# Aquaphotomics & NIR hyperspectral imaging

*tools for understanding the role of water in foods*

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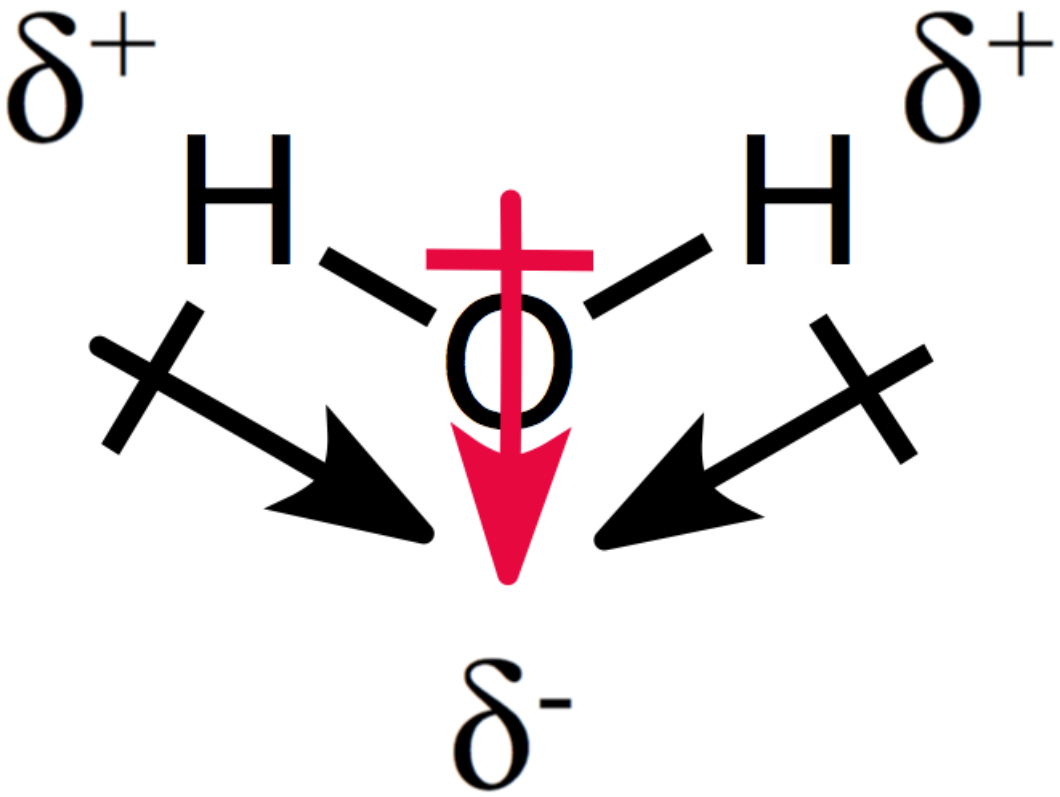
# Key Questions

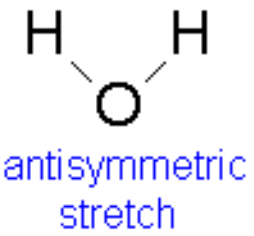
- What does water look like in the NIR?
- What can hyperspectral imaging show us?
- How can we use this to understand the role of water in foods?

# $H_2O$ molecule

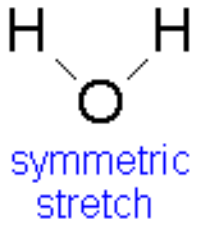


# $H_2O$ molecule

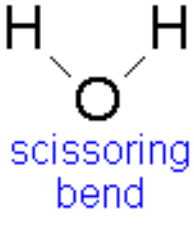
- Nonlinear molecule: 3N-6 vibrational modes
- 3 vibrational degrees of freedom




$\nu_{as}$
3657 $cm^{-1}$
2735 nm



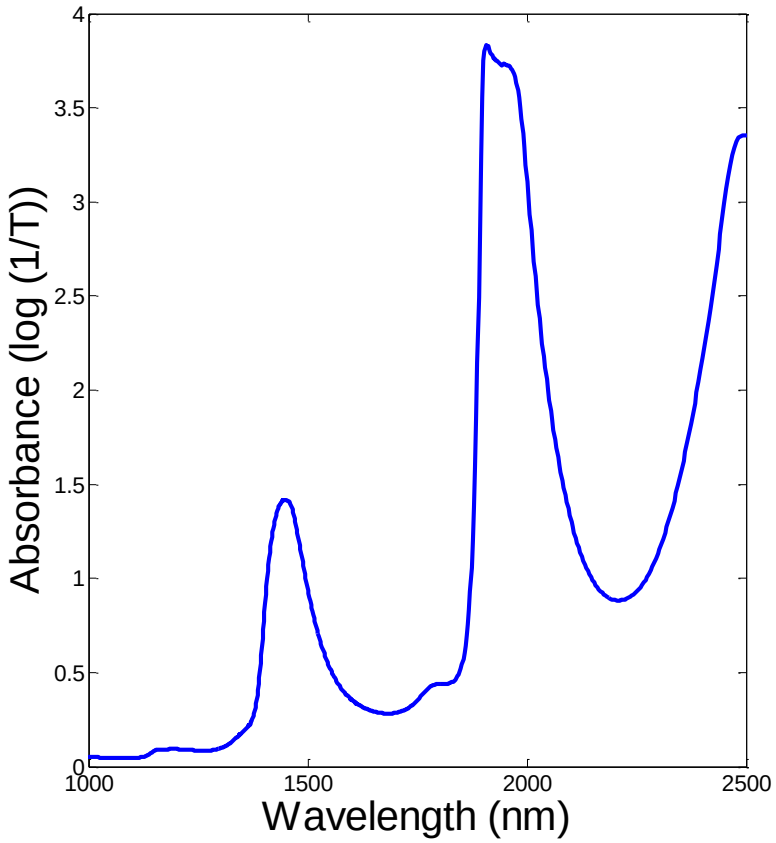

$\nu_s$
3756 $cm^{-1}$
2662 nm

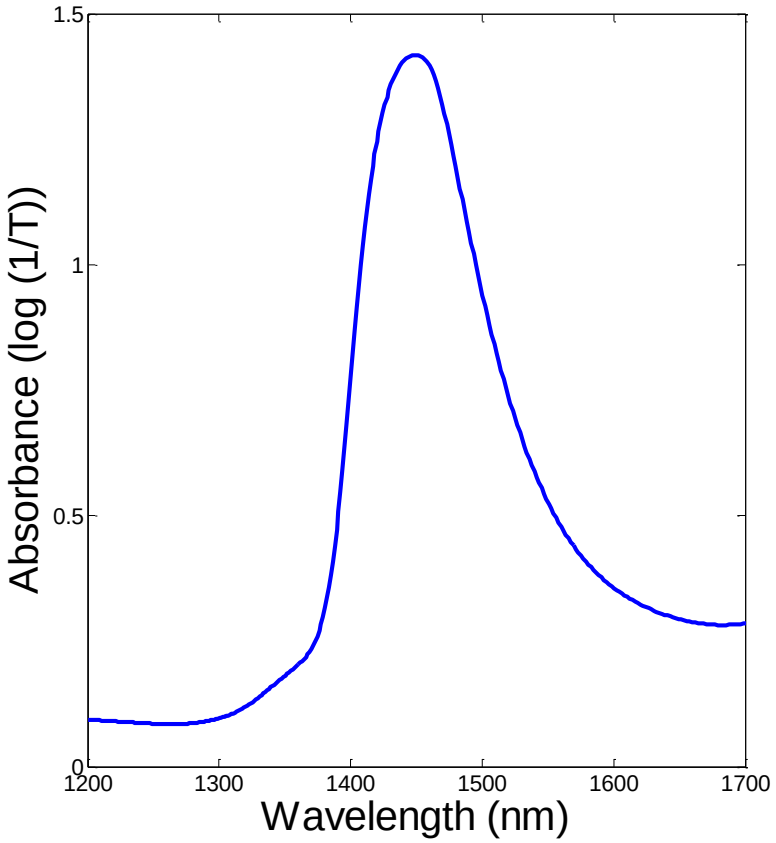



$\delta_s$
1595 $cm^{-1}$
6270 nm

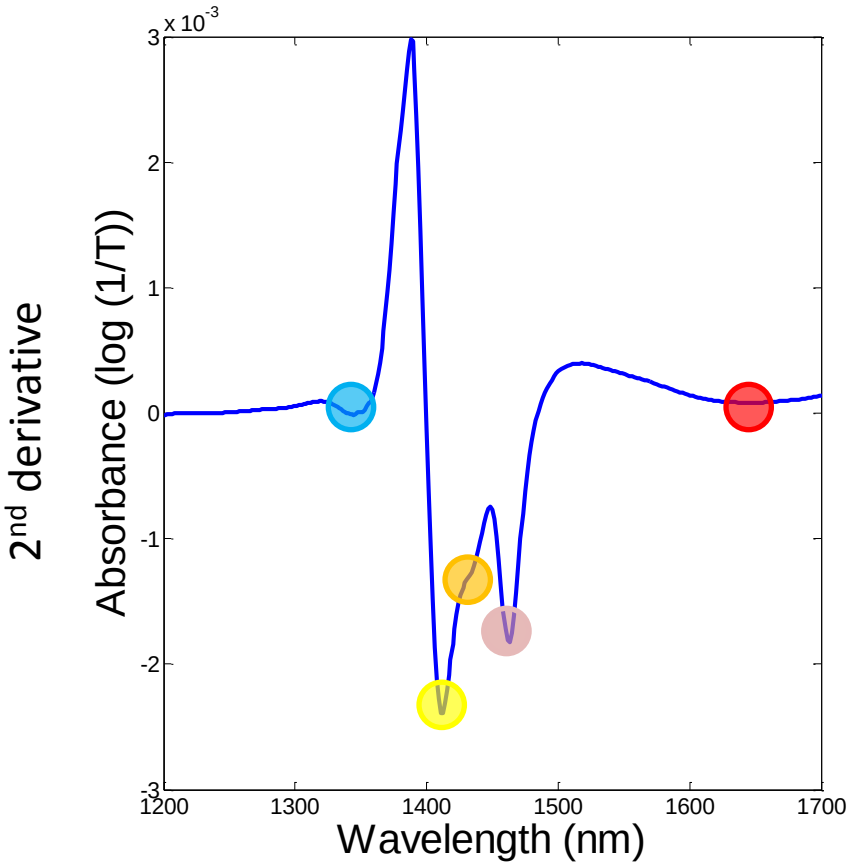
*Band locations from* Chs 1 & 9 in Siesler , Ozaki, Kawata and Heise, 2002, Near-Infrared Spectroscopy: Principles, Instruments and Applications, Wiley
GIFs from http://chemwiki.ucdavis.edu/Physical_Chemistry/Spectroscopy/Vibrational_Spectroscopy

# NIR spectrum of Water



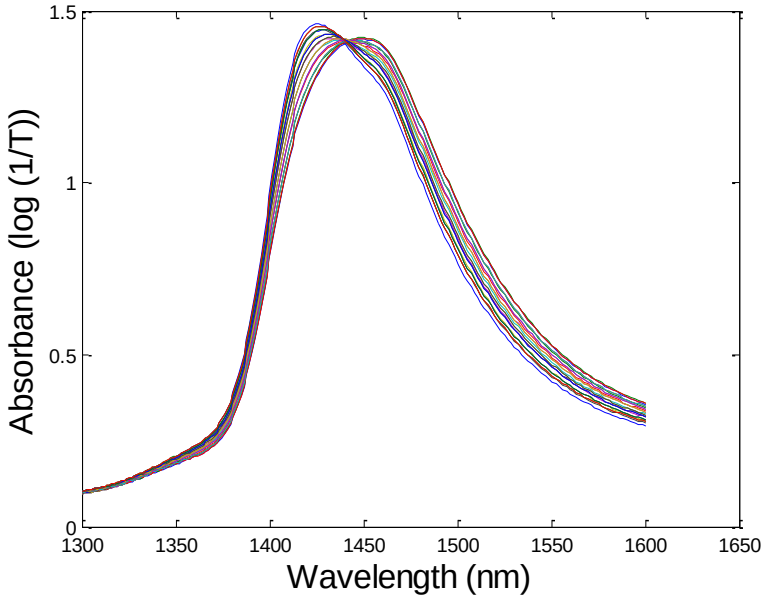
# NIR spectrum of Water

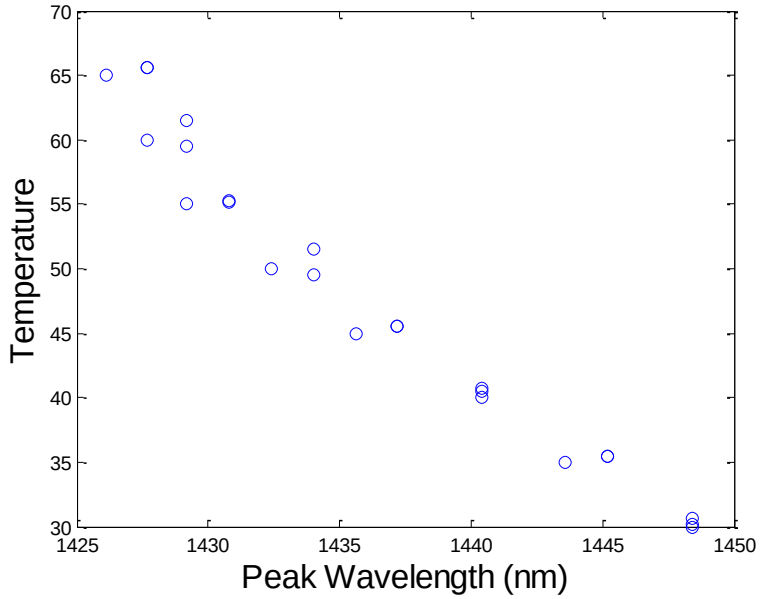



| λ(nm) | Tentative assignment [expected λ] |
|---|---|
| 1343 | $2\nu_s$ [1331-1371] |
| 1412 | $2\nu_{as}$ [1367-1408] |
| 1431 | $2\nu_{\delta s} + \nu_s$ [1440-1502] |
| 1463 | $\nu_{as} + 2\nu_{\delta s}$ [1460-1525] |
| 1647 | $\nu_{as} + 2\nu_{\delta s}$ [1460-1525] |

Siesler , Ozaki, Kawata and Heise, 2002, Near-Infrared Spectroscopy: Principles, Instruments and Applications, Wiley

# NIR spectra of Water: temperature

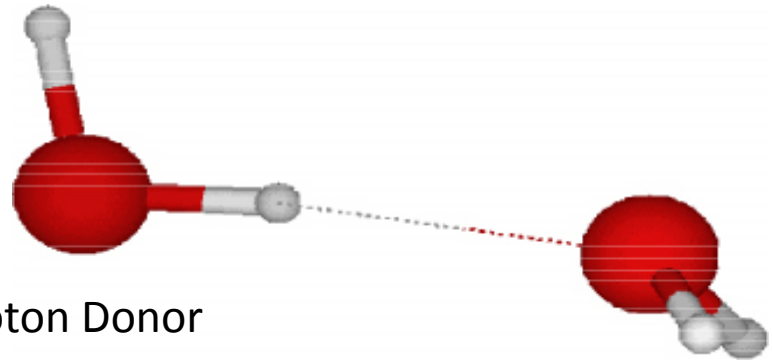



"**Blue** Shift": as T ⬆ λ ⬇

# Hydrogen Bonding

- Normal modes are perturbed when the vibrating **O-H bond 'senses'** another water molecule oriented so that its lone e's face H of the vibrating bond.



Proton Donor

Proton Acceptor

- Frequency of $\mathbf{v_s}$ **decreases from 3707** $cm^{-1}$ in the isolated molecule to 3628 $cm^{-1}$ in liquid water and to 3277 $cm^{-1}$ in ice.



**Adapted from* Ch 2 (p. 14) in Franks, 2000, Water: A matrix of life

# Suggested Assignments of Water Bands

| Assignment | $2v_s$ | $2v_{as}$ |
|---|---|---|
| Free OH in free water molecule | 1342-1383 | 1373-1415 |
| Free ("Dangling") OH | 1351-1392 | 1381-1423 |
| H bond in dimer | 1410-1453 | 1455-1499 |
| H bond in trimer | 1424-1467 | - |
| H bond in tetramer | 1475-1519 | 1533-1580 |

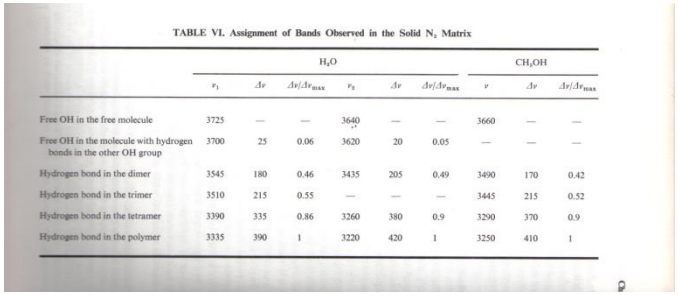


TABLE VI. Assignment of Bands Observed in the Solid $N_2$ Matrix

| | $H_2O$ | | | | | | $CH_3OH$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\nu_1$ | $\Delta\nu$ | $\Delta\nu/\Delta\nu_{max}$ | $\nu_3$ | $\Delta\nu$ | $\Delta\nu/\Delta\nu_{max}$ | $\nu$ | $\Delta\nu$ | $\Delta\nu/\Delta\nu_{max}$ |
| Free OH in the free molecule | 3725 | — | — | 3640 | — | — | 3660 | — | — |
| Free OH in the molecule with hydrogen bonds in the other OH group | 3700 | 25 | 0.06 | 3620 | 20 | 0.05 | — | — | — |
| Hydrogen bond in the dimer | 3545 | 180 | 0.46 | 3435 | 205 | 0.49 | 3490 | 170 | 0.42 |
| Hydrogen bond in the trimer | 3510 | 215 | 0.55 | — | — | — | 3445 | 215 | 0.52 |
| Hydrogen bond in the tetramer | 3390 | 335 | 0.86 | 3260 | 380 | 0.9 | 3290 | 370 | 0.9 |
| Hydrogen bond in the polymer | 3335 | 390 | 1 | 3220 | 420 | 1 | 3250 | 410 | 1 |

*Indicates blue shift of bands when less H bonding between $H_2O$ molecules*

**Observed in solid N2 matrix. Adapted from* Luck, W.A.P. Infrared studies of Hydrogen Bonding in Pure Liquids and Solutions, in Franks, 1973, Water: A comprehensive treatise (Ch 4 p. 276-9)

# Water bands in NIR

| Assignment | Min | Max |
|---|---|---|
| $3v_s$ | 887 | 1021 |
| $v_{as}+2v_s$ | 895 | 913 |
| $2v_{as}+v_s$ | 903 | 921 |
| $3v_{as}$ | 911 | 1048 |

887-1048 nm

| Assignment | Min | Max |
|---|---|---|
| $2_{vs}$ | 1331 | 1371 |
| $2V_{as}$ Free OH | 1343 | 1383 |
| $v_{as}+v_s$ | 1349 | |
| $2v_{as}$ Dangling OH | 1352 | 1392 |
| $2v_{as}$ | 1367 | 1408 |
| $2v_s$ Free OH | 1374 | 1415 |
| $2v_s$Dangling OH | 1381 | 1423 |
| $2v_{as}$ H bond in dimer | 1411 | 1453 |
| $2v_{as}$ H bond in trimer | 1425 | 1467 |

1331-1467 nm

| Assignment | Min | Max |
|---|---|---|
| $2v_{as}$ H bond in tetramer | 1475 | 1519 |
| $2v_{as}$ H bond in polymer | 1500 | 1544 |
| 2 $v_s$ H bond in tetramer | 1534 | 1580 |
| 2 $v_s$ H bond in polymer | 1553 | 1599 |

1475-1599 nm

1098-1150 nm

| Assignment | Min | Max |
|---|---|---|
| $v_{ds}+2v_s$ | 1098 | 1125 |
| $v_{as}+v_{ds}+v_s$ | 1110 | |
| $2v_{as}+v_{ds}$ | 1122 | 1150 |

1440-1525 nm

| Assignment | Min | Max |
|---|---|---|
| $2v_{ds}+v_s$ | 1440 | 1502 |
| 2 $v_s$ H bond in dimer | 1456 | 1499 |
| $v_{as}+2v_{ds}$ | 1460 | 1525 |

