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Nanosecond Time-Domain Fluorescence: Application of Three-Way Analysis Methods to Elucidate Composition

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Overview

- Introduction and motivation for research
- Previous research
- Hardware
- Experiment
- Methods of data analysis
  - Two-way and three-way treatments
- Data processing
- Results
- Conclusions and future directions

Introduction

- Numerous R&D areas employ fluorescence lifetime to characterize fundamental molecular properties.
  - However, analytical applications of fluorescence lifetime technology remain quite rare.
- Multiway fluorescence measurements provide greater analytical power than single modalities
- Two-way wavelength-time matrices (WTM) collected at different excitation wavelengths can produce three-way data
  - Time-Resolved Excitation Emission Matrix (TREEM).
- Fluorescence decay curves collected with time-correlated single photon counting (TCSPC) require a significant time commitment.
  - Innovative methods for rapid collection of WTMs has reduced data collection times dramatically, making this a viable and valuable tool for analytical chemists

Motivation

- Time domain combined with emission wavelength measurements can provide information-rich data
  - Varying excitation wavelengths can yield high-quality three-way data
- Rapid data collection coupled with fast data analysis create an analytical tool that is practical in the high-throughput laboratory
- Evaluate two-way analysis methods on three-way data
  - What does a three-way method buy you?
- Demonstrate the power of PARAFAC for analyzing data from new fluorescence lifetime technology
  - Should we use PARAFAC2 or PARAFAC for data with shifts?
- Introduce a novel three-way method specifically designed for analysis of TREEMs.
  - Can we make use of knowledge of the physics underlying the data?

Related Published Research

- H.-B. Wang, Y.-J. Zhang, X. Xiao, S.-H. Yu, W.-Q. Liu, Application of excitation-emission matrix fluorescence combined with second-order calibration algorithm for the determination of five polycyclic aromatic hydrocarbons simultaneously in drinking water, Analytical Methods, 3 (2011) 688-695.
- H. Wang, Y. Zhang, X. Xiao, Quantification of Polycyclic Aromatic Hydrocarbons in Water: a Comparative Study Based on Three-dimensional Excitation-emission Matrix Fluorescence, Anal. Sci., 26 (2010) 1271-1276.
- H.-B. Wang, Y.-j. Zhang, J.-B. Duan, X. Xiao, S.-H. Yu, K. Zhang, Simultaneous determination of benzo[k]fluoranthene and perylene using excitation-emission matrix fluorescence, in: Y. Zhang, J. Sasian, L. Xiang, S. To (Eds.), SPIE, Dalian, China, 2010, pp. 76561F-76566.
- S.A. Bortolato, J.A. Arancibia, G.M. Escandar, Chemometrics-Assisted Excitation−Emission Fluorescence Spectroscopy on Nylon Membranes. Simultaneous Determination of Benzo[a]pyrene and Dibenz[a,h]anthracene at Parts-Per-Trillion Levels in the Presence of the Remaining EPA PAH Priority Pollutants As Interferences, Anal. Chem., 80 (2008) 8276-8286.
- [1] M.V. Bosco, M.S. Larrechi, PARAFAC and MCR-ALS applied to the quantitative monitoring of the photodegradation process of polycyclic aromatic hydrocarbons using three-dimensional excitation emission fluorescent spectra: Comparative results with HPLC, Talanta, 71 (2007) 1703-1709.
- E. Selli, C. Zaccaria, F. Sena, G. Tomasi, G. Bidoglio, Application of multi-way models to the time-resolved fluorescence of polycyclic aromatic hydrocarbons mixtures in water, Water Res., 38 (2004) 2269-2276.
- P.D. Wentzell, S.S. Nair, R.D. Guy, Three-Way Analysis of Fluorescence Spectra of Polycyclic Aromatic Hydrocarbons with Quenching by Nitromethane, Anal. Chem., 73 (2001) 1408-1415.

