Chemometrics for Improved Limits of Detection and Dynamic Range in Flow Injection Analysis with Spectrometric Detection

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SEATTLE, WA

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Special Thanks

National Environmental Monitoring Conference

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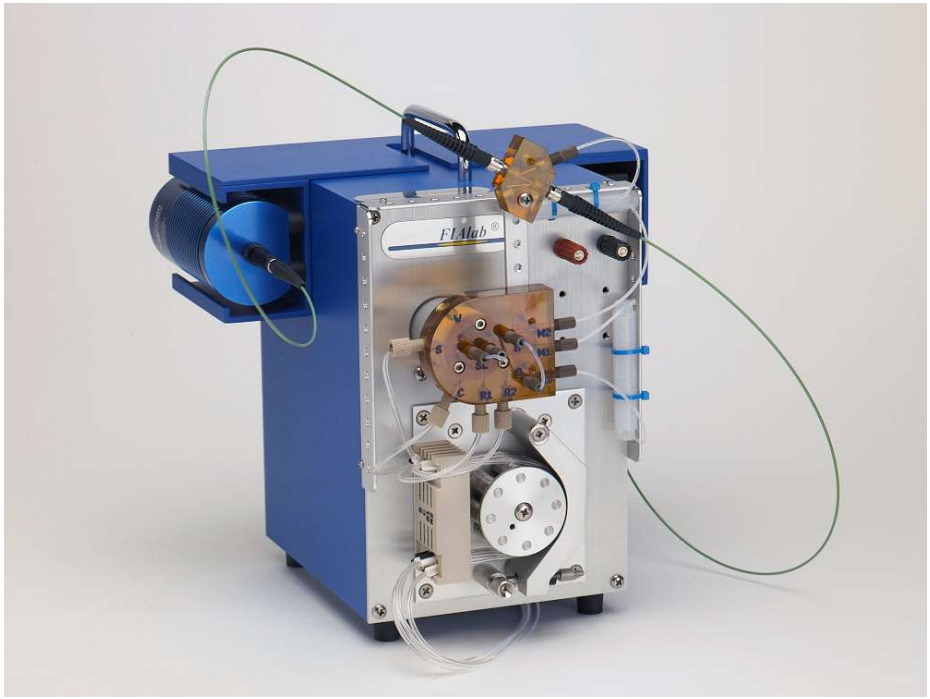
Introduction – Purpose

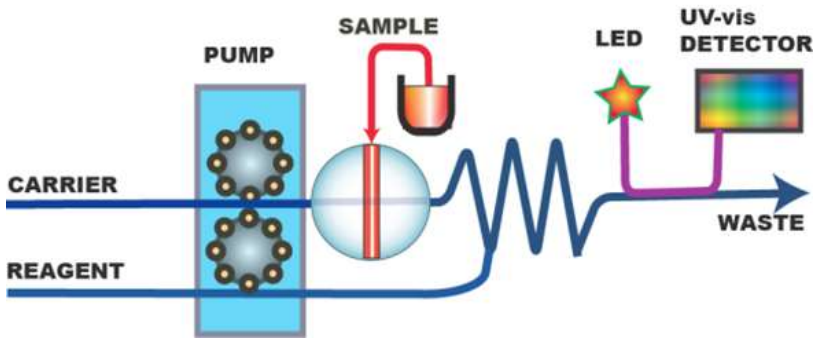
- Demonstrate how multivariate chemometric techniques can improve detection limits in common environmental assays
  - FIAlyzer 1000 flow injection analyzer
  - UV-VIS spectrophotometer (CCD array or PDA based)
- Technique leverages the wide spectral range of spectrophotometers and real time data processing algorithms
- 3-5 fold noise reduction in common assays