$\lambda$

# Aquaphotomics

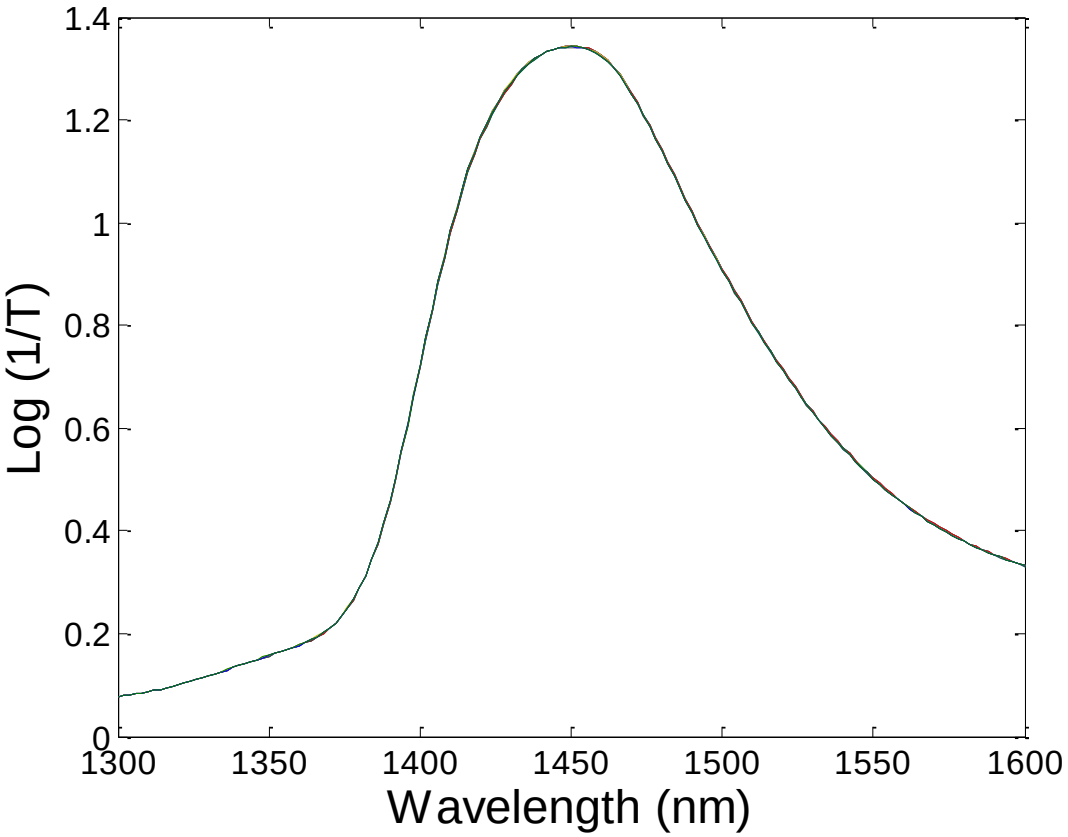
Prof. Roumiana Tsenkova
Kobe University, Japan

www.aquaphotomics.com

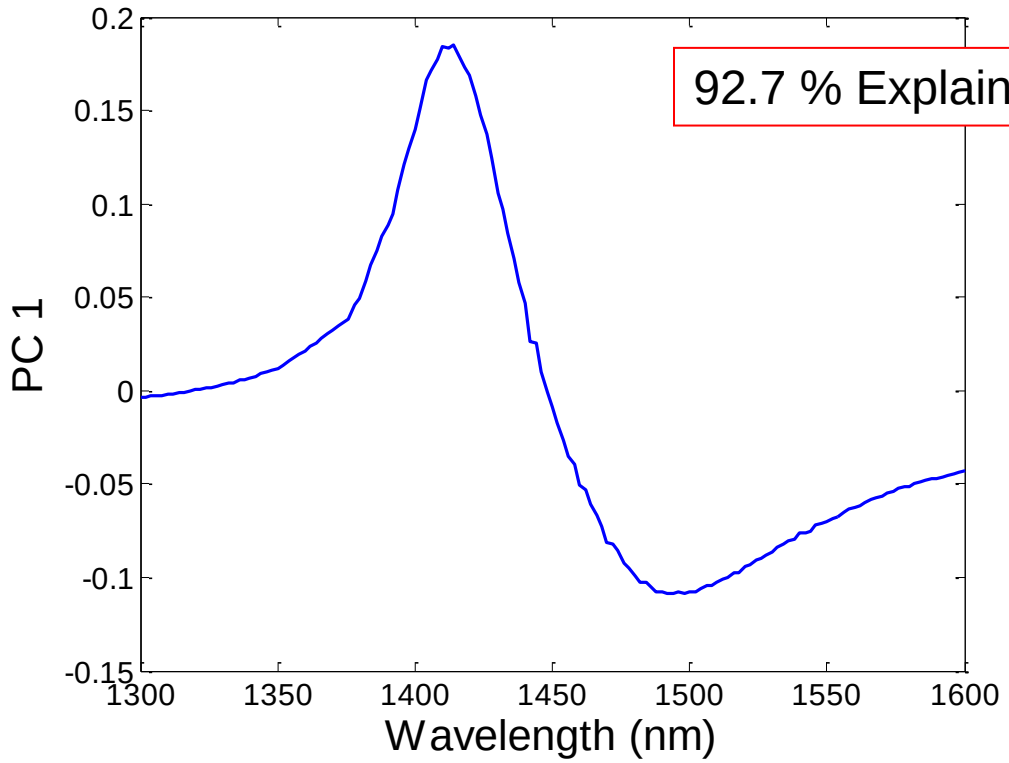
Aims to extract **water absorbance patterns** to describe biological systems e.g. animals, plants…

How to extract **water absorbance pattern (WAP) from NIR Spectrum**?

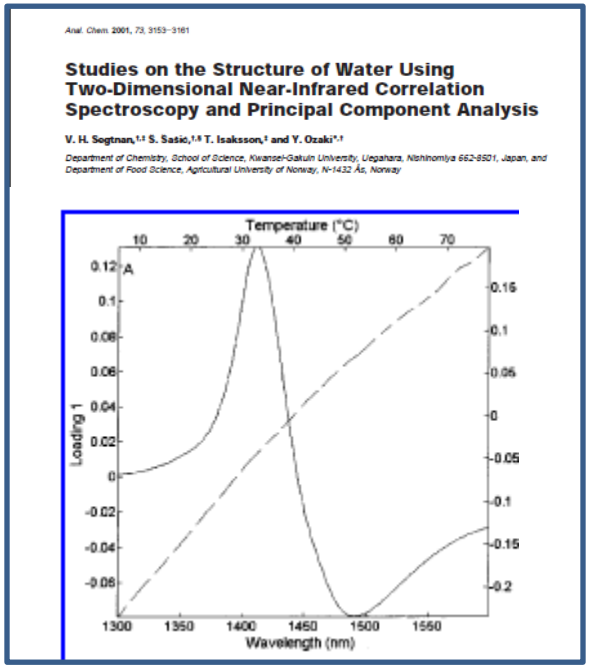
## Spectra of water over 1 hour at "constant" temperature of 28 °C



# PC1 of water over 1 hour at "constant" temperature of 28 °C




92.7 % Explained Variance



Anal. Chem. 2001, 73, 3153−3161

**Studies on the Structure of Water Using Two-Dimensional Near-Infrared Correlation Spectroscopy and Principal Component Analysis**

V. H. Segtnan,[†,‡] S. Šašić,[†,§] T. Isaksson,[‡] and Y. Ozaki*[,†]

*Department of Chemistry, School of Science, Kwansei-Gakuin University, Uegahara, Nishinomiya 662-8501, Japan, and Department of Food Science, Agricultural University of Norway, N-1432 Ås, Norway*

# Expt. 1: Salts in Water

- **4 salts:**

  NaCl, KCl, $MgCl_2$ or $AlCl_3$

- **Concentration range**:

  0.002 – 0.1 $Mol.L^{-1}$

- **Control:** Millipore water

**x 3**

Gowen et al, Talanta, 2014

# Experimental Design





**A. Gowen** (Dublin)
NIR Systems 6500





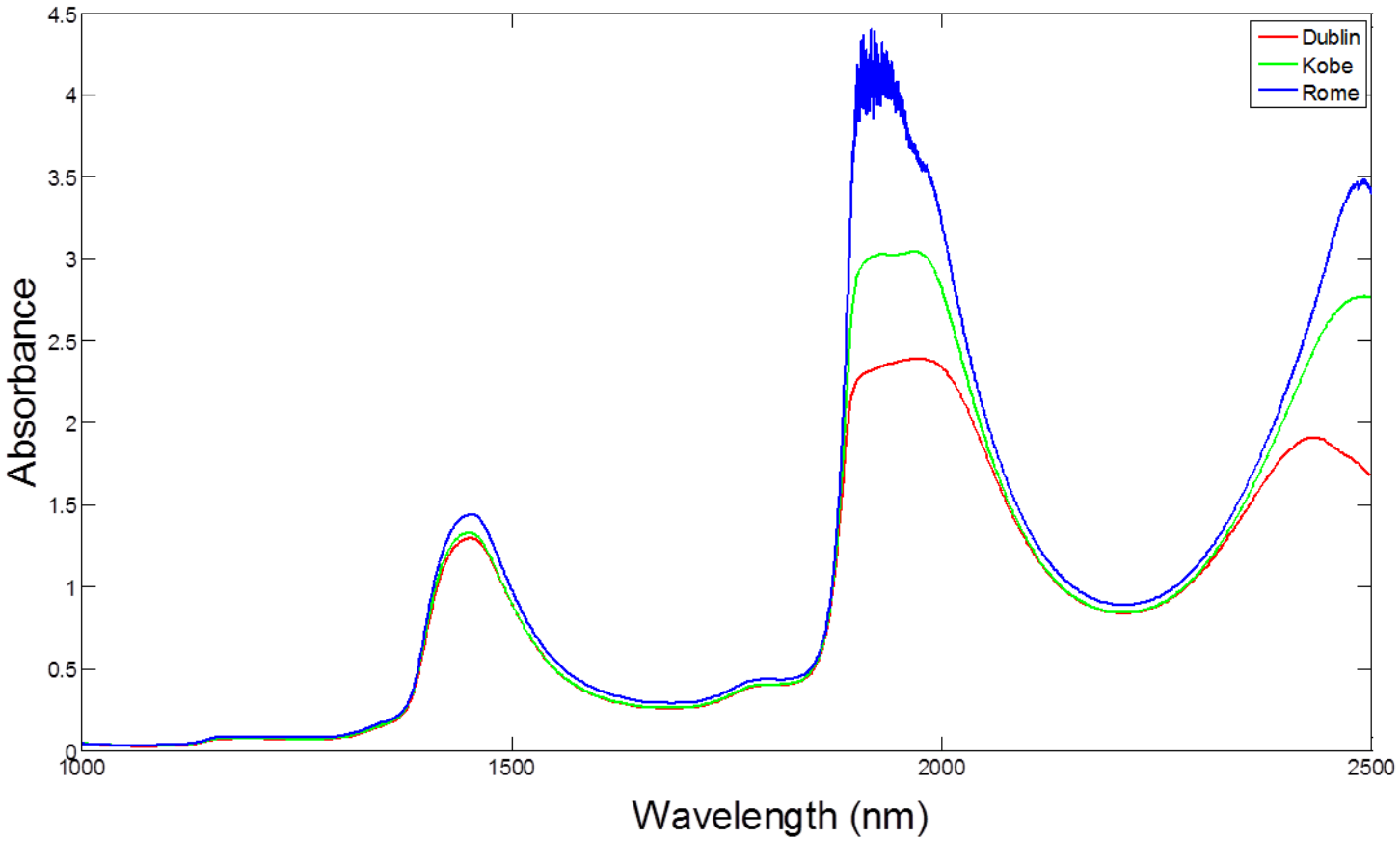
**S. De Luca** (Rome)
Nicolet 6700 FT-NIR



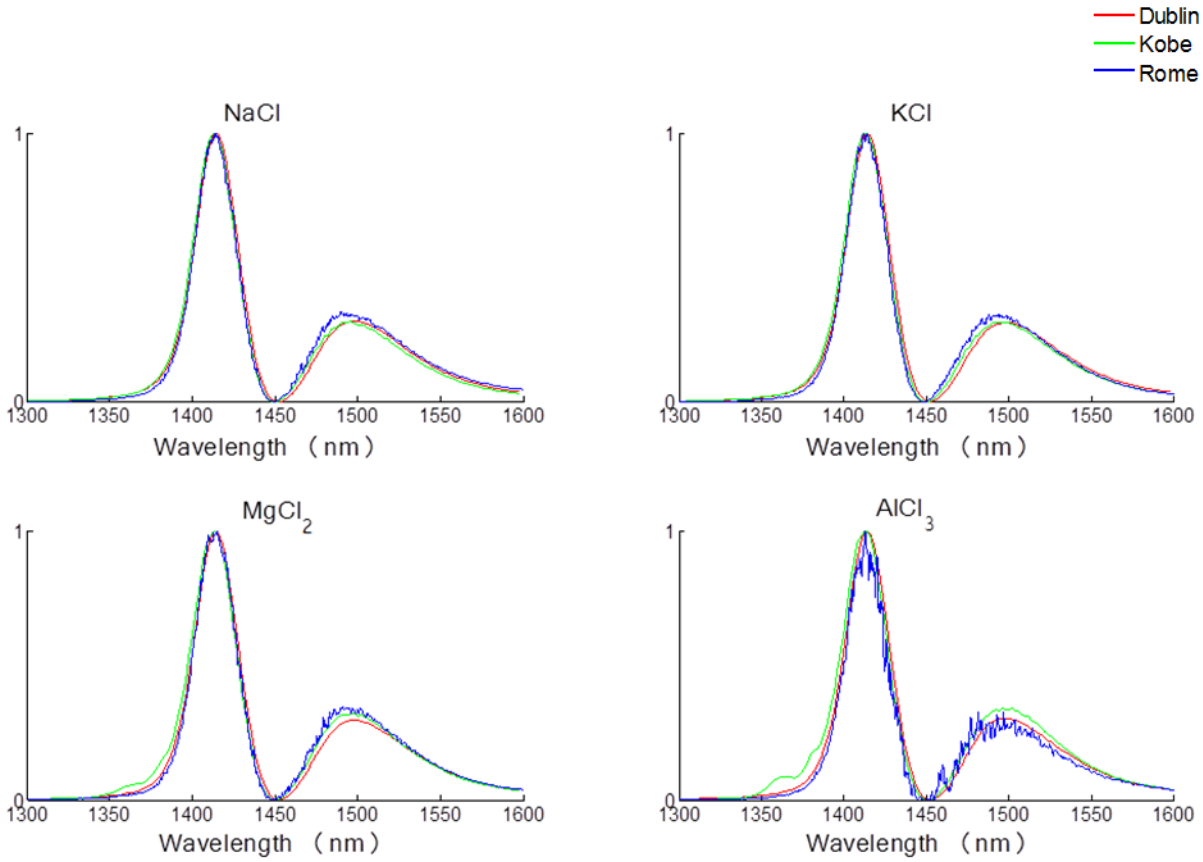


**Y. Tsuchisaka** (Kobe)
NIR Systems 6500

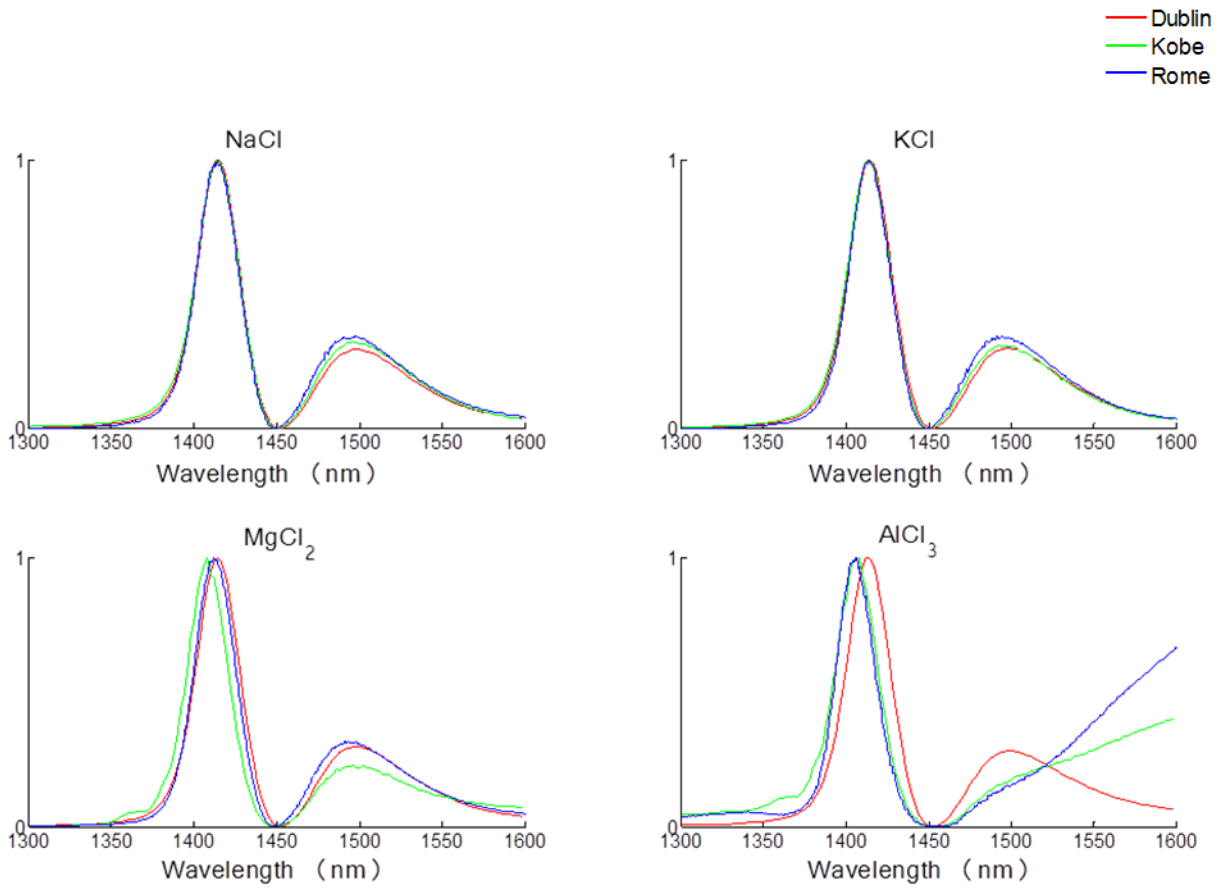
# Water spectra



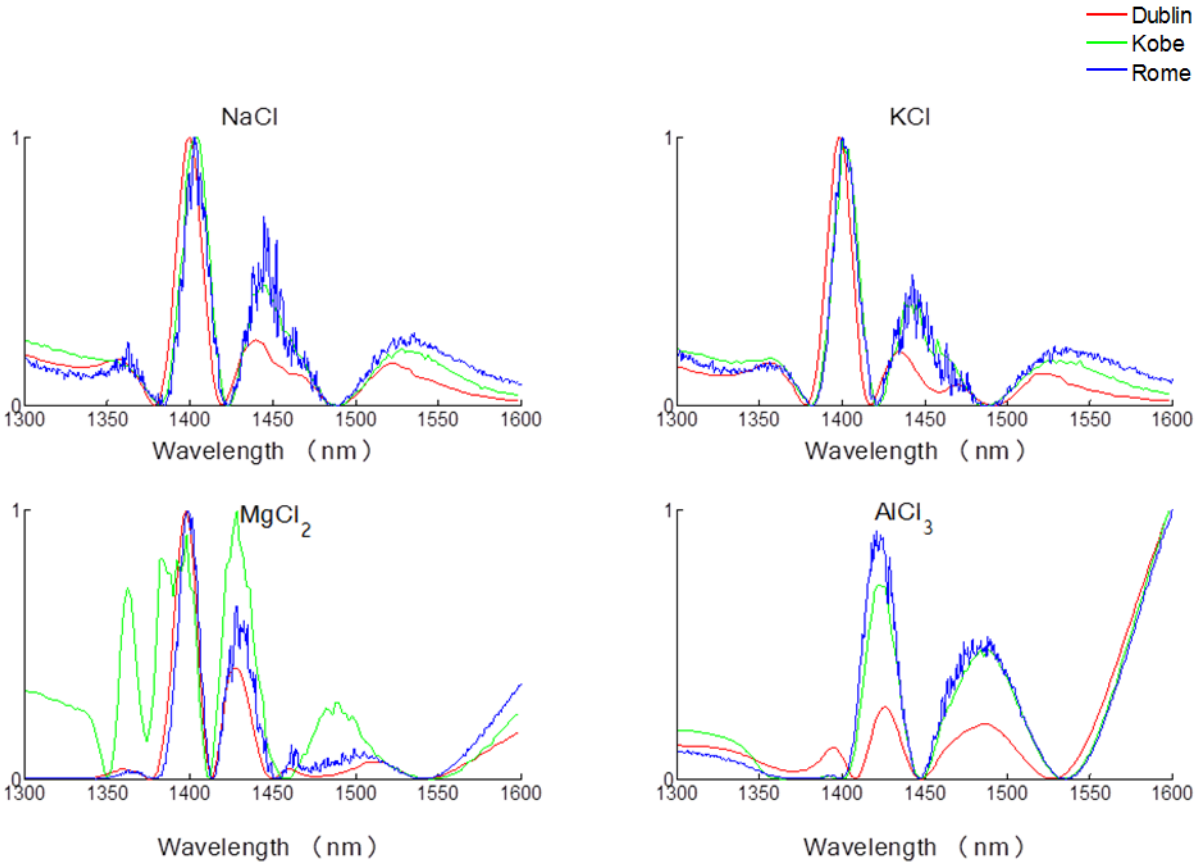
# PC 1 Water only



# PC 1 Salts


Dublin
Kobe
Rome
NaCl
KCl
MgCl2
AlCl3
1
0
1300
1350
1400
1450
1500
1550
1600
Wavelength （nm）

# PC 2 Salts


Dublin
Kobe
Rome
NaCl
KCl
MgCl2
AlCl3
1
0
1300
1350
1400
1450
1500
1550
1600
Wavelength （nm）

# Question

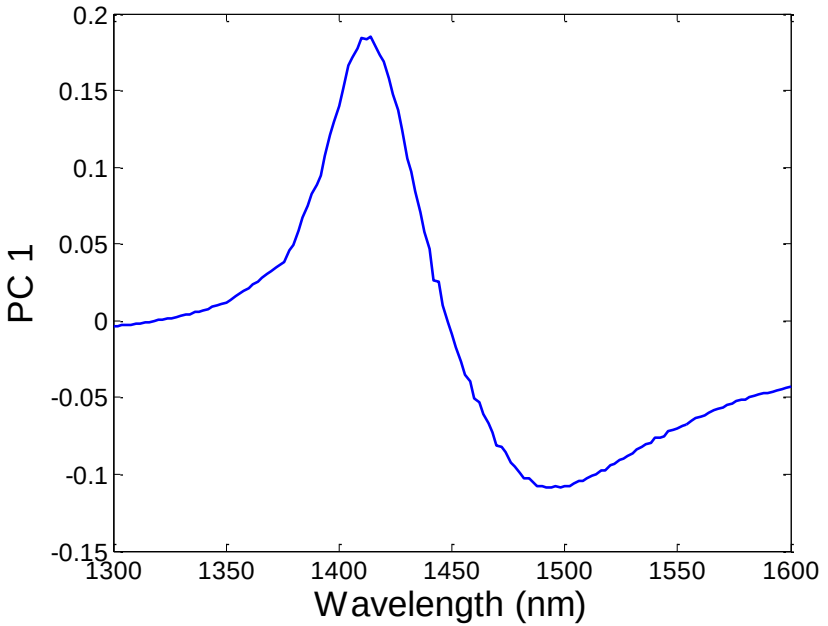
- How to remove effect of temperature in spectra?

- Extended Multiplicative Signal Correction!