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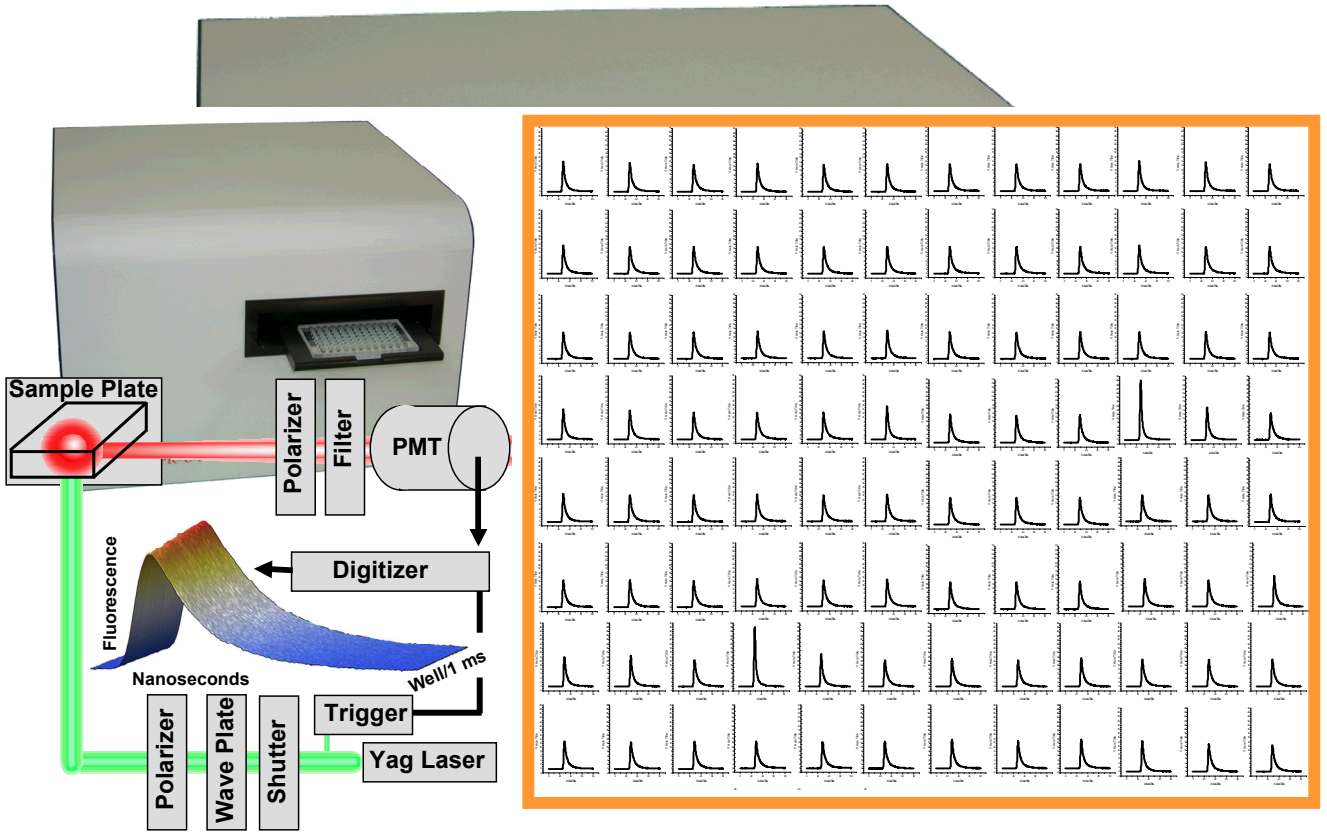
NovaFluor PR-UV



