Aquaphotomics: Water Spectral Pattern as a Biomarker for Diagnosis

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Contents

1. Aquaphotomics concept
2. Near Infrared Spectroscopy
3. Water Spectral Patterns as a biomarker of water
4. Water spectral pattern of urine as biomarker for estrus in Giant Panda
5. Water Spectral Patterns of soy bean plant as a biomarker of biotic and abiotic stress
1. Aquaphotomics concept

- *From biosystems to water* -
Water, Biosystem, NIRS

- Water in tissues change from one conformation to another, when biological reaction change from steady state to no steady state.


- The spectral difference at water bands, i.e. 1440 nm (S1), 1398nm could provide valuable information for mammary gland inflammation diagnosis, but WHY???

R. Tsenkova et al., Near Infrared Analysis, 2(1), 59-66, 2001
Water spectral changes in *bio systems* under various perturbation
Bio monitoring

Accumulating real time spectral data base

Fractals???
**Aquaphotomics: a new field of science dawn to biological sciences and engineering**

“Aquaphotomics” is a new term introduced by Prof. Roumiana Tsenkova opens a new area in biological sciences and engineering. It describes a new way for exploring biological systems through a non-destructive monitoring of their interaction with VIS-NIR light. Multivariate spectral analysis reveals that changes with the water matrix under perturbation reflect, like a mirror, the rest of the molecules surrounded by water. As a result, characteristic water absorbance patterns are used to measure very small concentrations of solutes and for disease diagnosis.

**WAMACS** = Water Matrix Coordinates, i.e. water absorbance bands in VIS-NIR range

R. Tsenkova *J. NIR infrared spectroscopy*, 17, 303-314 (2009)
**Aquaphotomics: in bio monitoring and diagnosis**

**Mastitis disease**

“Altered milk components trace signals influence the bonding structure of water measured by NIRS all at once and provide rapid and accurate mastitis diagnosis”


**Prion disease**

“...consecutive exposure of hydrogen bond network, in the body of mouse, to NIR light allowed successful diagnosis of fatal prion disease”


**Soybean Mosaic disease**

“Monitoring of Second Overtone of Water Absorbance Bands Reveals Hypersensitive Response from Virus Infected Plants”


**Virus diagnosis - HIV**

“Regression vector coefficients reveals variations in water structures related to functionality of HIV”

**Aquaphotomics: in micro particle analysis**

**Micro particles in water**

“Better prediction of polystyrene mesoscopic particle concentration is obtained by multivariate analysis (a) based on water absorbance bands than univariate method based on polystyrene absorbance (b) band at 1680nm”

**Aquaphotomics: A NIRS Investigation into the Perturbation of Water Spectrum in an Aqueous Suspension of Mesoscopic Scale Polystyrene Spheres. Asia Near Infrared conference, Tsukuba 2008**

**Heavy metal in water**

“Mg/L concentration measurement of Cd, Mg, Mn, Zn in water solution possible because of the interaction with water “seen” by the NIR light at various water absorbance bands.

**Aquaphotomics: Sakudo, A., Tsenkova, R., Tei, K., Onozuka, T., Ikuta, K., Yoshimura, E., and Onodera, T.: Comparison of Vibration Mode of Metals in HNO3 by Partial Least Squares Regression, Bioscience, Biotechnology and Biochemistry, 2006.**

<table>
<thead>
<tr>
<th>Particle size</th>
<th>R²(a)</th>
<th>R²(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1-0.01%</td>
<td>0.69</td>
<td>0.363</td>
</tr>
<tr>
<td>0.01-0.001%</td>
<td>0.75</td>
<td>0.014</td>
</tr>
<tr>
<td>0.001-0.0001%</td>
<td>0.64</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Microbial organism detection (bacteria)**

On going work
AQUAPHOTOMICS*

Aqua - : water
Photo - : light
Omics - : all about,
complement of something

*Proposed by Roumiana Tsenkova in 2005
The **PROTEOME** is the entire complement of **proteins** expressed by a genome.

