, for respective perturbations or conditions of the systems. In Figure 3, the aquagram of water and vapour is shown, emphasising the difference in water molecular spectra. Aquaphotomics has developed in the following directions: answering basic questions related to phenomena and applications. One of them was in understanding the role of water in biotic and abiotic stress of plants.[4] It was found that less hydrogen-bonded water structures diminish with the biotic stress and the strength to withstand low temperatures of genetically-modified plants could be precisely identified by their water spectral changes under low temperature perturbation. This suggested the idea to look in further detail about DNA—water interaction. Together with the group of Professor Nishigori at the Medical Faculty of Kobe University, we studied UV light influence on water in DNA and published a paper in *Scientific Reports*[5] showing that light does change the water structure and it might cause the DNA damage. Our results were consistent with high-performance liquid chromatography (HPLC) results of measuring DNA dimer produced as a result of DNA damage. This article illustrates a paradigm shift from the previous understanding that NIR spectroscopy cannot be used to measure low concentrations because of the often very low absorbance. Aquaphotomics results prove the opposite when the water NIR spectrum is used as a molecular mirror of the constituent present at low concentration.

In the few last years, we have tried to systematise the knowledge about water molecular changes under perturbations of different kinds. One direction is the molecular perturbation. In collaboration with Keio University we are further developing the water absorbance bands data base, the aquaphotome, by systematically perturbing water with the main biomolecules at physiological concentration. Having done that, we might be able to understand a lot about different phenomena in biology. Another direction is expanding the knowledge about protein fibrillation. Together with Professor E. Chatani from the Faculty of Science at Kobe University, Japan, in 2014, we published a paper in *PLOS One*[6] observing and understanding the role of water molecular changes in the process of insulin fibrillation in salt solution and at high temperature. Recently, at the system level, we are interested in using water spectral changes for diagnosis of oestrus. In collaboration with Kyoto University, and Awajishima



$$A'_\lambda = \frac{A_\lambda - \mu}{\sigma}$$

A : Absorbance after EMSC (1300-1600 nm)
μ : Mean of Averaged spectra
σ : SD of absorbance each wave length

Figure 3. Water and vapour spectra and Aquagram.

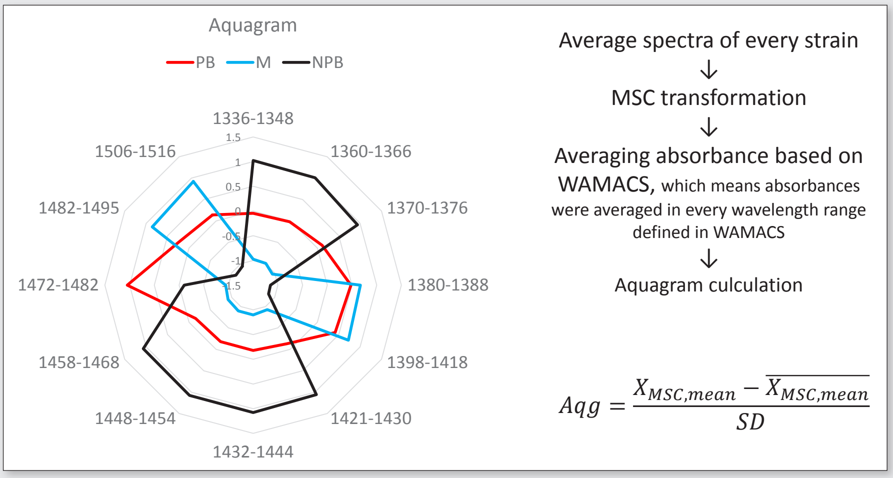


Figure 4. Aquagram of lactic acid bacteria (probiotic, moderate and non-probiotic strains averaged spectra at around the 8–11 hours of the development).

Agricultural Center, Japan, we published papers about successful oestrus diagnosis in pandas and dairy cows.[7] Another exciting direction is using water spectrum to understand the differences between and mechanisms of probiotic lactic acid bacteria, Figure 4. In this figure, differences in the specific water absorbance patterns can be seen for the respective strains. Together with Professor A. Slavchev at the Food Technology University in Plovdiv, Bulgaria, we investigated the behaviour of different lactic acid bacterial strains. We have started this research in collaboration with Tiziana Cattaneo from Italy and her group. In the near future, in relation to other already observed patterns of living systems, this might help in understanding the role of water in biological systems in general. Zoltan Kovacs, now at Kobe University, following A. Gowen's paper in *Talanta*,[8] developed an algorithm[9] using water spectral pattern as an instant biomarker for water quality evaluation. G. Bazar from Kaposhvar University wrote a paper in *PLOS one*[10] about how important the NIR instrument is in aquaphotomics having in mind that we are measuring and analysing 10^{-4} absorbance units changes.

Water has been studied by different disciplines in many different ways. All of them use their own particular terminology and sometimes it is quite difficult to translate scientific findings from one area in to another. Aquaphotomics provides an opportunity to start building up a "water vocabulary" in order to translate findings about water between different disciplines. Experimental identification of water bands in the NIR wavelength range relies on multivariate data analysis. These can be related to overtone bands calculated from observed fundamental bands. Once a large database, *aquaphotome*, of characteristic water absorbance bands has been acquired, they will be related to specific biological functions, structures etc., and used for understanding biology, chemistry and physics of water and other aqueous systems. In order to describe the natural phenomena related to water and for their better understanding, Aquaphotome development in the NIR range could be described as writing the alphabet of "water–light" interactions, where WAMACS would be the letters and the WAPS would be the words. Having such an "alphabet", any perturbation (chemical, physical or of any other nature) could be well defined using the "letters" of water. Within the water matrix the vast amounts of hydrogen bonds that could be "seen" with NIR light reflects any molecule embedded in it like a mirror. We call it a three-dimensional water mirror (Aquamirror on molecular level) as with the contemporary technology, it is becoming possible to acquire a pixel spectrum in a 3D matrix.

Aquaphotomics, as an "omes and omics" concept, is a result of high throughput experimental analyses. Through an understanding of water–light interaction dynamics and their relation to biological functions, aquaphotomics brings together the knowledge acquired by other -omics disciplines such us genomics, proteomics, metabolomics etc. that describe single elements of biological systems. Aquaphotomics upgrades this knowledge to a system level as water does in biology and aqueous systems.

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