Introduction – Flow Injection Analysis

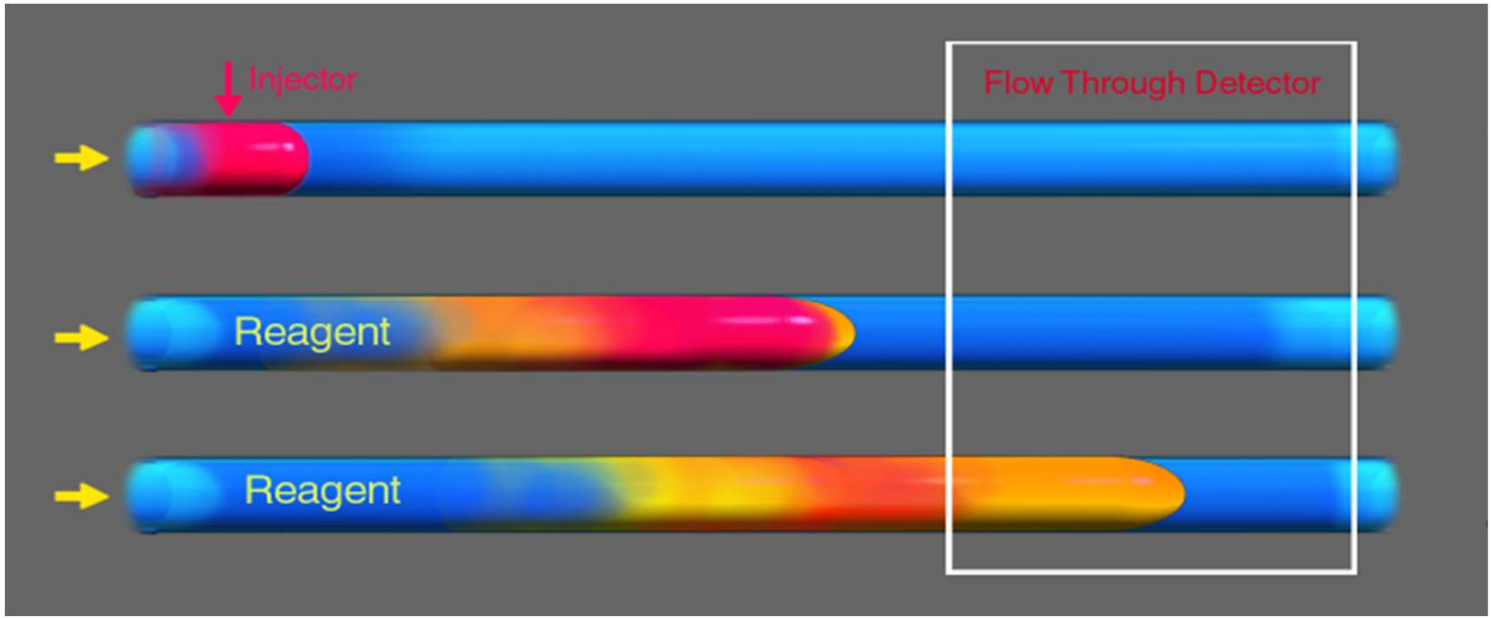
- Flow Injection Analysis (FIA):
  - Automated technique for sample injection, reagent mixing, and signal detection by moving streams
    - Sample injected into a continuously moving stream
    - Reagent merged with sample, generating colored product
    - Color intensity measured in detector