# EMSC Model

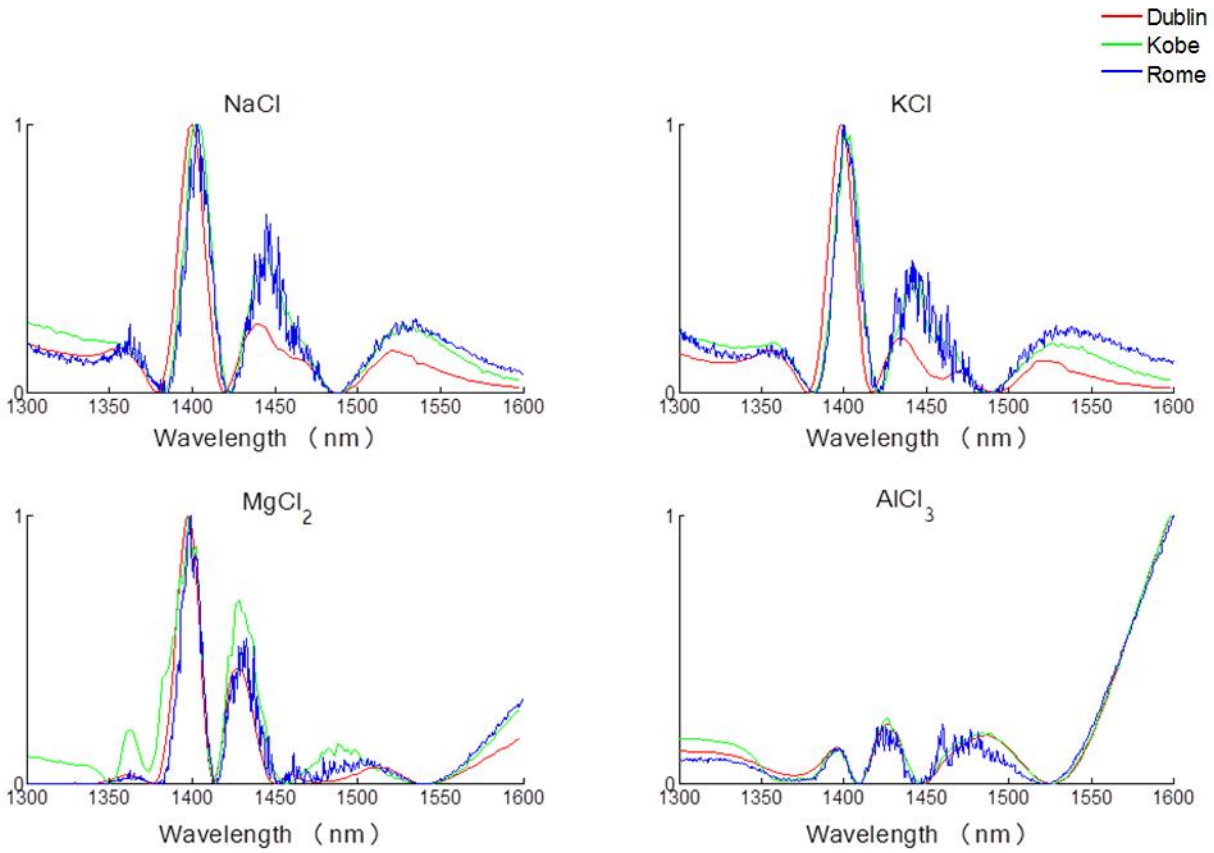
$$X = b_0 + b_1\bar{X} + b_2 I_1$$

Interference Spectra



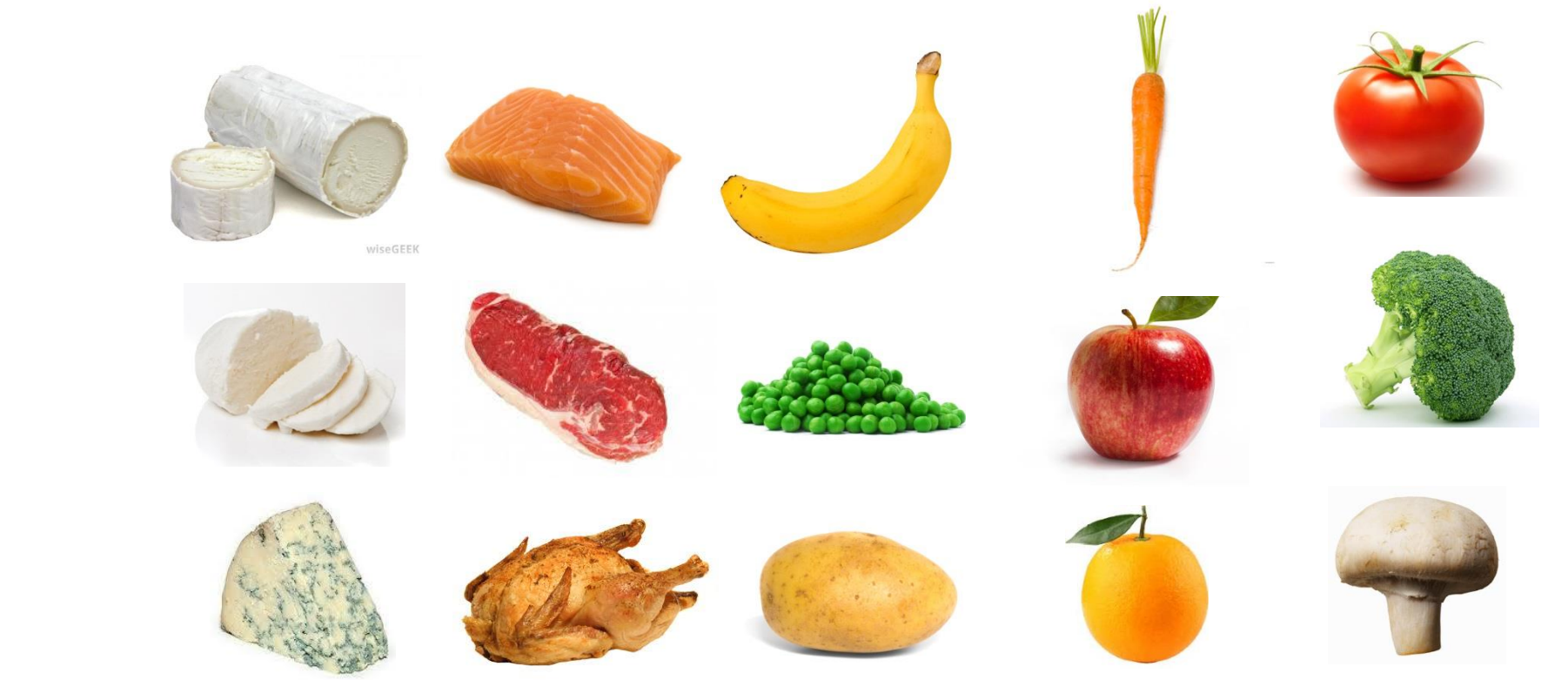

Corrected spectrum → $$\hat{X} = \frac{X - b_0}{b_1} - \frac{b_2 I_1}{b_1} - \frac{b_3 I_2}{b_1}$$

# PC 1 EMSC Salts



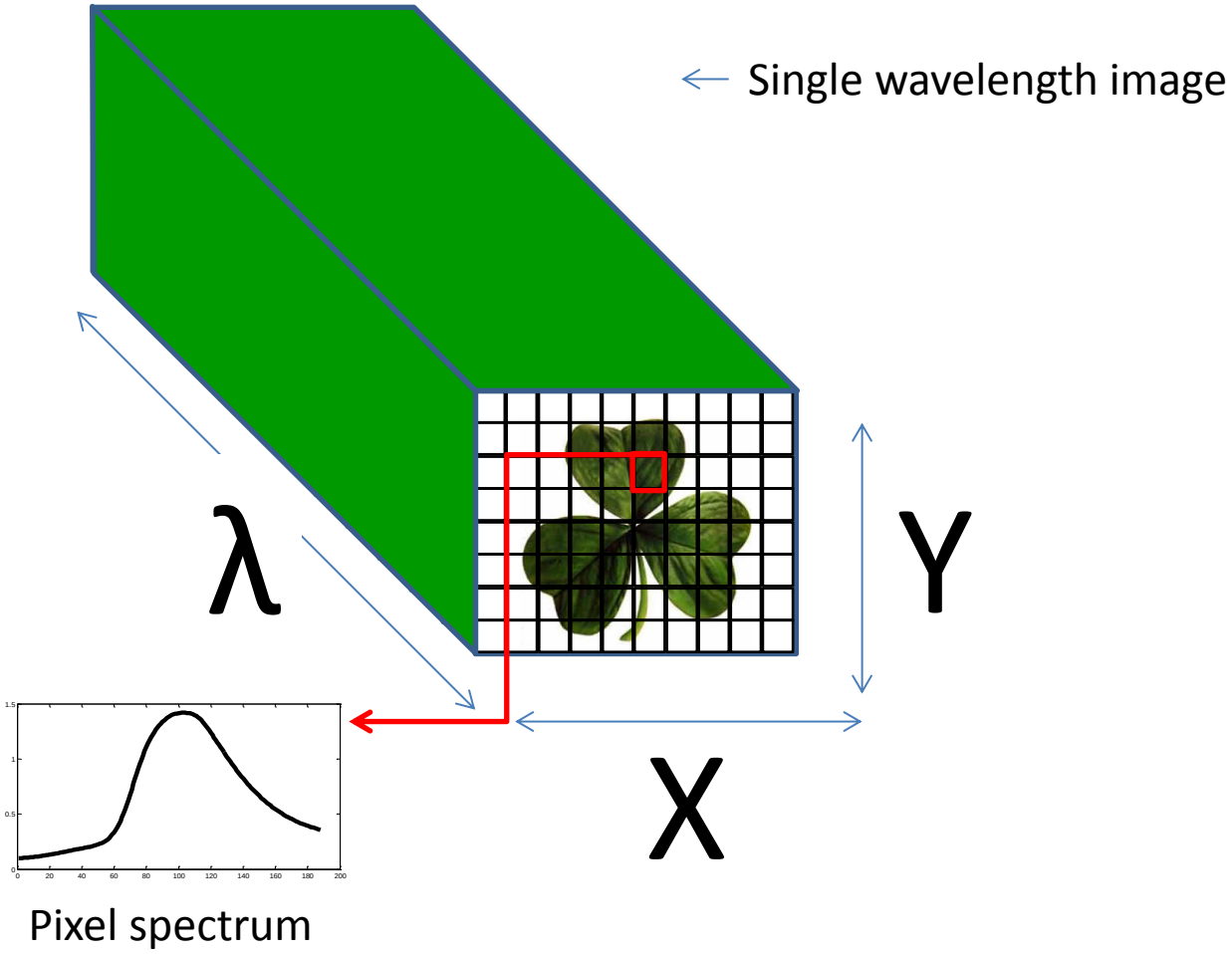

LOD ~ 0.1 % (mass/mass)

# How watery is food?

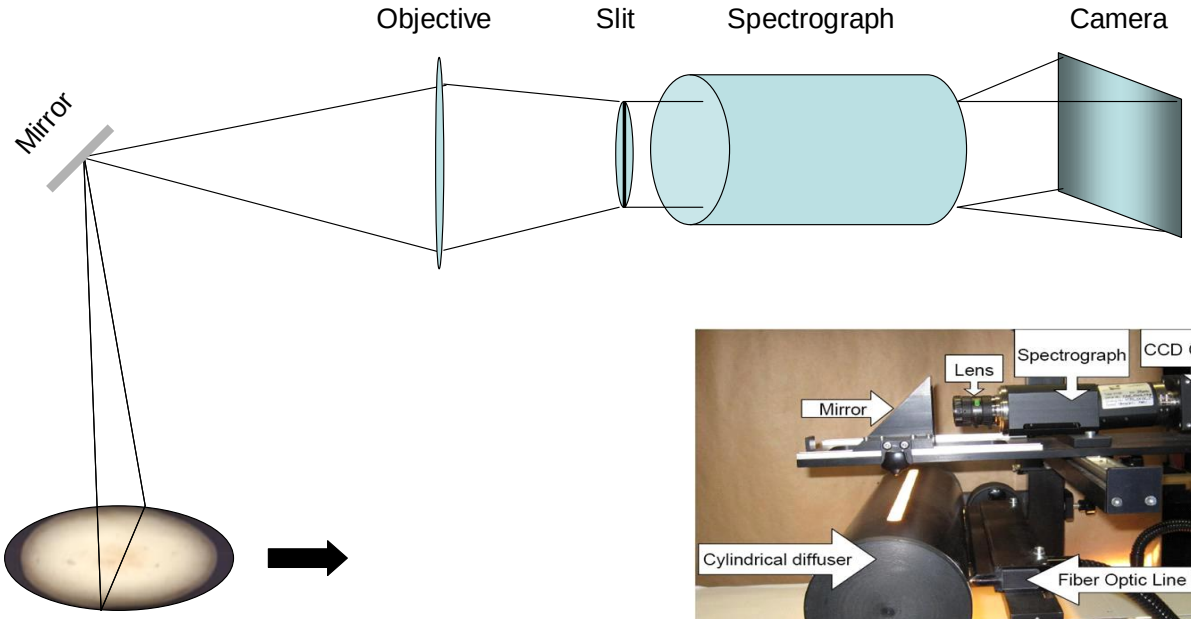



Google images

# Hyperspectral Imaging

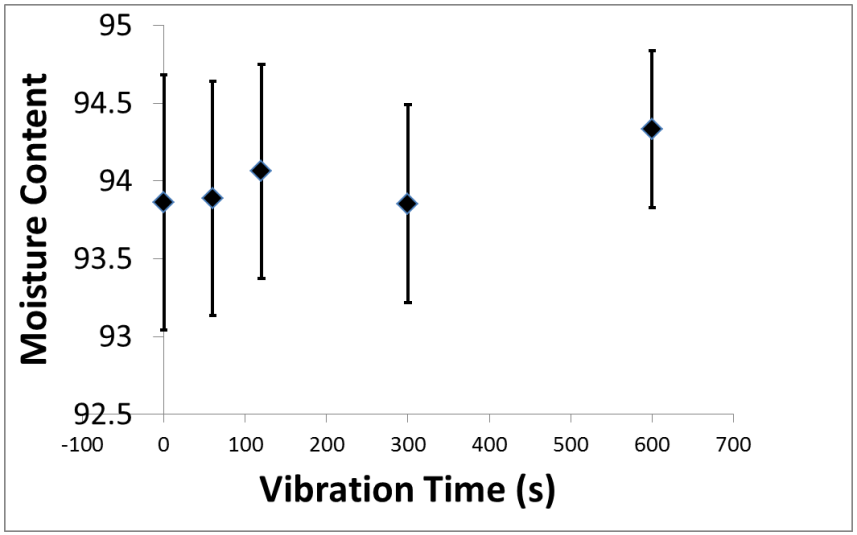


# Hyperspectral Imaging Equipment



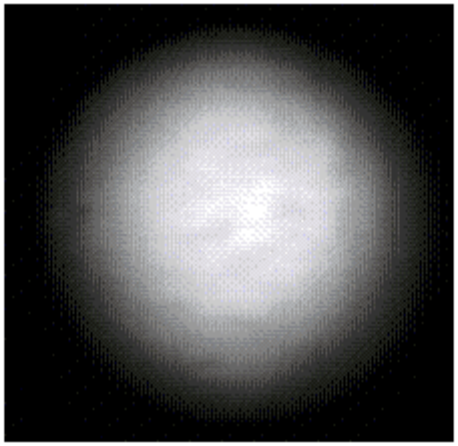

| | |
|---|---|
| **Wavelength range (nm)** | 950–1650 |
| **Spectral resolution (nm)** | 7 |
| **Detector** | InGaAs |

