Joint Brown and Caldwell – Horiba Webinar

Integrated Characterization of Organic Matter for Water Treatment Optimization

Katherine Y. Bell, Ph.D., PE, BCEE
Director of Water Strategy, Brown and Caldwell, KBell@BrwnCald.com

Martha J.M. Wells, Ph.D.
Owner, EnviroChem Services, info@envirochemservices.net

Consulting Chemist to Brown and Caldwell, MWells1@BrwnCald.com
Emeritus Professor of Chemistry, Tennessee Technological University, mjmwells@tntech.edu

Agenda

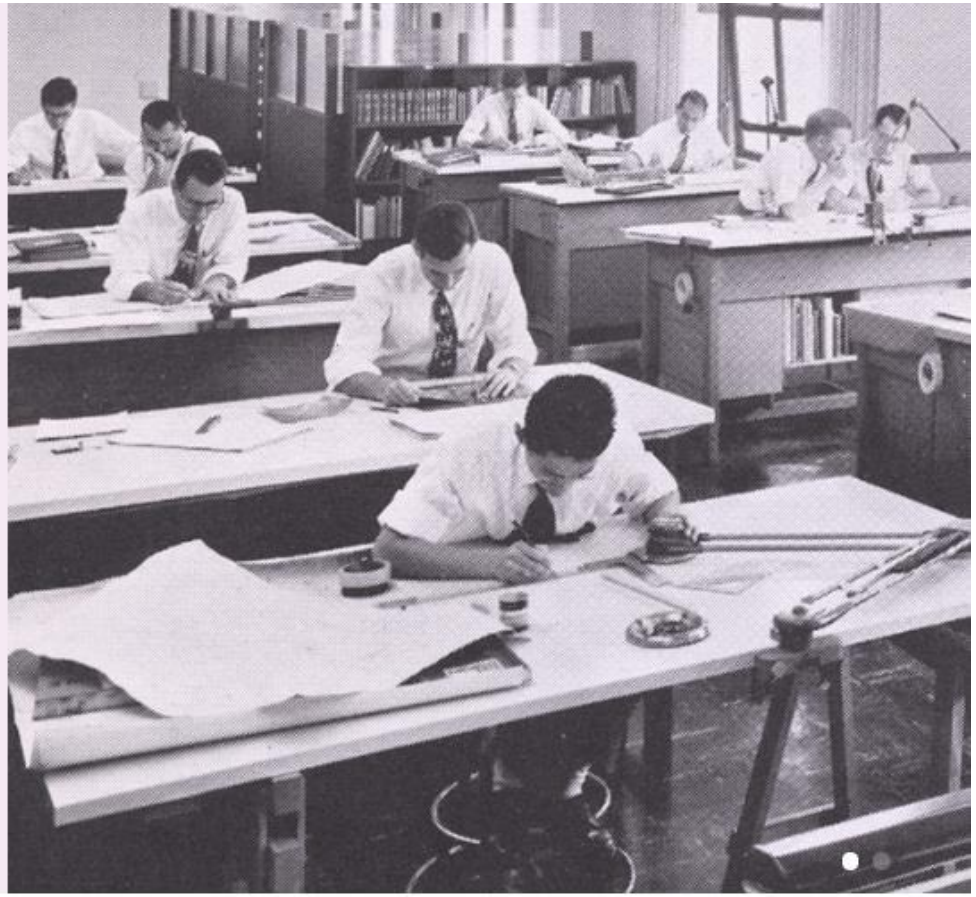
- BC water research and treatability
- Operationalization—from fundamental to applied research
- Aqueous organic carbon
- Sampling and sample preparation
- Advances in instrumentation
  - EEM—PARAfac (Horiba Aqualog)
  - DLS (Horiba SZ-100)
  - Laser diffraction (Horiba 960A)
  - Zeta-potential (Horiba SZ-100)

Brown and Caldwell is a 100% environmental, employee-owned company with the purpose and passion of safeguarding water, maintaining infrastructure, and restoring habitats to keep our communities thriving.

Our history

We are passionate about delivering exceptional service, collaborating with clients, adding value through innovation and building relationships that last. This passion dates back to Ken Brown and Dave Caldwell, who, since founding the company in 1947, stood out for their ability to solve engineering challenges, apply technology to emerging environmental problems, and serve their community.

Learn more >

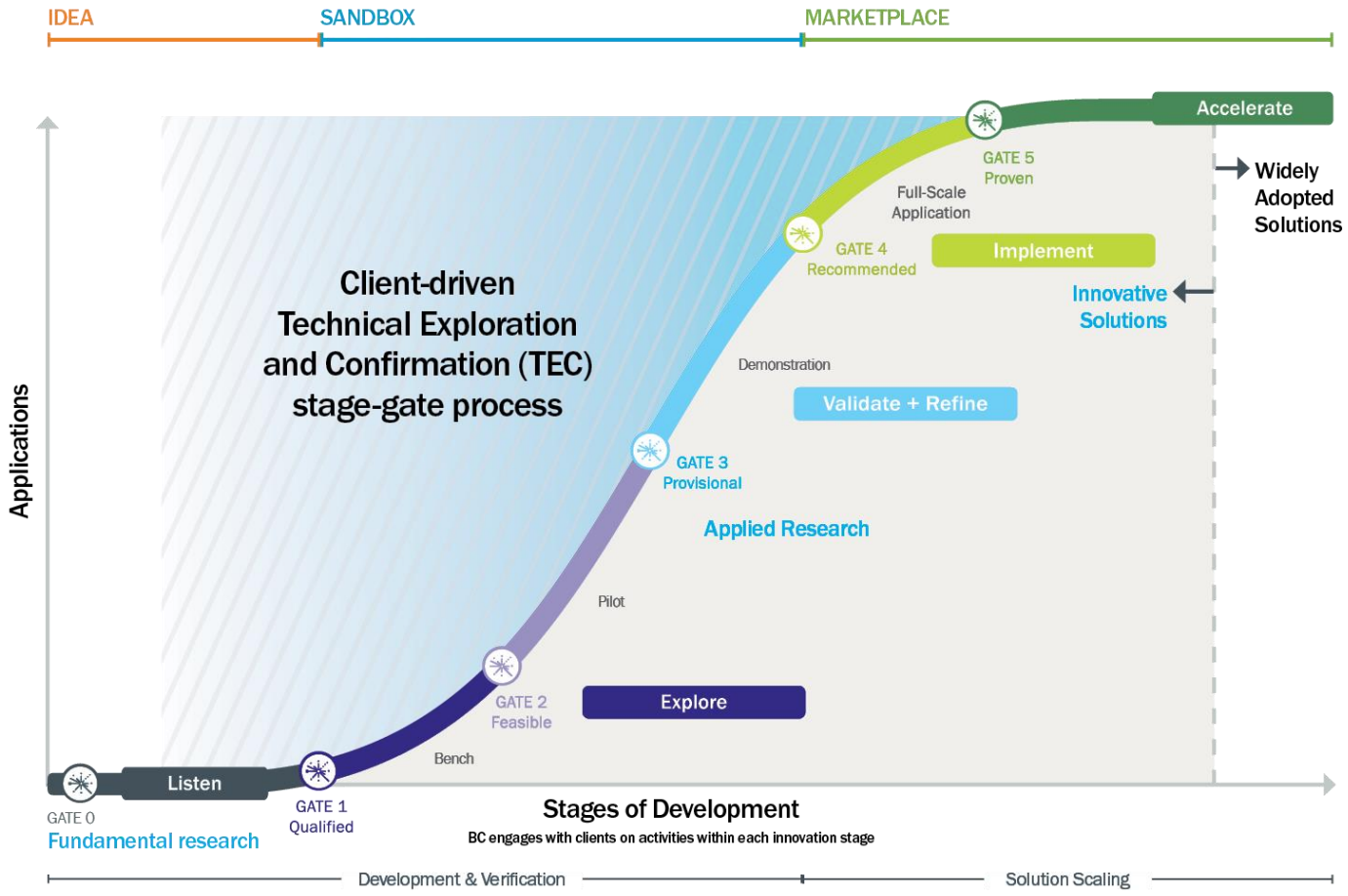


BC research helps clients address unsolved challenges

Research and innovation work together to de-risk solutions for earlier implementation

Early pilots and demonstrations with client partners tests the viability and feasibility of innovation and identifies questions or needs for refinement.

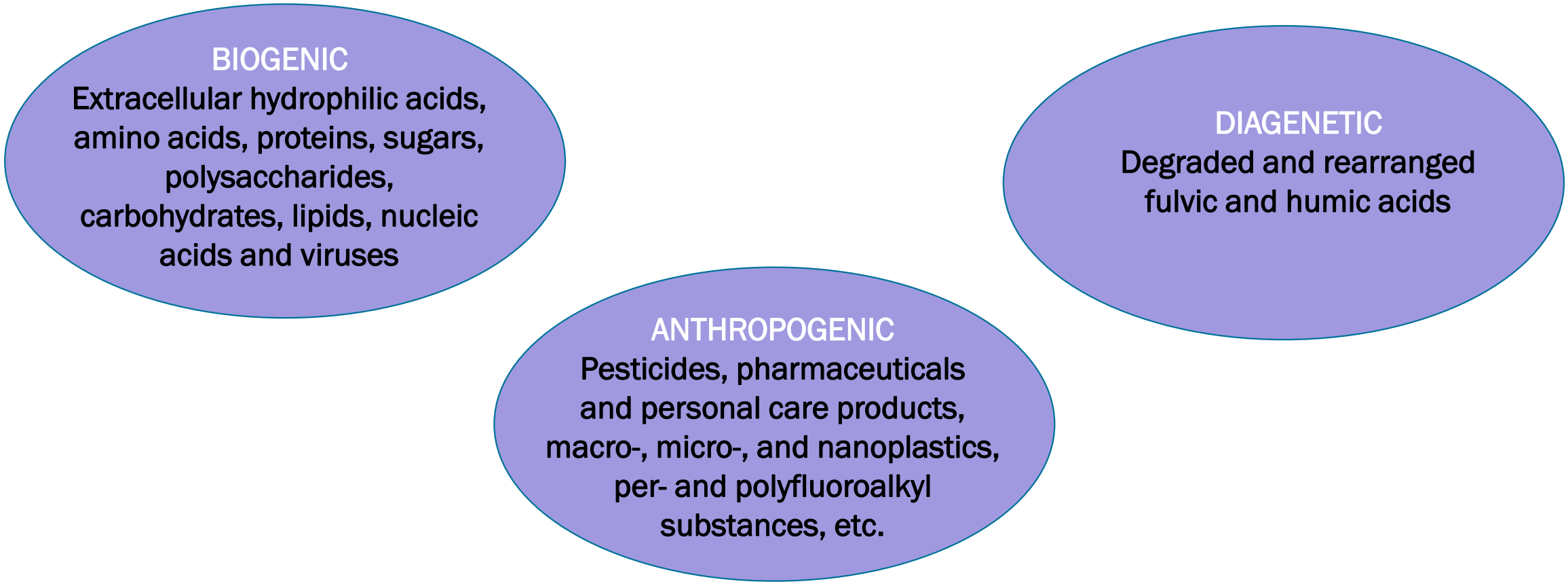
BC treatability testing laboratory is a platform to support bench- and pilot-scale investigations for conventional and innovative solutions.



The problem of aqueous organic carbon…

- Surrogate measures (UVA254, TOC/DOC, and COD) indicate trends but inadequately portray character OM removed and/or transformed at each treatment stage (Bieroza et al., 2009).
- The presence of OC impedes water treatment by decreasing effectiveness of additives such as oxidants and disinfectants (Baghoth et al., 2011)
- All water treatment processes are affected by DOM (Ishii and Boyer, 2012)…
- Thus, unit processes are integrated into treatment trains for OC removal (Sillanpaa, 2015).
- If present, OM leads to formation of potentially hazardous DBPs (Abouleish and Wells, 2015)
- Analytical tools are emerging to monitor and characterize AOM in treatment (Wells et al., 2017).
- Past knowledge of water treatment stages is of a train of black boxes (Li et al., 2020)
- No single treatment method—coagulation/flocculation, sedimentation/flotation, filtration, ion exchange, activated carbon, AOPs, or biofilms—can individually remove OC fractions present in raw water. (Wells, Bell, et al., *in preparation*).

Characterization of “nonliving” organic carbon in water



Diagenetic denotes the partial degradation, rearrangement, and recombination of biogenetic molecules into fulvic and humic acids. Schwarzenbach, R.P., P.M. Gschwend, and D.M. Imboden. 2017. Environmental Organic Chemistry. John Wiley & Sons, Hoboken, NJ. Wells, M.J.M. 2019. Supramolecular Answers to the Organic Matter Controversy. J. Environ. Qual. 48:1644-1651.

An old story of organic matter…

"Although soil scientists have studied the chemical nature of soil humic substances for two centuries, little is known about their chemical structure and composition due to their complexity and diversity."

- A. Zaujec and S.S. Gonet, in *The Role of Humic Substances in the Ecosystems and in Environmental Protection,* ed. J. Drozd, S.S. Gonet, N. Senesi, J. Weber, IHSS - Polish Society of Humic Substances, Wroclaw, Poland, 1997, pp. 517 - 522.

The term "humus" is attributed to the doctoral thesis of Wallerius in 1761.

- Wallerius, J.G., 1761. Agriculturae Fundamenta Chemica. Doctoral Thesis, Upsaliae.
- Waksman, S.A., 1936. Humus: Origin, Chemical Composition, and Importance in Nature. Williams & Wilkins, Baltimore.
- Bleam, W., 2017. Soil and Environmental Chemistry, second edition, Academic Press/Elsevier, Amsterdam.

Current thinking…

Is “nonliving” organic carbon in water

- Molecular?
- Macromolecular?
  - polymers
- Supramolecular?
  - Coined by Lehn—the chemistry of the intermolecular bond

The answer to all is YES!

Combined into supramolecular soft matter compared to that of condensed systems—Condensed systems refer to polystyrene beads, gold nanoparticles, clay, etc.