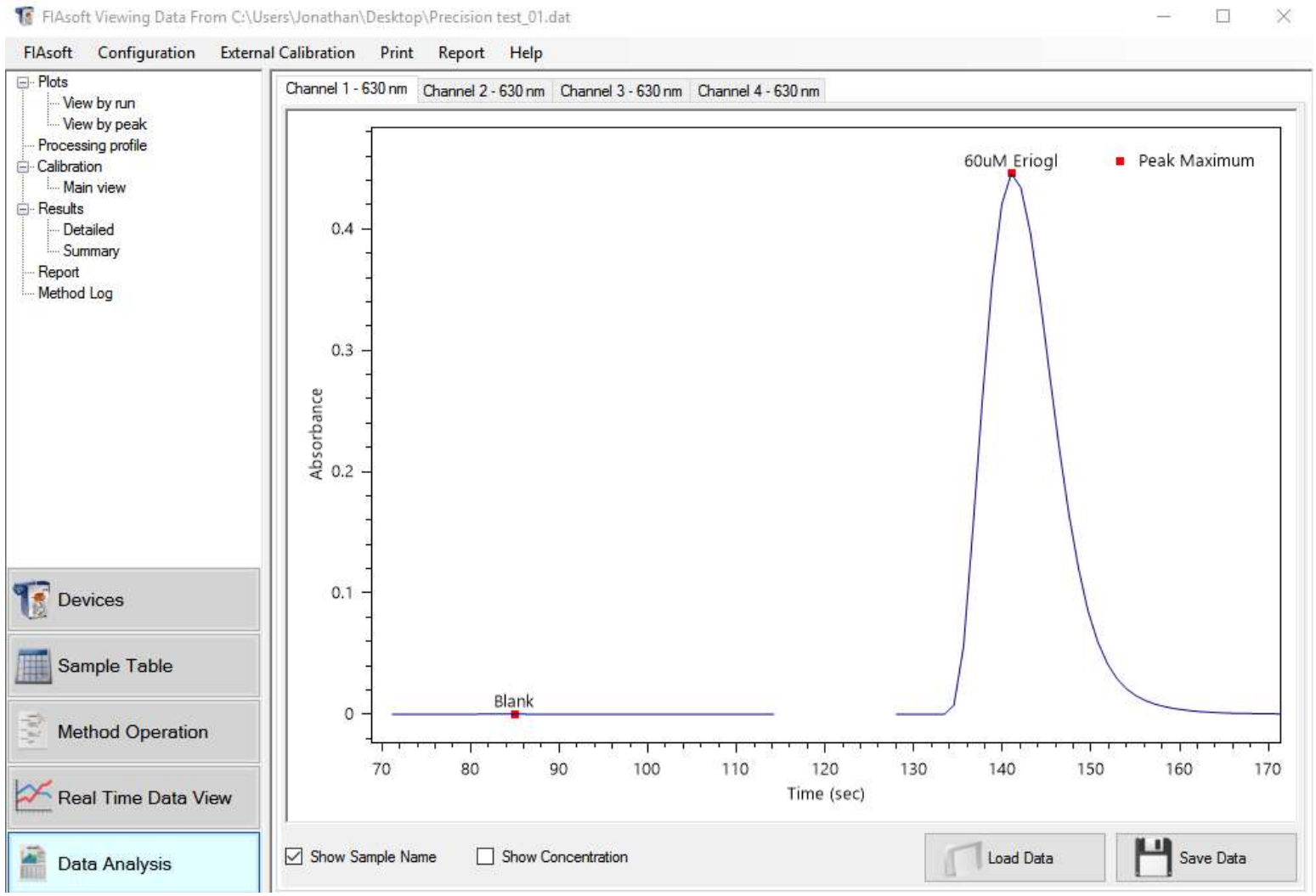
Introduction – Flow Injection Analysis





Introduction – Flow Injection Analysis

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Configuration
External Calibration
Print
Report
Help
Plots
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View by peak
Processing profile
Calibration
Main view
Results
Detailed
Summary
Report
Method Log
Devices
Sample Table
Method Operation
Real Time Data View
Data Analysis
Channel 1 - 630 nm
Channel 2 - 630 nm
Channel 3 - 630 nm
Channel 4 - 630 nm
60uM Eriogl
Peak Maximum
Blank
Absorbance
0
0.1
0.2
0.3
0.4
70
80
90
100
110
120
130
140
150
160
170
Time (sec)
Show Sample Name
Show Concentration
Load Data
Save Data

Introduction – Chemometrics

- Application of data analysis techniques from statistics, mathematics, and computer science to analytical chemistry
- Examples: Multivariate analysis, classification & clustering, curve resolution