# Expt. 2: Mushrooms



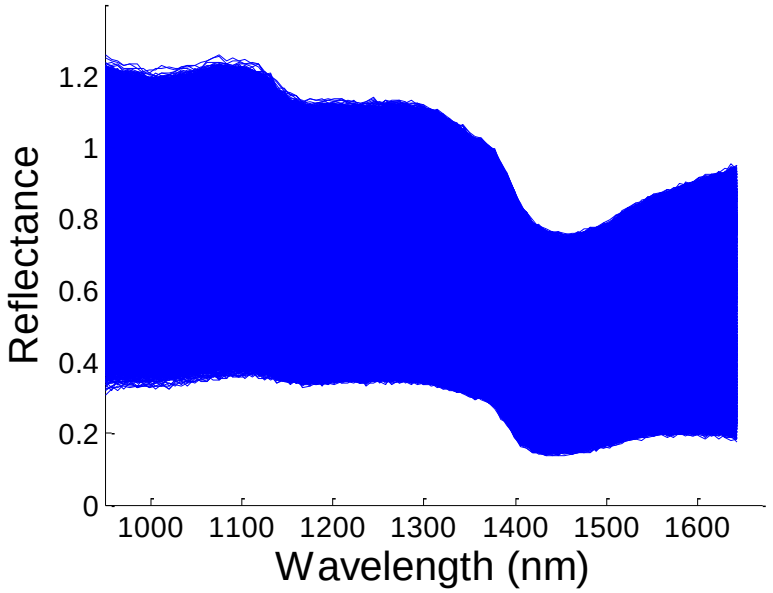

- 5 vibration times
- => induce surface damage
- x 24 mushrooms
- x 3 reps

# Mushroom Curvature

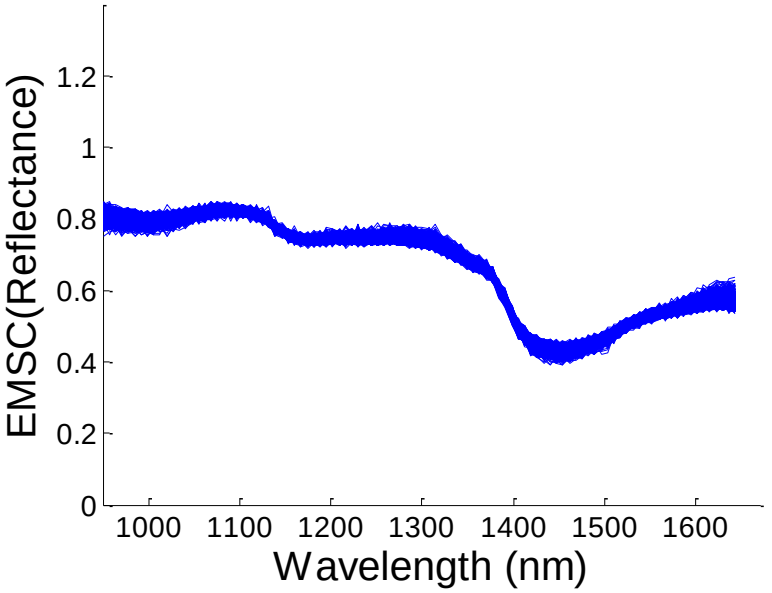


# EMSC on Mushrooms

Raw Spectra: 1 mushroom

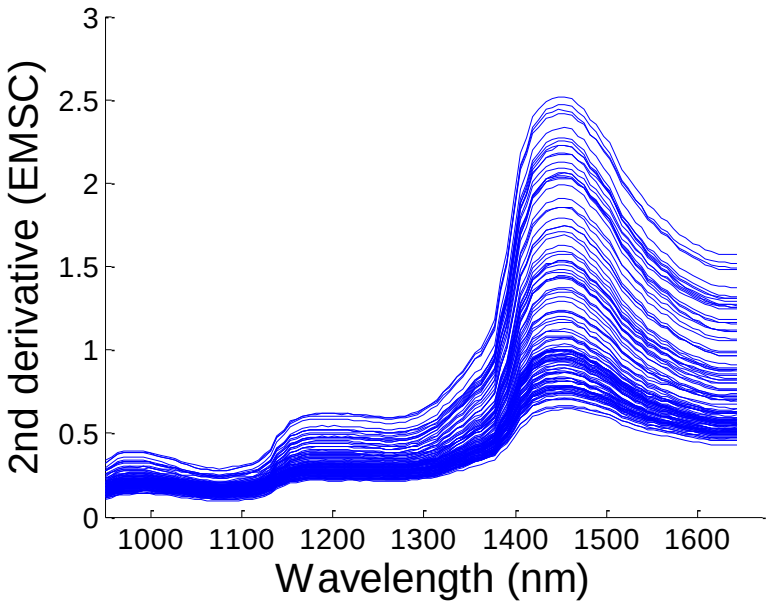



EMSC Spectra: 1 mushroom

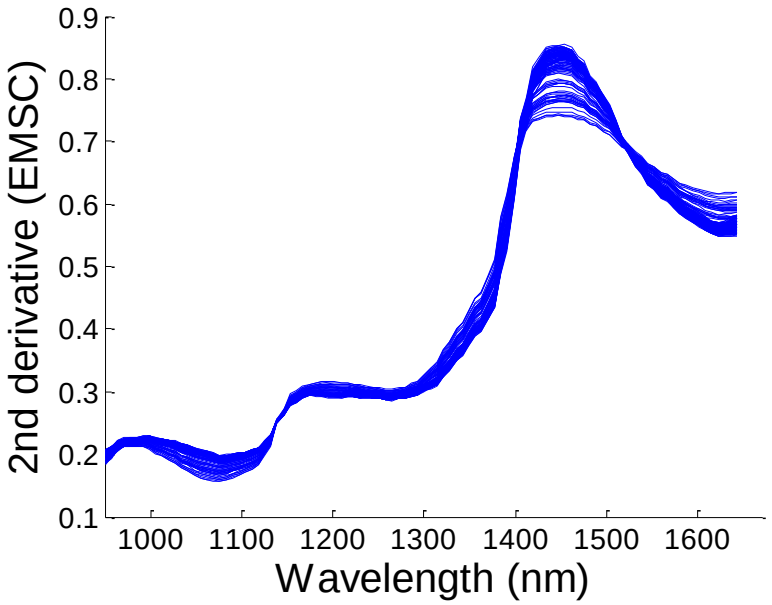


# EMSC on Mushrooms

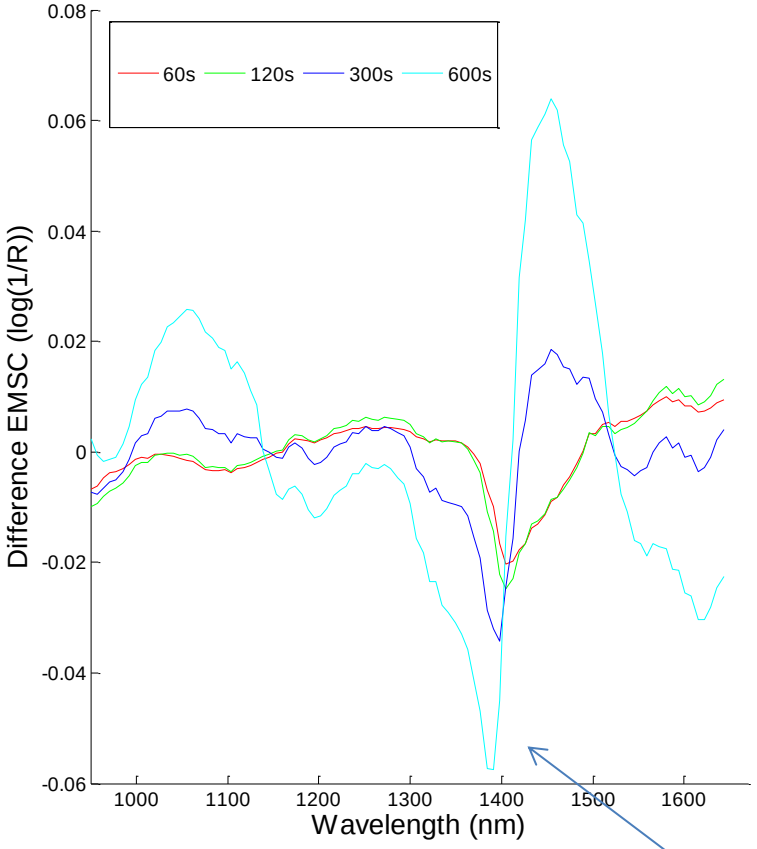
Mean Spectra: 120 mushrooms

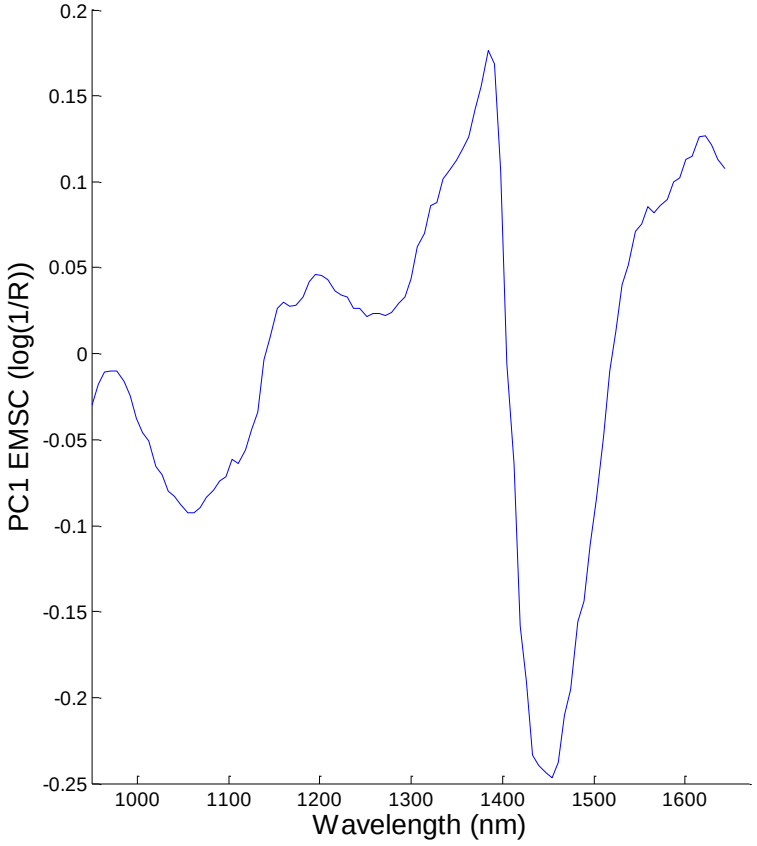



Mean EMSC Spectra: 120 mushrooms



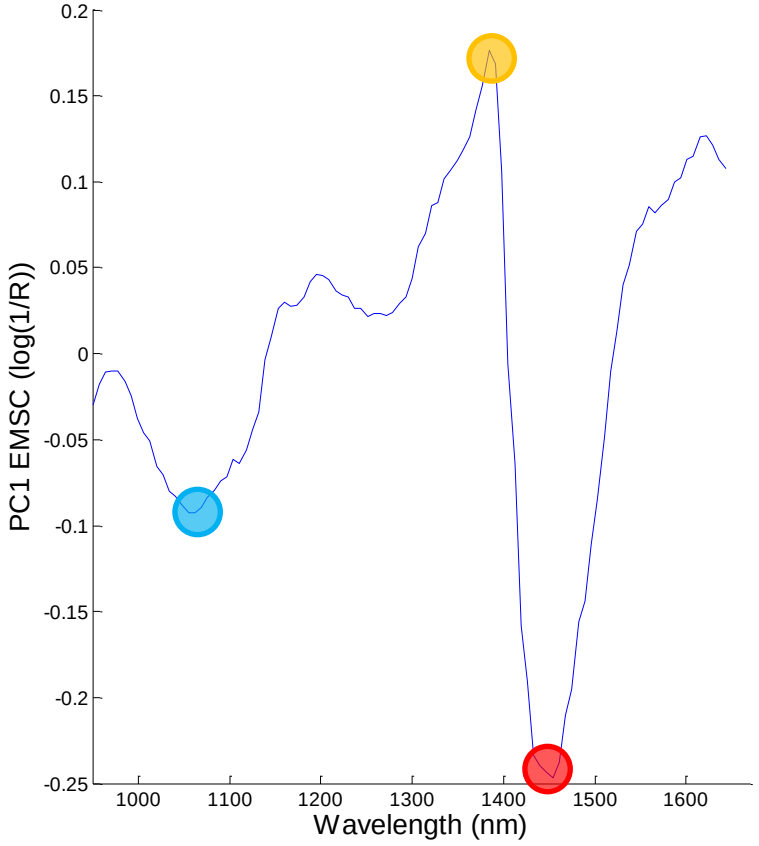
# EMSC Mushrooms: difference spectra



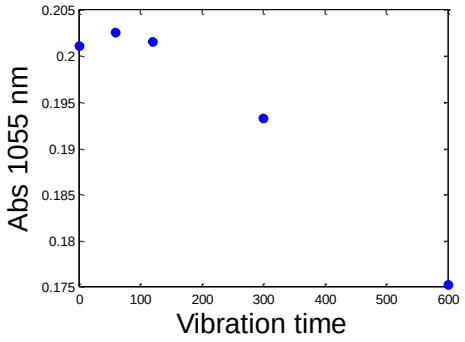

Blue shift indicating more free water as damage proceeds

# PC1 EMSC Mushrooms

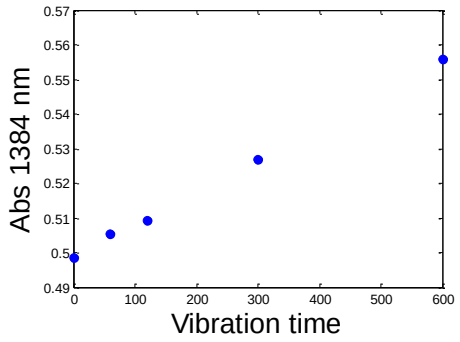



| λ (nm) | 2D | Tentative assignment |
|---|---|---|
| 1055 | -I | $3v_{as}$ |
| 1384 | -I | $2v_{as}$ dangling OH<br>or<br>$2v_s$ Free OH in free water molecule |
| 1454 | -I | $2v_{ds}+v_s$<br>or<br>$v_{as}$ in dimer |

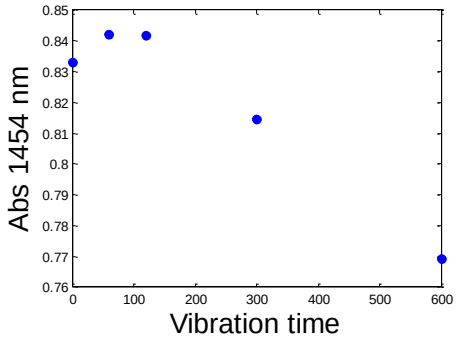
# Abs Peaks Mushroom




$3v_{as}$

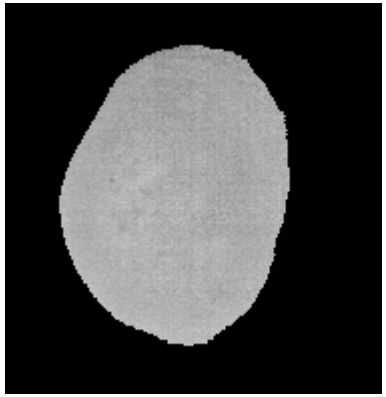



$2v_{as}$ dangling OH

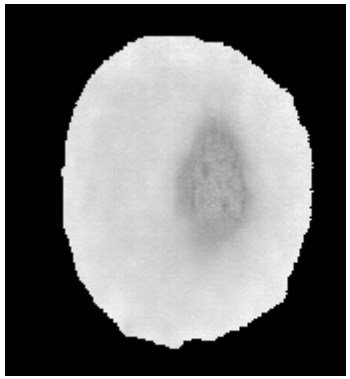



$2v_{ds}+v_s$ or $v_{as}$ in dimer

EMSC corrected images at 1384 nm



Undamaged



Damaged

# Free v's Bound water

- **Free water:** can be extracted easily from foods by squeezing or cutting or pressing
- **Bound water:** cannot be extracted easily
- Bound water molecules can't escape as vapor

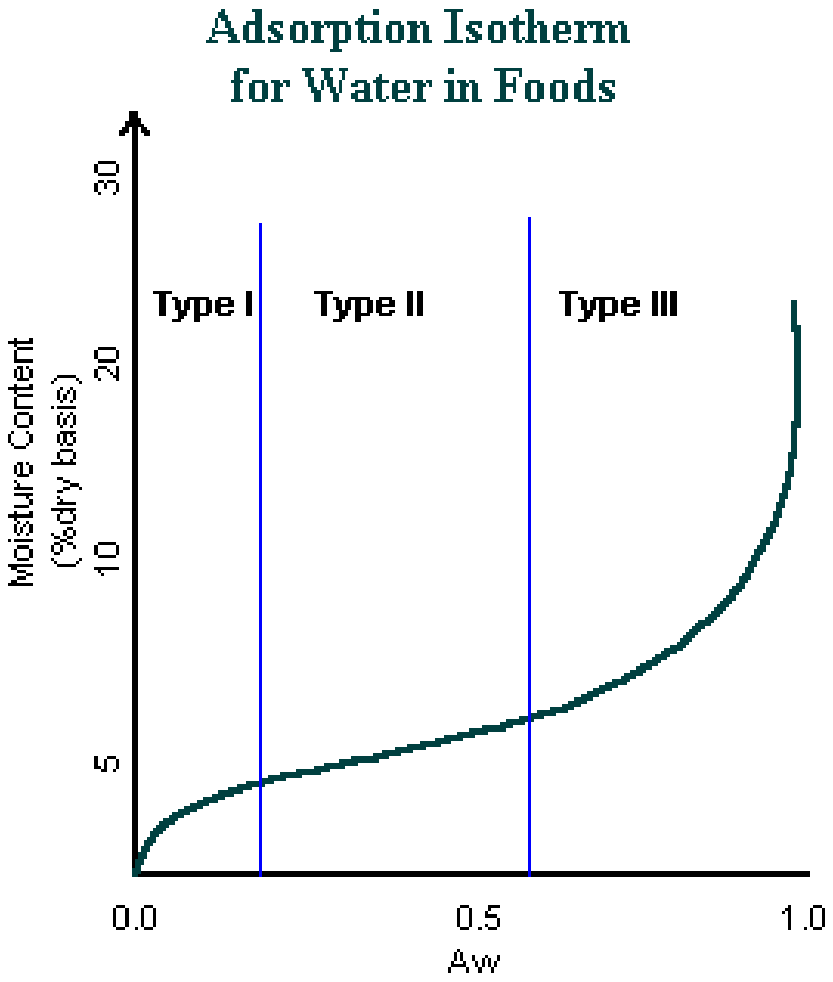
=> Even upon dehydration food contains bound water.

# Water activity

- W J Scott (1952) established that it was not water content that correlated with bacterial growth in foods, but

- **Water activity:** ratio of the water vapor pressure of the food to the water vapor pressure of pure water under the same conditions

# Moisture Content Vs Water Activity

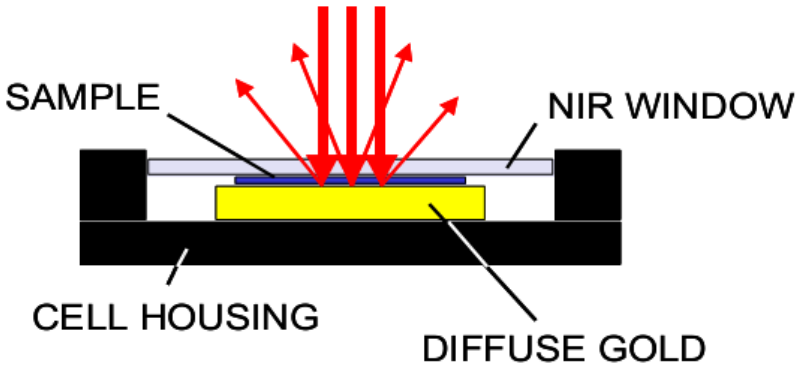


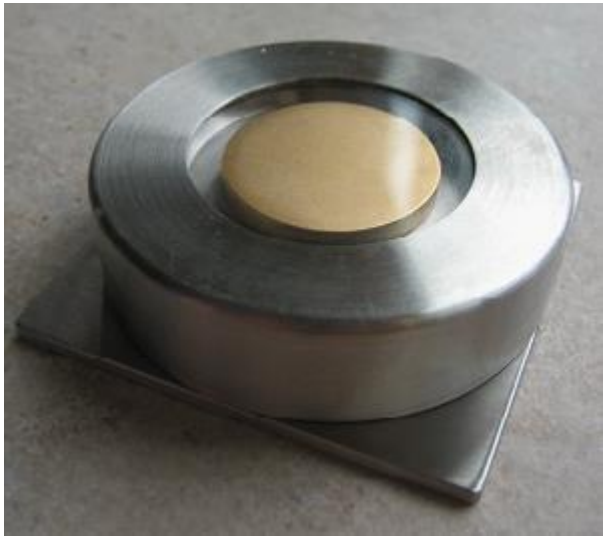

http://vssweb1.landfood.ubc.ca/courses/fnh/301/water/wprin.htm

# Expt 3. Aqueous solutions

- Transflectance Cell
- 300 µL
- Aqueous solutions:

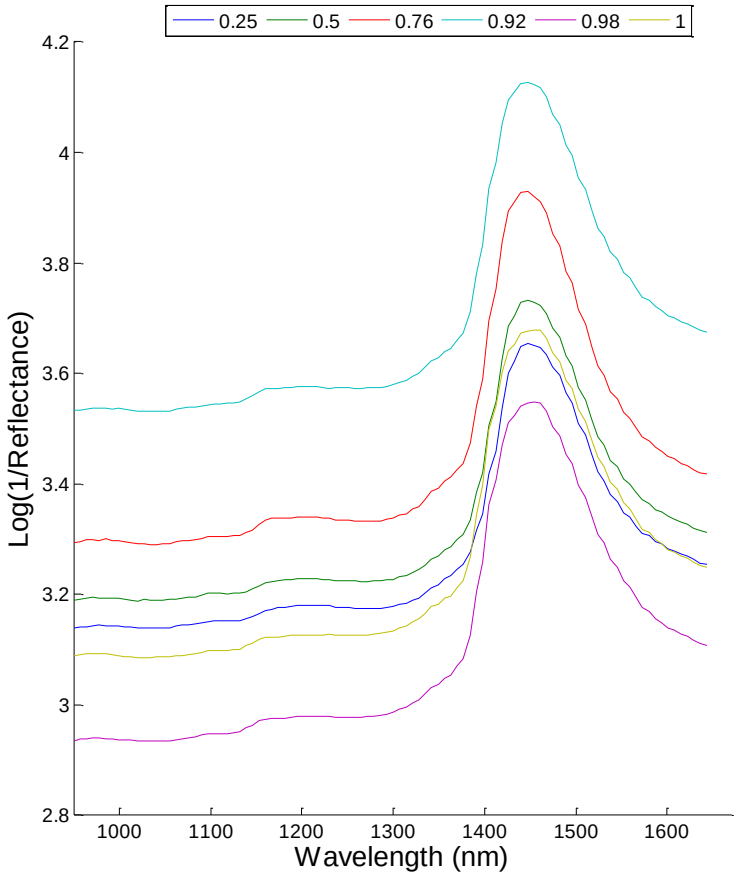
| Salt | Aw |
|---|---|
| LiCl | 0.25 |
| LiCl | 0.5 |
| NaCl | 0.76 |
| NaCl | 0.92 |
| KCl | 0.98 |
| W | 1 |