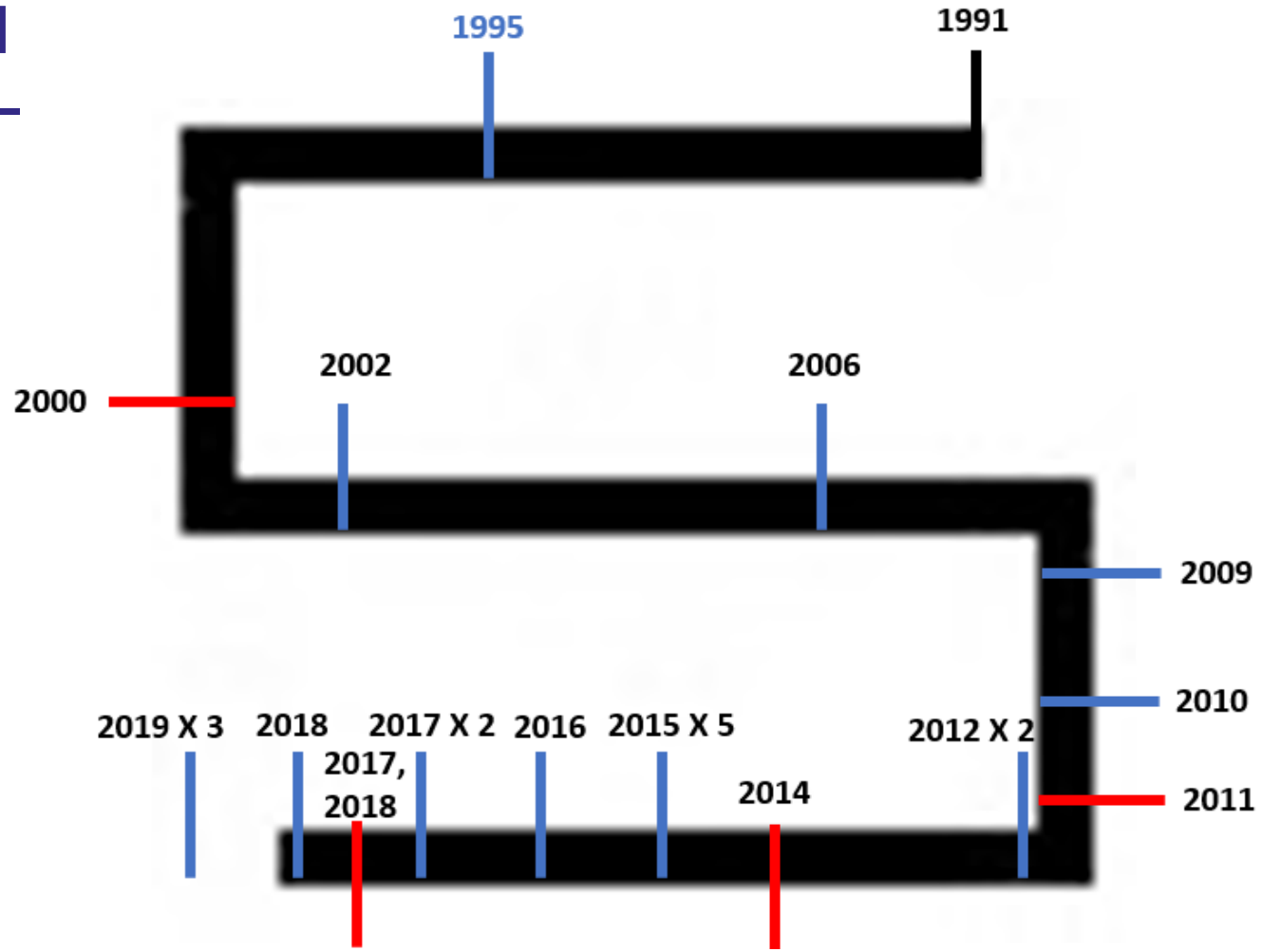
Important distinction when we talk about dynamic light scattering (DLS)

Wells, M.J.M. and Stretz, H.A., 2019. *Science of the Total Environment, 671:1125*–1133.
Wells, M.J.M. 2019. Supramolecular Answers to the Organic Matter Controversy. J. Environ. Qual. 48:1644-1651.

Improvements in analytical measurements

Form and function –

Martha's humic timeline



"Never undervalue forward progress on a tough problem"

—attributed to George R. Aiken

Humic research initiated

Humic peer refereed publications

Epiphany moments!

BC uses organic carbon characterization techniques

Total/Dissolved Organic Carbon (TOC/DOC)
UV-Vis/EEM/PARAFAC (Horiba Aqualog)
Zeta Potential (Horiba SZ-100)
Particle Size Distribution (PSD) DLS (Horiba SZ-100) Laser Scattering (Horiba LA-960)
pH
Conductivity
Size exclusion chromatography

BC Treatability Laboratory (Nashville, TN)

Organic carbon sampling considerations

Sample containers

- Samples for organic carbon characterization analyses shall be collected in virgin, EPA-certified contaminant-free amber glass bottles with appropriate closures (Standard Methods 5310, EPA Method 415.3, and EPA 540/R-93). Clear glass may be used if protected from light (i.e., bottles wrapped in aluminum foil).
- PTFE-lined caps (i.e., Teflon)
- Possess certificate of conformance to EPA “Specifications and Guidance for Contaminant-Free Sample Containers.”

Blanks

- Liquid chromatography-mass spectrometry (LC-MS) grade water
- Trip Blank: A clean sample of a matrix that is taken from the laboratory to the sampling site and transported back to the laboratory without being exposed to sampling procedures (U.S. EPA).
- Field Blank: A sample of analyte free water poured into the container in the field, preserved and shipped to the laboratory with field samples (U.S. EPA).

Hold Times

- DOC/EEM/DLS: Within 7 days (filter within 48 h)
- No pH adjustment for EEM/DLS/PSD
- TOC: pH $\leq$ 2 in the field

Organic carbon sample preparation

Filtration

- Dissolved organic carbon (DOC) is operationally defined as that passing through a 0.45 µm filter
- 0.45 µm polyethersulfone membrane filter (syringe filter or filtration apparatus)

Dilution

If samples must be diluted use liquid chromatography-mass spectrometry grade water

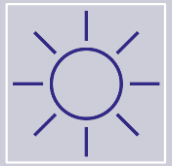
Quartz cuvettes

- Scrupulously clean
- nitric acid and detergent—Aqualog manual
- Aqua regia

Preservation

- Sodium azide (0.03%)—biocide, sample becomes a hazardous waste
- Add sodium azide or not to DLS experiments; don’t add to EEM samples
- Generates abiotic, nonliving, aqueous organic carbon

A tale of three phenomena in organic carbon…



FLUORESCENCE and SCATTERING

(Horiba Aqualog)

UV-Visible ABSORBANCE and SCATTERING

(Horiba Aqualog)

SCATTERING—with a smattering of electrophoretic mobility

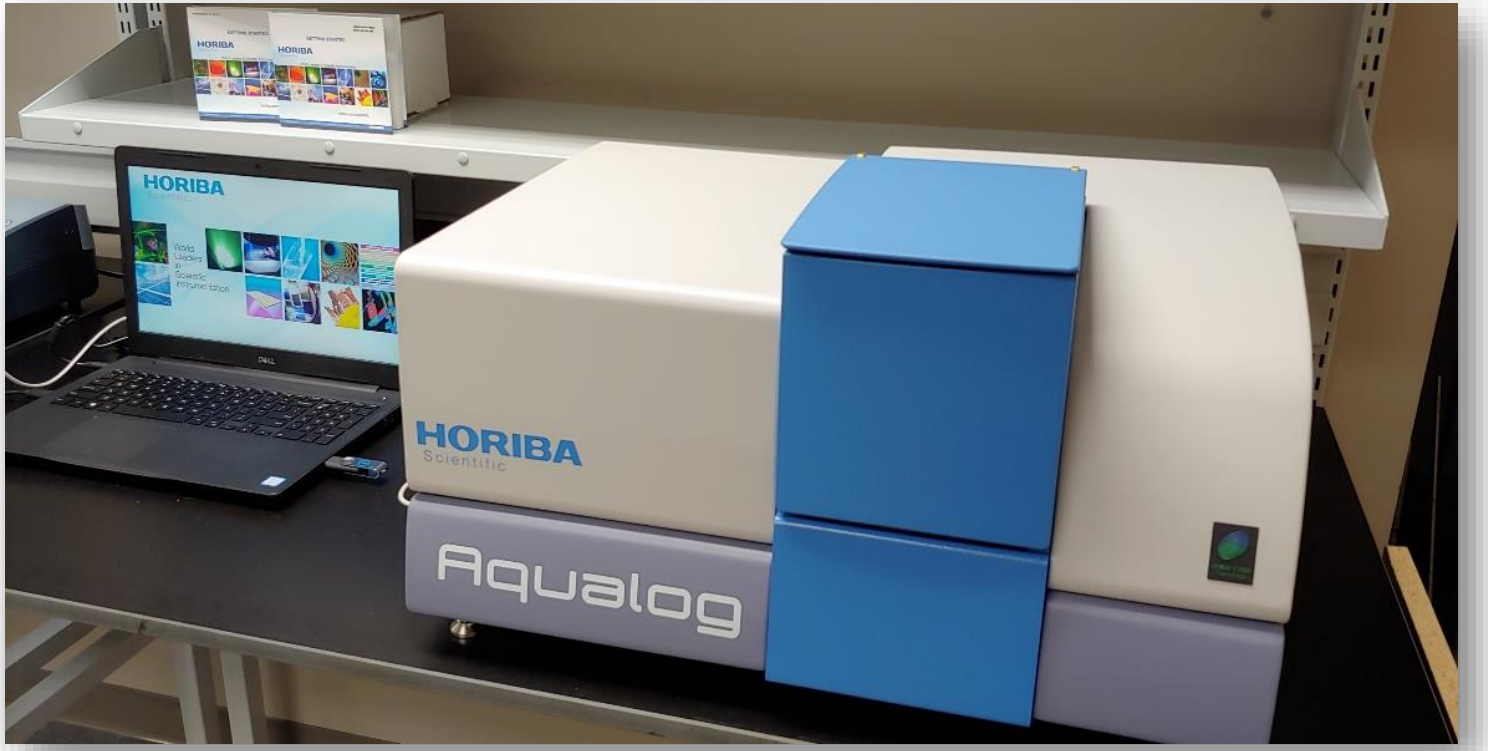
(Horiba SZ-100 and Horiba LA-960)

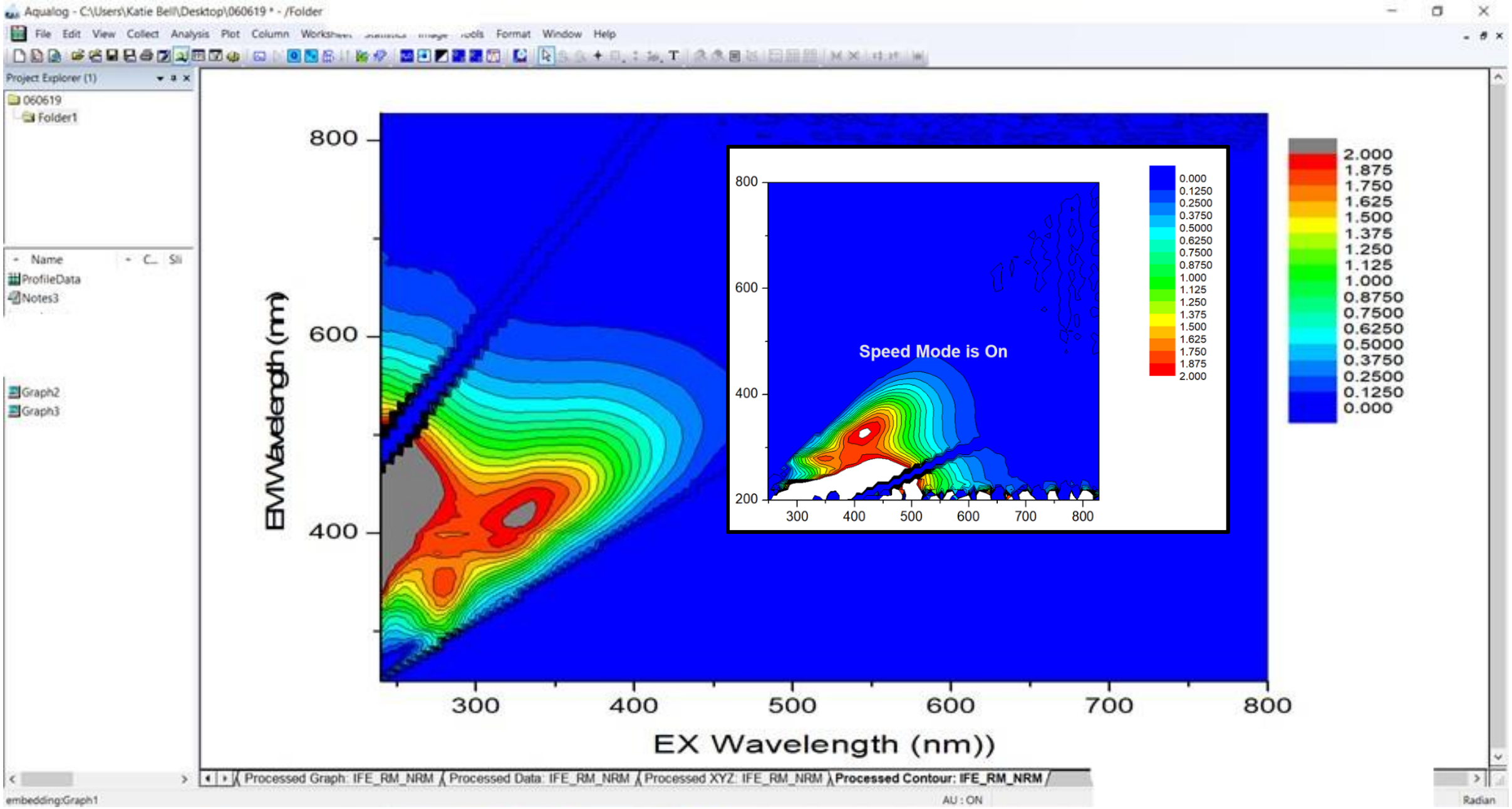
EEM-PARAFAC with the Horiba Aqualog

- Excitation-Emission Matrix Fluorescence Spectroscopy (EEM)
- Ultraviolet absorbance (UVA)/Ultraviolet-visible transmittance (UVT)