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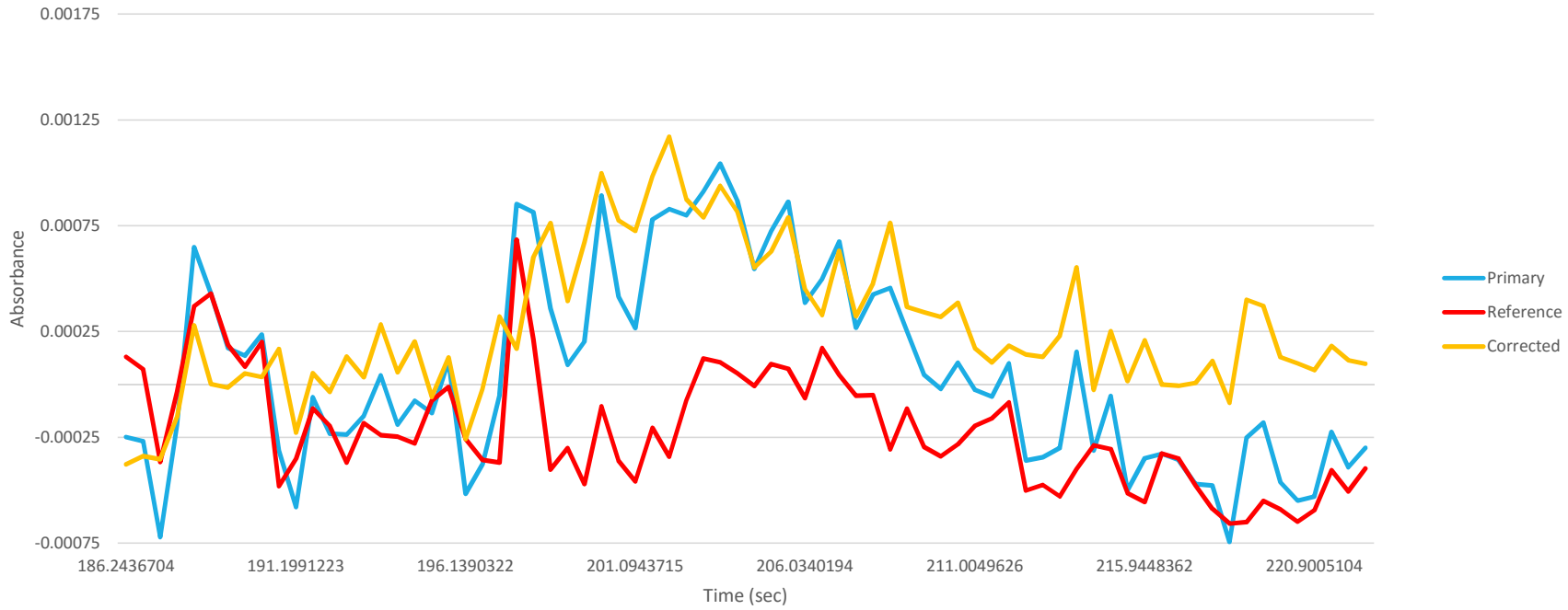
Current Methodology