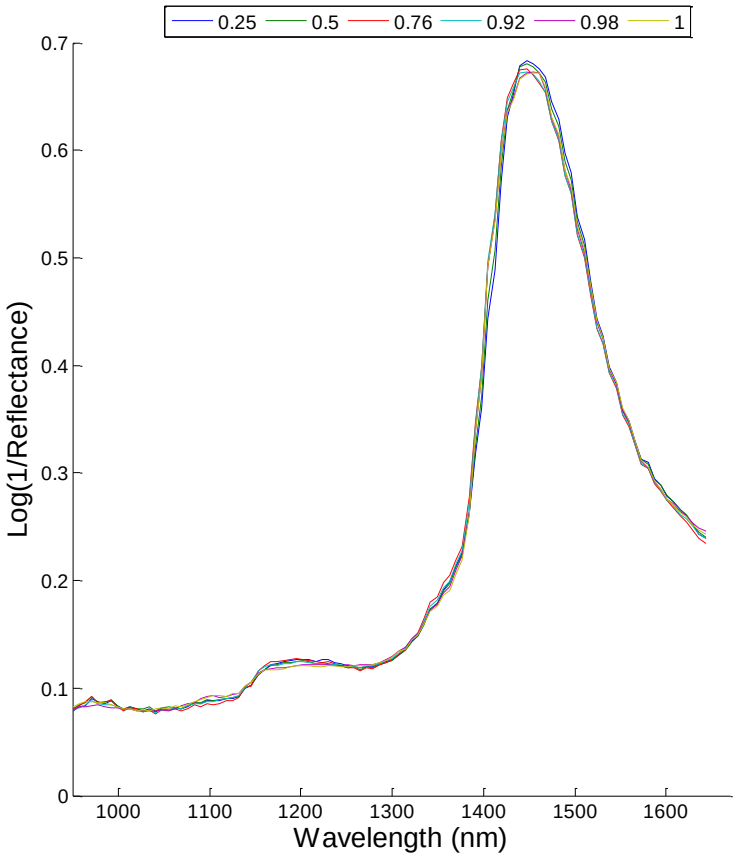



# Mean Sample Spectrum

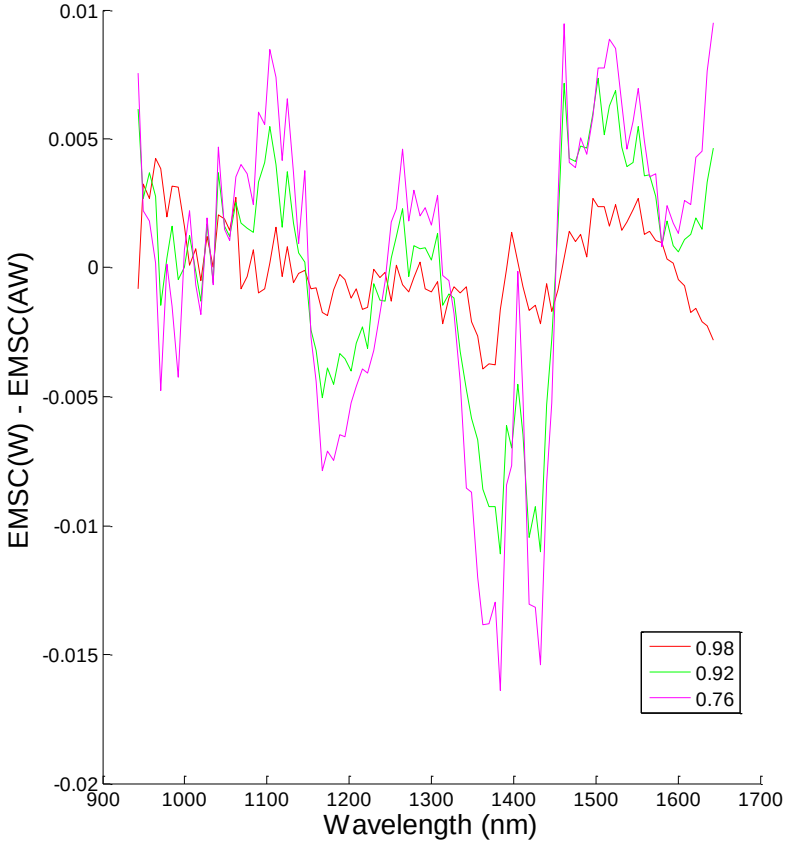
No pretreatment

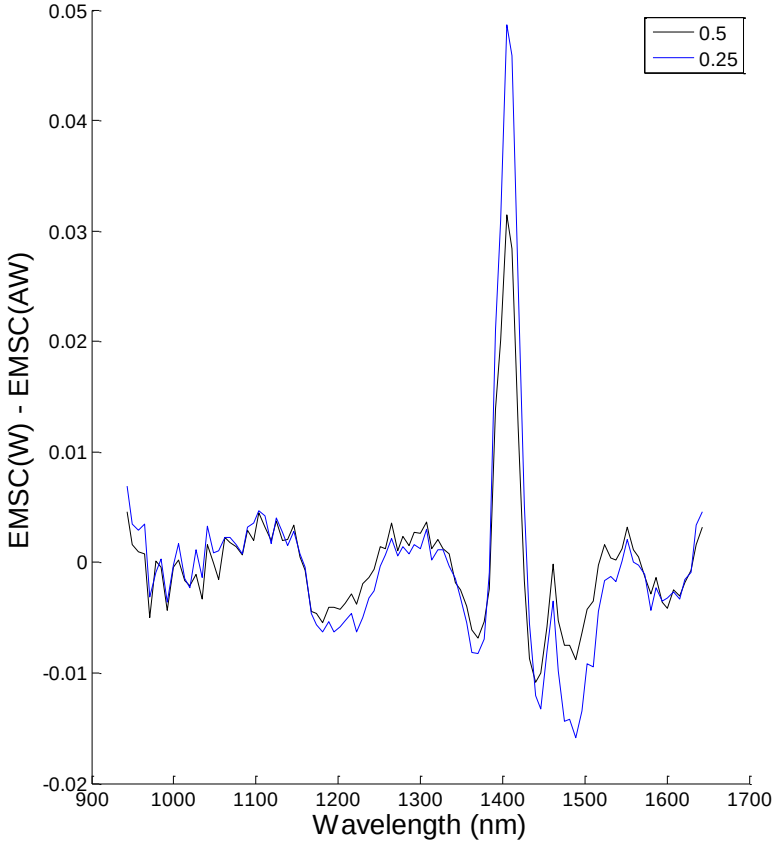



EMSC

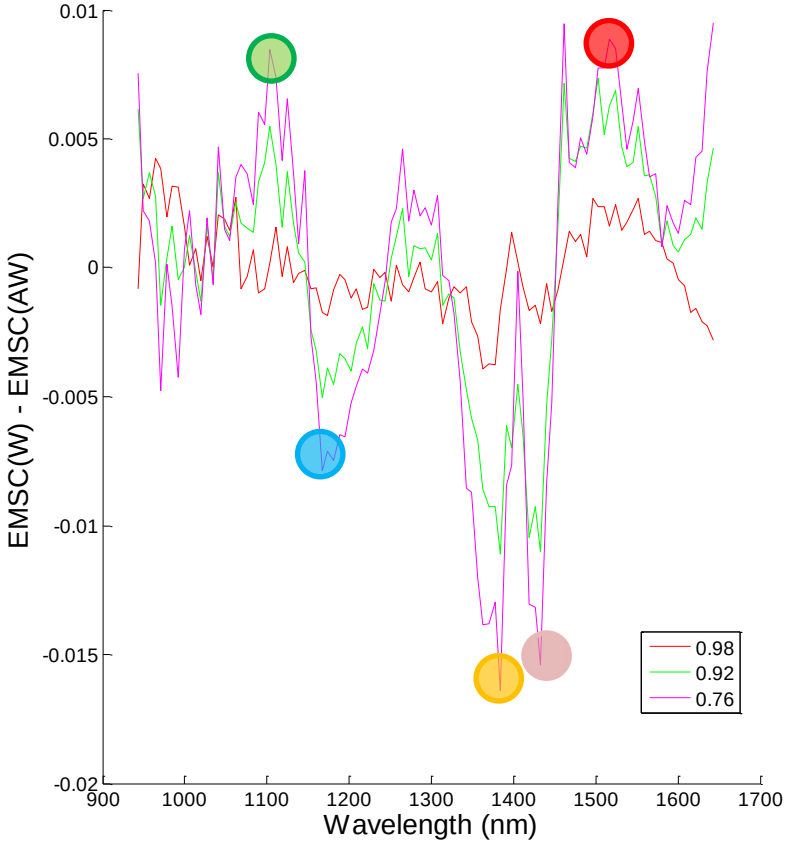


# Difference Spectrum (EMSC (Water -))

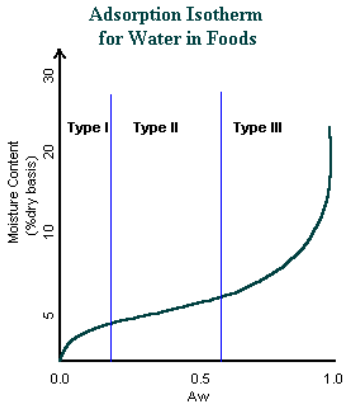


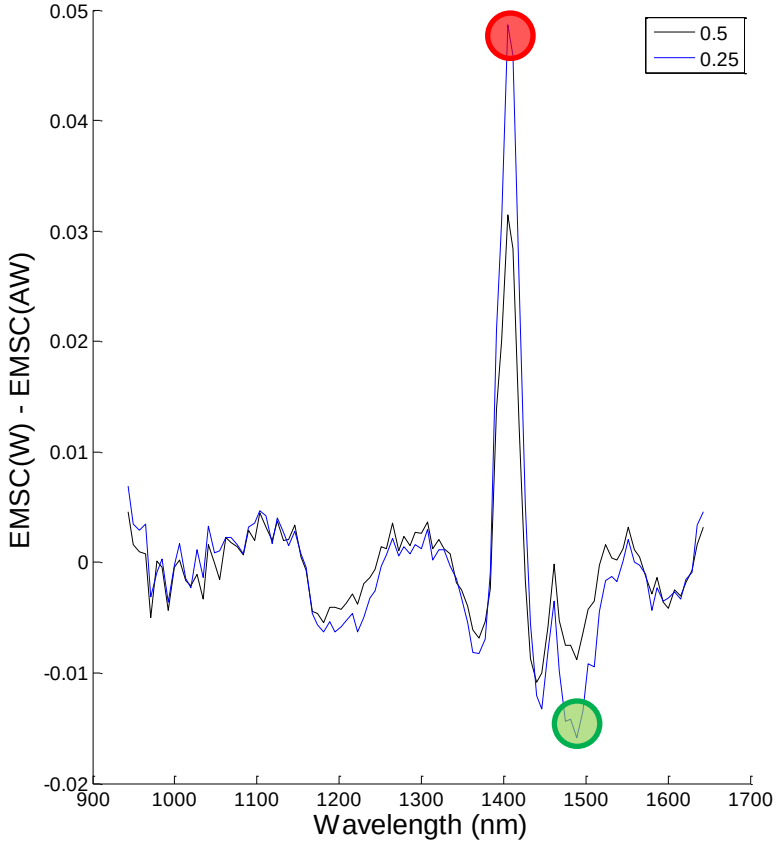
# Difference Spectrum EMSC Aw > 0.5



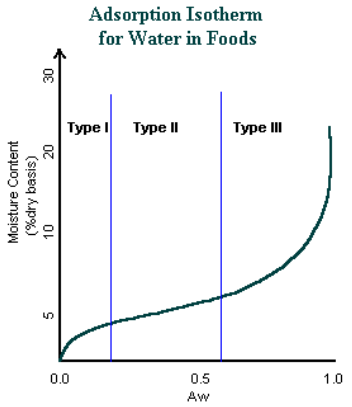

| 2D | λ (nm) | Tentative assignment |
|---|---|---|
| I+ | 1104 | $v_{\delta s}+2v_s$ |
| - | 1167 | $2v_{as}+ v_{\delta s}$ |
| + | 1384 | $2v_{as}$ or $2v_s$ dangling OH |
| M- | 1433 | $2v_{as}$ in dimer or trimer |
| I+ | 1517 | $v_{as}+ 2v_{\delta s}$ or $2v_{as}$ in tetramer |



# Difference Spectrum EMSC Aw<=0.5

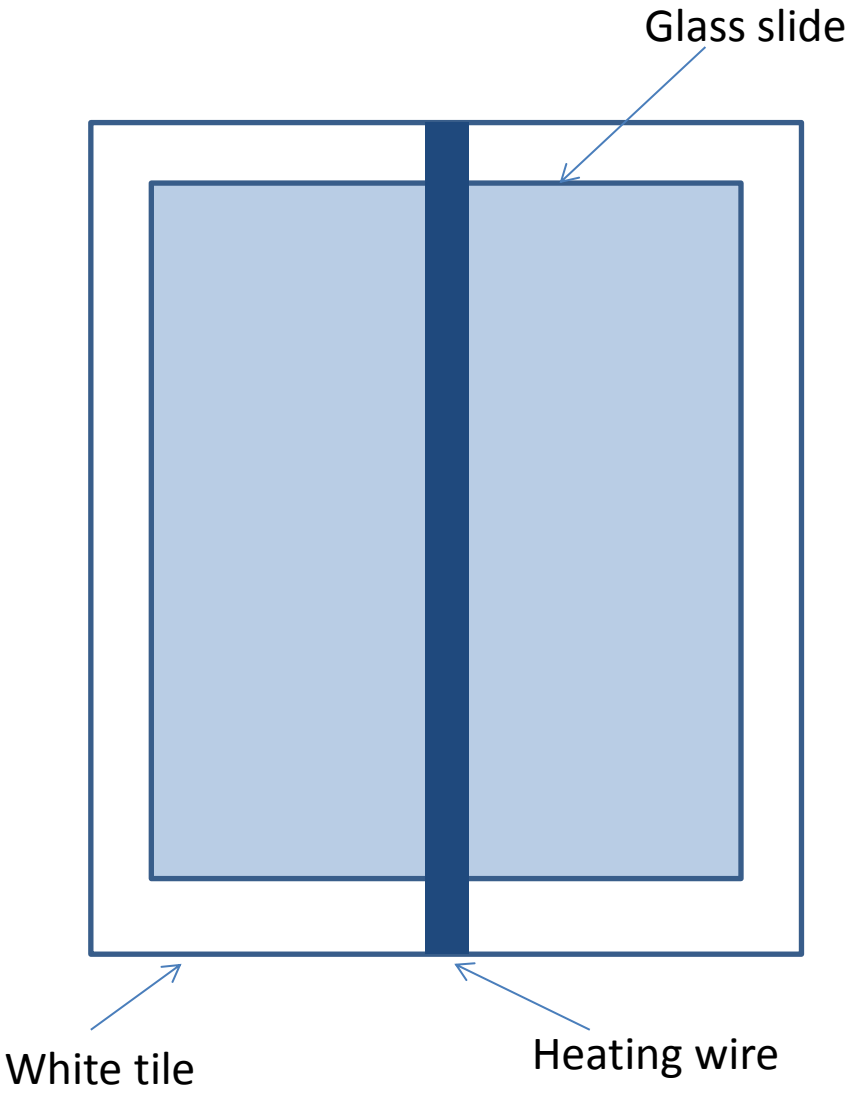



| 2D | λ (nm) | Tentative assignment |
|---|---|---|
| -I | 1405 | $2v_{as}$ or $2v_s$ dangling OH |
| -I | 1489 | $2v_{ds}+v_s$ or 2 $v_s$ H bond in dimer or $v_{as}+2v_{ds}$ |

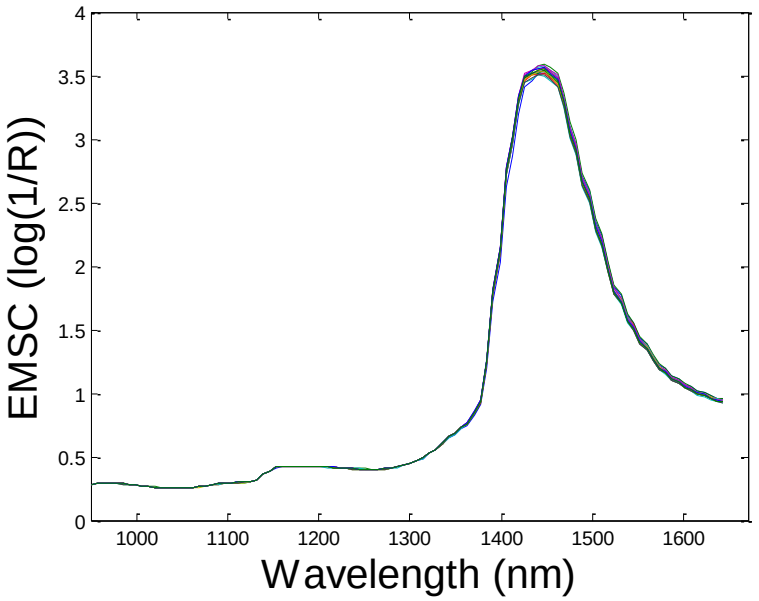


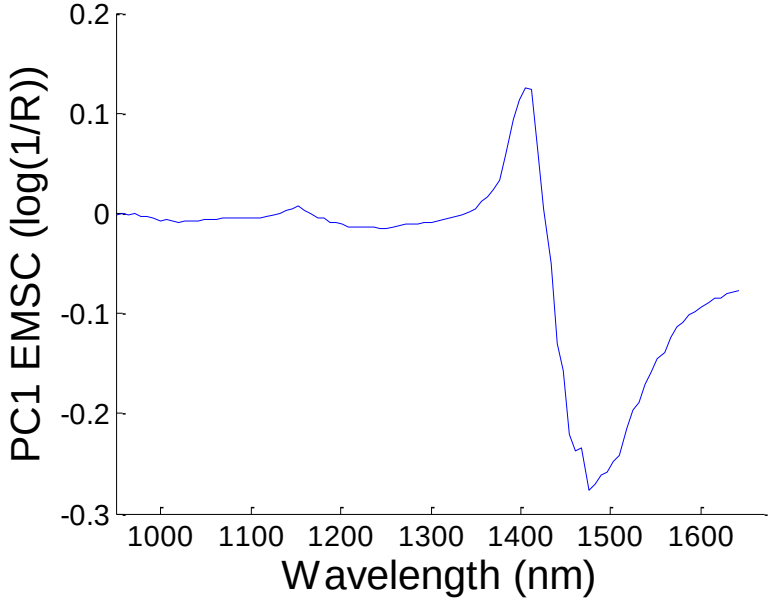
# Expt. 4: Water temperature

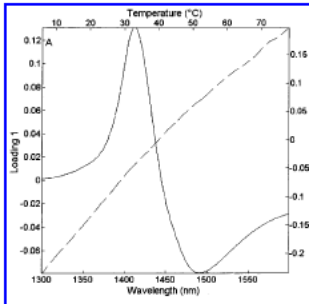
- 1ml water pipetted on ceramic tile
- heating wire placed through centre of drop
- Glass slide placed on top =>thin layer of water
- Heating wire switched on
- Images obtained every minute for a total of 15 mins.



# Expt. 2: Water temperature

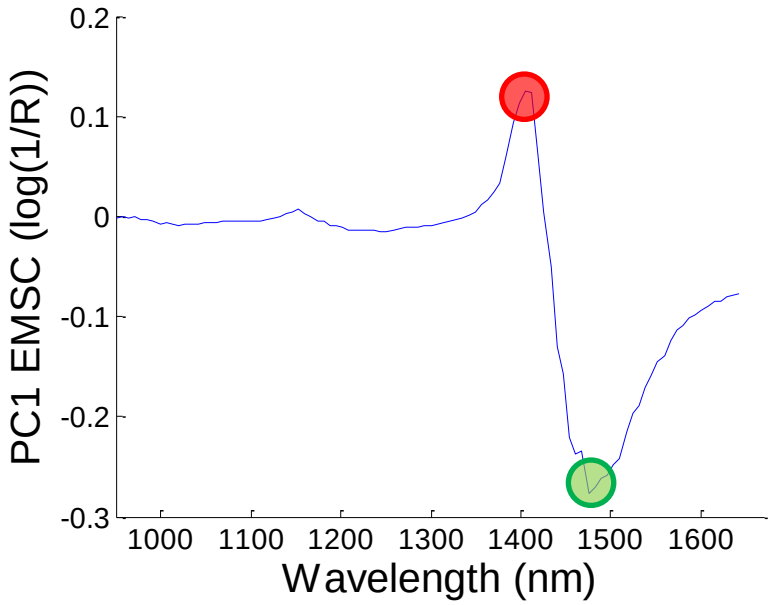


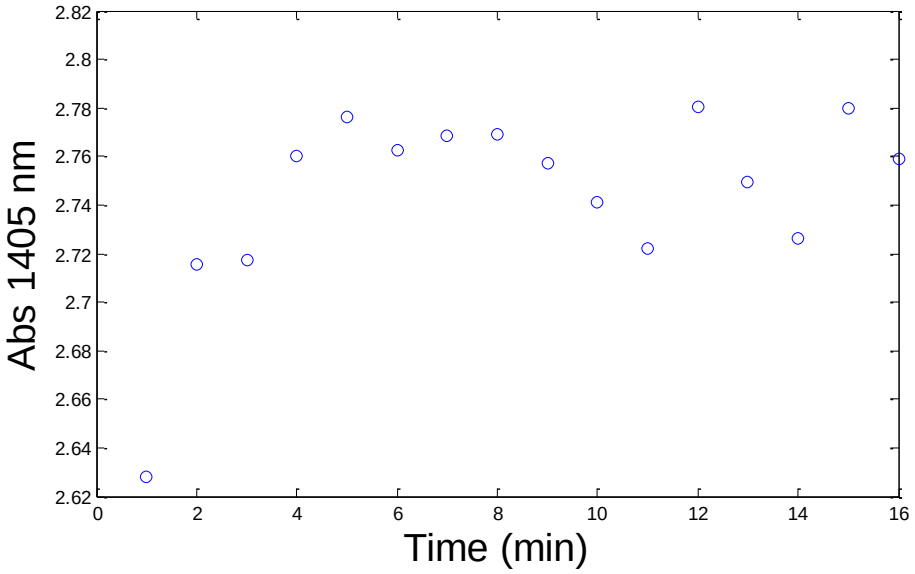

Classic “effect of temperature”

# PC 1 EMSC

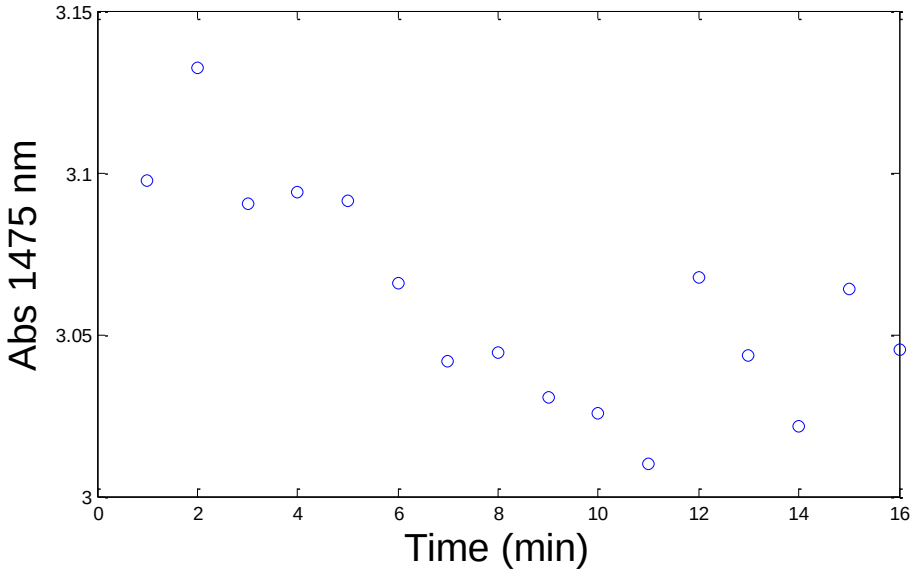



| λ (nm) | 2D | Tentative assignment |
|---|---|---|
| 1405 | I- | $2v_{as}$ dangling OH |
| 1475 | I- | $v_{as}+2v_{ds}$ Or $2v_{as}$ in tetramer |