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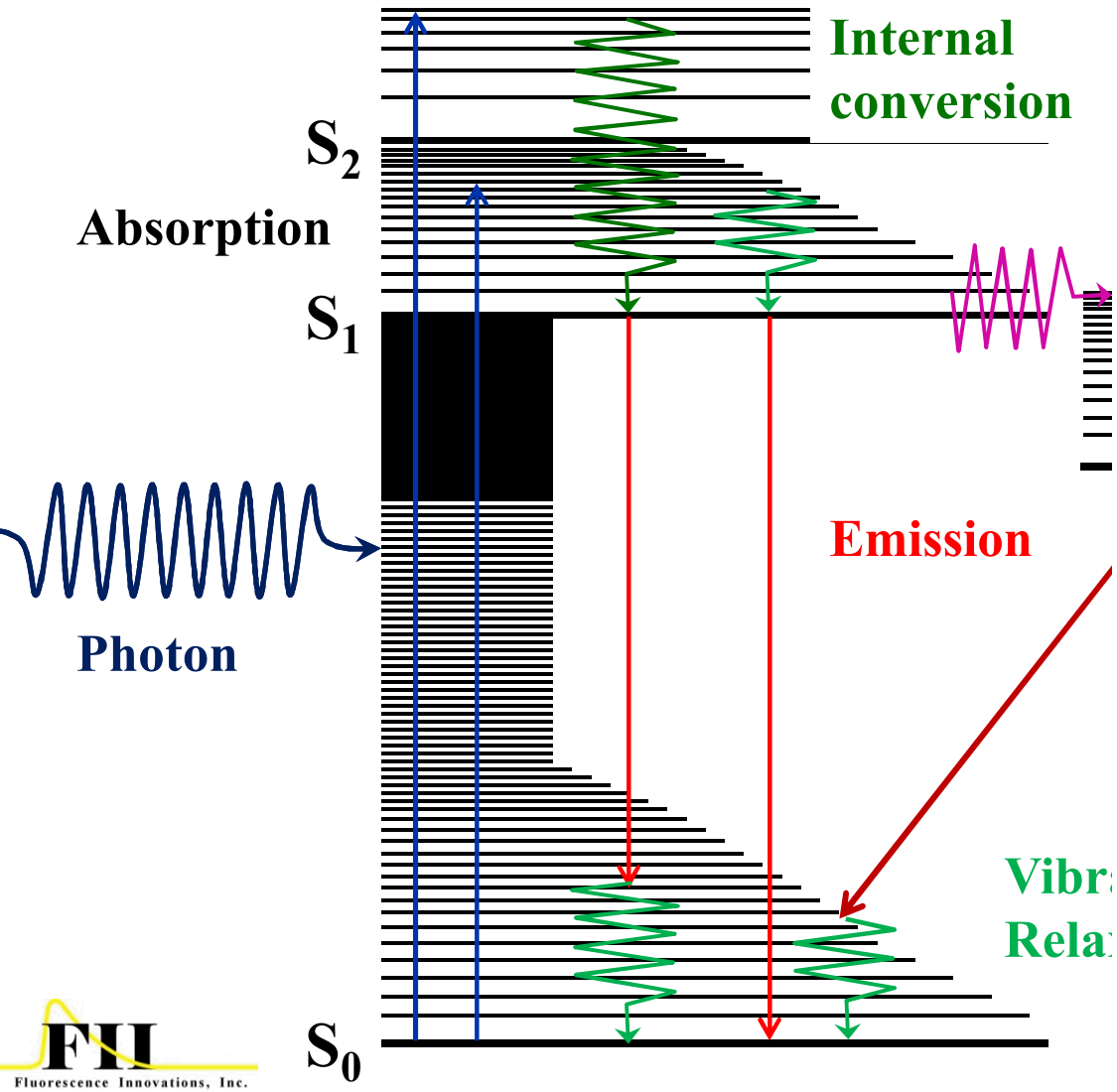
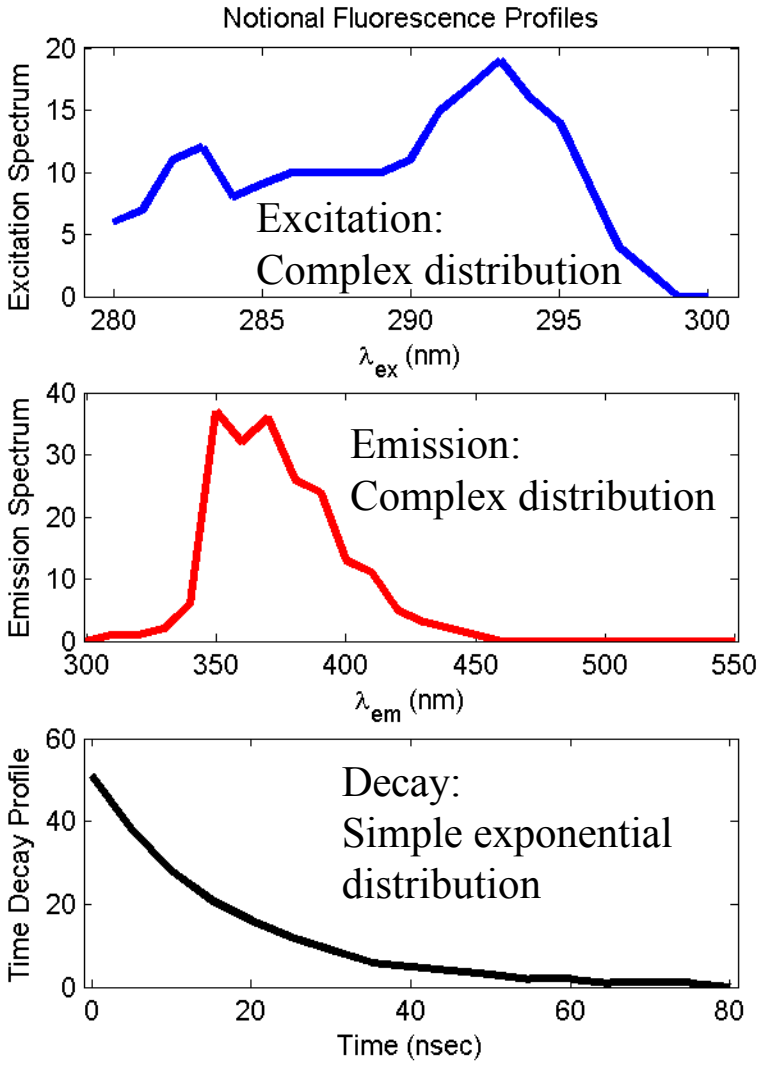
How does fluorescence arise?
The Jablonski Diagram