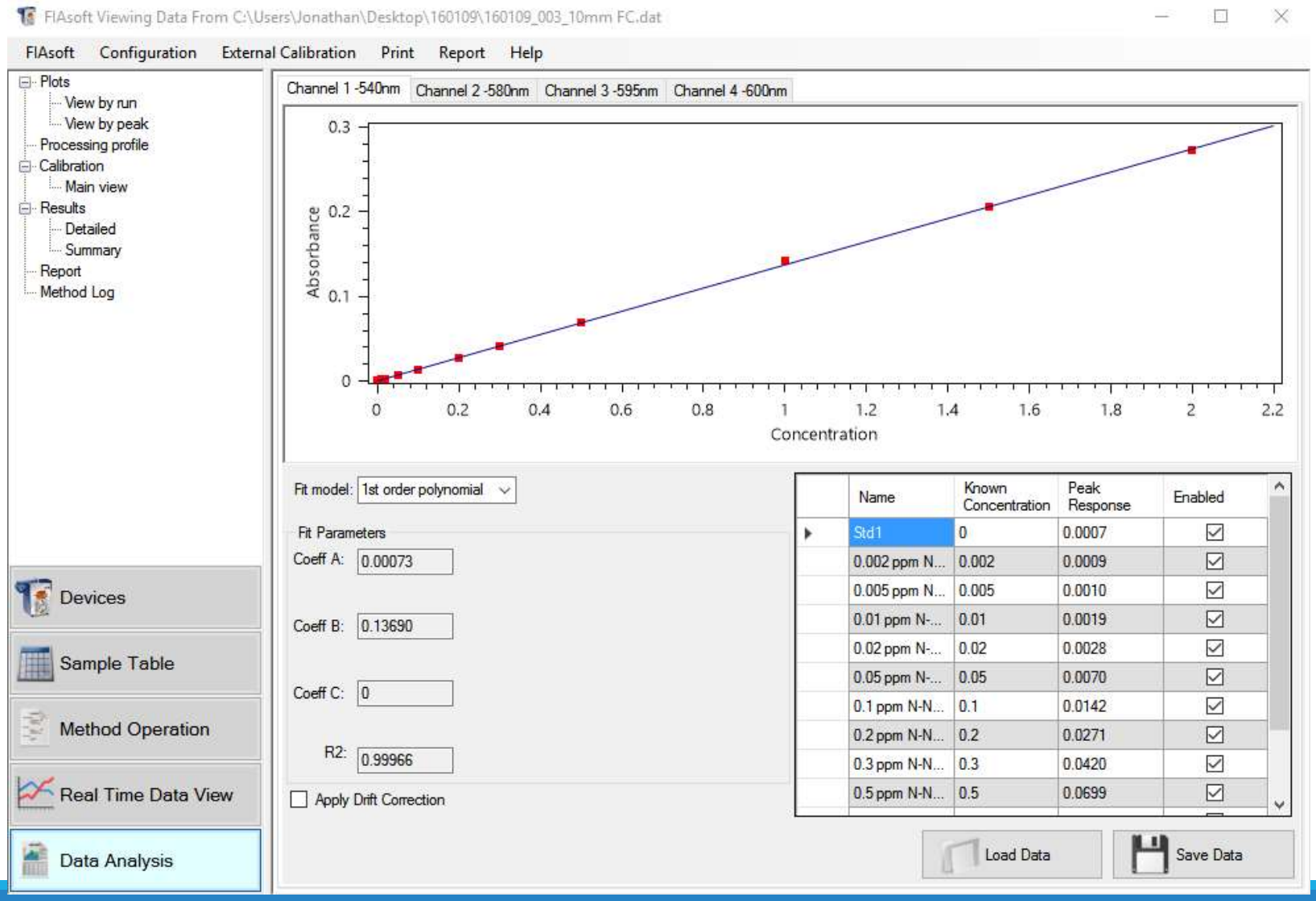
- As each sample passes through a flowcell, a spectrophotometer measures absorbance:
  - $A = \log_{10} \frac{I_0}{I}$
  - Measured at a primary wavelength – typically peak wavelength of analyte's absorbance spectrum
  - Corrected using a reference wavelength – a wavelength where no absorbance is expected. Fluctuations due to measurement noise.

Reference Correction Example



Current Methodology - Continued

- Absorbance and concentration are related by Beer's Law:
  - $A = \log_{10} \frac{I_0}{I} = \varepsilon l c$
    - $\varepsilon = \text{absorptivity}, l = \text{optical pathlength}, c = \text{concentration}$
- Construct calibration curve from absorbance and concentration of known standards
- Use calibration curve and absorbance of unknown samples to interpolate concentration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Report
Help
Plots
View by run
View by peak
Processing profile
Calibration
Main view
Results
Detailed
Summary
Report
Method Log
Devices
Sample Table
Method Operation
Real Time Data View
Data Analysis
Channel 1 -540nm
Channel 2 -580nm
Channel 3 -595nm
Channel 4 -600nm
Absorbance
Concentration
0.3
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
Fit model: 1st order polynomial
Fit Parameters
Coeff A: 0.00073
Coeff B: 0.13690
Coeff C: 0
R2: 0.99966
Apply Drift Correction
Name
Known Concentration
Peak Response
Enabled
Std1 0 0.0007
0.002 ppm N... 0.002 0.0009
0.005 ppm N... 0.005 0.0010
0.01 ppm N-... 0.01 0.0019
0.02 ppm N-... 0.02 0.0028
0.05 ppm N-... 0.05 0.0070
0.1 ppm N-N... 0.1 0.0142
0.2 ppm N-N... 0.2 0.0271
0.3 ppm N-N... 0.3 0.0420
0.5 ppm N-N... 0.5 0.0699
Load Data
Save Data

Current Methodology - Improvement

- Current methodology is widely used and simple
- Our application uses a spectrophotometer for a detector
  - Sensitive across a wide spectral range (typical 200nm – 800nm UV-VIS range)
  - Spectral peaks are usually broad (Nitrate FWHM ~80nm)
  - However, we were only monitoring a single wavelength
- Can we improve detection limits by using multivariate data we are already collecting? Yes!