# Absorbance at PC1 peaks

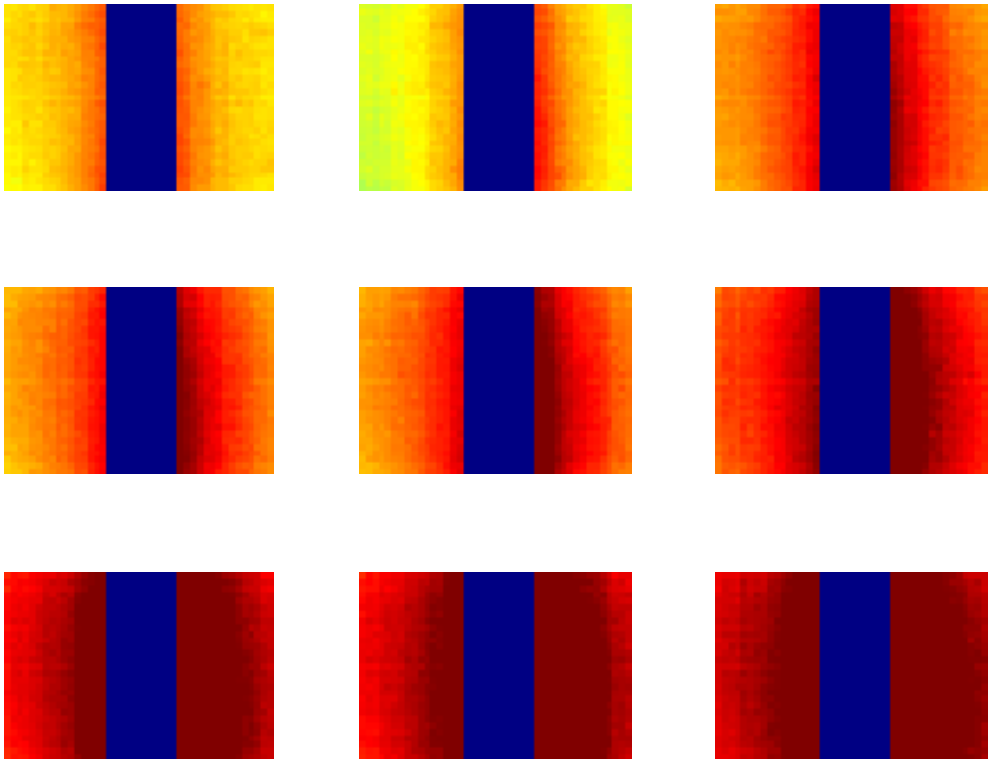



$2v_{as}$ dangling OH




$v_{as}+2v_{ds}$
or
$2v_{as}$ in tetramer

# PC1 Score images



# Expt. 5: Hydration of Dry foods

Coffee

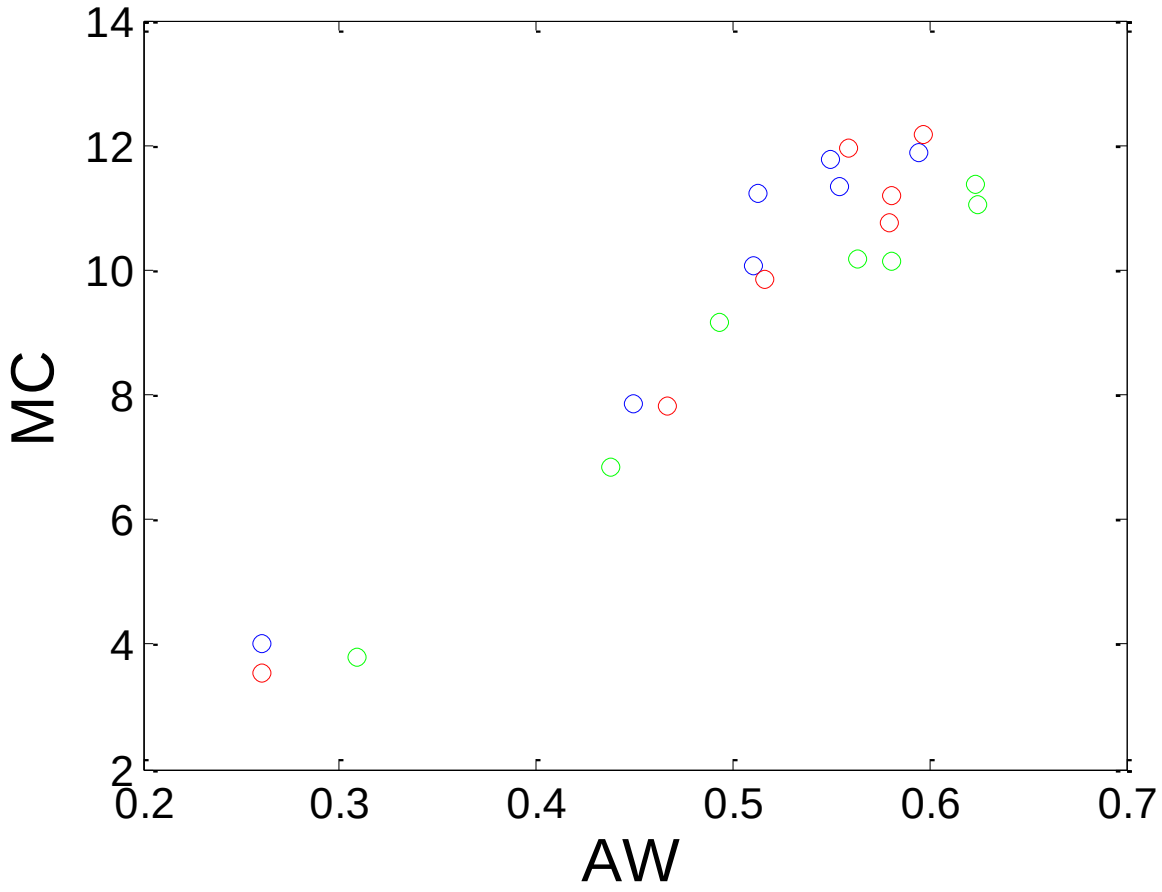
Wafer Soybeans

**Hydrate** to different MC & AW

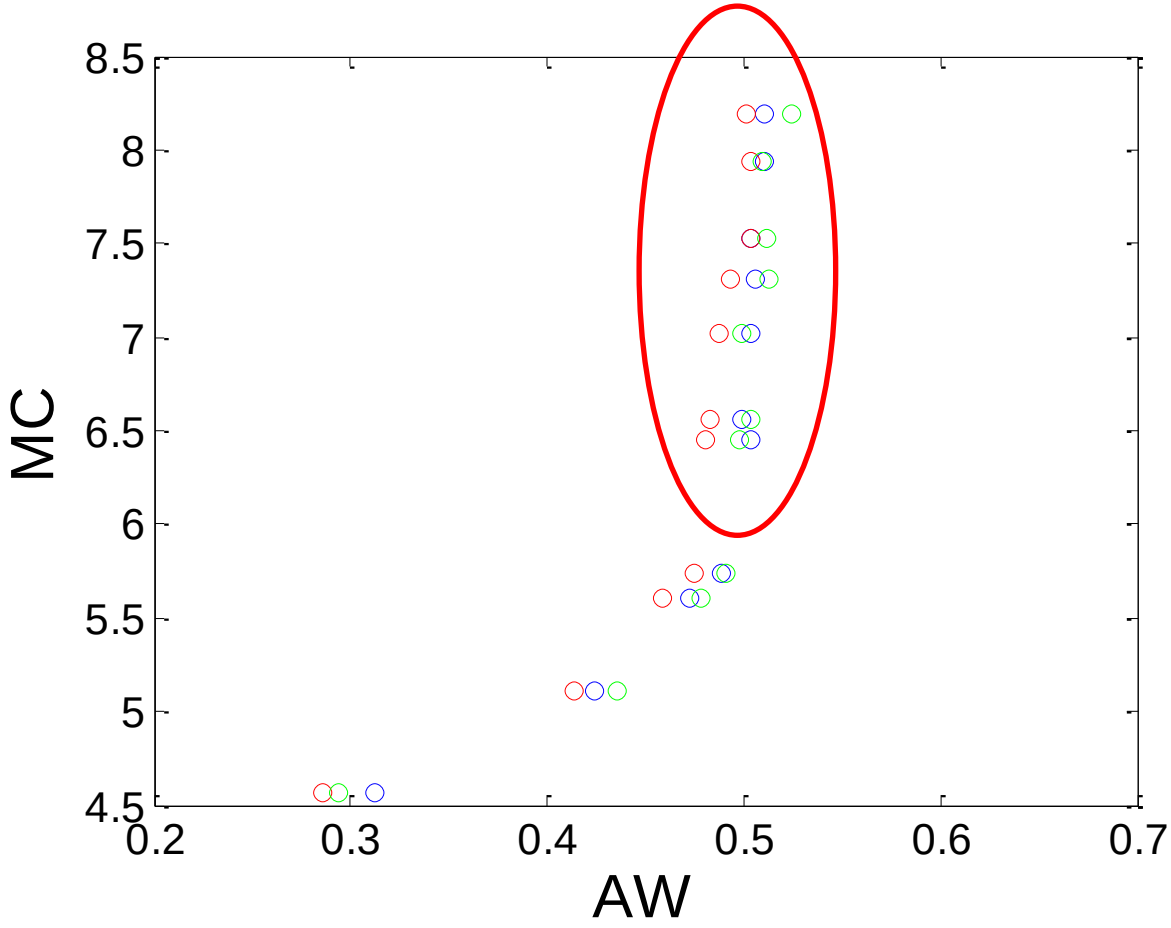
**Measure**
NIR HSI, MC, AW

| Sample | # | Sample size |
|---|---|---|
| Coffee | 33 | 2g |
| Wafer | 21 | 0.6g |
| Soybeans | 42 | 20 kernels |