Two Instruments in One

- PARAllel FACtor (PARAFAC)
- Recommend purchase of the remote software license




Aqualog - C:\Users\Katie Bell\Desktop\060619 * - /Folder
File Edit View Collect Analysis Plot Column Format Window Help
Project Explorer (1)
060619
Folder1
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Notes3
Graph2
Graph3
EM Wavelength (nm)
EX Wavelength (nm))
800
600
400
300
400
500
600
700
800
2.000
1.875
1.750
1.625
1.500
1.375
1.250
1.125
1.000
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0.6250
0.5000
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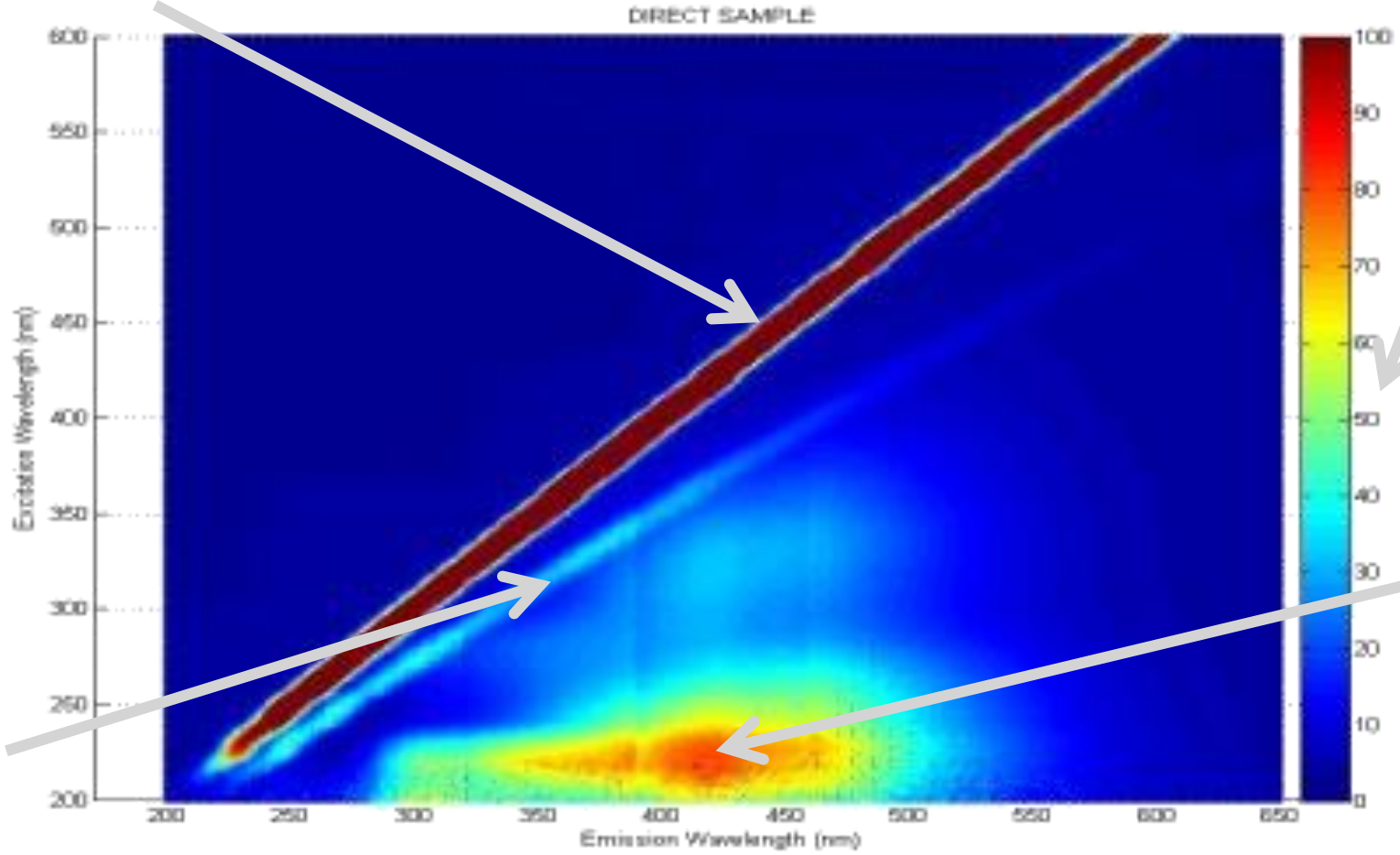
Radian

3-dimensional fluorescence

Relative Scale

First Order Rayleigh Scatter ($\lambda_{Em} = \lambda_{Ex}$)



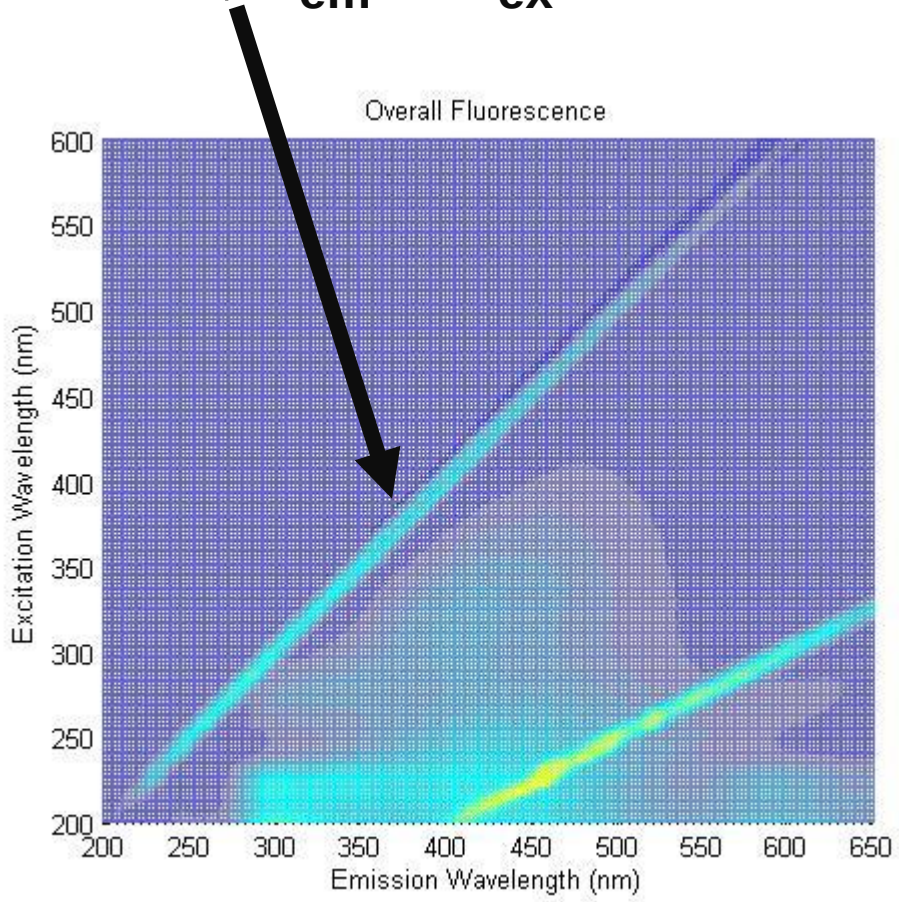
Fluorophore centers or "fingerprints"

Water Raman

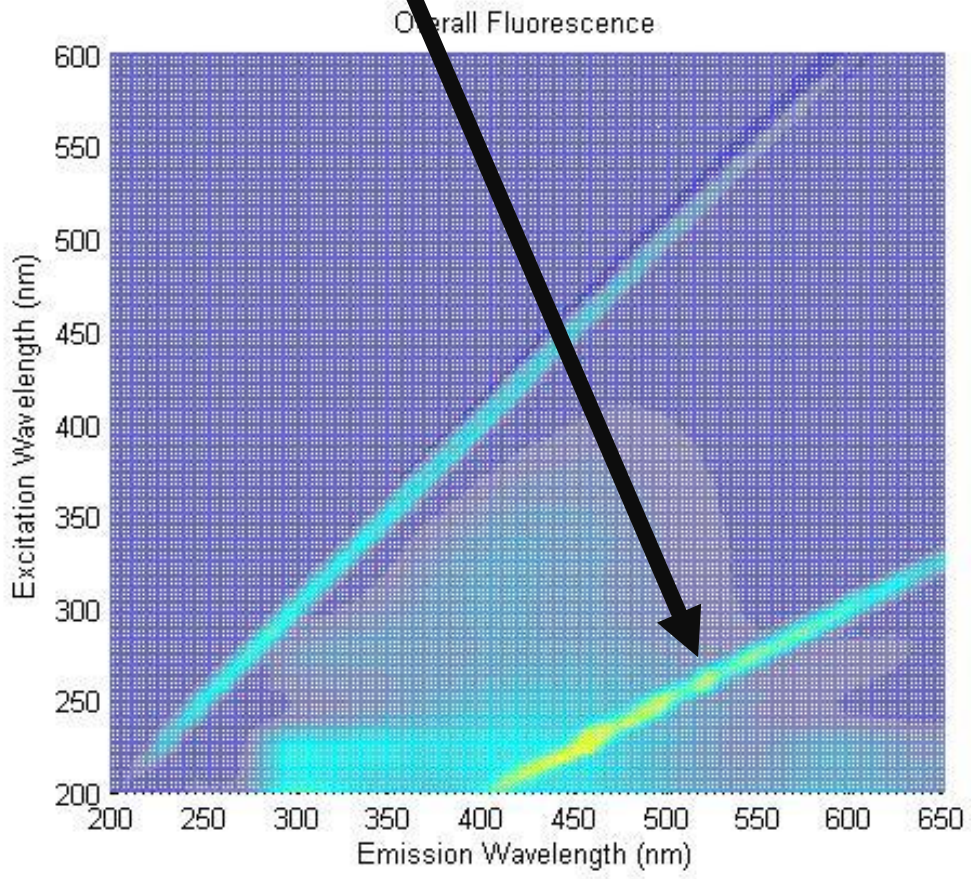
Not a Horiba Aqualog spectrum

Why the sky is blue – Rayleigh scattering

First Order, $\lambda_{em} = \lambda_{ex}$

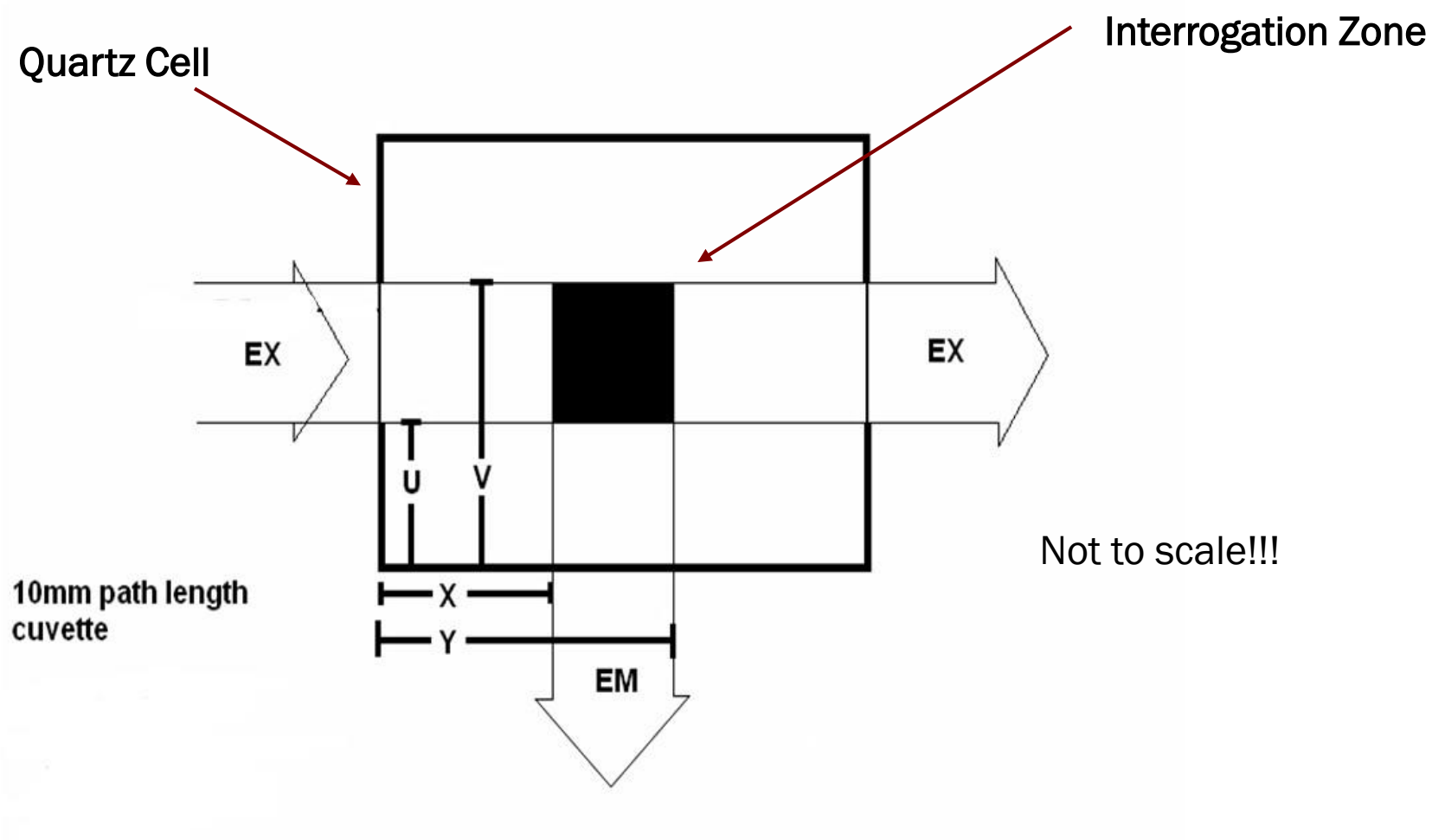


Second Order, $\lambda_{em} = 2\lambda_{ex}$



Not Horiba Aqualog spectra

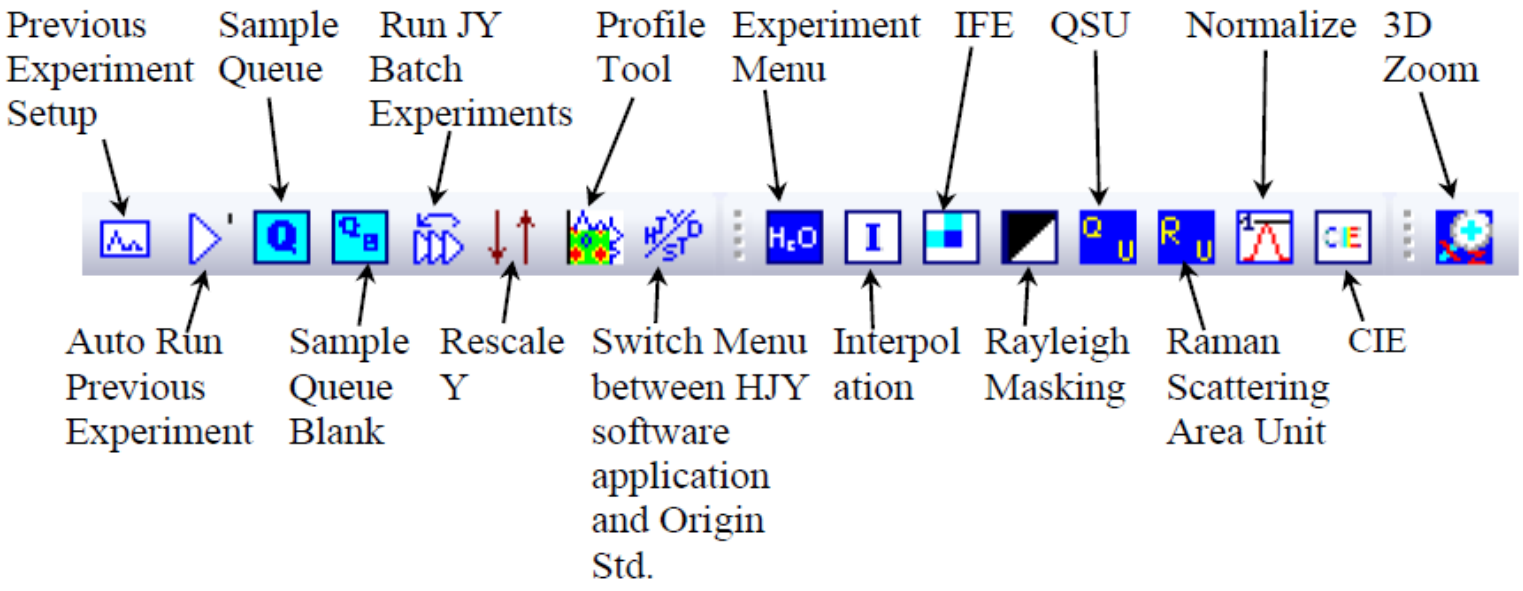
Primary and secondary inner filtering effects (IFEs)



S.A. Tucker, V.L. Amszi, and W.E. Acree, Jr. 1992. Journal of Chemical Education, 69(1):A8, A11, A12.

