HORIBA

A-TEEM Direktor

Your Guide to Multivariate Analysis

horiba.com/fluorescence

Revolutionize A-TEEM™ Data Analysis

Introduction

A-TEEM Direktor Multivariate Analysis (MVA) is a cutting-edge software suite designed to streamline and simplify the analysis of fluorescence A-TEEM data. With its intuitive interface and guided workflow, A-TEEM Direktor empowers researchers and analysts to extract meaningful insights from their data.

It is a tool designed specifically for fluorescence A-TEEM data analysis. The guided workflow takes a user step-by-step through data import, visualization, variable selection, preprocessing, and modeling A-TEEM calibration data sets. Prediction tools facilitate the application of calibration models to predict class or concentration information from new sample data.

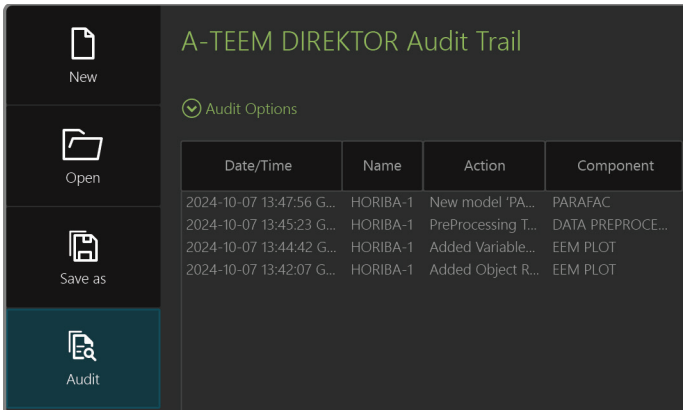
Drag and drop functionality and a graphically driven user interface help to make MVA for A-TEEM a better experience. For GMP labs, A-TEEM Direktor includes a full audit trail, PDF report generation, and user login authentication.

The Power of A-TEEM

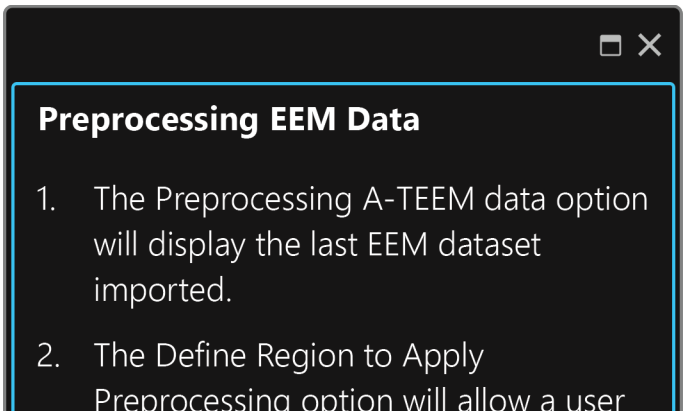
A-TEEM Spectroscopy simultaneously captures absorbance, transmittance, and fluorescence excitation-emission matrix data, while correcting for the inner-filter effect on the fly. This produces a three-dimensional plot of the sample under study. All this is non-destructive and takes a matter of seconds to produce.

Traditional fluorescence adheres to Beer-Lambert linearity, meaning that fluorescence intensity is linear with concentration and absorbance. Fluorescence spectra (and EEMs) can change non-linearly due to high optical density/high concentration and inner-filter effects.

This means that traditional fluorescence of solutions can only be quantitative if concentrations of fluorescing species have low optical density. At higher concentrations, the use of the absorbance spectra to correct for inner-filter effects allows fluorescence A-TEEMs to be used quantitatively and reproducibly over a wider range of samples and concentrations.