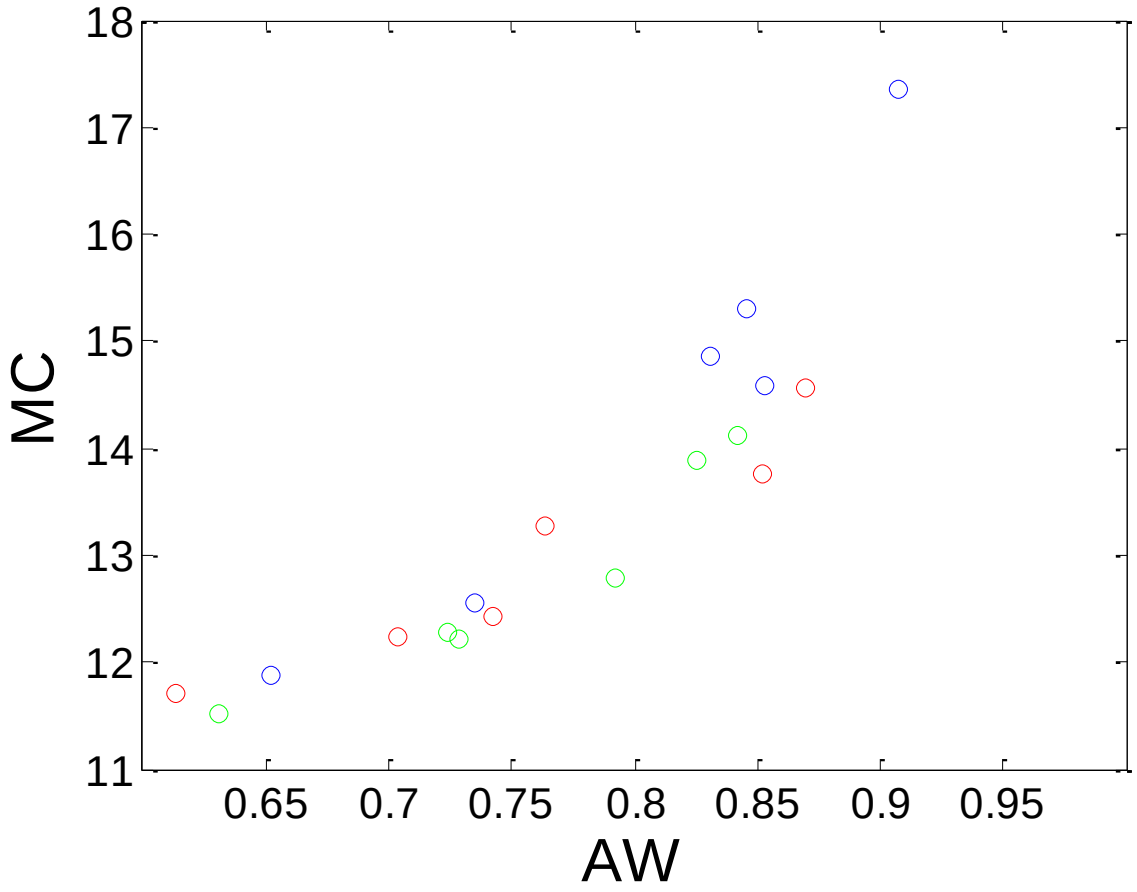
# AW/MC Wafer



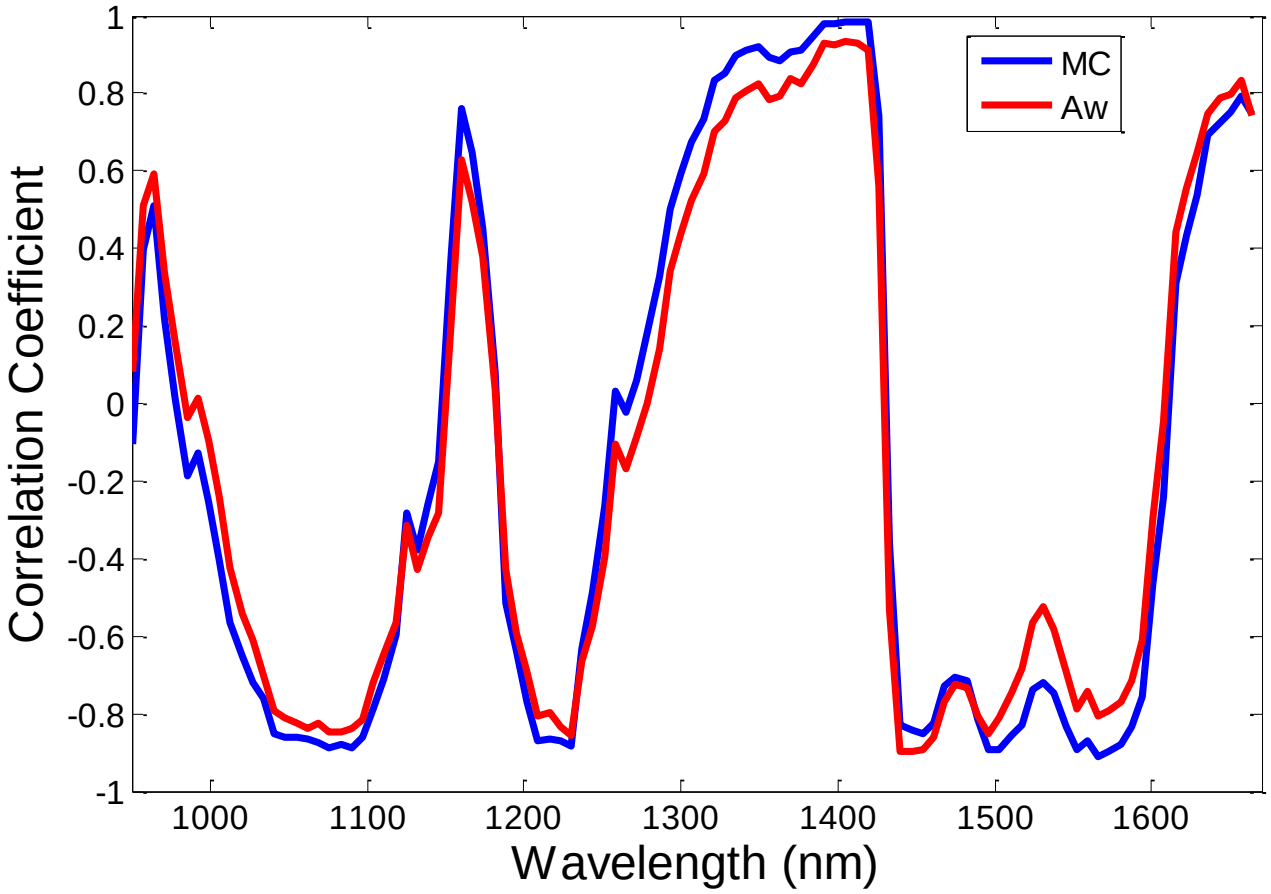
# AW/MC Coffee



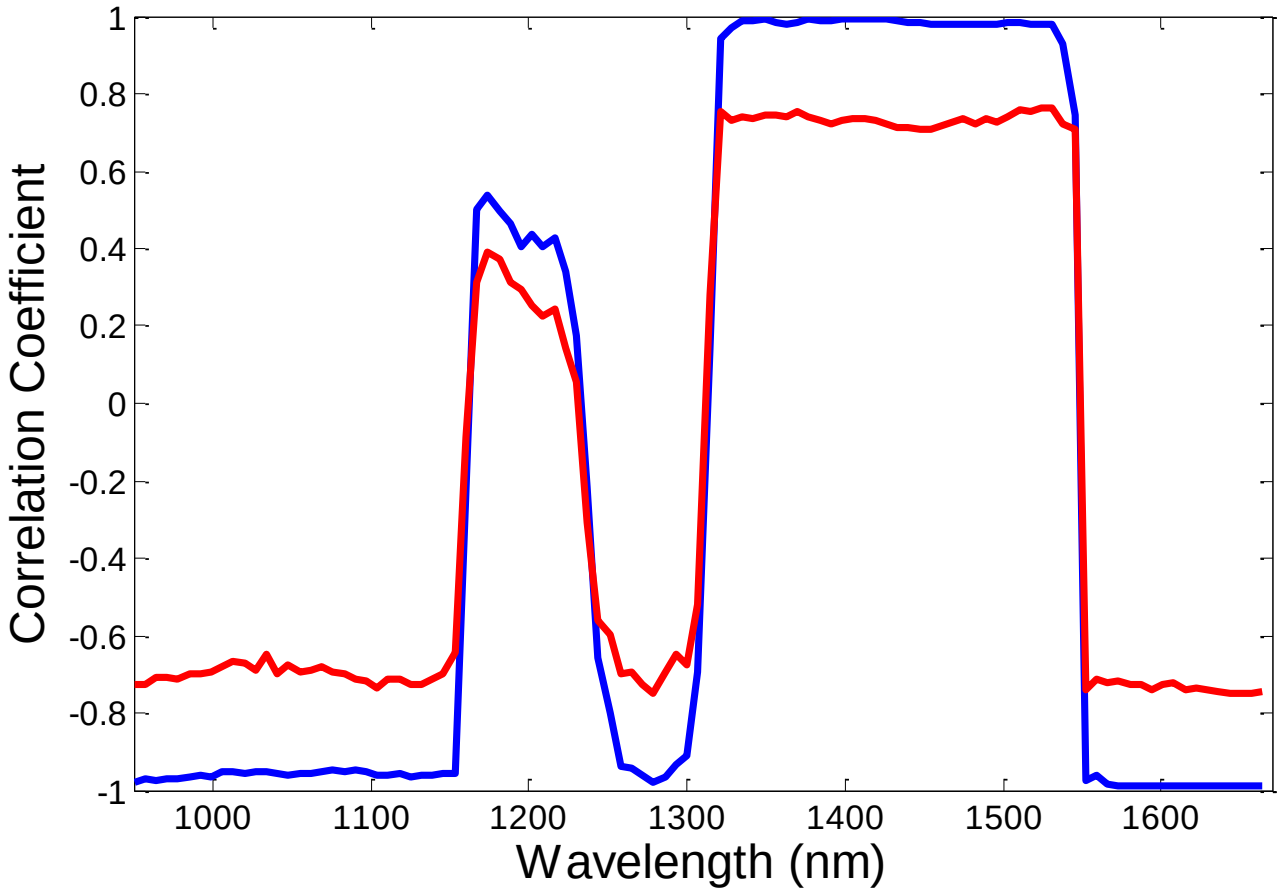
# Soybean



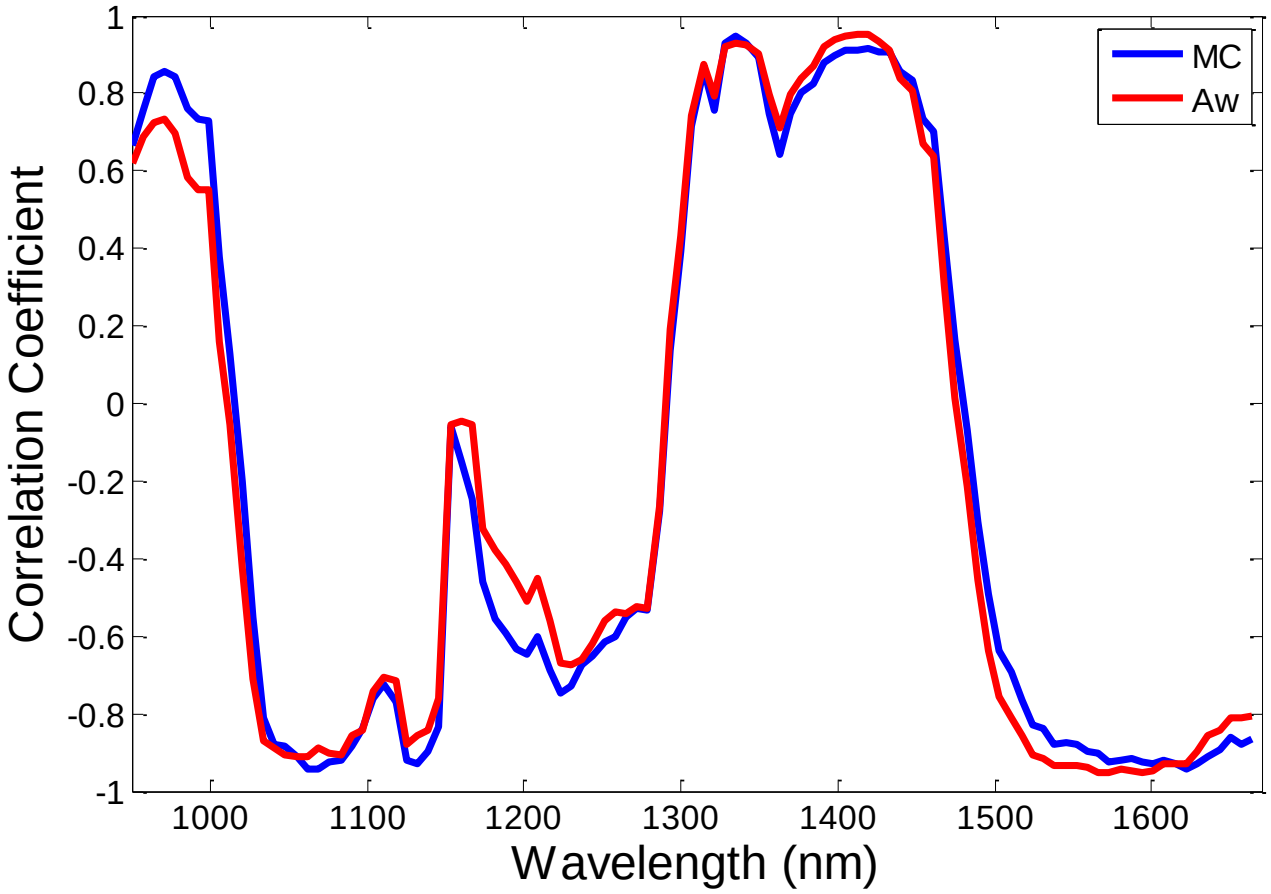
# Correlation between Aw, MC: wafer



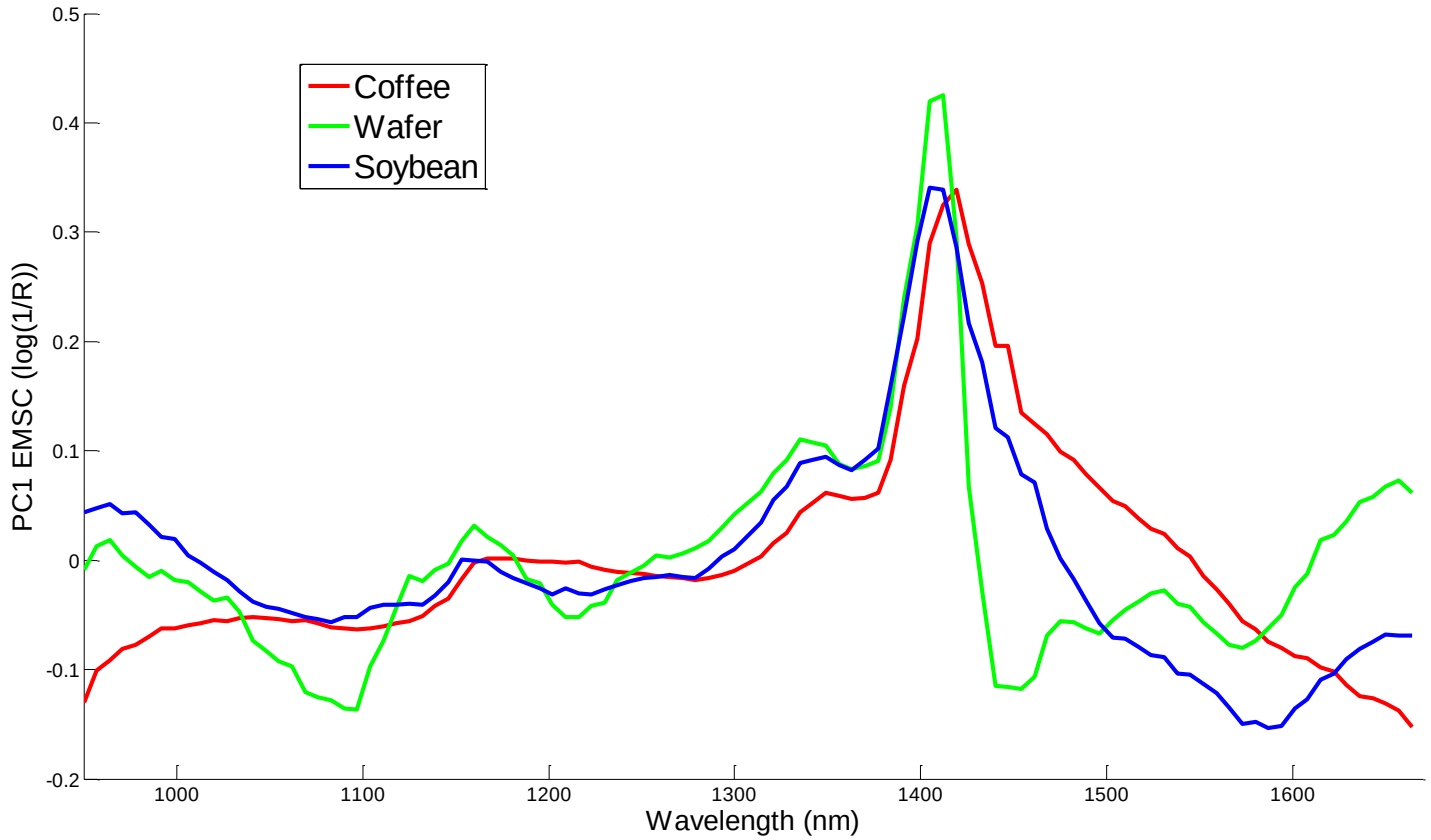
# Correlation between Aw, MC: coffee



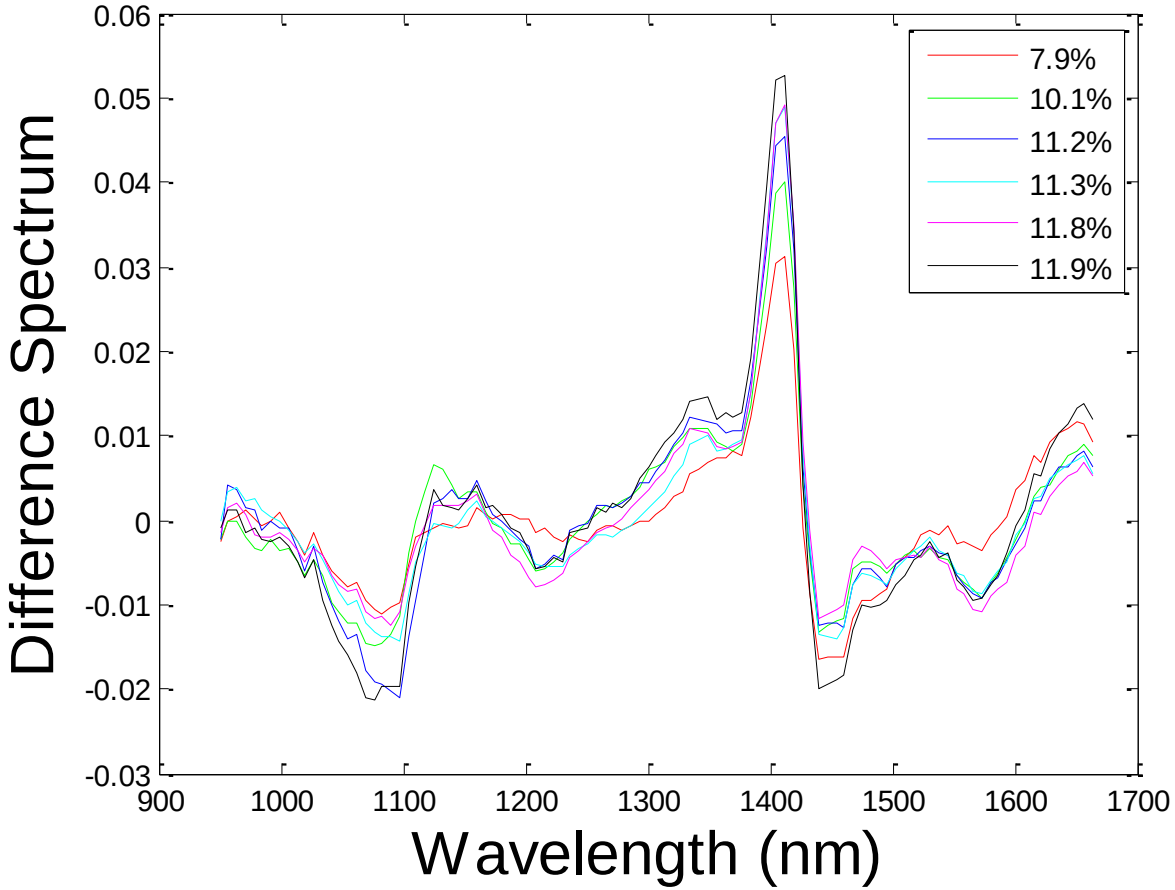
# Correlation between Aw, MC: soybean



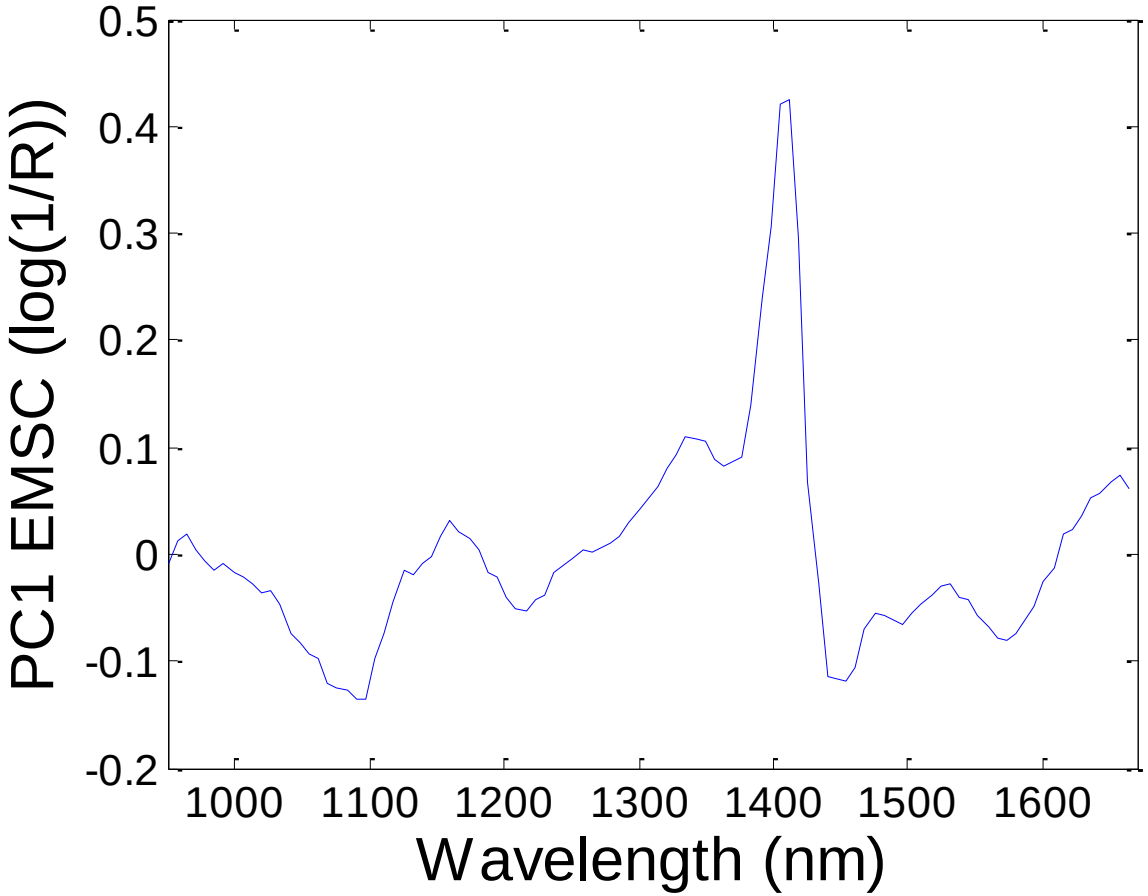
# PC 1 EMSC Spectra



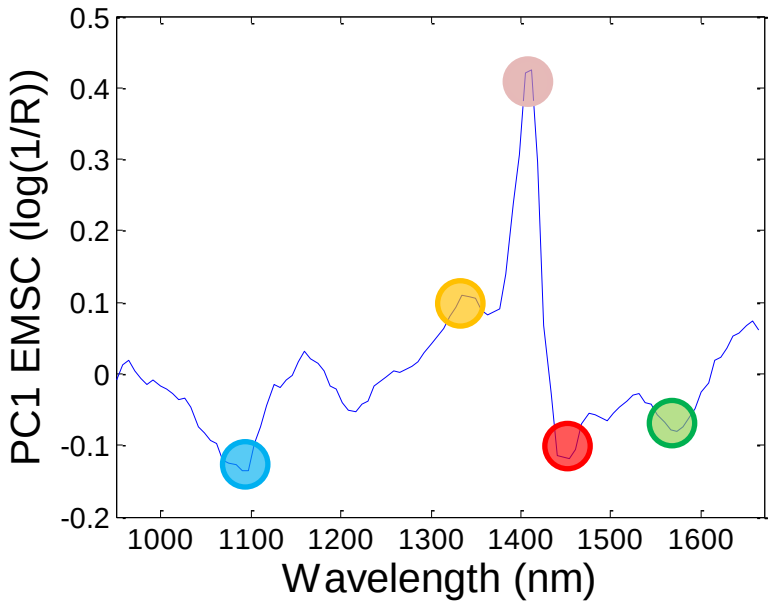
# Difference EMSC (subtracting T0) Wafer



# Wafer: PC1 EMSC loading

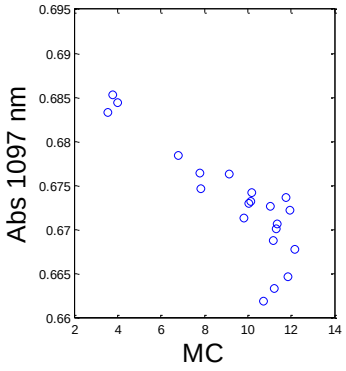


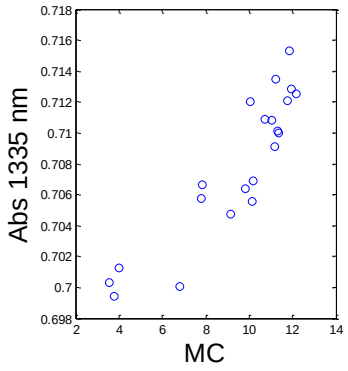
# PC1 EMSC Wafer

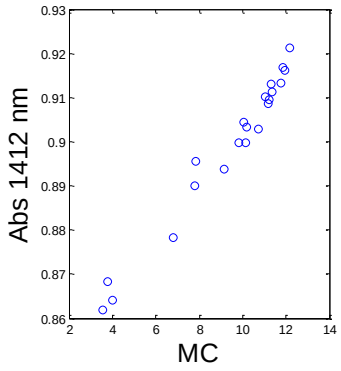


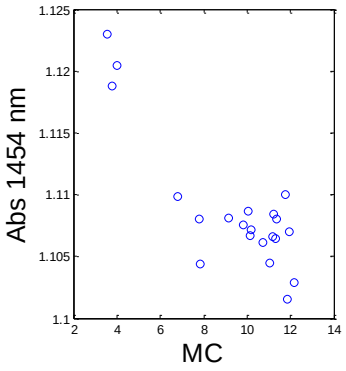

| 2D | λ (nm) | Tentative assignment |
|---|---|---|
| -M | 1097 | $v_{\delta s}+2v_s$ |
| +I | 1335 | $2v_s$ |
| -I | 1412 | $2v_s$ Free OH in free water molecule or $2v_s$ Dangling OH |
| -M | 1454 | $2v_s$ in dimer or $2v_{as}$ in dimer |
| -M | 1573 | $2v_{\delta s}$ in tetramer |

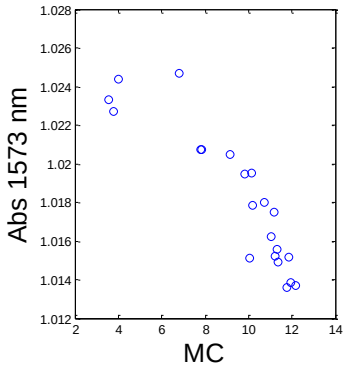
# Abs Peaks Wafer

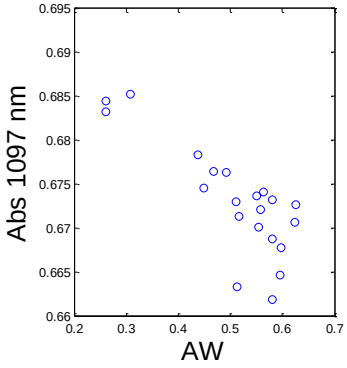


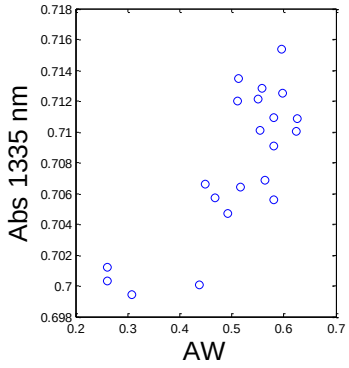



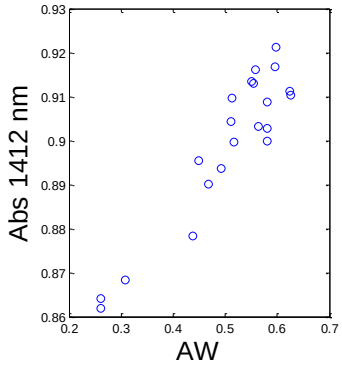



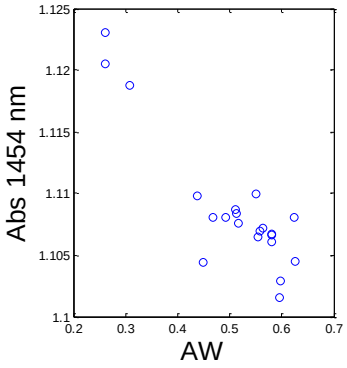



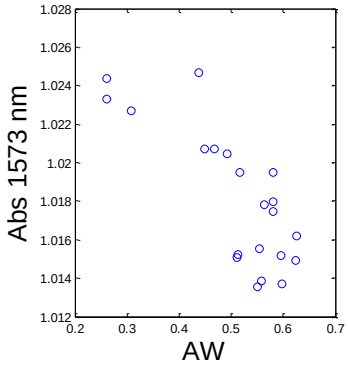




$v_{\delta s}+2v_s$

$2v_s$

$2v_s$ Free OH in free water molecule

or

$2v_s$ Dangling OH

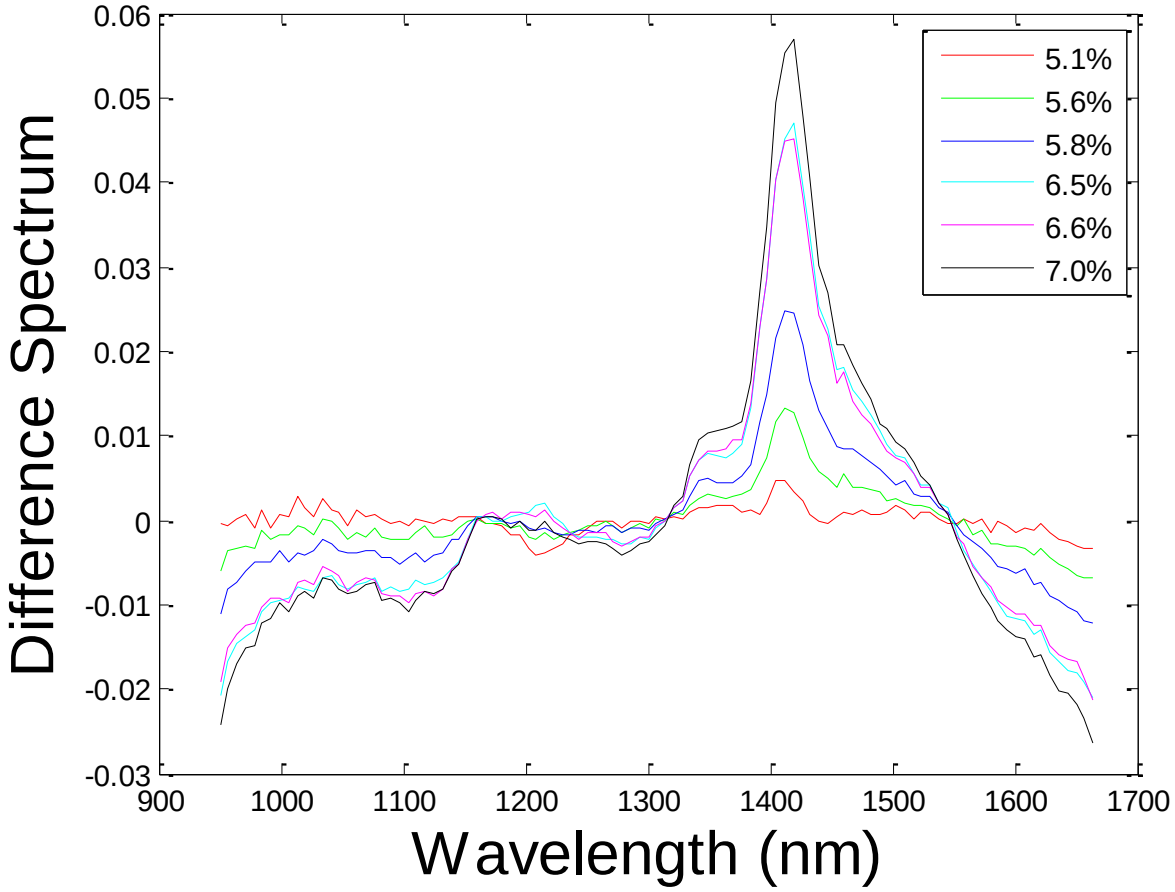
$2v_s$ in dimer

or

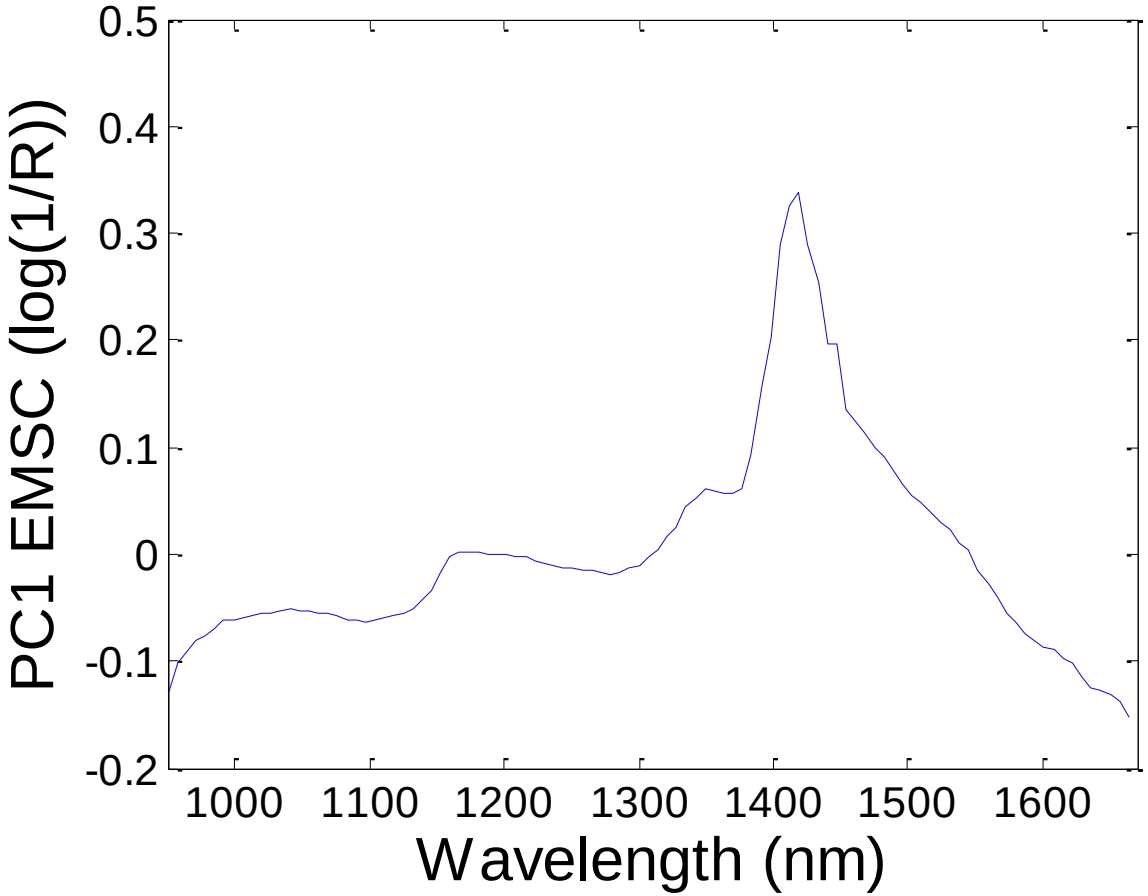
$2v_{as}$ in dimer

$2v_{\delta s}$ in tetramer

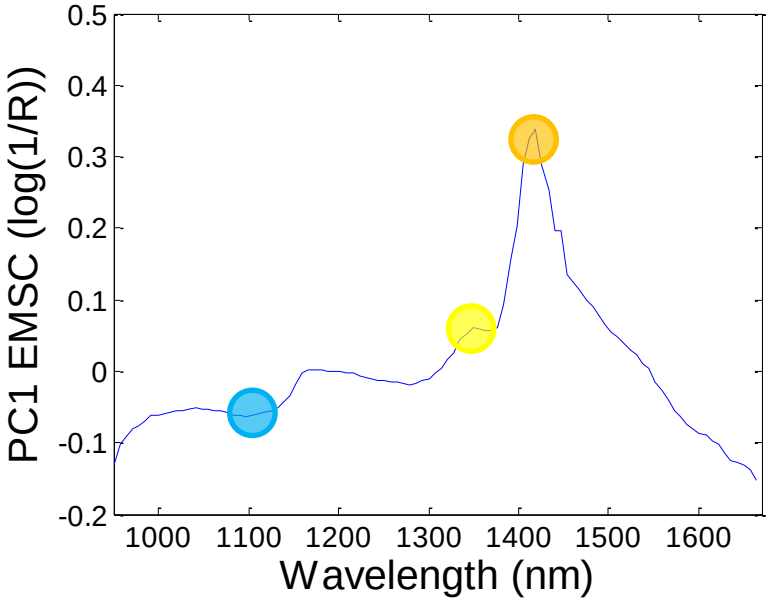
# Difference EMSC (subtracting T0) Coffee