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New Technique

- Goal: Use full absorbance spectrum data rather than single wavelength to reduce noise and increase detection limit
  - Most analytes have a well-defined absorbance spectrum
  - If we know what the absorbance spectrum looks like, we can use this as a model to fit experimental data against
    - Derive model spectrum from a matrix-matched standard
    - Fit all other experimental data against this spectrum
  - From the fitted spectrum, interpolate the absorbance at the wavelength of interest and continue as in the previous methodology

New Technique - Steps

1. Derive model spectrum from known, matrix-matched standard
2. For each spectrum, fit experimental spectrum using model spectrum
3. Interpolate the absorbance at the primary wavelength from fitted spectrum
4. We can now use the interpolated data, rather than raw data, to construct calibration curve and derive maximum absorbance for each sample
5. Continue as in the previous methodology

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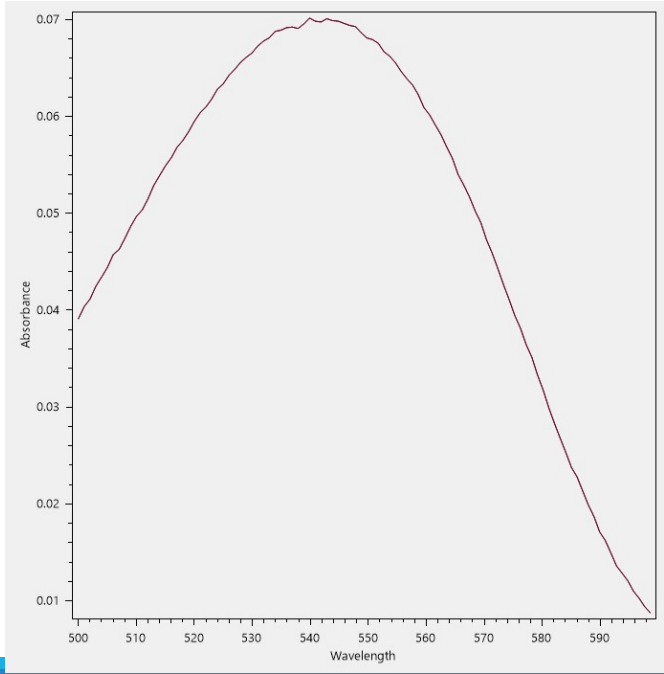
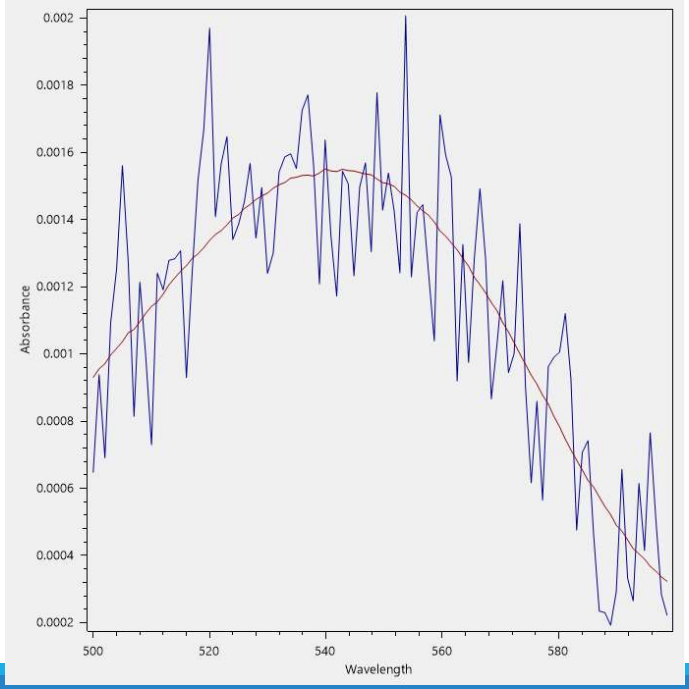
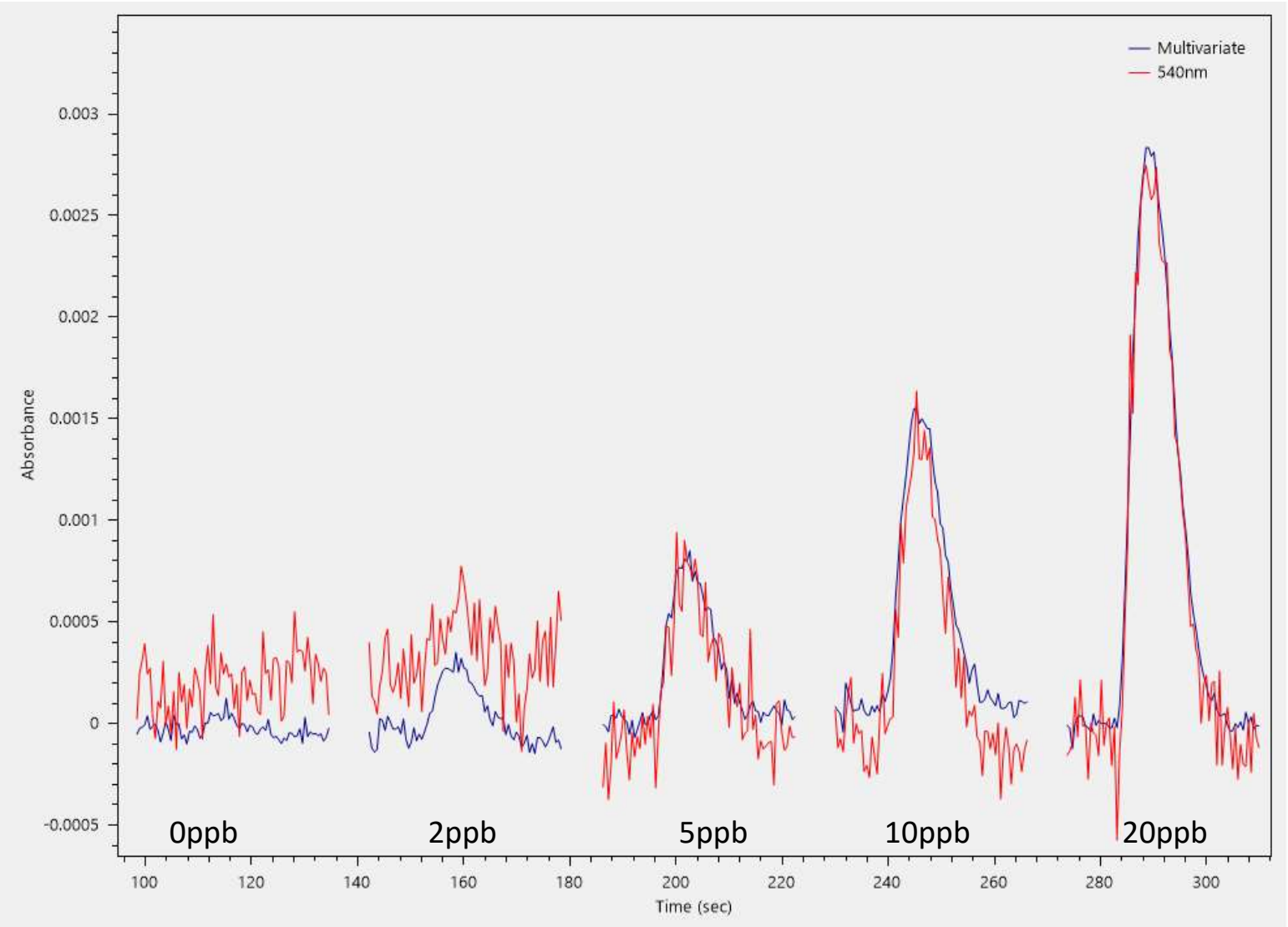
Results – Model and Fitted Data, Nitrate