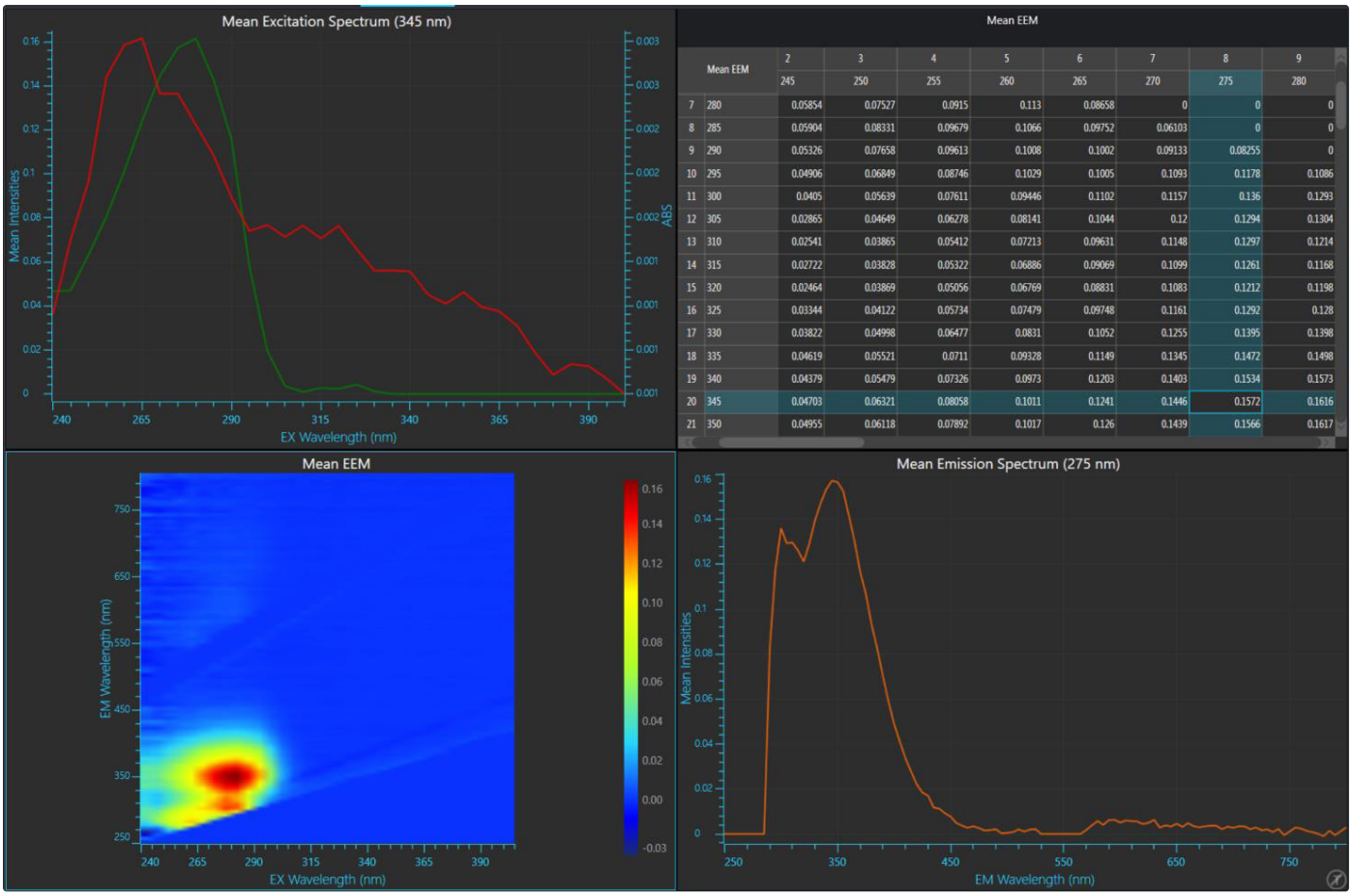
Audit Trail for GMP and 21 CFR Part 11



Context-Sensitive Tool Tips

Key Benefits

- **Ease of Use:** Benefit from a user-friendly interface and drag-and-drop functionality, making A-TEEM Direktor accessible to users of all levels.
- **Flexibility:** While A-TEEM Direktor is optimized for A-TEEM data, it can also be used to analyze other data types and spectra.
- **Guided Workflow:** Follow a step-by-step process for seamless data import, visualization, variable selection, preprocessing, modeling and prediction.
- **GMP Compliance:** Ensure data integrity and regulatory compliance with built-in audit trails, PDF report generation, and login authentication.
- **Versatile Modeling:** Choose from a range of multivariate models, including PARAFAC, PCA, PLSR, PLS-DA, and more, to suit your specific analysis needs.
- **Context-Sensitive Help:** Extensive tool tips and help menus are integrated throughout the software for quick explanations and user guidance.