# Coffee – PC1 EMSC loading

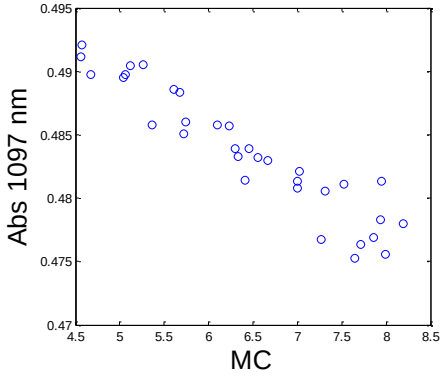


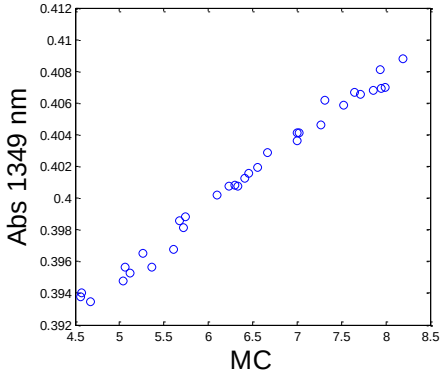
# PC1: EMSC Coffee

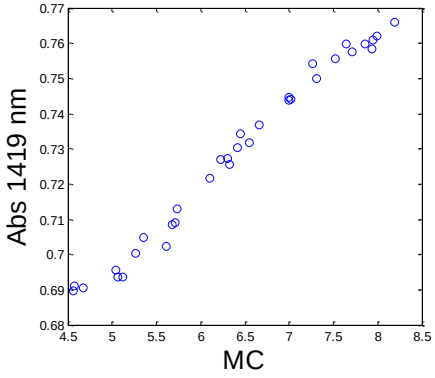


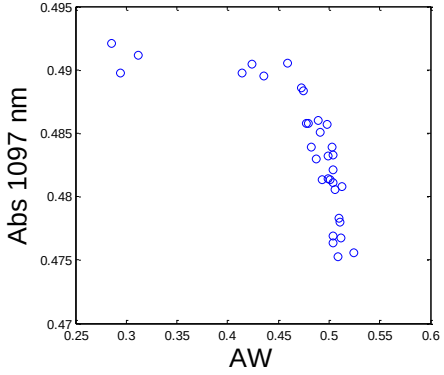

| D2 | λ (nm) | Tentative assignment |
|---|---|---|
| M- | 1097 | $v_{\delta s}+2v_s$ |
| I+ | 1342 | $2v_s$ or $2v_{as}$ Free OH in free water molecule |
| I- | 1419 | $2v_s$ dangling OH or $2v_{as}$ H bond in dimer |

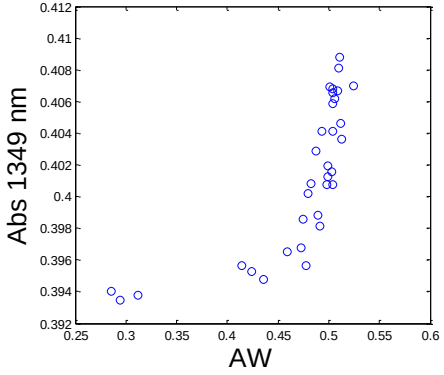
# Abs Peaks Coffee

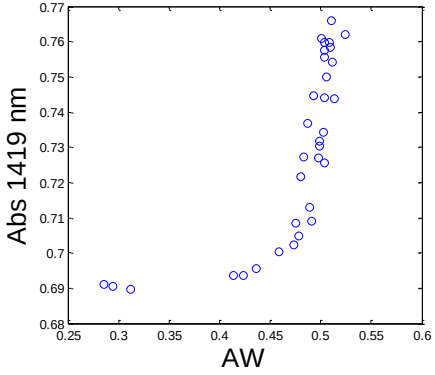


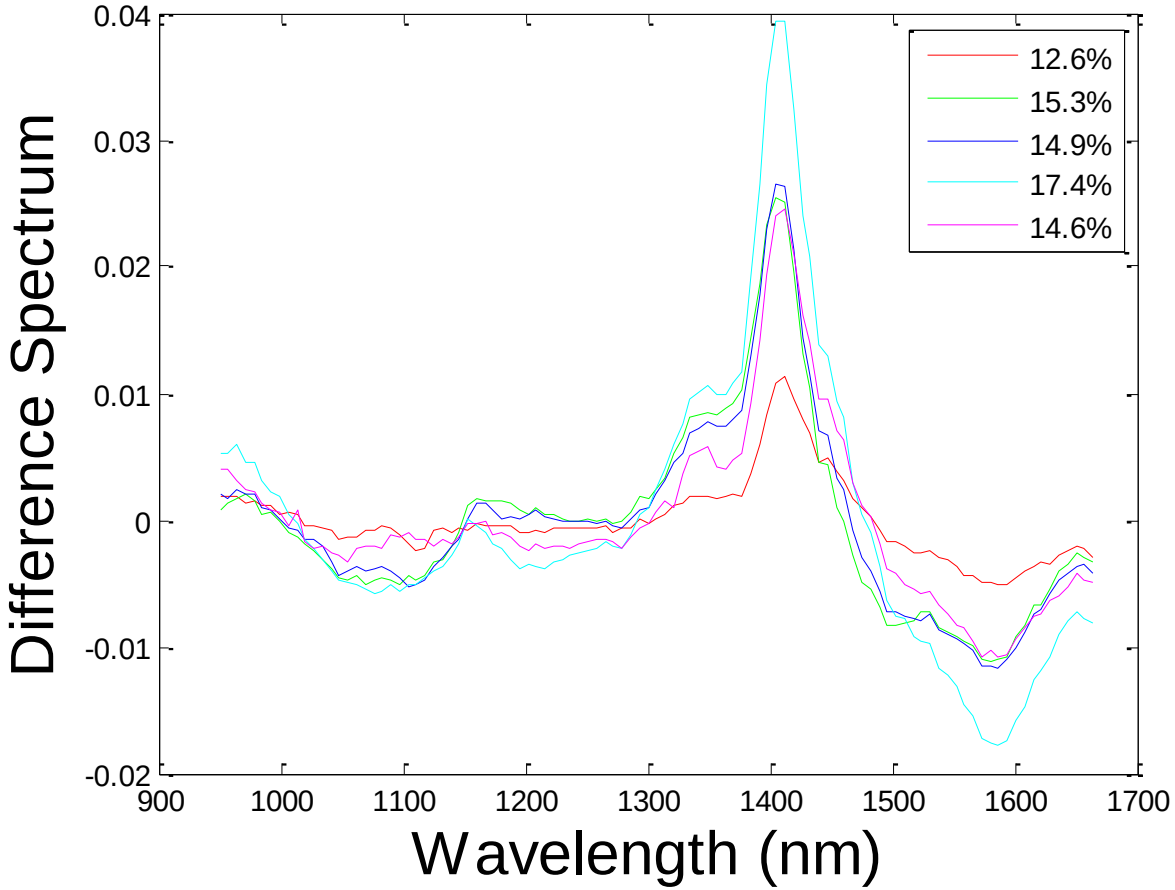




$v_{\delta s}+2v_s$

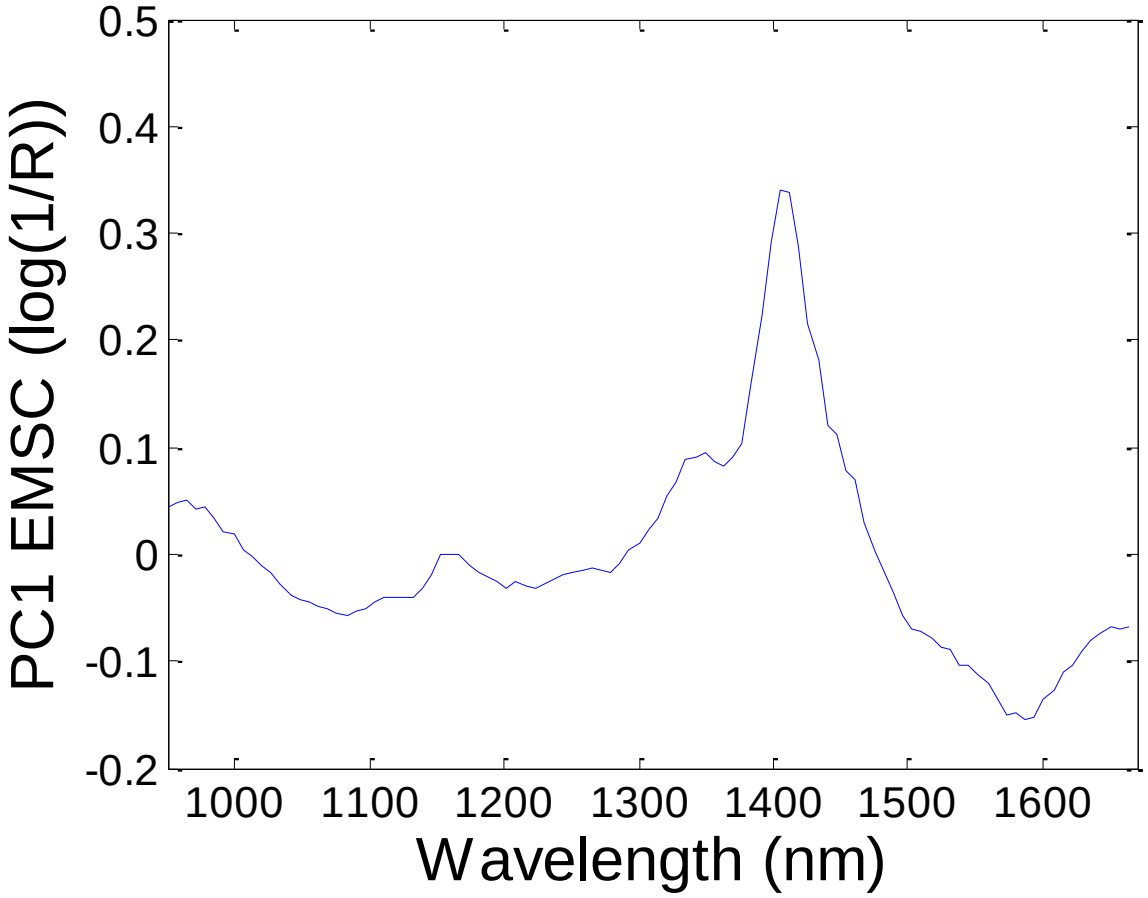
$2v_s$ or
$2v_{as}$ Free OH in free water molecule

$2v_s$ dangling OH or
$2v_{as}$ H bond in dimer

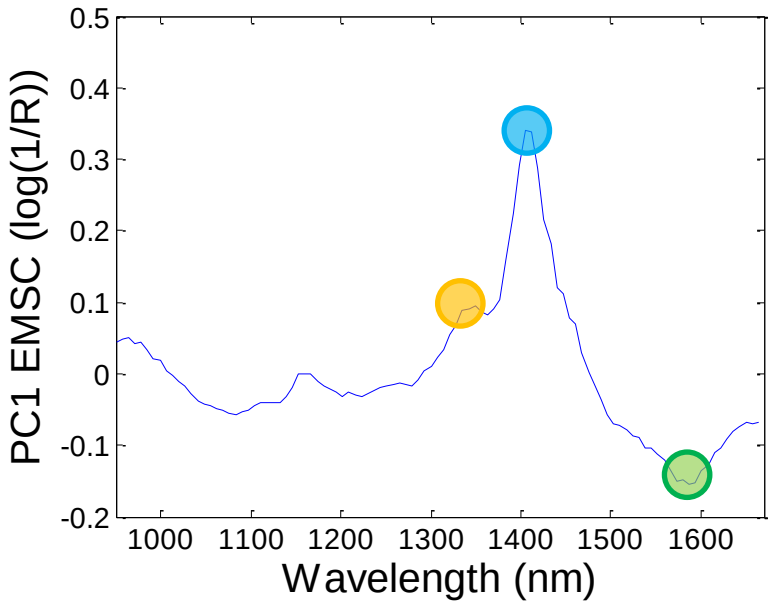
# Difference EMSC (subtracting T0) Soybeans



# Soybean: PC1 EMSC loading

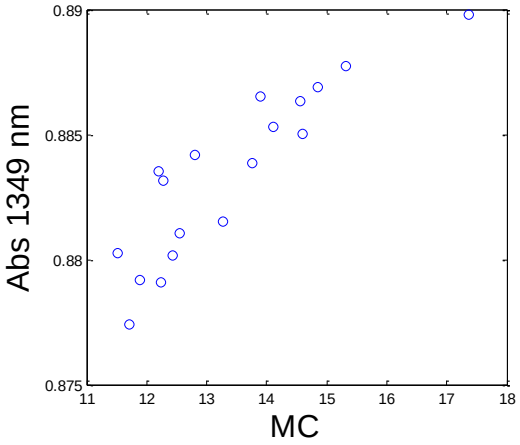


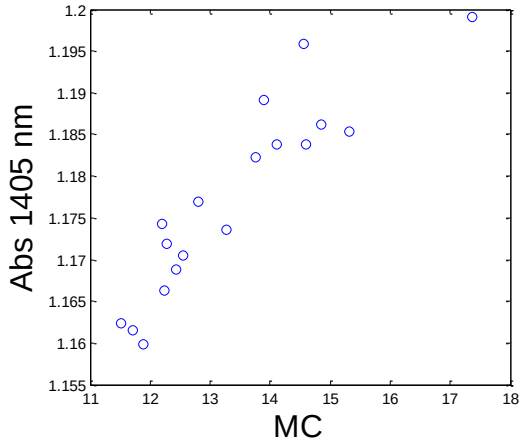
# PC1 EMSC Soybean

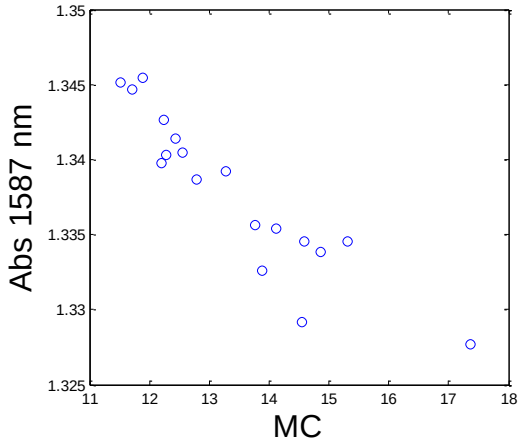


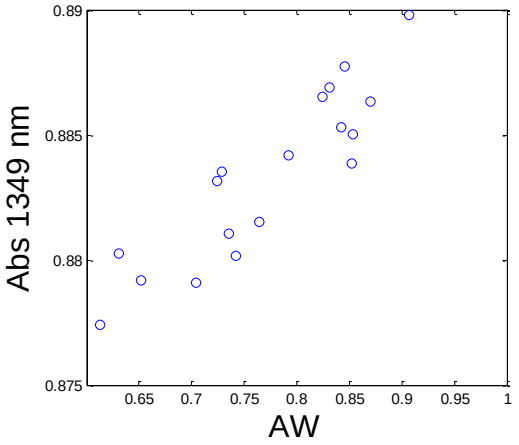

| 2D | λ (nm) | Tentative assignment |
|---|---|---|
| I+ | 1335 | $2v_s$ |
| -I | 1412 | $2v_s$ Free OH in free water molecule or $2v_s$ Dangling OH |
| -M | 1587 | $2v_{\delta s}$ in tetramer |

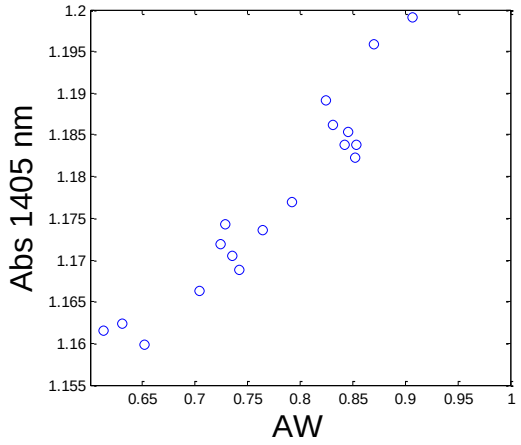
# Abs Peaks Soybean

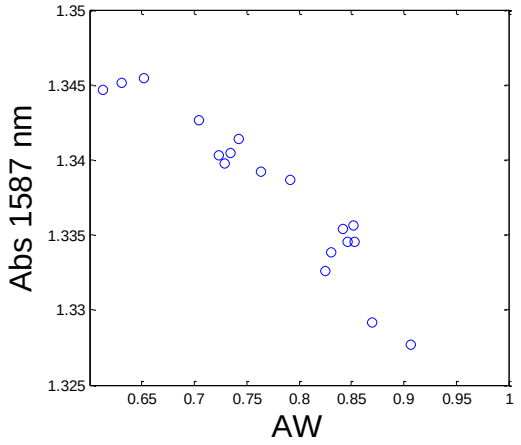



$2v_s$

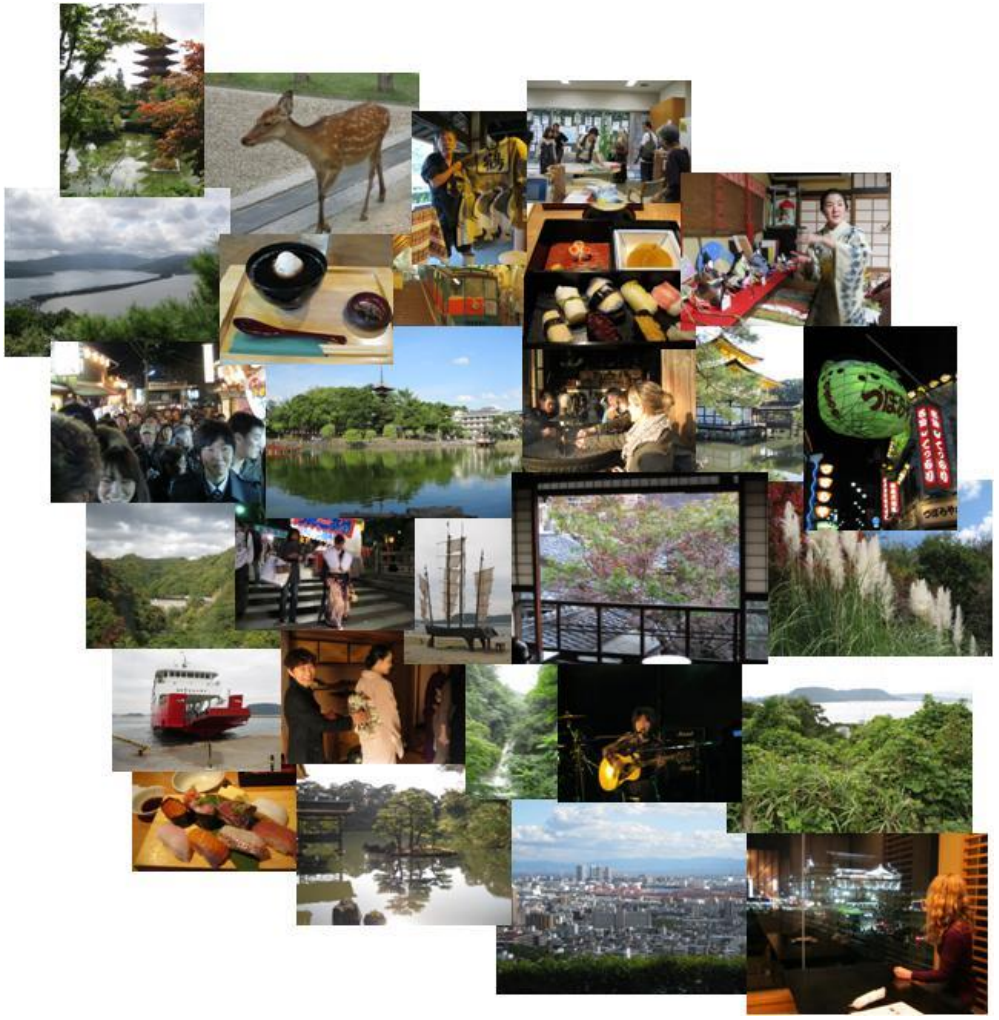
$2v_s$ Free OH in free water molecule or $2v_s$ Dangling OH

$2v_{\delta s}$ in tetramer

# Key Questions

- What does water look like in the NIR?
- What can hyperspectral imaging show us?
- How can we use this to understand the role of water in foods?

# Acknowledgements



MARIE CURIE ACTIONS

SEVENTH FRAMEWORK PROGRAMME

European Research Council

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