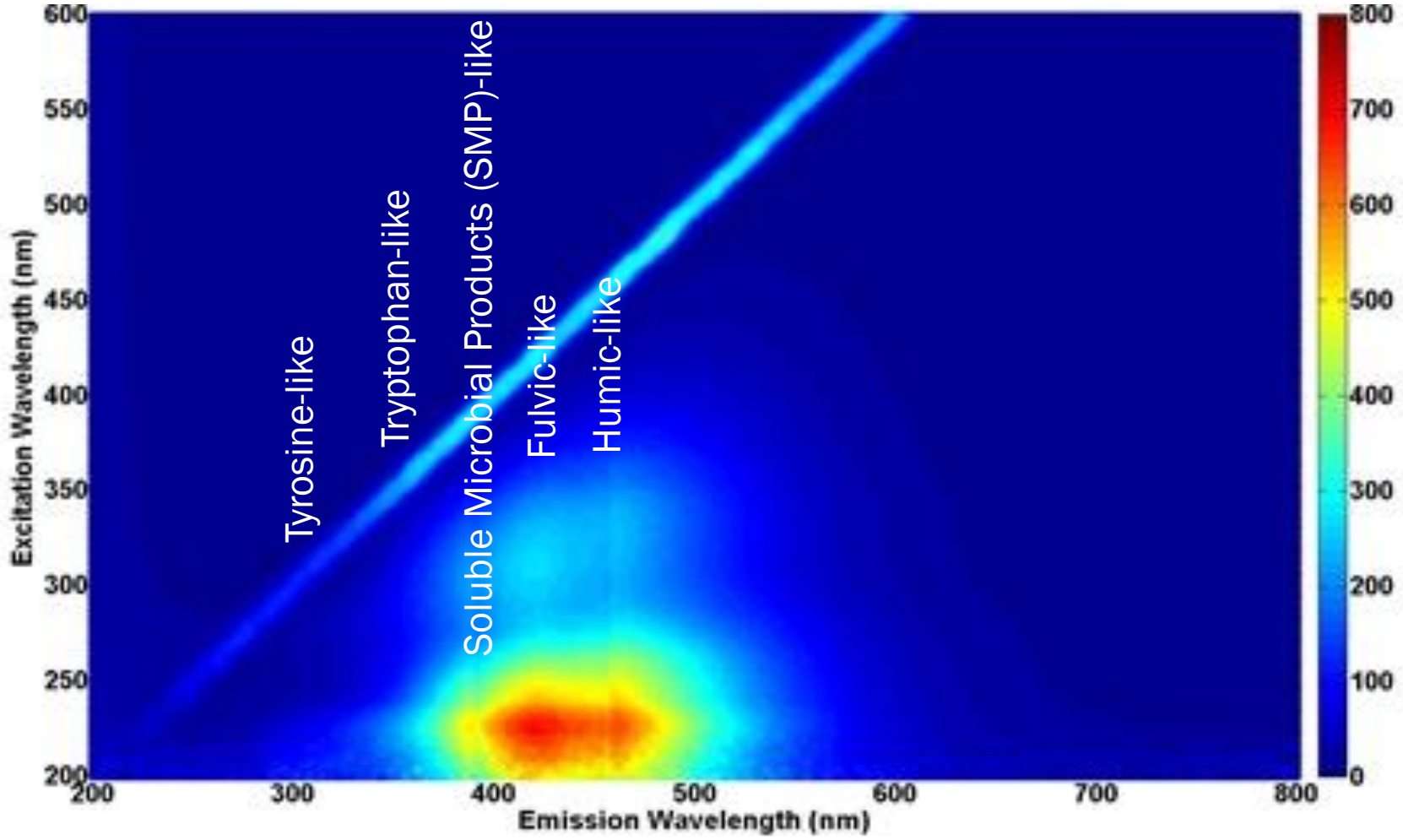
How to process EEMs with the Aqualog

- Correct for first- and second order Rayleigh scattering
- Subtract Raman scattering spectrum of water (sealed “superpure” water)
- Raman normalization (Normalize for temperature and lamp life)
- Correction for inner filter effects (IFEs)—all samples, all wavelength pairs
- Post-processing
- Fluorescence quenching:
  - Static
  - Dynamic (collisional)
  - Thermal
  - Inner Filter Effects (IFEs)



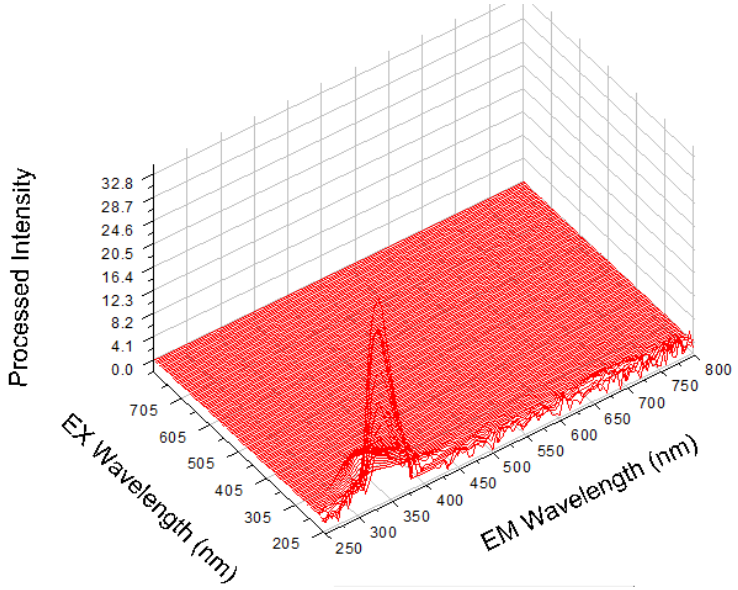
Spectrally separated by EEM

EEM Spectrum of Wastewater-Impacted River Water
(Not an Aqualog Spectrum)

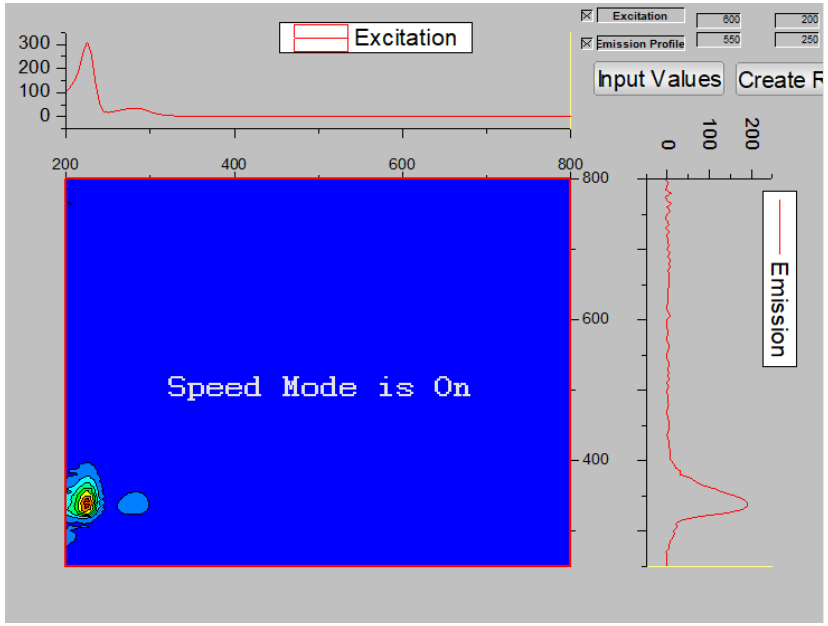


Wells, M.J.M., Mullins, G.A., Bell, K.Y. Da Silva, A.K., Navarrete, E.M. Environ. Sci. Technol. 2017, 51, 13592.

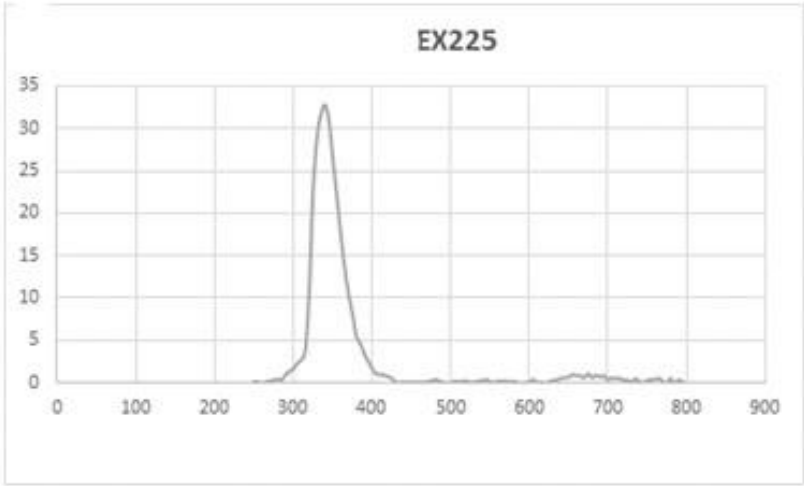
Potential sample contamination event (tryptophan)



3D EEM Spectrum



Profile Plot

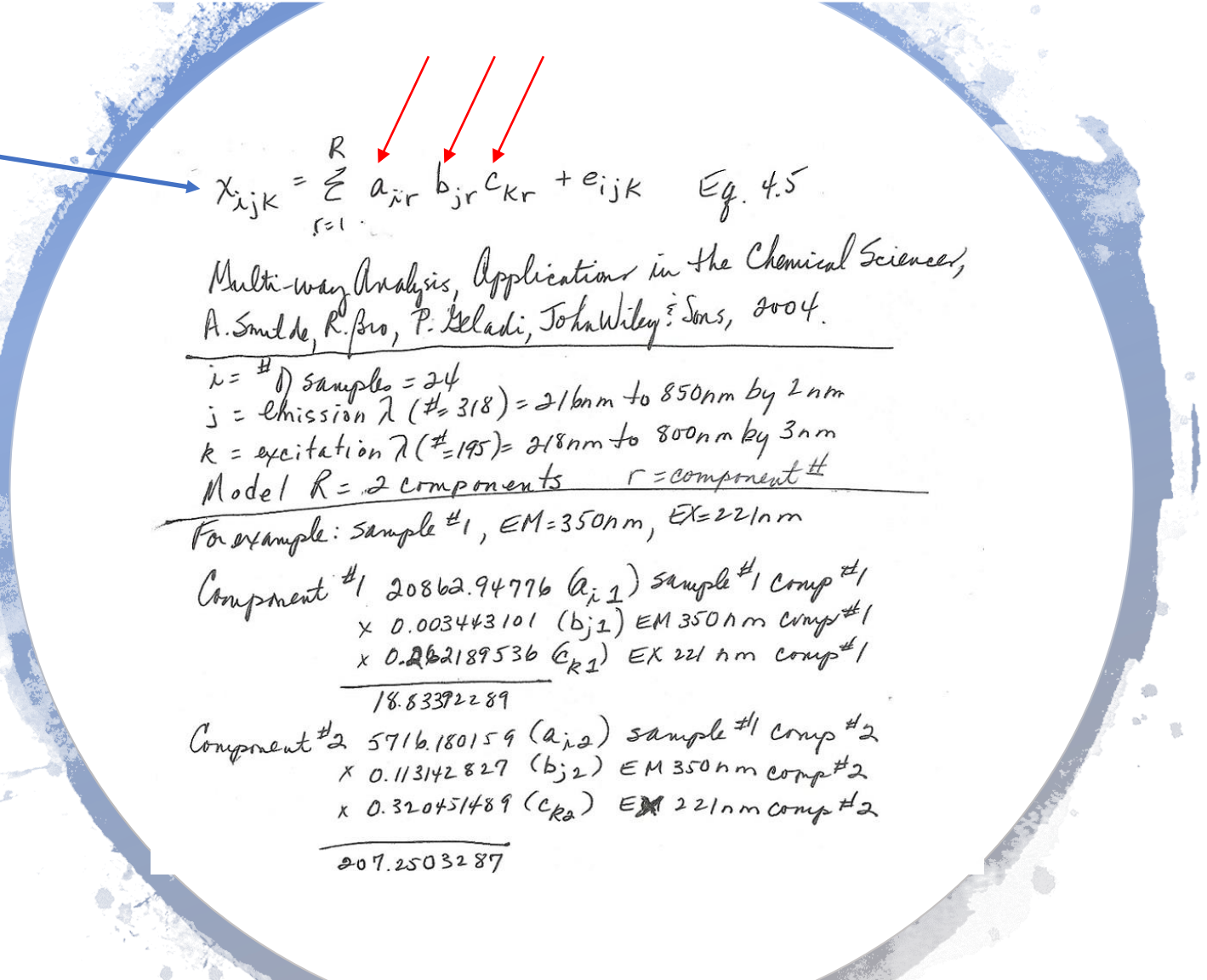


2D Excitation Spectrum at Constant EX = 225 nm

*A **profile plot** is a graphical data analysis technique for examining the relative behavior of all variables in a multivariate data set.* profile.pdf (nist.gov)

The PARAFAC model

Intensity of sample number i at EM = j and EX = k summed over all components R



$$x_{ijk} = \sum_{r=1}^{R} a_{ir} b_{jr} c_{kr} + e_{ijk} \quad \text{Eq. 4.5}$$

Multi-way Analysis, Applications in the Chemical Sciences, A. Smilde, R. Bro, P. Geladi, John Wiley & Sons, 2004.

i = # of samples = 24
j = emission λ (# = 318) = 216nm to 850nm by 2nm
k = excitation λ (# = 195) = 218nm to 800nm by 3nm
Model R = 2 components r = component #

For example: sample #1, EM = 350nm, EX = 221nm

Component #1 20862.94776 (a_{i1}) sample #1 comp #1
× 0.003443101 (b_{j1}) EM 350nm comp #1
× 0.262189536 (c_{k1}) EX 221nm comp #1
18.83392289

Component #2 5716.180159 (a_{i2}) sample #1 comp #2
× 0.113142827 (b_{j2}) EM 350nm comp #2
× 0.320451489 (c_{k2}) EX 221nm comp #2
207.2503287

PARAFAC Model

The PARAFAC Model (x_{ijk})* for fitting three-way data sets yields three important data elements or modes of information. In the jargon applied by the EEM/PARAFAC community, these three modes of information are referred to as **Sample Scores, Emission Loadings, and Excitation Loadings**. Within each mode, the three-way EEM data (i.e., intensity measured at emission and excitation wavelengths for each sample) are deconvoluted into components. Each component is representative of a family of fluorescing, carbon-containing compounds.

$$x_{ijk} = \sum_{r=1}^{R} a_{ir} b_{jr} c_{kr} + e_{ijk}$$

where x_{ijk} represents the PARAFAC Model;

a, *b*, and *c* are the three modes related to samples, emission wavelengths, and excitation wavelengths, respectively;

r is the component number and *R* is the total number of components described by the model;

i, *j*, and *k* are the individual sample number, the specific emission wavelength, and the specific excitation wavelength, respectively;

e is a residual term containing all the variation not explained by the model.