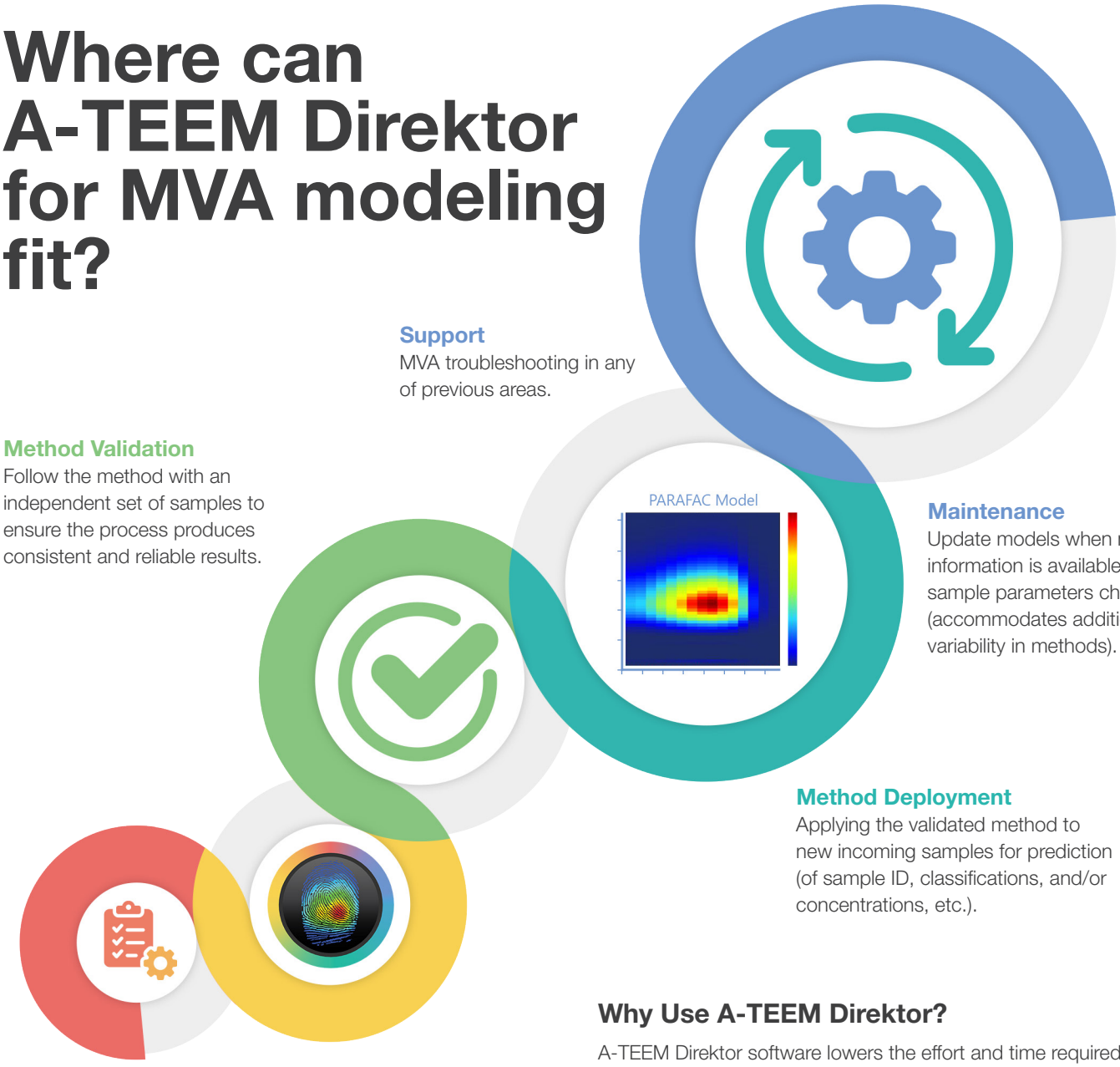
Mean EEM	2	3	4	5	6	7	8	9
	245	250	255	260	265	270	275	280
7 280	0.05854	0.07527	0.0915	0.113	0.08658	0	0	0
8 285	0.05904	0.08331	0.09679	0.1066	0.09752	0.06103	0	0
9 290	0.05326	0.07658	0.09613	0.1008	0.1002	0.09133	0.08255	0
10 295	0.04906	0.06849	0.08746	0.1029	0.1005	0.1093	0.1178	0.1086
11 300	0.0405	0.05639	0.07611	0.09446	0.1102	0.1157	0.136	0.1293
12 305	0.02865	0.04649	0.06278	0.08141	0.1044	0.12	0.1294	0.1304
13 310	0.02541	0.03865	0.05412	0.07213	0.09631	0.1148	0.1297	0.1214
14 315	0.02722	0.03828	0.05322	0.06886	0.09069	0.1099	0.1261	0.1168
15 320	0.02464	0.03869	0.05056	0.06769	0.08831	0.1083	0.1212	0.1198
16 325	0.03344	0.04122	0.05734	0.07479	0.09748	0.1161	0.1292	0.128
17 330	0.03822	0.04998	0.06477	0.0831	0.1052	0.1255	0.1395	0.1398
18 335	0.04619	0.05521	0.0711	0.09328	0.1149	0.1345	0.1472	0.1498
19 340	0.04379	0.05479	0.07326	0.0973	0.1203	0.1403	0.1534	0.1573
20 345	0.04703	0.06321	0.08058	0.1011	0.1241	0.1446	0.1572	0.1616
21 350	0.04955	0.06118	0.07892	0.1017	0.126	0.1439	0.1566	0.1617

2D and 3D A-TEEM Spectra

Steps of A-TEEM Method Development

The use of A-TEEM for robust and reliable analysis requires multiple steps which begin with feasibility, then method development, validation, deployment, and maintenance. Benchtop instrument and acquisition software are involved, but multivariate analysis tools, like A-TEEM Direktor, are critical in many, if not all parts of this process.

Where can A-TEEM Direktor for MVA modeling fit?



Support
MVA troubleshooting in any of previous areas.

Method Validation
Follow the method with an independent set of samples to ensure the process produces consistent and reliable results.

Maintenance
Update models when new information is available, or sample parameters change (accommodates additional variability in methods).

Method Deployment
Applying the validated method to new incoming samples for prediction (of sample ID, classifications, and/or concentrations, etc.).