MODEL SPECTRUM



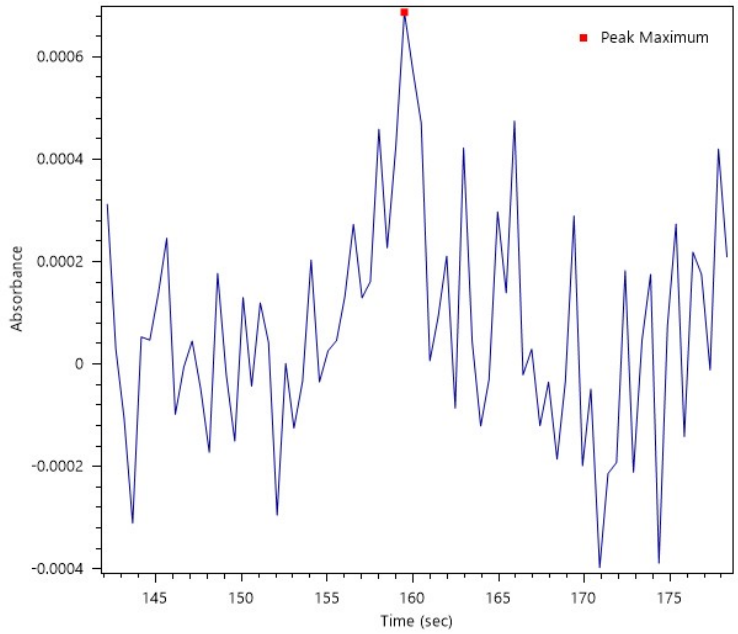
LOW CONCENTRATION FITTED DATA



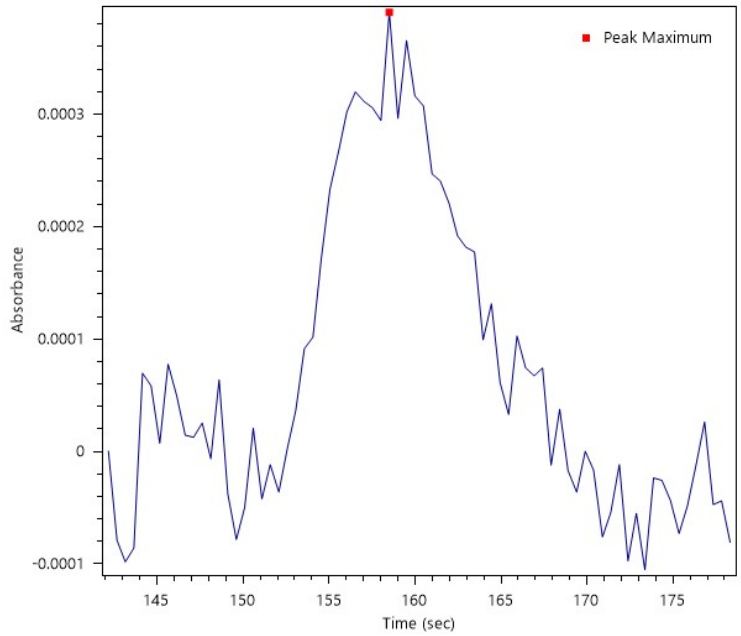

Multivariate
540nm
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
-0.0005
Absorbance
0ppb
2ppb
5ppb
10ppb
20ppb
100
120
140
160
180
200
220
240
260
280
300
Time (sec)

Results – Single vs Multivariate Data, Nitrate

SINGLE WAVELENGTH DATA, 2ppb



MULTIVARIATE FITTED DATA, 2ppb



Results – Concentration Estimates, Nitrate

True Concentration (ppb)	Single Wavelength Conc (ppb)	Multivariate Concentration (ppb)
0	1.679	0.661
2	3.087	2.489
5	5.960	5.631
10	10.66	10.32
20	19.34	20.21

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Conclusion

- Standard analysis techniques rely on measuring absorbance at a single wavelength of interest
- Our analytical instruments employ spectrophotometers that collect absorbance data across a wide spectral range
- Use the entire absorbance spectrum and multivariate analysis
  - Derive a “model spectrum” from a known matrix-matched standard
  - Fit experimental spectral data against this model spectrum
  - Interpolate the primary wavelength from the fitted spectrum
  - Using interpolated data, construct calibration curve and interpolate sample concentrations as usual
- 3-5 fold reduction in noise and lower detection limits

Thank You!

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