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Review of Relevant Two-Way Methods

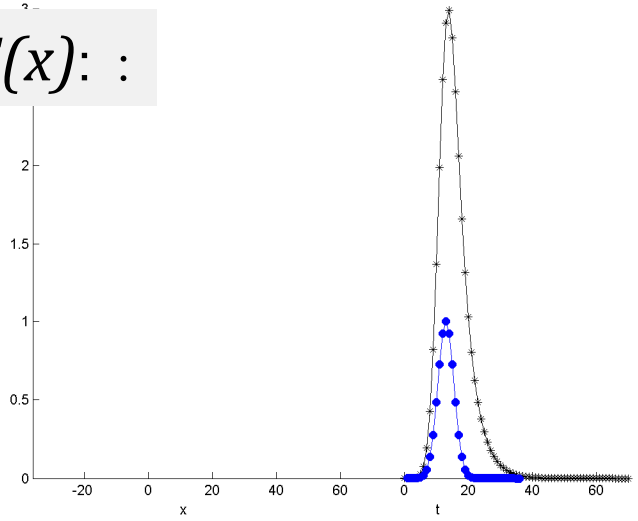
- Principal Component Analysis – PCA[1,2]
  - $D = TP^T$
  - Well established method of factor analysis
  - Provides abstract (orthogonal) factors
  - Great for de-noising data (use only significant factors)
- Multivariate Curve Resolution – MCR[3,4]
  - Varied implementations:
    - Factor rotations, evolving factor analysis, SIMPLISMA, and ALS
  - ALS, very common: $D = AS^T + E$
    - Employs constraints (nonnegativity, equality)
- Global Analysis[5,6]
  - Uses lifetime information in building model

1. Rummel, R.J. Applied Factor Analysis, 1970.
2. Harman, H.H. Modern Factor Analysis, 1976.
3. Lawton, W.H. and Sylvestre, E.A. (1971) Technometrics, 13 617-633.
4. Tauler, R. and Barcelo, D. TrAC, Trends Anal. Chem., 12 (1993) 319-327.
5. Knorr, F.J. and Harris, J.M. (1981) Analytical Chemistry, 53, 272-276.
6. Knutson, J.R. and J.M. Beechem, L. Brand, Chem. Phys. Lett., 102 (1983) 501-507.