The three types of mathematically deconvoluted graphs generally produced to describe a PARAFAC model illustrate the **Sample Scores, Emission Loadings, and Excitation Loadings** datasets.

Data output from an EEM-PARAFAC model

SCORES (Mode 1)

- One number per each sample per each component
- Scores are akin to but not equivalent to concentration (they cannot be equivalent to concentration because the components are not composed of pure compounds).

LOADINGS—Emission (Mode 2)

- One number per each emission wavelength per each component

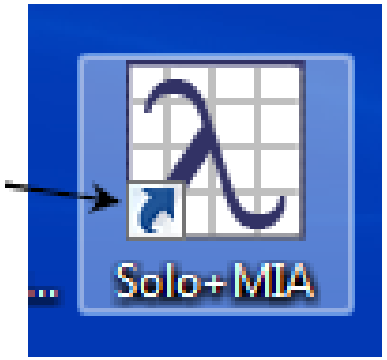
LOADINGS—Excitation (Mode 3)

- One number per each excitation wavelength per each component

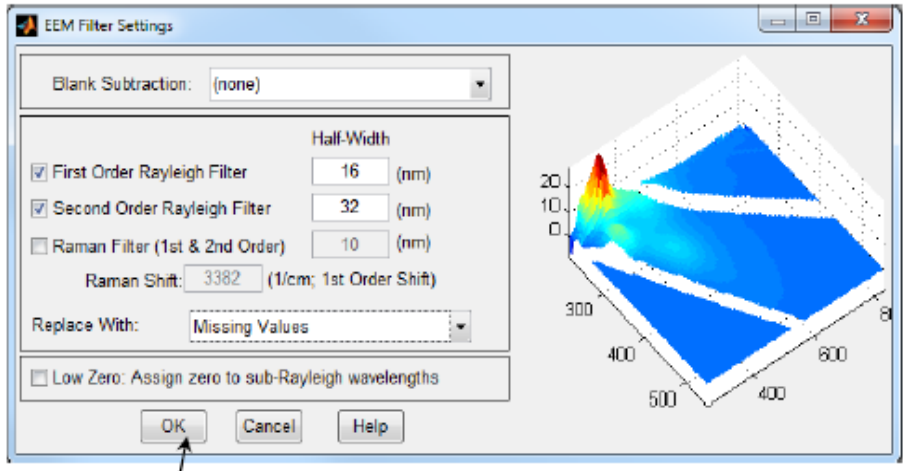
Always export these data as Excel (*.csv files)

- Then these data can be used to make 1D, 2D, and 3D graphs in any graphics software

How to perform PARAFAC with Aqualog



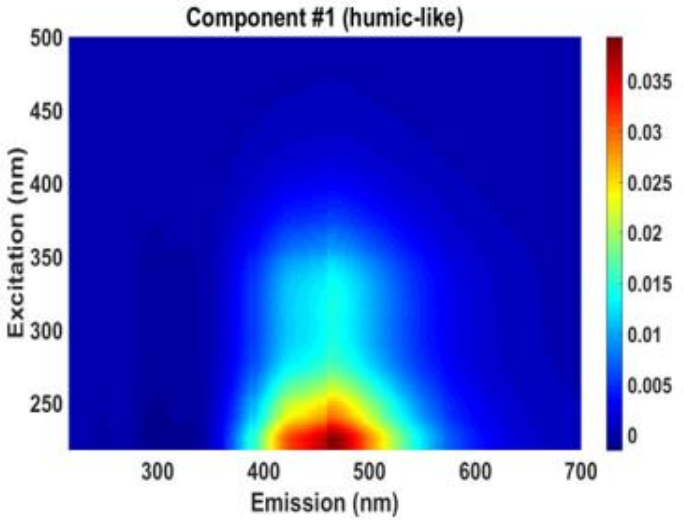
- Randomize data by sample name *(Martha's opinion: do not organize input data by treatment train—data are later unrandomized for sample scores)*
- Click on Eigenvector lambda symbol (λ) and refer to the Aqualog manual
- If scattering is bad, remove data below EX 250 nm
- Perform a non-negativity (i.e., constrained) model (or other models, if you are adventurous)
- Record the time stamp and identify the sample set for any models you create, *you will thank me later*
- Evaluate core consistency and split-half analyses for model validation

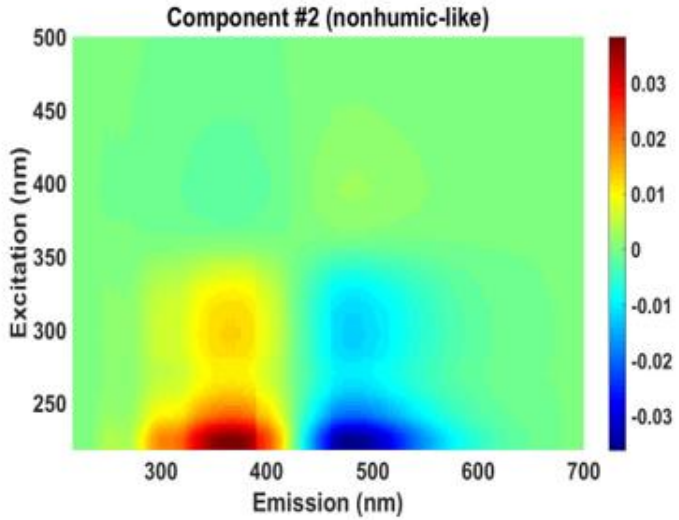


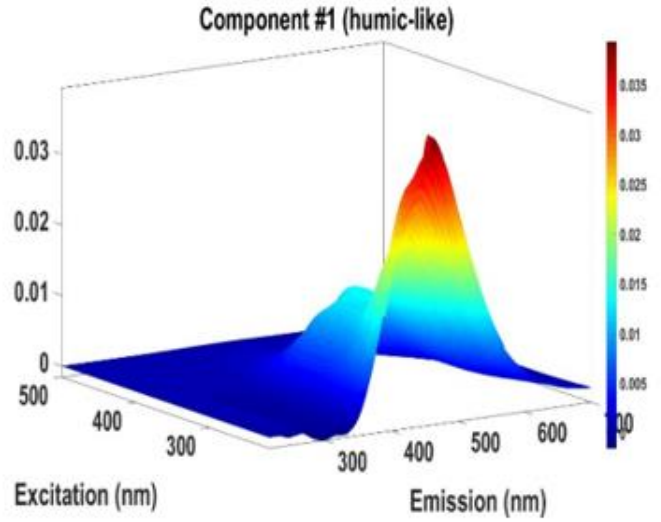
Click the OK button when all desired filters are set properly.

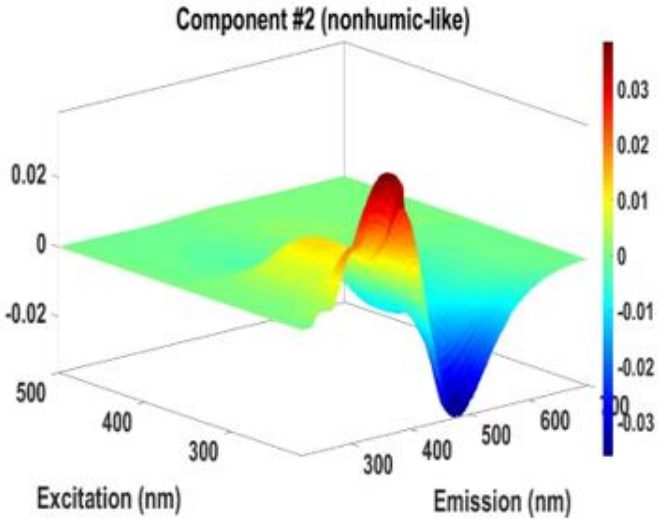
Modeled PARAFAC Components in 2D and 3D

- Mathematically separated by PARAllel FACtor (PARAFAC) analysis









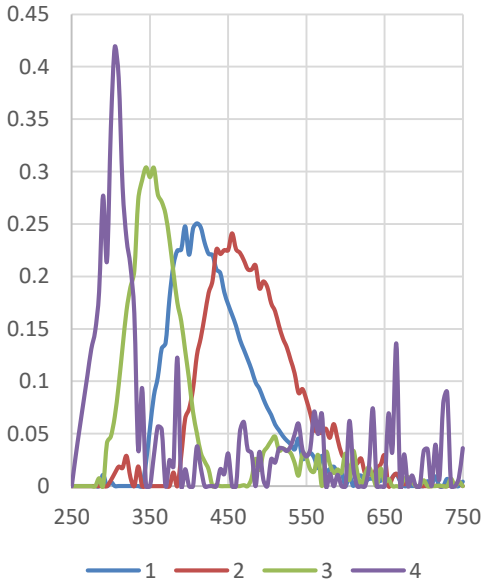
Wells, M.J.M., Mullins, G.A., Bell, K.Y. da Silva, A.K., Navarrete, E.M. ES&T. 2017, 51, 13592.

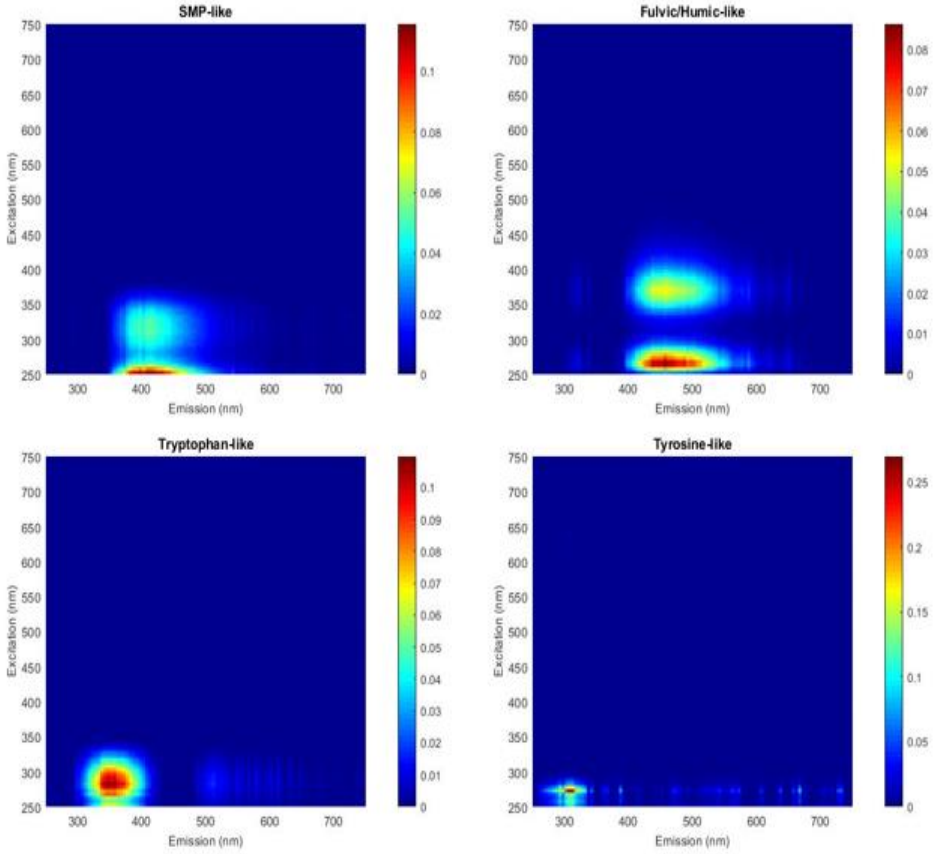
Data output from an EEM-PARAFAC model

- Components are numbered (ranked) according to percent variance explained by each component in the model; the larger the percentage explained, the more important the component. Be careful – rankings vary from matrix to matrix
- Emission loadings are best way to quickly assign the components, Martha's assignments:
  - EM ~ 300 nm = tyrosine-like
  - EM ~ 350 nm = tryptophan-like
  - EM ~ 400 = soluble microbial product-like
  - EM ~ 425 = fulvic-like
  - EM ~ 475 = humic-like

- *X-axis is emission*
- *Y-axis is excitation*
- *Relative intensities refer to emission and NOT to rank of the component*