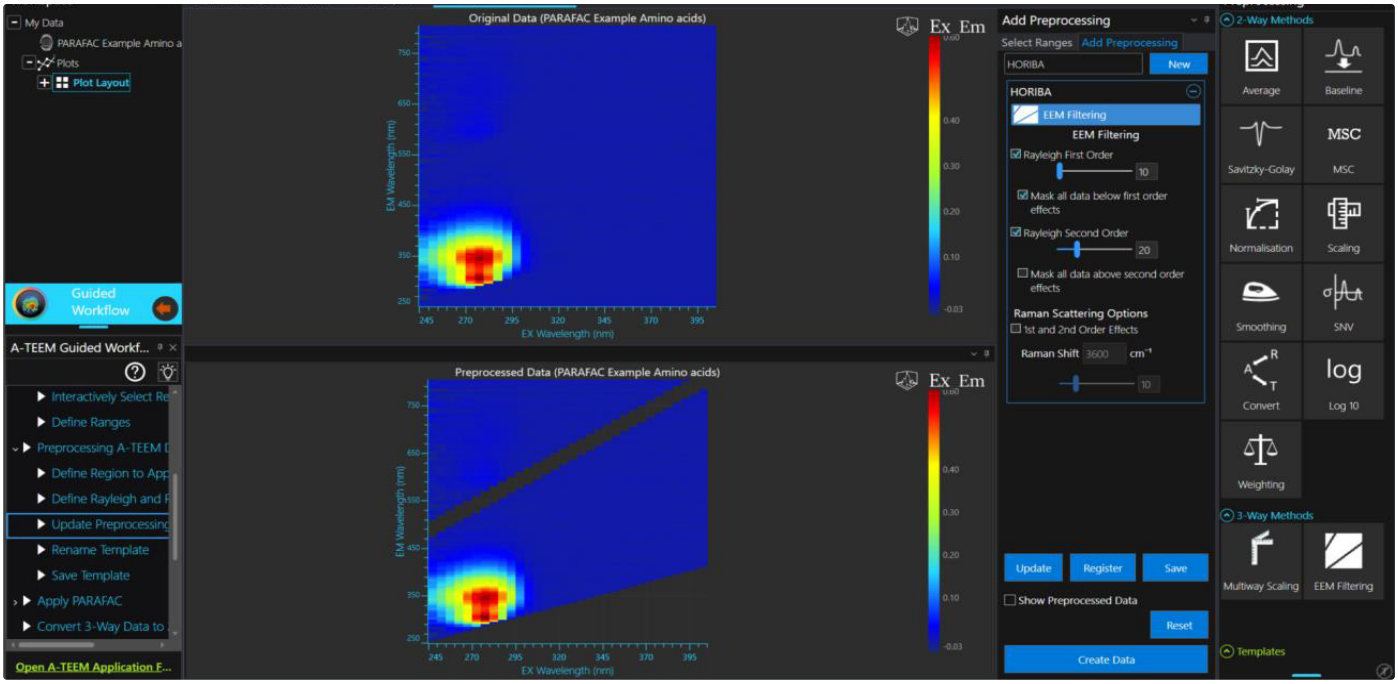
Feasibility
A-TEEM Direktor can be used to analyze a variety of data types and spectra in order to determine if A-TEEM spectroscopy can work for those samples.

Method Development
Testing and planning of acquisition parameters, calibration sets (what samples to include) and defining model parameters. Choose and develop a model type (PARAFAC, PCA, or PLS) based on goals determined in Feasibility.

Why Use A-TEEM Direktor?

A-TEEM Direktor software lowers the effort and time required to get EEM and A-TEEM data into a multivariate analysis solution, and get answers from the data. Guided workflows enable a user to navigate data import, visualization, variable selection, pre-processing, modeling and prediction step-by-step in an order that's sensible for 3-way data, and highlights modeling data in a way that gets answers more clearly from potentially complex data sets.

All algorithms used in A-TEEM Direktor are referenced and validated, meaning that the results can be trusted. As a bonus, A-TEEM Direktor enables working in a GMP environment by having software login authentication, a full audit trail, and protection of original data.



PARAFAC Model Examing Amino Acids

A-TEEM Guided Workflow Step-by-Step

1. Import A-TEEM Data

Drag and drop folders of A-TEEM data files and Absorbance spectra data files into a project. The import tool reads the PEM.dat and ABS.dat files into the project, along with the meta data contained in the log files associated with each SampleQ data output folder, bringing traceability of the data into multivariate analysis going forward.

2. Define Class Variables and Y-Reference Data

Edit or copy and paste class variables or concentrations for each sample data into an easy to manage table. Use the class variables and Y-reference data for regression or classification models or for plotting unsupervised model outputs to associate known meta data with each sample.

3. Plot Data and Define Ranges

Plot the sample A-TEEM or EEM and absorbance spectral data for each sample, or plot the mean (average) A-TEEM or EEM. Define the excitation and emission wavelength ranges that are usable for modeling. Save unique variable ranges to be used in a model.

4. 3-Way Preprocessing

Set Rayleigh and/or Raman scatter masking regions so that analysis is done only on fluorescence data in the regions of interest.

5. 3-Way Modeling

Parallel Factor Analysis (PARAFAC) to decompose mixtures into pure samples via 3-way spectral components.

6. Unfold/Matricize 3-Way Data to 2-Way Data

7. 2-Way Preprocessing (scaling, normalization, etc.)

Models for 2-way Data

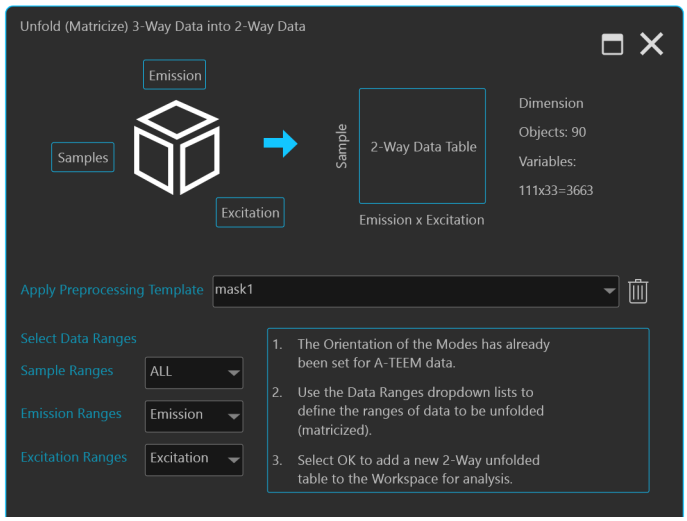
Models Supported by A-TEEM Direktor

Guided Workflow

- **Principal Components Analysis (PCA)** to investigate the structure of the data and find interesting patterns.
- **Partial Least Squares Regression (PLSR)** to generate quantitative predictive model of concentrations or other properties of the samples.
- **Partial Least Squares Discrimination Analysis (PLS-DA)** to define classification rules to classify new samples into known groups or identities.