The **GENOME** of an **organism** is its whole hereditary information and is encoded in the **DNA**.
AQUAPHOTOME is the entire complements of water matrix absorbance bands found under various perturbations.
WAMACS

Water Matrix Coordinates:

Water absorbance bands corresponding to the water structural equilibria

(coherent domains???)
### Water Matrix Coordinates, WAMACS

<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C10</th>
<th>C11</th>
<th>C12</th>
<th>WAMACS (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1344</td>
<td>1364</td>
<td>1372</td>
<td>1382</td>
<td>1398</td>
<td>1410</td>
<td>1438</td>
<td>1444</td>
<td>1464</td>
<td>1474</td>
<td>1492</td>
<td>1518</td>
<td>(nm)</td>
</tr>
</tbody>
</table>

**Temperature**
- Standard Deviation
- PCA
- PCA (2nd D)

**Nano particles**
- 2nd D
- PCA (MSC)
- SD
- PLS

**Sucrose**
- PCA (2D)
- PLS (MSC)
- PLS (2D)

**Citric acid**
- PCA (2D)
- PLS (MSC)
- PLS (2D)

**Aquaporin**
- PCA (2D)
- PLS-DA (MSC)
- PLS-DA (2D)
- PCA (2D)
- SD
- PLS AQP-WT
Aquagram was devised to visualize the WASP.

The aquagram displays normalized absorbance values at specific water bands on the axes originating from the center of the graph.

Absorbance values at the WAMACs are placed on the respective radial axes.
Change in temperature

Absorbance

Wavelength (nm)

65℃ 60℃ 55℃ 50℃ 45℃ 40℃ 35℃ 30℃
Water Vapor Spectrum

1362nm, free water OH stretch (OH-H2O) 1374nm, 1371nm O2-. (H2O)4
1382nm, 1383nm interwater DD stretch (OH-(H2O)4
Water and Water Vapor

Water vapor

Absorbance

Wavelength (nm)

1362
1382
1452

MPA

water vapor

water
EXTENDED WATER MIRROR

http://www.gallery-sakura.com/search/higashiyama_kaii/gs1026.html
EXTENDED WATER MIRROR

http://www.gallery-sakura.com/search/higashiyama_kaii/gs1026.html
EXTENDED WATER MIRROR with near infrared light, NIR

As WATER is a molecular NETWORK, NIR light can penetrate, get partially absorbed and bring information for the surrounding molecules making water a mirror on molecular level.
Why Extended Water Mirror: Example

- Cd\textsuperscript{2+} has no spectral features in NIR...
- Prediction of Cd in water possible!!! HOW?
- Cd\textsuperscript{2+} alters water structure
- We detect Cd\textsuperscript{2+} indirectly, using the interaction of water and NIR light

![Graph showing predicted concentration vs. concentration](image)
NaCl in Water
2. Near Infrared Spectroscopy
Overtone Vibrational Spectroscopy: a powerful tool for non-invasive water observation: immense information about water molecular conformations and respective function for better understanding of the biological world.
X-rays, UV, VIS, Infrared, NIR, IR

Wavelengths: 0.01, 0.4, 0.6, 1.0, 2.5, 50

R: small, big
Abs: small, small
T: big, small

NIR range
## Relative intensities of C-H stretch bands for infrared and the overtone NIR regions

<table>
<thead>
<tr>
<th>Band</th>
<th>Wavelength region</th>
<th>Relative Intensity</th>
<th>Pathlength</th>
<th>Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundamental band (n)</td>
<td>3380 - 3510 nm</td>
<td>100</td>
<td>0.01 mm</td>
<td>2</td>
</tr>
<tr>
<td>1st overtone (2n)</td>
<td>1690 - 1755 nm</td>
<td>1</td>
<td>1.0 mm</td>
<td>2</td>
</tr>
<tr>
<td>2nd overtone (3n)</td>
<td>1127 - 1170 nm</td>
<td>0.1</td>
<td>1.0 cm</td>
<td>2</td>
</tr>
<tr>
<td>3rd overtone (4n)</td>
<td>845 - 878 nm</td>
<td>0.01</td>
<td>10.0 cm</td>
<td>2</td>
</tr>
<tr>
<td>4th overtone (5n)</td>
<td>690 - 780 nm</td>
<td>0.005</td>
<td>10.0 cm</td>
<td>1</td>
</tr>
</tbody>
</table>
Why Near Infrared Spectroscopy?

• Long path lengths
• Non-destructive
• Fast
• Immense amount of information in one spectrum
Bio Fluid Analysis in Test Tubes
PORTABLE INSTRUMENTS and FIBER PROBES FOR

BIO MONITORING AND
BIO DIAGNOSIS
Water spectra
NIR range
SOLAR IRRADIANCE OUTSIDE ATMOSPHERE

DIRECT SOLAR IRRADIANCE AT SEA LEVEL
AIRMASS = 1.5
WATER VAPOR = 2.0 cm
OZONE = 0.34 cm
\( \tau \) aerosol 550nm = 0.126
\( \tau \) exponent = 0.66

MODIS BANDS

SOLAR SPECTRAL IRRADIANCE (W m\(^{-2}\) \(\mu\)m\(^{-1}\))

WAVELENGTH (nm)
NIR and H₂O

- Vibration frequency of hydrogen bonds in water are highly influenced by nearby molecules & environment.