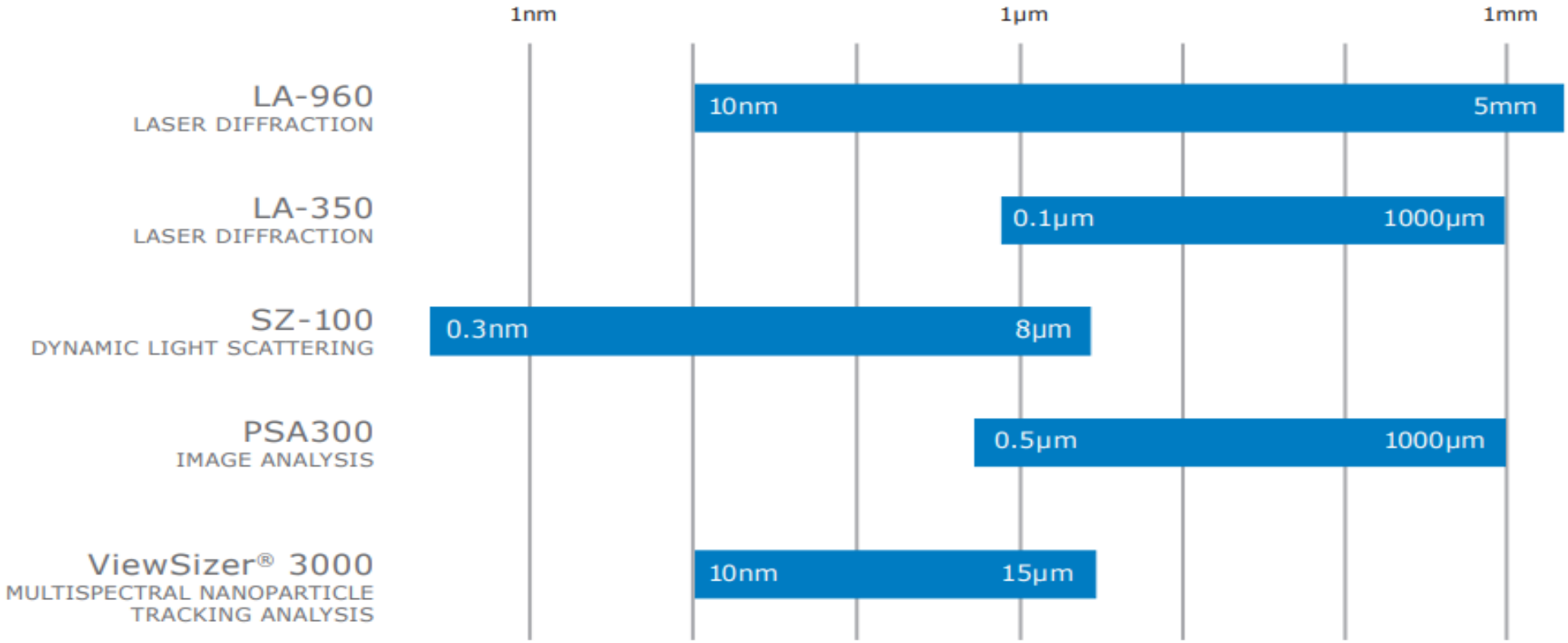
	Groundwater Four-component model (% Model Fit)			
Source ID (variance of data explained)	Tyrosine-like	Tryptophan-like	Soluble Microbial Product-like	Fulvic/Humic-like
94%	2	7	80	11



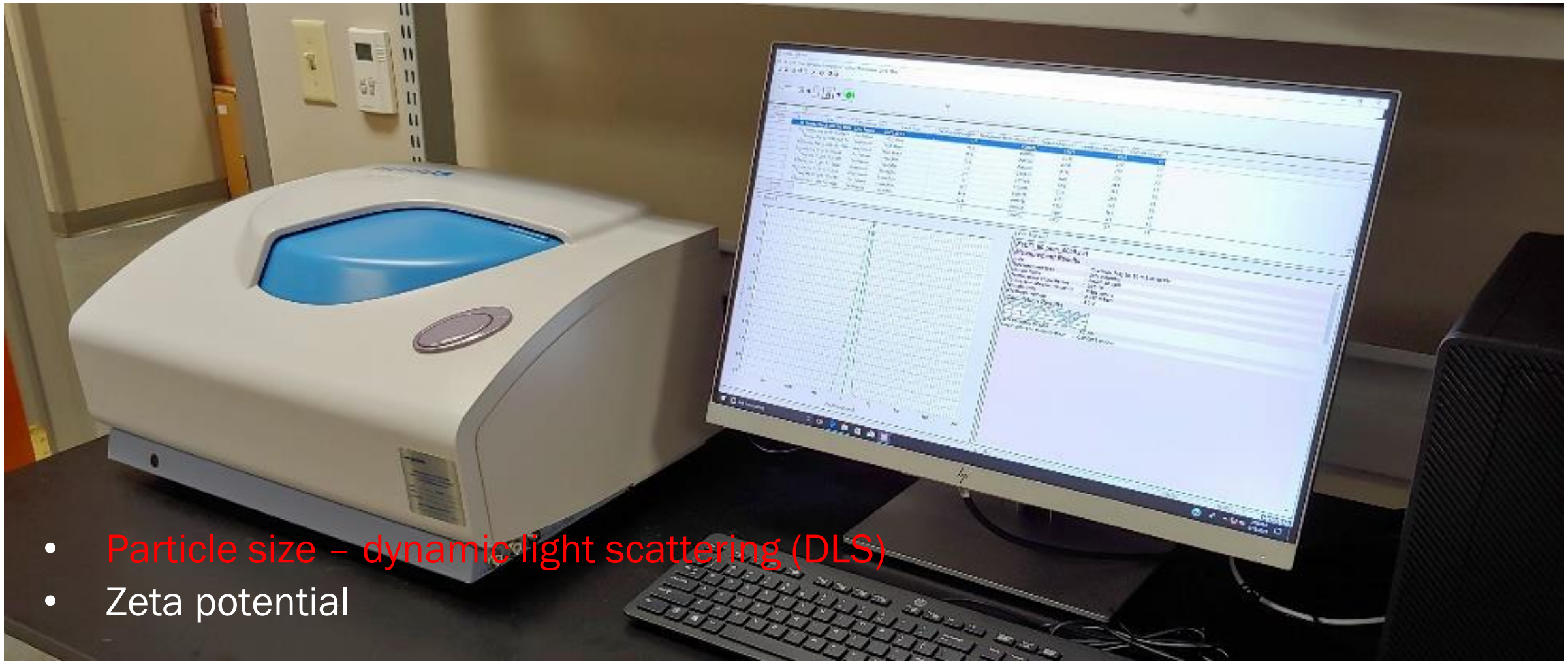


This treated groundwater DOC is below detection limit (0.04 mg/L)

Dynamic range of the Horiba Systems



Horiba SZ-100 with automatic titrator



- Particle size – dynamic light scattering (DLS)
- Zeta potential

Dynamic light scattering (DLS) using the SZ-100

Measurement of particle size distribution is based DLS, which measures Brownian motion.

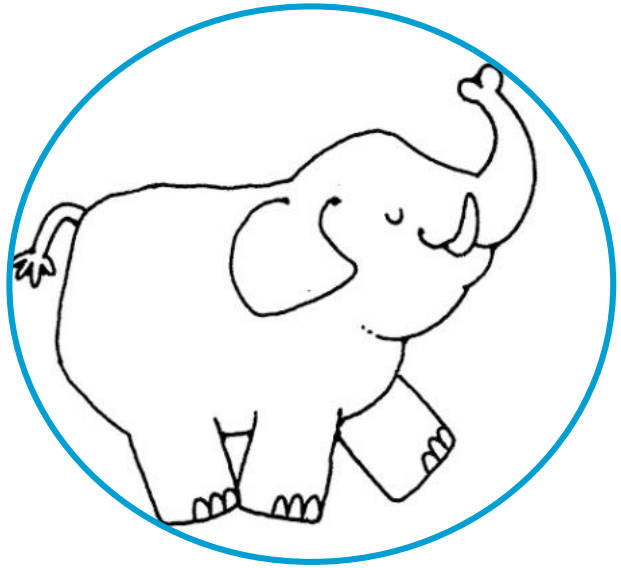
- Bigger particles move slowly
- Smaller particles move quickly

The Stokes-Einstein equation is used to convert DLS data to particle size (hydrodynamic volume), assuming every particle is a sphere (Horiba Instruments, Inc., 2017) and particle size distributions (PSD) are calculated on:

- intensity
- volume
- number

The key reading is intensity distribution (Z-average) and the polydispersity index (PDI) describes distribution width.

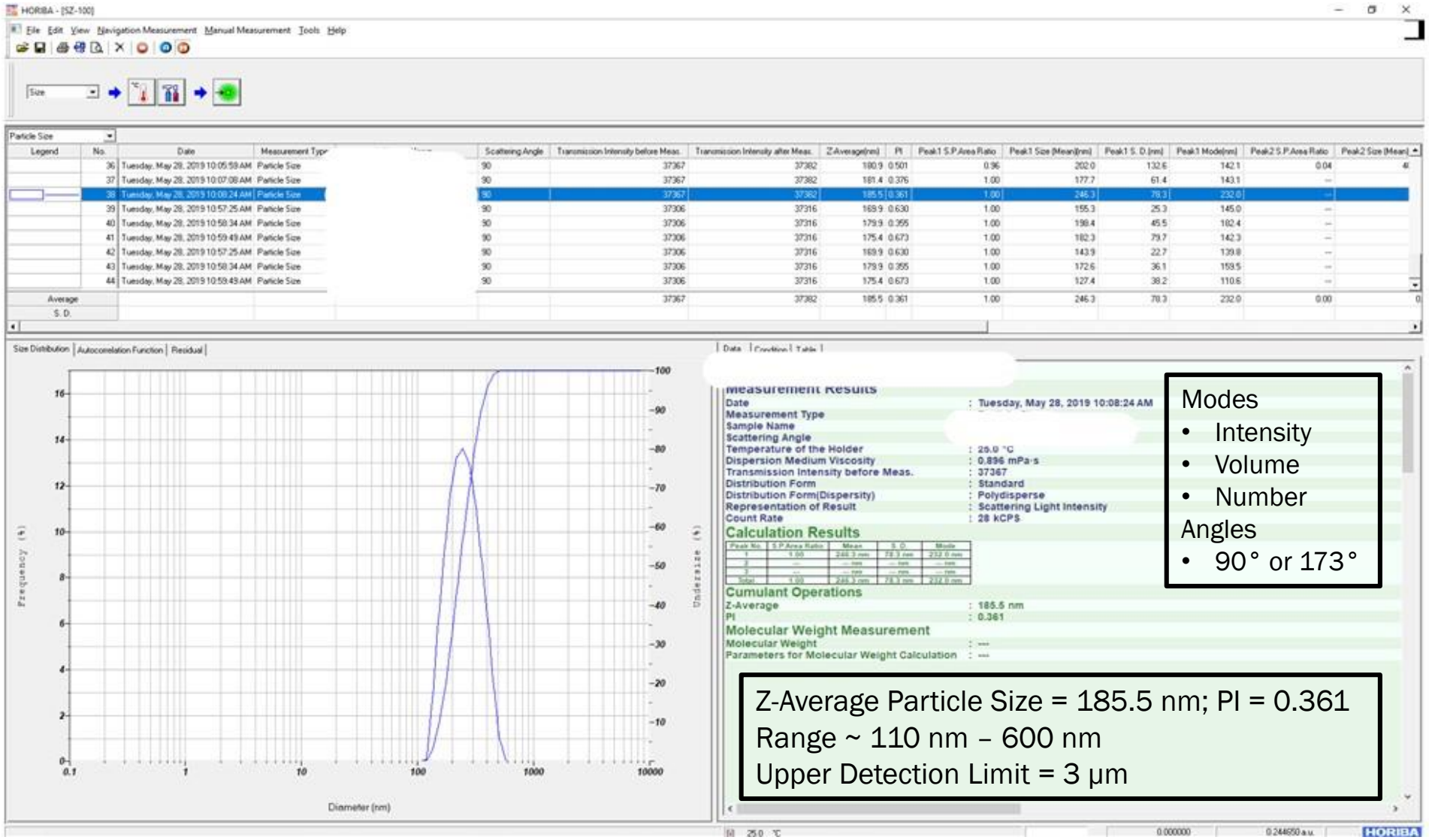
DLS can be used to measure PSD for condensed phases (metal nanoparticles, polystyrene latex standards) and soft which is easily deformed (e.g., by thermal or mechanical stress), NOM is such a material.

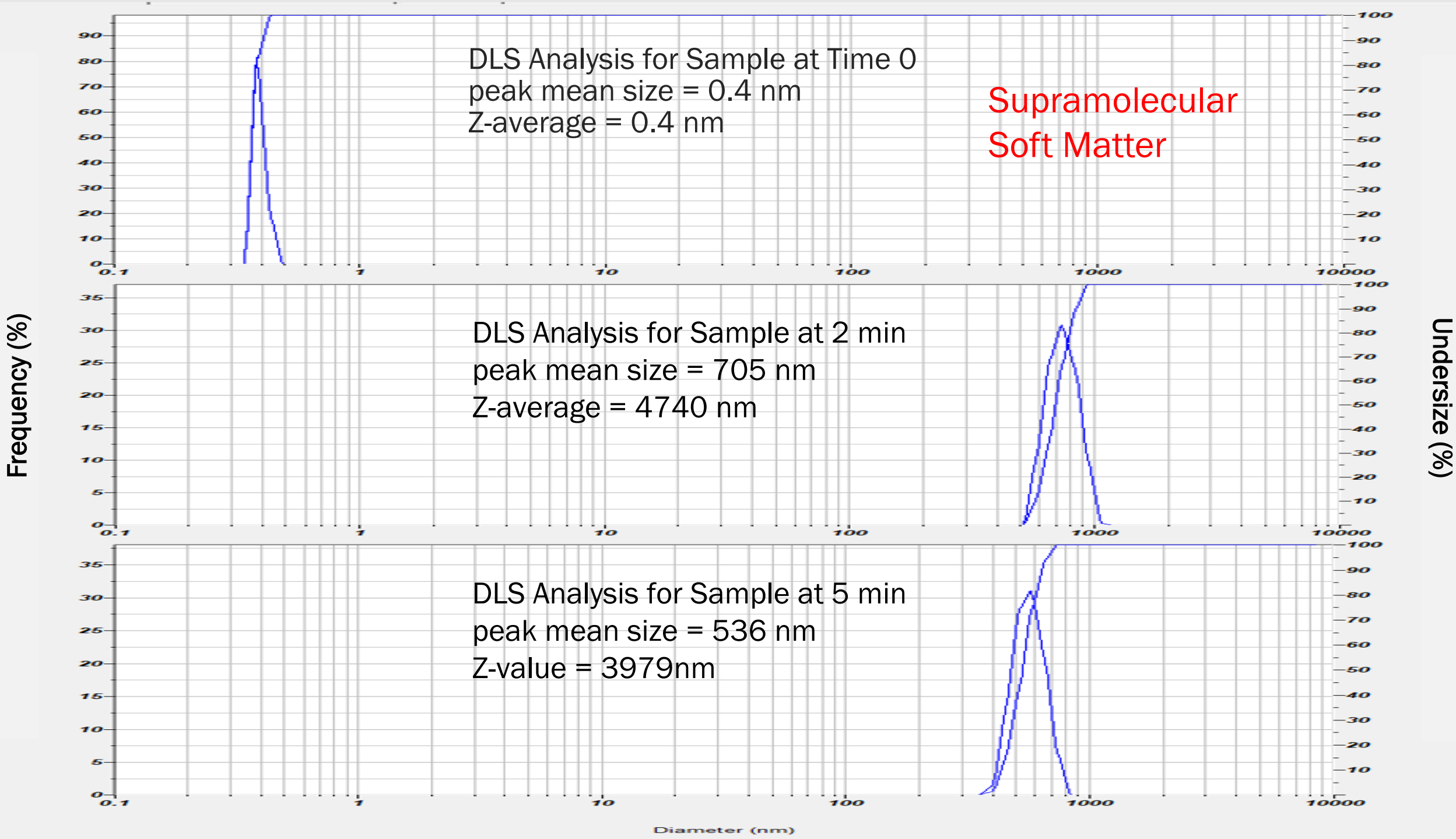


However, for aquatic NOM, which continuously aggregates and disaggregates by dynamic equilibrium, Brownian motion also occurs simultaneously, complicating interpretation of results (*Wells, Joint Webinar, 2021*).