Prediction

A-TEEM Direktor applies calibration models to new data sets for predicting classification or concentration of the new samples.



Converting 3-Way Data into 2-Way Data

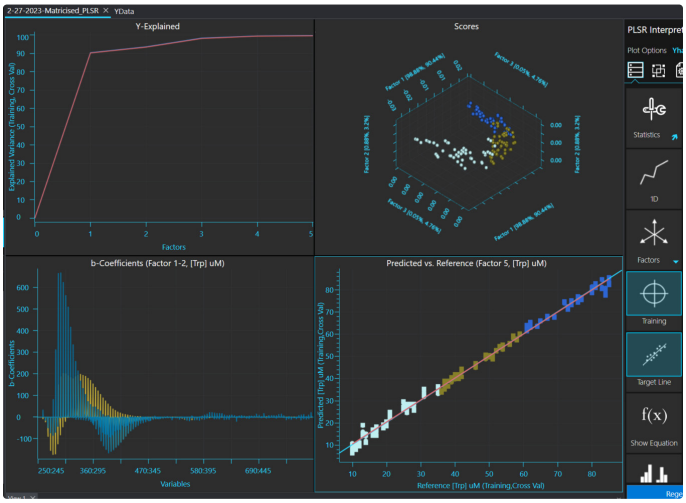
Important Analysis Methods Outside the Guided Workflow

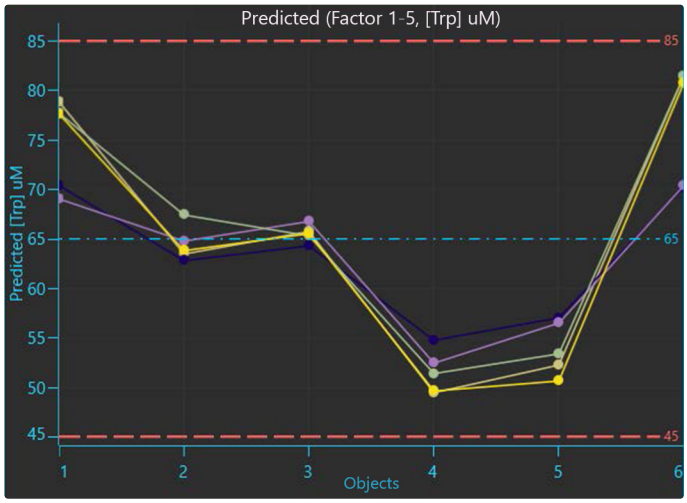
Univariate Analysis

- ANOVA
- Distance Measures
- F-Test
- Normality Test
- Statistical Overview
- t-Test

Multivariate Analysis

- Multiple Linear Regression (MLR)
- Support Vector Machine Regression (SVMR)
- Support Vector Machine Classification (SVMC)
- Extreme Gradient Boost Regression (XGBoostR)
- Extreme Gradient Boost Classification (XGBoostC)
- Soft Independent Modeling of Class Analogies (SIMCA) Libraries