Global Analysis for Convoluted Data

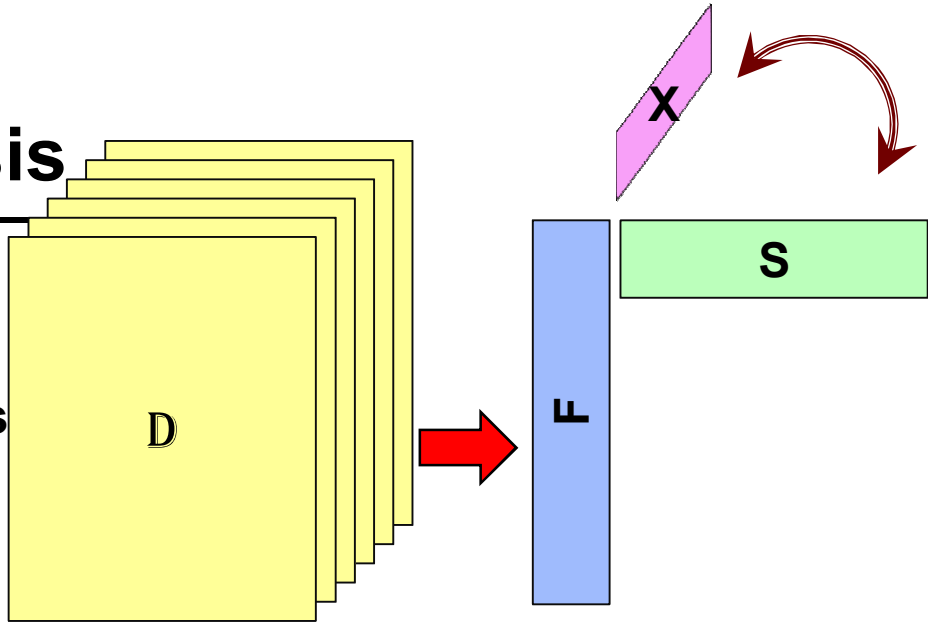
- **Objective**
  - **Minimize $D = FS^T$**
- **Nonlinear Part - F**
  - **Initialize with lifetime estimates and calculate decay $y(t) = e^{-t/\tau}$**
  - **Convolve y(t) with laser-electronics input profile E(t)**
- **Linear Part – S**
  - **Using the decay profiles in F, solve for S**
  - **Can use nonnegative constraint**
  - **$\hat{S} = F^{+}D$**
- **Update lifetimes using nonlinear least squares algorithm**

F1 Input function $E(x)$: :



- **Convolution:**
  - **Fluorescence in bulk shows exponential decay**
  - **"Input" function involves laser profile and electronics response**
  - **$F(t) = \sum_{x=0}^{t} E(t-x)y(x)$**