Horiba Instruments, Inc., A Guidebook to Particle Size Analysis, 2017, Irvine, CA.

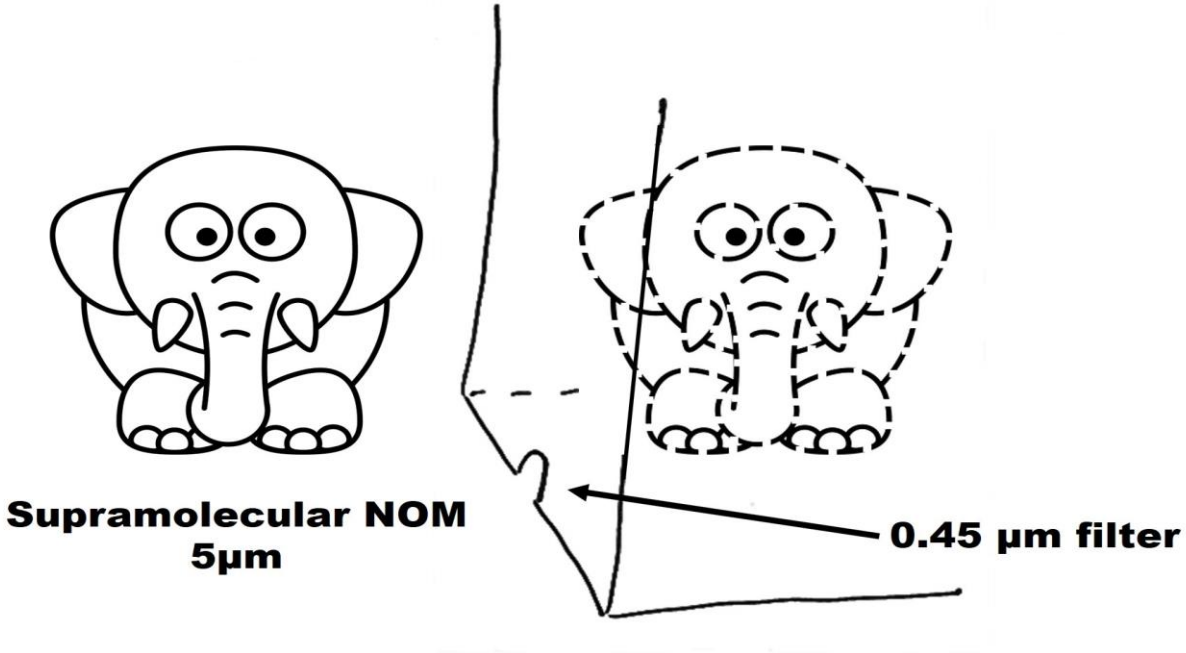
Particle size distribution




DLS Analysis for Sample at Time 0
peak mean size = 0.4 nm
Z-average = 0.4 nm
Supramolecular
Soft Matter
DLS Analysis for Sample at 2 min
peak mean size = 705 nm
Z-average = 4740 nm
DLS Analysis for Sample at 5 min
peak mean size = 536 nm
Z-value = 3979nm
Frequency (%)
Undersize (%)
Diameter (nm)

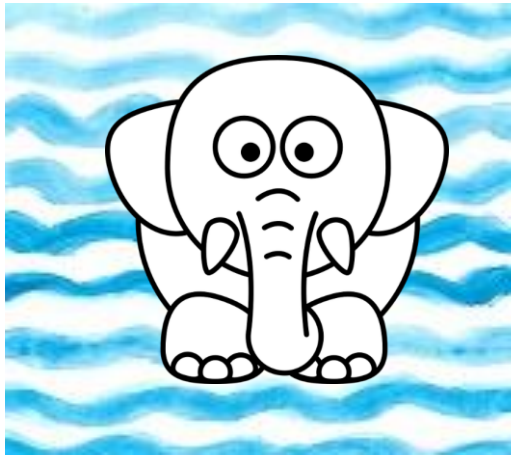
“Dynamic” DLS – beyond Brownian motion

How does a supramolecular elephant fit through a mouse hole?



$$\text{Disaggregation} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Aggregation}$$

How can a supramolecular elephant float?



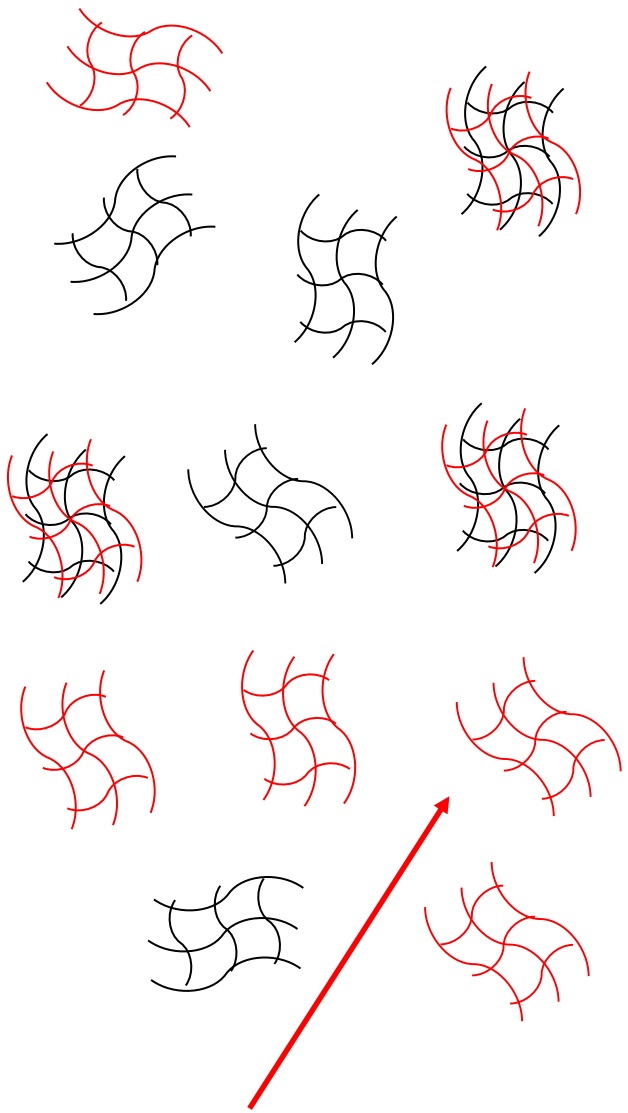
Particles are:

- large in volume,
- few in number
- 10 times > the nominal filter size (0.45 µm)

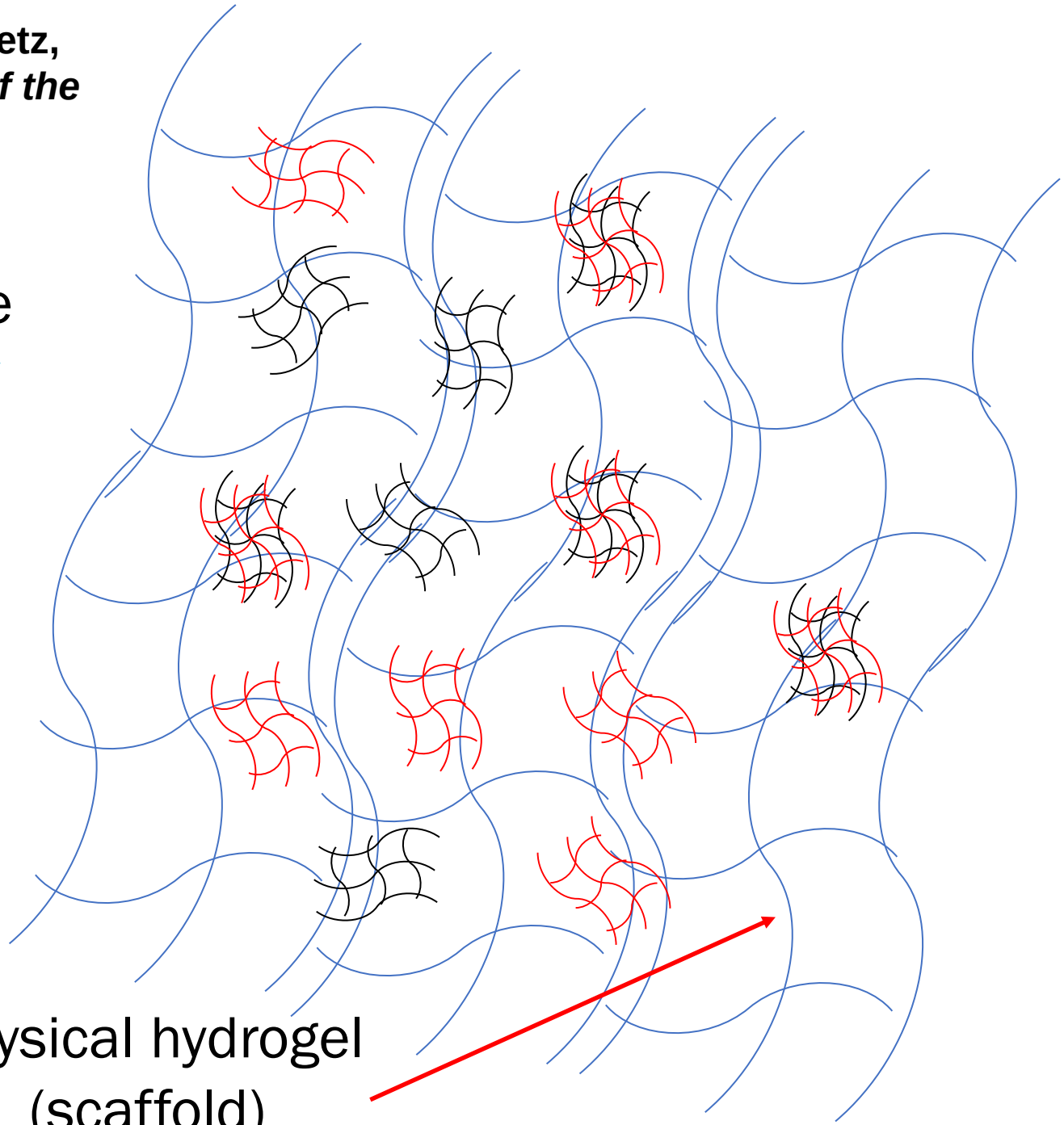
Wells, M.J.M. and Stretz, H.A., 2019. *Science of the Total Environment, 671:1125*–1133.

quiescence

turbulence



metachemical hydrogel



physical hydrogel
(scaffold)

Supramolecular associations of HSs are held together…

"…by intermolecular forces (van der Waals, hydrogen bonding, ionic effects) other than covalent bonding."

Esfahani, M.R., Stretz, H.A. and Wells, M.J.M. 2015. Science of the Total Environment, 537:81–92

"…as physical hydrogels by non-covalent weak intermolecular forces (van der Waals, weak hydrogen bonding, ionic effects) and as metachemical hydrogels by near-covalent strong hydrogen bonding in a hierarchical 'supramolecular within supramolecular' self-assembling architecture."

Wells, M.J.M. and Stretz, H.A., 2019. *Science of the Total Environment, 671:1125*–1133.

Horiba LA-960 particle size analyzer uses laser diffraction

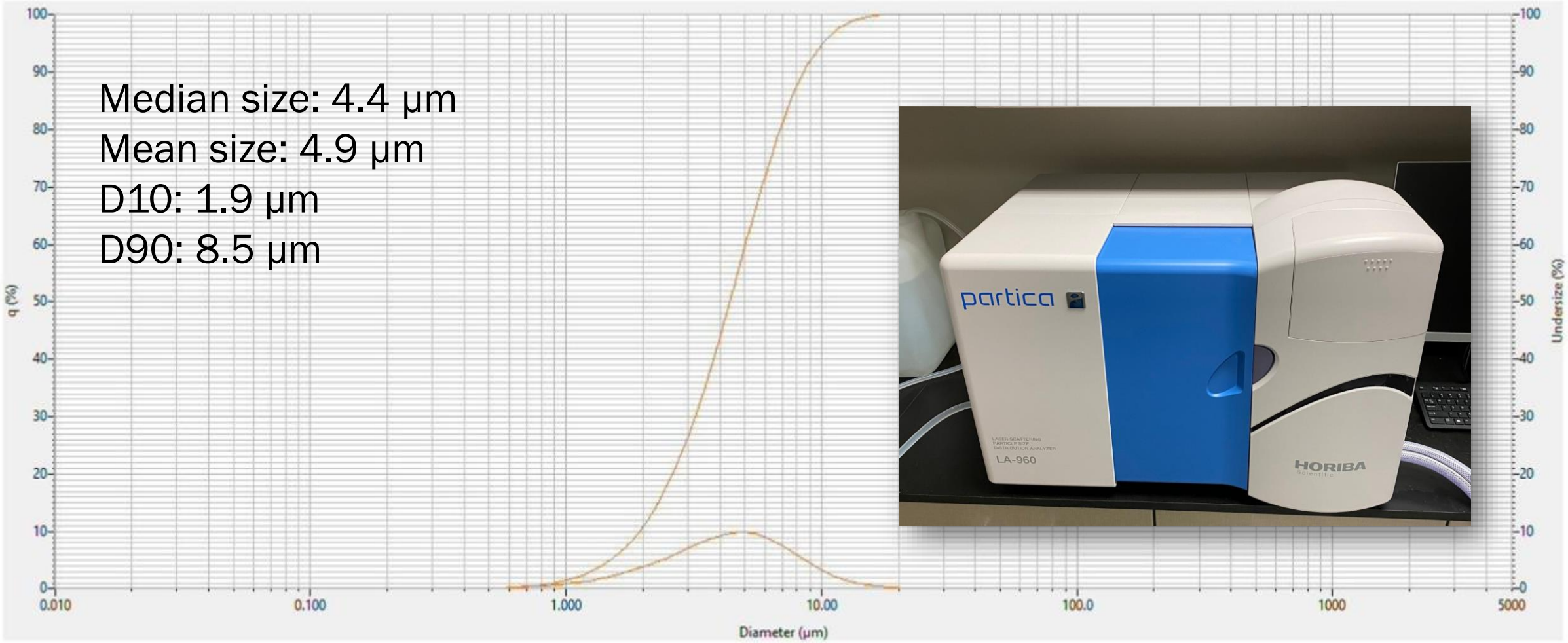
Laser diffraction uses first principles to calculate size via light scattered off the particle (edge diffraction) and through the particle (secondary scattering refraction).

The LA-960 incorporates full Mie scattering theory to cover the widest size range available.