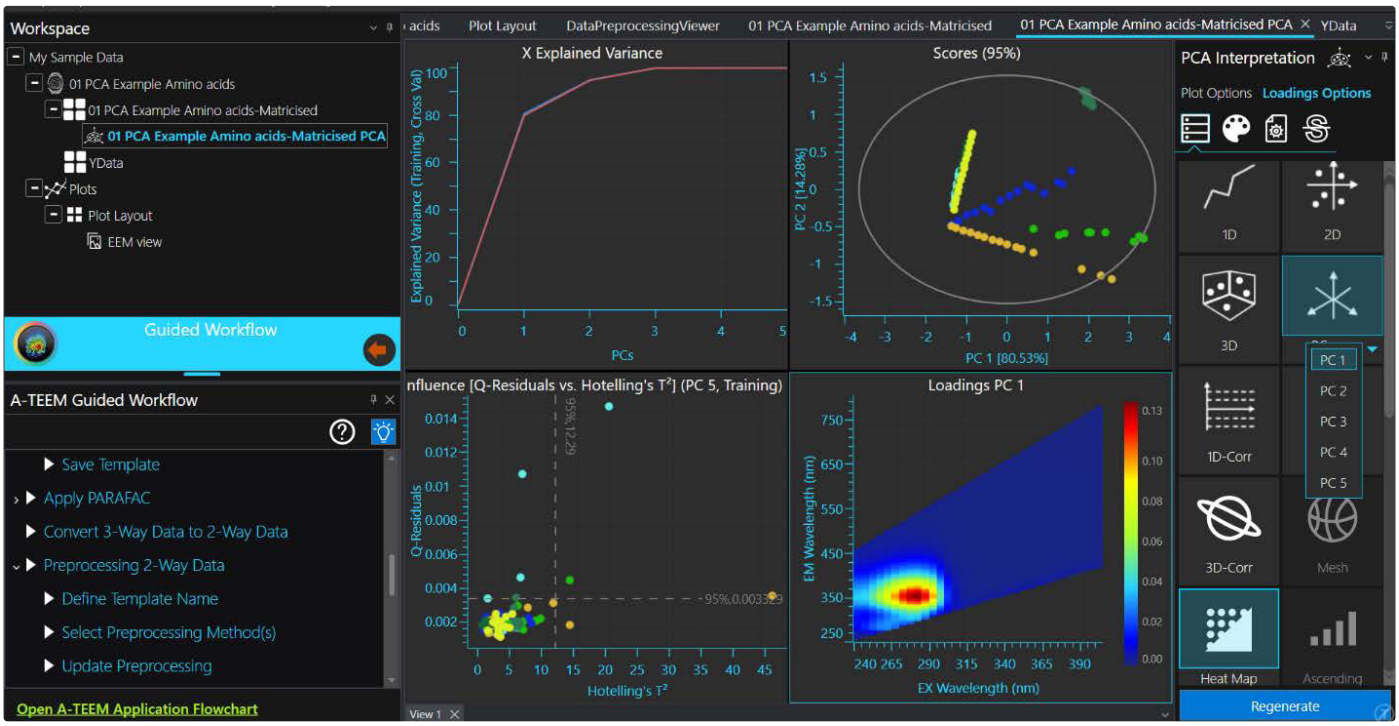


Experience the Power of A-TEEM Direktor

Example loadings from a PARAFAC model showing the decomposition of the sample mixtures into three distinct fluorescent spectral components. Mode 1 = samples, Mode 2 = emission spectra, Mode 3 = excitation spectra. Loadings can also be plotted as heatmaps, showing each pure component EEM.

Predict New Data

Use multivariate models calibrated and validated to predict, project, or classify properties of new data sets. Import data sets and apply the same variable selection and pre-processing templates, apply a previously created model. Create upper and lower thresholds for output parameters to see if new data “passes or fails” expectations.



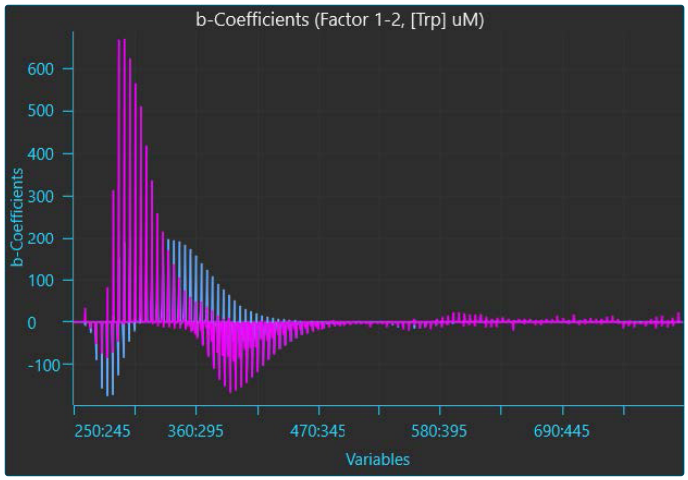
Results from a PCA model showing X-Explained Variance, Scores plot, Influence plot, and heatmap of PCA loadings of A-TEEM data where PC1 can be assigned to the tryptophan amino acid.

The Power of Interactive Plotting

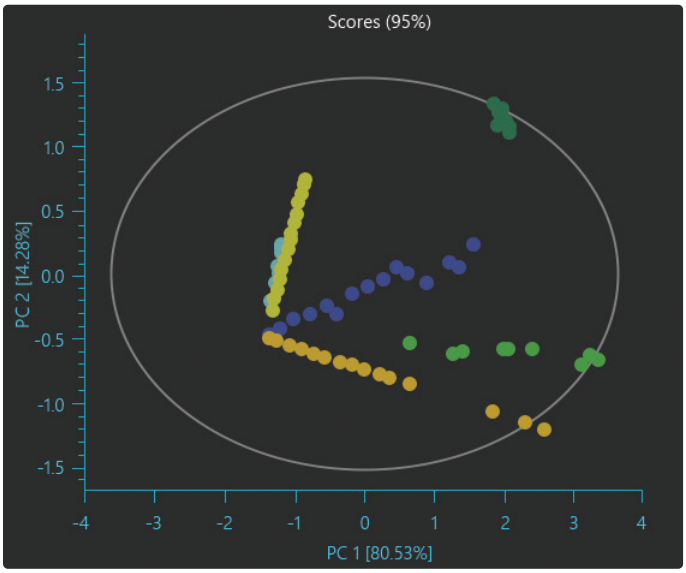
Classification Table (95%, Factor 5, Training)

Classification	F only	W only	Y only	Result
2024-01-11-12-42-30-B1S2W2PEM	0	1	0	W only
2024-01-11-12-48-39-B1S3W3PEM	0	1	0	W only
2024-01-11-12-54-50-B1S4W4PEM	0	1	0	W only
2024-01-11-13-00-59-B1S5W5PEM	0	1	0	W only
2024-01-11-13-07-08-B1S6W6PEM	0	1	0	W only
2024-01-11-13-13-17-B1S7W7PEM	0	1	0	W only
2024-01-11-13-19-26-B1S8W8PEM	0	1	0	W only
2024-01-11-13-25-36-B1S9W9PEM	0	1	0	W only
2024-01-11-13-31-45-B1S10W10PEM	0	1	0	W only
2024-01-11-13-37-54-B1S11W11PEM	0	1	0	W only