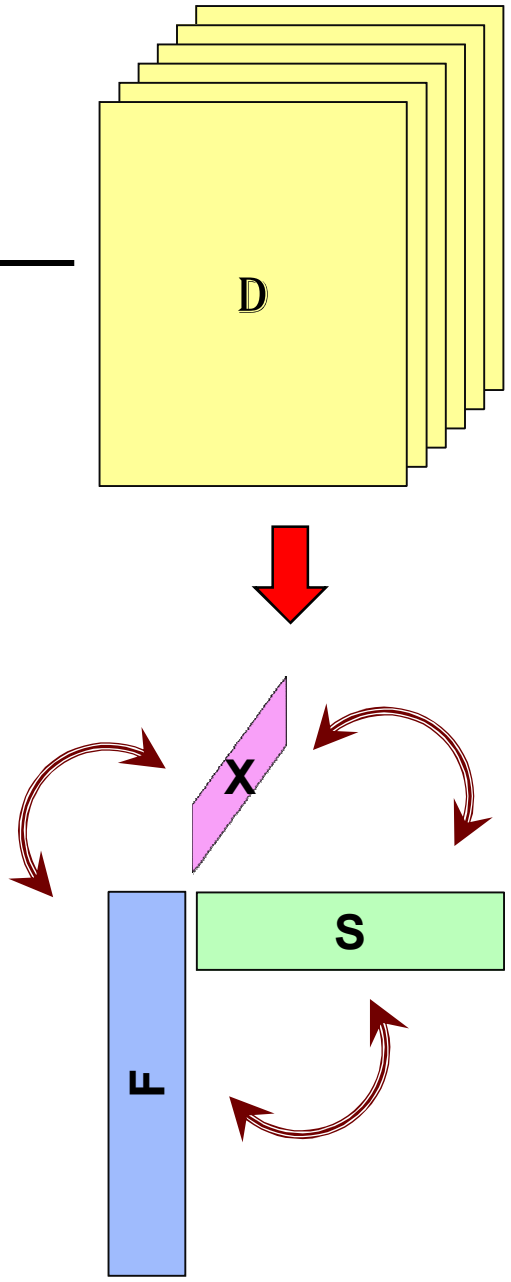
Three-way Global Analysis



- **Objective**
  - L.S. model of $\mathbb{D} = \otimes\{F,S,X\}) + \mathbb{E}$
  - F matrix of time-mode decay profiles
  - S matrix of emission mode profiles
  - X matrix of excitation mode profiles
- **Nonlinear Part - F**
  - Initialize with lifetime estimates and calculate decay $y(t) = e^{-t/\tau}$
  - Convolve y(t) with laser-electronics input profile E(t) to generate F
- **Linear Parts – S and X**
  - Using the decay profiles in F and estimate for X, solve for S
  - $S = (F^T F * X^T X)^{\ddagger} (F \odot X)_{FX} D_S$
  - Using the decay profiles in F and estimated S, solve for X
  - $X = (F^T F * S^T S)^{\ddagger} (F \odot S)_{FS} D_X$
  - Iterate until convergence
- **Update lifetimes using nonlinear least squares algorithm**

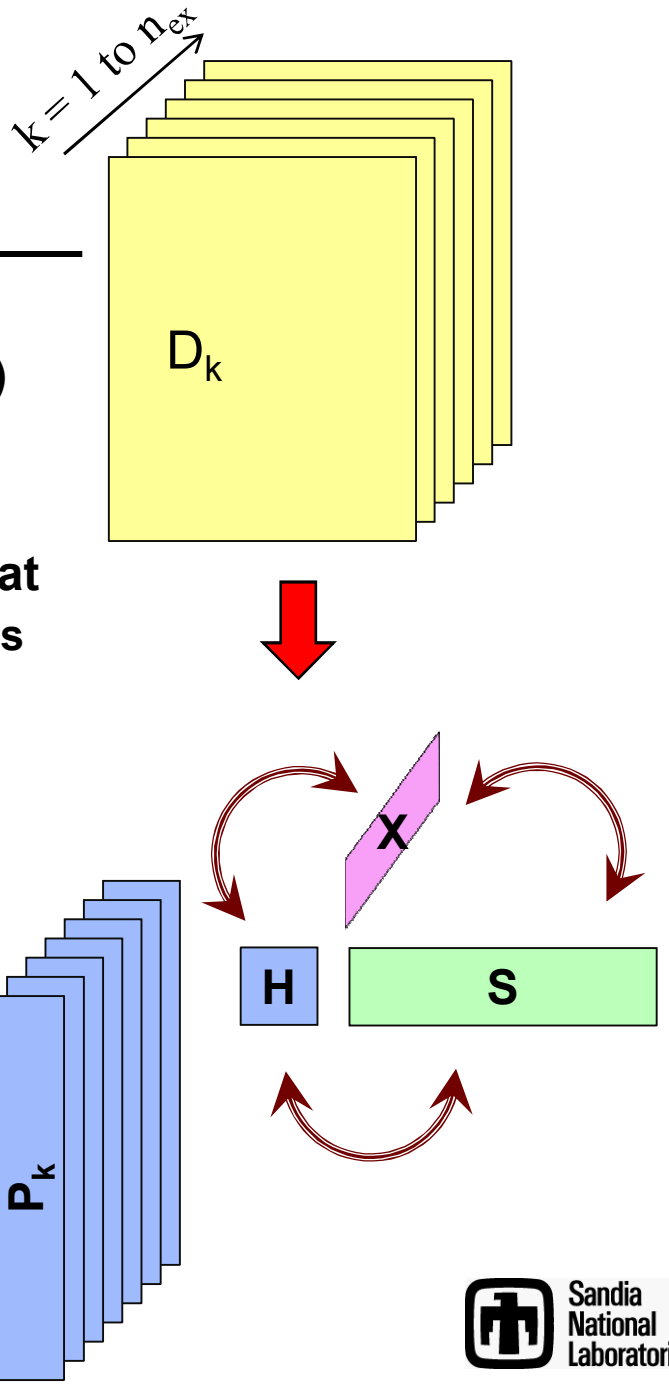
PARAFAC (Conceptual)

- Objective
  - Least squares model of $\mathbb{D} = \otimes\{F,S,X\}) + \mathbb{E}$
  - F matrix of time-mode decay profiles
  - S matrix of emission mode profiles
  - X matrix of excitation mode profiles
- Decomposition of $\mathbb{D}$
  - Initialize with some guesses for F and S
  - Using estimates for F and X, solve for S
  - $S = (F^T F * X^T X)^{\ddagger} (F \odot X)\ {}_{FX}D_S$
  - Using estimates for S and F, solve for X
  - $X = (S^T S * F^T F)^{\ddagger} (S \odot F)\ {}_{SF}D_X$
  - Using estimates for F and S, solve for X
  - $F = (X^T X * S^T S)^{\ddagger} (X \odot S)\ {}_{XS}D_F$
- Iterate until convergence