Horiba Instruments, Inc., A Guidebook to Particle Size Analysis, 2017, Irvine, CA.

Horiba LA-960 particle size distribution analyzer

Median size: 4.4 µm
Mean size: 4.9 µm
D10: 1.9 µm
D90: 8.5 µm



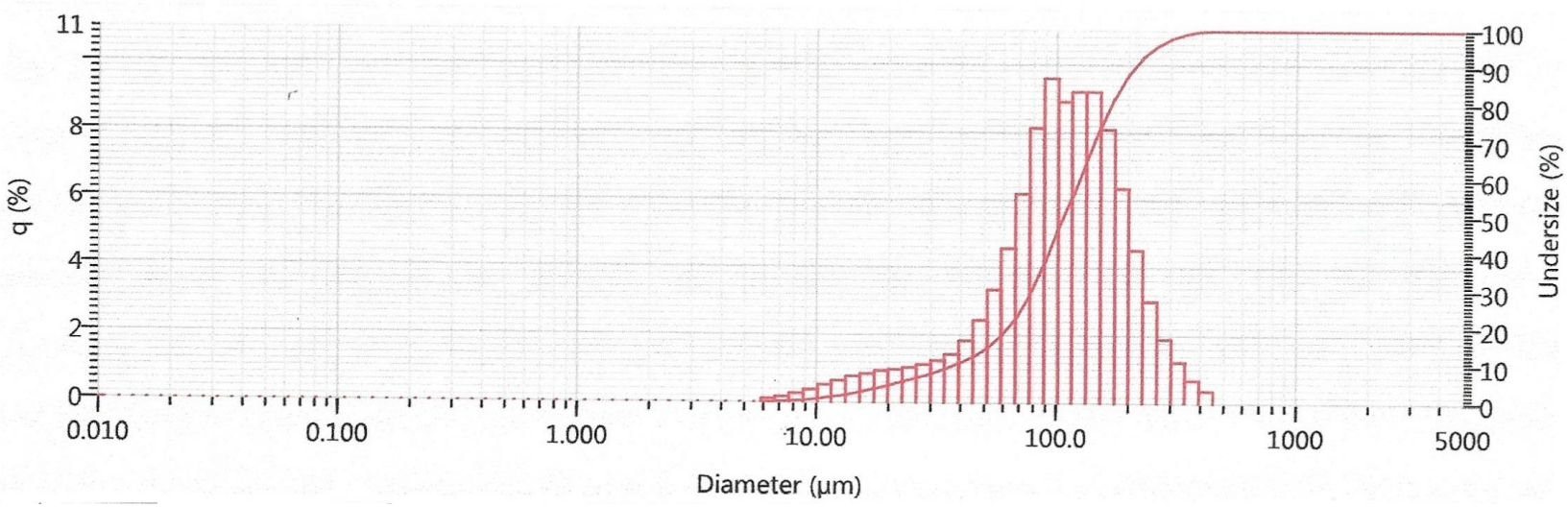
Particle Size Distribution in wastewater treatment

HORIBA

Laser Scattering Particle Size Distribution Analyzer LA-960

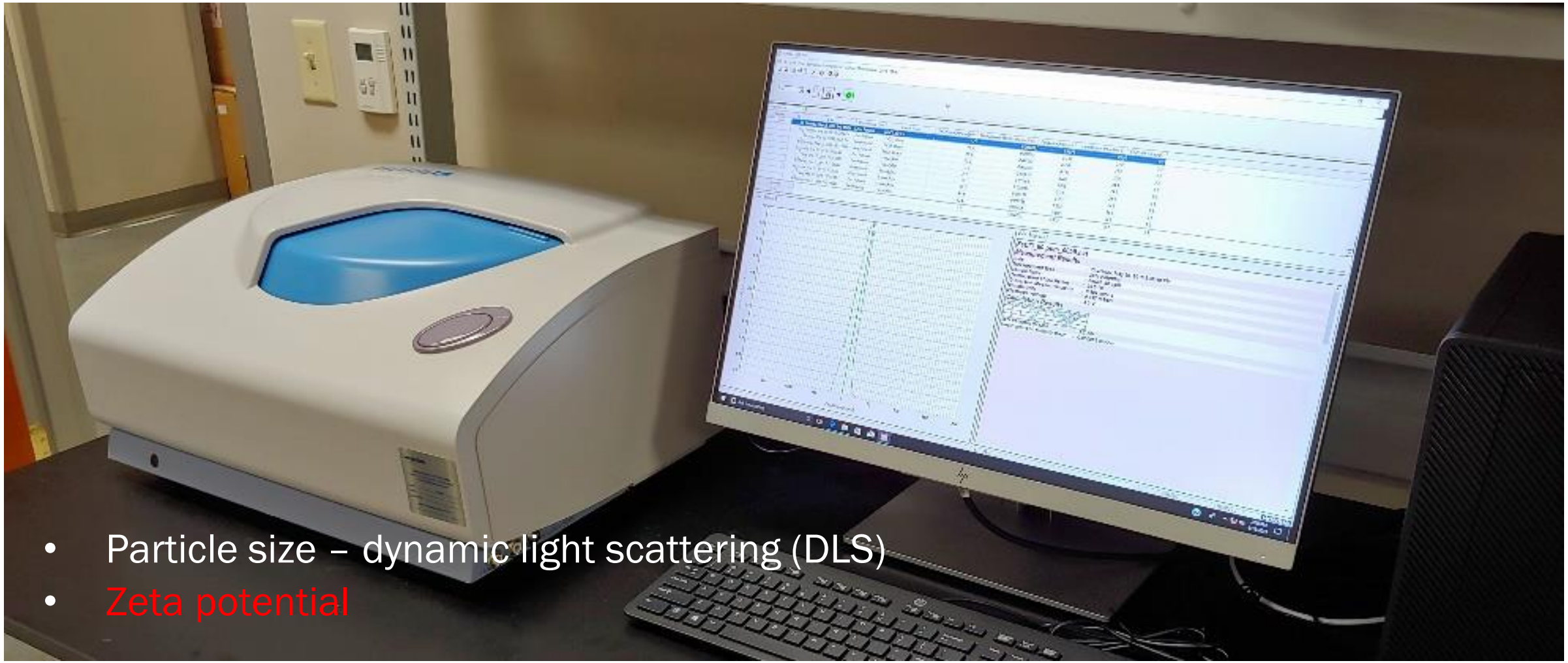
Brown AND Caldwell

Data name	:	Median size	: 106.96520 (µm)
Sample name	:	Mean size	: 119.01536 (µm)
Transmittance (R)	: 85.4 (%)	Mode size	: 95.3232 (µm)
Transmittance (B)	: 89.5 (%)	Span	: 1.6181
Circulation speed	: 3	Diameter on cumulative %	: (1)10.00 (%)- 37.4722 (µm)
Agitation speed	: 2		: (2)20.00 (%)- 62.4475 (µm)
Ultrasound	: Off		: (3)30.00 (%)- 78.8862 (µm)
Convergence factor	: 15		: (4)40.00 (%)- 92.4961 (µm)
Distribution base	: Volume		: (5)50.00 (%)- 106.9652 (µm)
Refractive index (R)	: Wastewater		: (6)60.00 (%)- 124.2982 (µm)
	[Wastewater(1.450 - 0.000i),water(1.333)]		: (7)70.00 (%)- 144.0614 (µm)
			: (8)80.00 (%)- 169.0553 (µm)
			: (9)90.00 (%)- 210.5480 (µm)



The use of the laser diffraction system for PSD is a useful tool to examine wastewater effluent samples to determine whether UV disinfection is a good fit for a particular system.

Horiba SZ-100 with automatic titrator

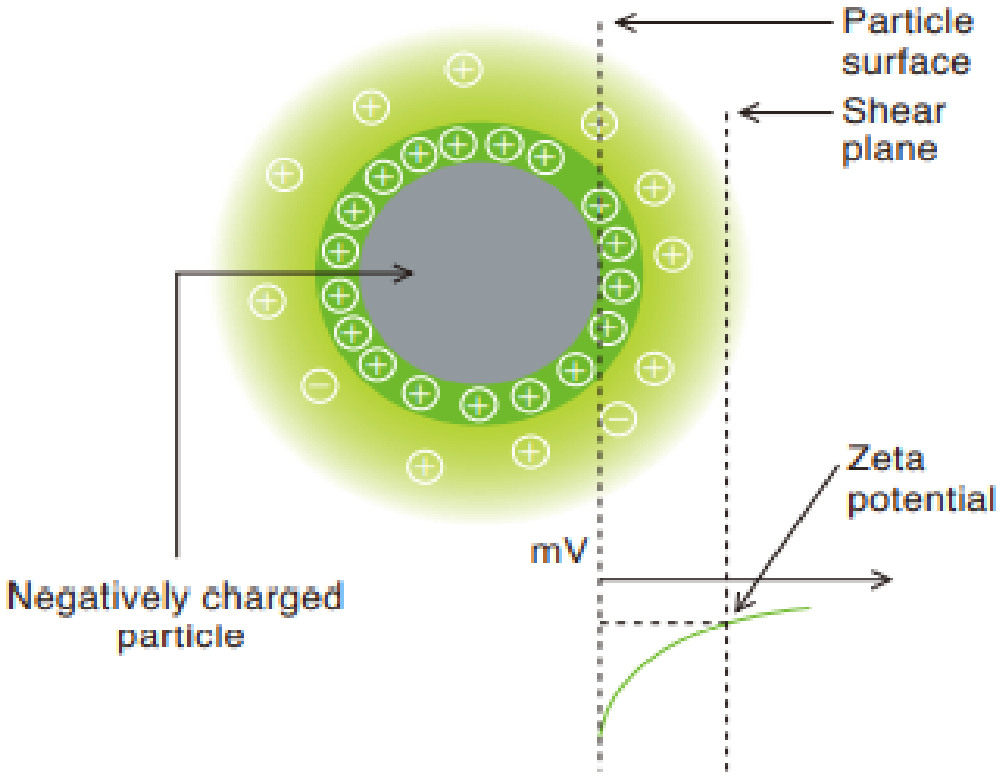


- Particle size – dynamic light scattering (DLS)
- Zeta potential

Horiba SZ-100 (Bulletin: HRE-3677C)

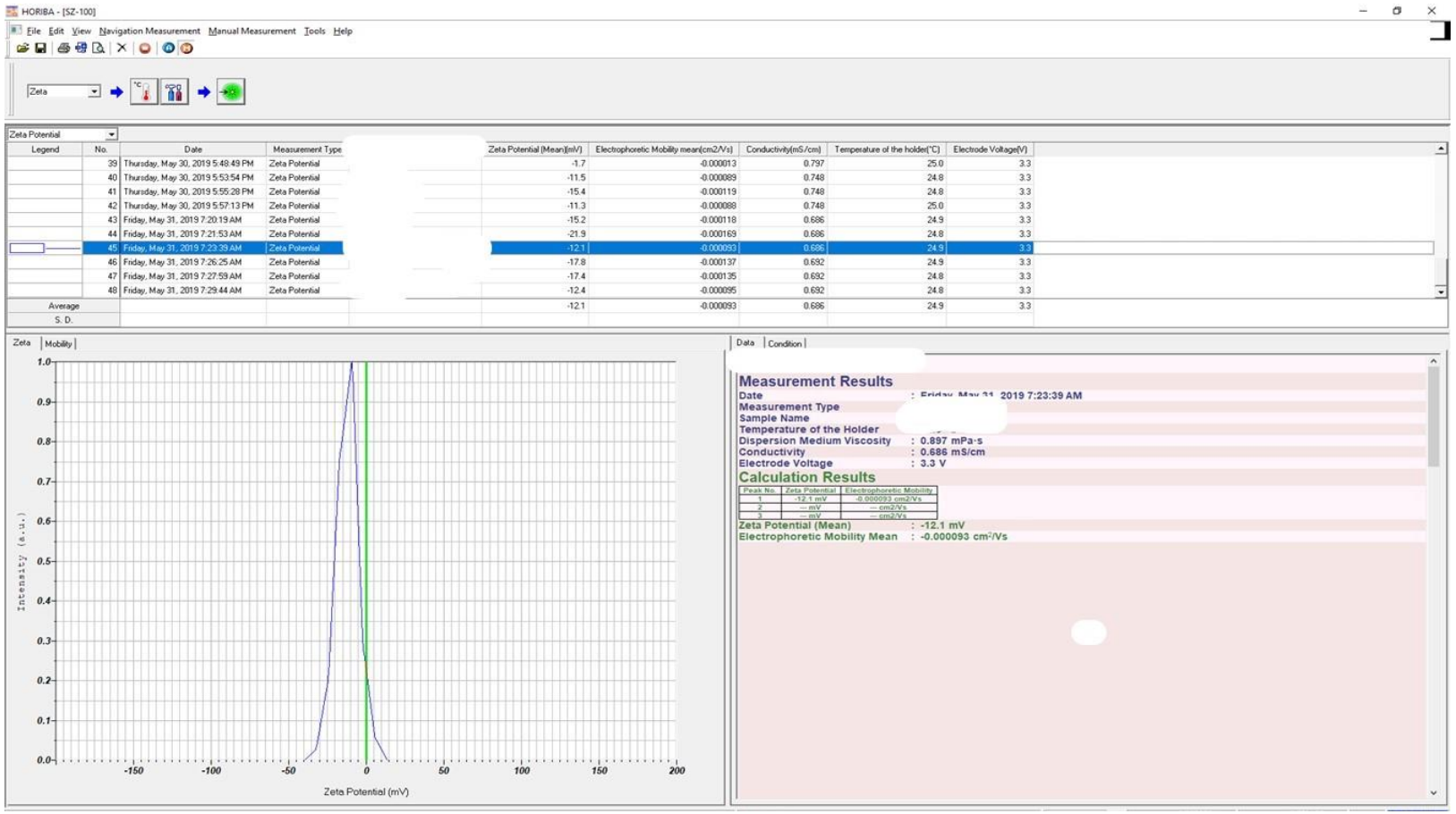
Zeta Potential Measurement Principle
(Laser Doppler Electrophoresis)

Many nanoparticles or colloidal particles have a surface charge when they are in suspension. When an electric field is applied, the particles move due to the interaction between the charged particle and the applied field. The direction and velocity of the motion is a function of particle charge, the suspending medium, and the electric field strength. Particle velocity is then measured by observing the Doppler shift in the scattered light. The particle velocity is proportional to the electrical potential of the particle at the shear plane which is the zeta potential. Thus, this optical measurement of particle motion under an applied field can be used to determine zeta potential.



Zeta potential in water/wastewater treatment

Zeta potential is an indicator of dispersion stability



Identifying conditions at which the zeta potential is zero (the isoelectric point) indicates optimal conditions for coagulation/flocculation for separating particles.

Zeta potential can be used to optimize coagulant and polymer doses to minimize cost.

Acknowledgements

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