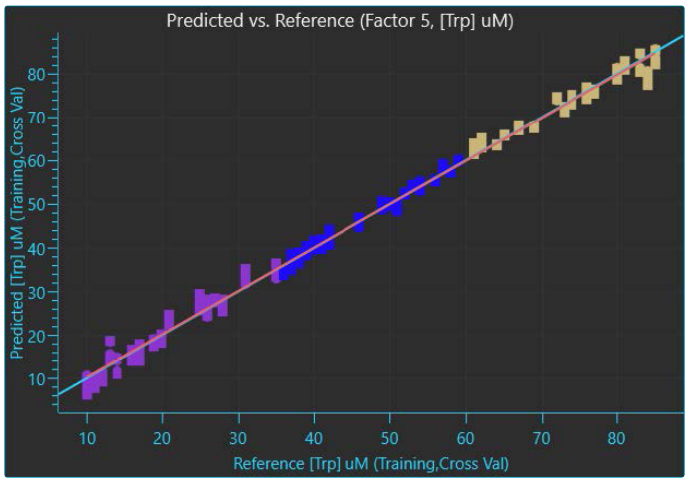
Classification Table from a PLS-DA model result for a training data set.



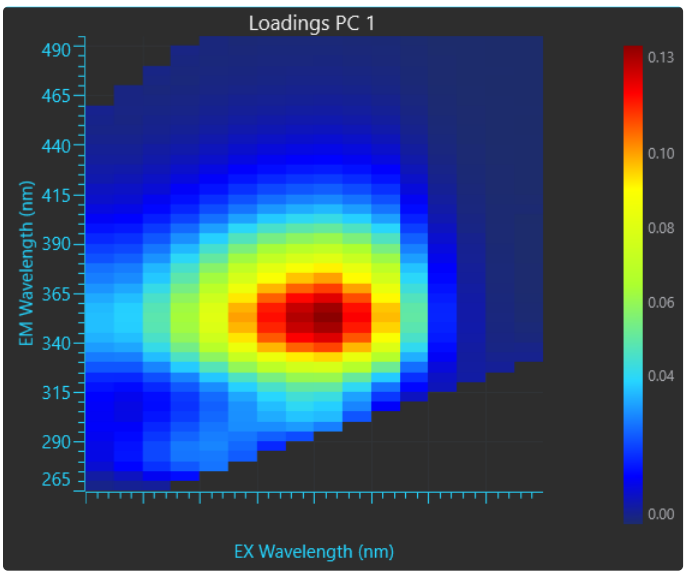
b-coefficients plotted vs. wavelength variables for factors 1 and 2 of a PLSR model predicting amino acid concentrations.