- Changes in these bonds may be used to gain information on the changing environments causing them (e.g. Presence of solutes, changing temperature).
Structures of the OH·(H2O)3,4 clusters showing (A) OH·(H2O)3, the completed first solvation shell, and (B) OH·(H2O)4, the onset of the second solvation shell.

Spectroscopic Determination of the OH Solvation Shell in the OH·(H2O)n Clusters

OH·(H2O)2 1366 nm

OH·(H2O) 1480 nm

William H. Robertson, Eric G. Diken, Erica A. Price, Joong-Won Shin, Mark A. Johnson*

WATER DYNAMIC SPECTRA
2D and 3D Synchronous Correlation Maps

represent negative correlation
represent positive correlation
WATER DYNAMIC SPECTRA

Power Spectra at 1530nm
3. Water Spectral Patterns as a \textit{biomarker of waters}
Total 315 spectra
Methods for Aquagram Calculations

Calculating formula

\[ A'_\lambda = \frac{A_\lambda - \mu_\lambda}{\sigma_\lambda} \]

\( A' \): Value of Aquagram  
\( A \): Absorbance after MSC applied on 1\textsuperscript{st} overtone region  
\( \mu \): Mean of all spectra  
\( \sigma \): SD of all spectra  
\( \lambda \): 12 Wavelengths(*)

\(<1342, 1364, 1372, 1382, 1398, 1410, 1438, 1444, 1464, 1474, 1492, 1518> \)

Aquagram of Various Waters

Preprocessing:
Smooth (21) + Autoscaling
Aquagram = Sample (avg) – Pure water (avg)
4. Water spectral pattern of urine as biomarker for estrus in Giant Panda
Fig. 1 Water matrix coordinates (WAMACs) related to the estrus in a female giant panda. (A) Smoothed raw difference spectra of urine calculated as a difference between a veraged spectra from the previous to the next day of $E_2\,G$ peak and the other averaged spectra. (B) Second derivative spectra of (A). (C) Second derivative spectra of 3 years’ urine samples after smoothing. From this plot, 12 absorbance bands were selected as WAMACs.


Fig. 2 Aquagrams of urine spectra from a female giant panda in (A: 2007, B: 2008, and C: 2009) and the Aquagram of median values (D) classified by hierarchical cluster analysis (HCA). Normalized absorbance values at the water matrix coordinates (WAMACs) (1344, 1364, 1372, 1386, 1410, 1424, 1444, 1456, 1464, 1474, 1494, and 1510 nm) were plotted on each axis. Red color is used to show high estrus aquagram.
5. Water Spectral Patterns of soy bean plant as a *biomarker of biotic and abiotic stress*
Mosaic Virus Diagnosis

Method

- Soybean, 20 plants were grown in a greenhouse.
- Only 10 plants were inoculated with SMV
- All the plants were kept under similar conditions.

SIMCA discrimination approach

**Method**

Accumulated, 20 plants x 10 spectra per plants x 12 days = 2400

**USED ELISA reference method data**
- to confirm the infection,
- in the model building

**Disease diagnosis sensitivity** = \(\frac{\text{Number of disease plant identified}}{\text{Actual number of disease plants}}\)

**Disease diagnosis specificity** = \(\frac{\text{Number of healthy plant identified}}{\text{Actual number of healthy plants}}\)
Healthy and infected plants were clearly differentiated after averaging.

Principal component space

**PCA specifications**
- **Preprocessing:** Mean-center
- **Transforms:** 2\(^{nd}\) derivative
- **Factors:** 8
Highly variable wavelengths with the progress of disease

Standard deviation profiles of two plant types

$946\ nm = (S_0)$ Non hydrogen bonded water
$976\ nm = (S_2)$ Bonded water (with two hydrogen bonds)  (Abe et al., 1995)

Water index $WI = \frac{R_{970}}{R_{900}}$  (Penuelas and Filella, 1998)
Specific water absorbance patterns \((WAP)\) at 870 nm, 912 nm, 936 nm, 952 nm specific for SMV infection.

\[(Jinendra \text{ et al.}, 2010)\]

The blue shift at 970 nm virus influence on common leaf water stress.

\[(Penuelas and Filella, 1998)\]
Homework

1. Development of new multivariate methods for spectral data analysis and assignment

2. Understanding the 12 or more absorbance ranges in the water spectrum (coherent domains?? or molecular structures)
Sound of a stream,
Sunlight dancing on waters,
Life wakes up again.

R. Tsenkova, 2004