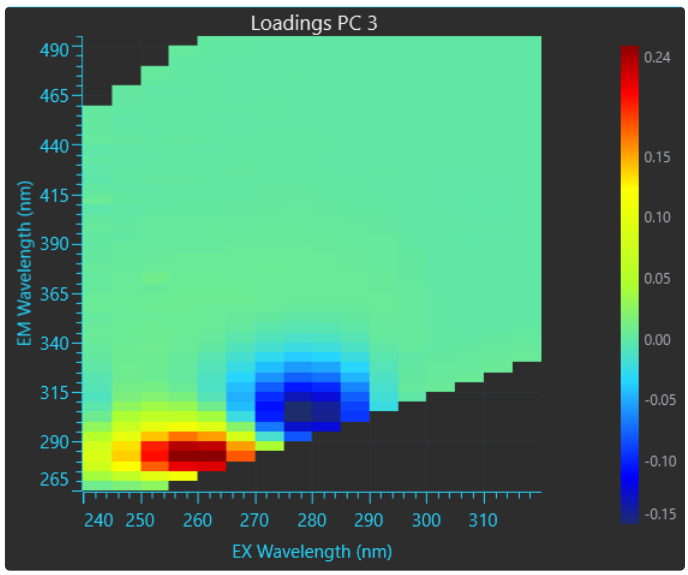
PCA Score Plot



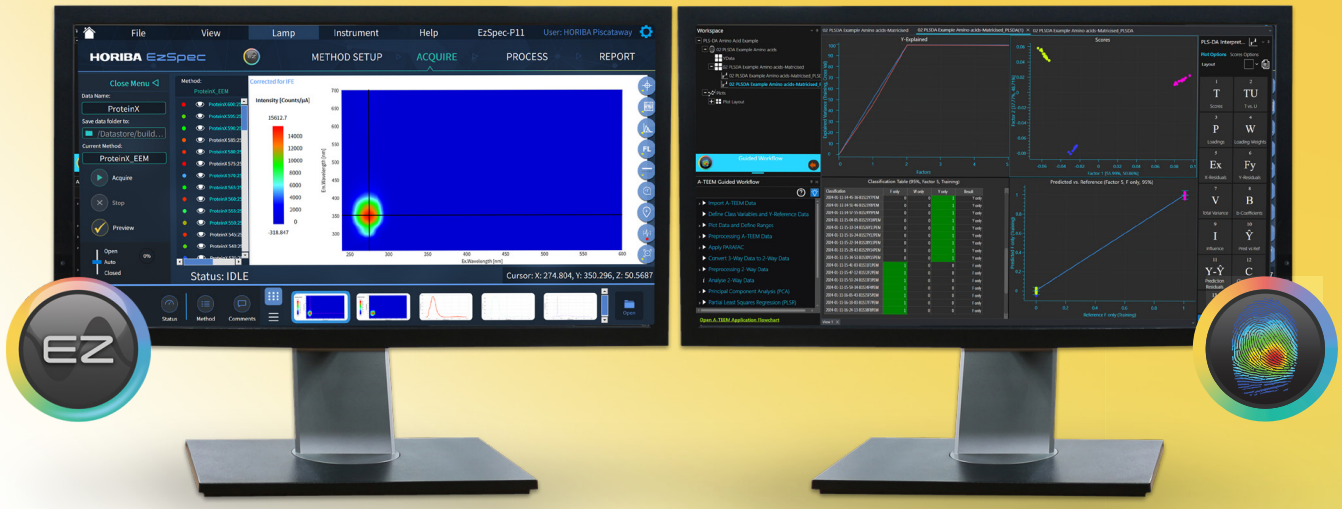
Predicted vs. Reference Plot for Training and Cross Validation Sample Data sets to indicate quality of the model to predict concentration.



PCA Loadings 1 Heatmap

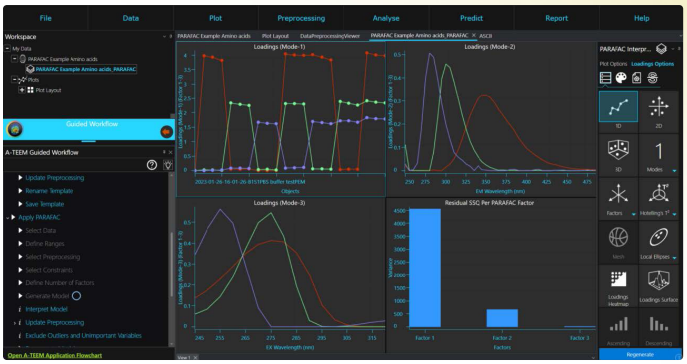


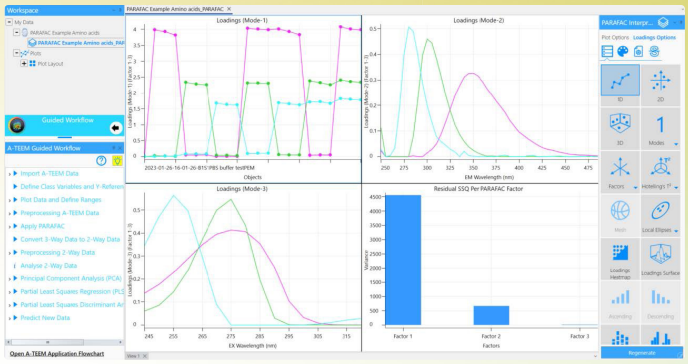
PCA Loadings 2 Heatmap



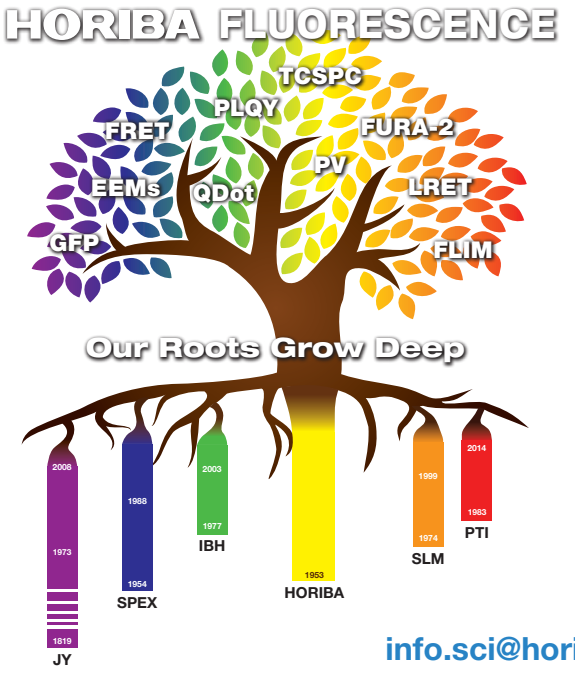
From Acquisition to Analysis

A-TEEM Direktor is capable of seemlessly managing A-TEEM data collected with HORIBA software.





Results and Graphs can be saved or exported to multiple data and image formats, in both light and dark modes for publication and presentation-worthy figures.



A-TEEM Direktor Specifications

Feature	Minimum	Recommended
Processor	Intel i5, i7, i9, and Xeon	Intel i7 for most tasks Intel i9 or Xeon for data intensive applications
Operating System	Windows 10 and later (64-bit)	Windows 10 64-bit
Memory (RAM)	8 GB	16 GB
Graphics Card	Direct 9	Direct 9
Hard Disk Storage	1 GB	500 GB to 1TB
File Support	Multiple open source formats, proprietary formats, and HORIBA .dat files from A-TEEM instrumentation	

Discover how A-TEEM Direktor can enhance your A-TEEM data analysis capabilities.

Contact us today to learn more and request a demo.

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