PARAFAC2 (Conceptual)

- Objective
  - Least squares model of $D_k = P_k H Z_k S + E_k \quad (\forall\ k)$
    - k - the index into the excitation mode
  - P_k the k^{th} orthogonal matrix of the time mode
  - H a square-n_{ex} transformation matrix, such that
    - $F_k = P_k * H$ matrix of k^{th} time-mode decay profiles
  - S matrix of emission-mode profiles
  - Z_k diagonal matrix of the k^{th} row of X
    - X matrix of excitation-mode profiles
- Decomposition of $\mathbb{D}$
  - Initialize with some guesses for H, X, and S
  - Using H, X, S, solve for each P_k
  - Form each term $Q_k = P_k^T D_k$
  - Perform PARAFAC on $\mathbb{Q}$ for H, X, and S
- Iterate until convergence

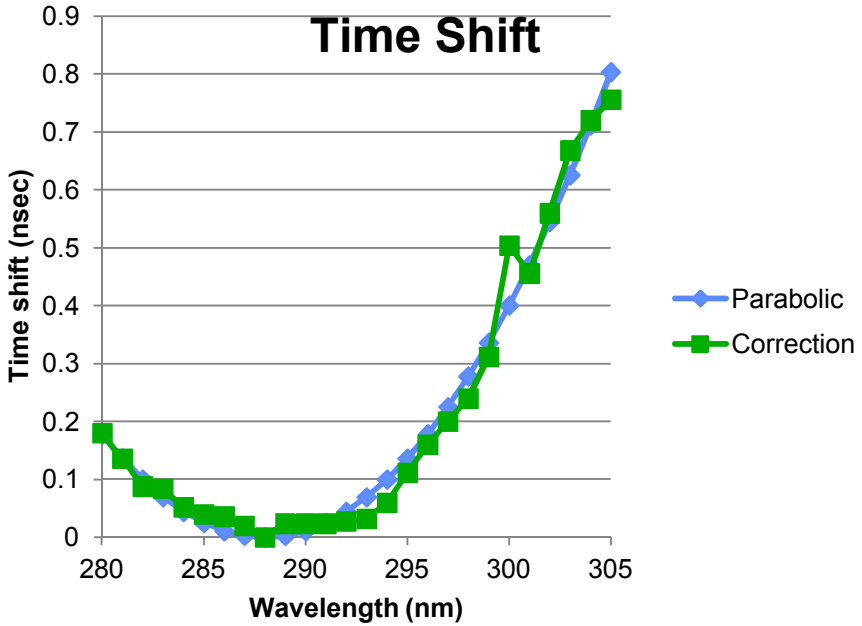


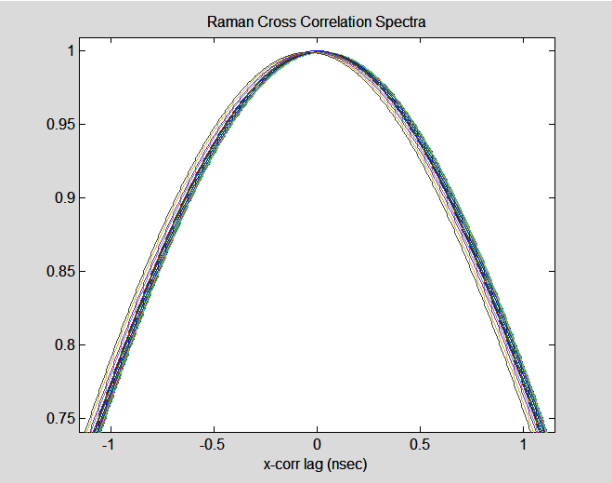
Experiment

- Solution of six EPA priority pollutants in methanol
  - Chemical Species:
    - Fluorene, Naphthalene, Pyrenene, Phenanthrene, Benzo[k]fluoranthrene, Benzo[b]fluoranthrene
    - From Sigma-Aldrich used without further purification
    - Solutions prepared in HPLC grade methanol.
- Collect a single TREEM
  - Original data size:
    - 640 time-mode elements
    - 251 emission wavelength-mode elements
    - 26 excitation wavelength-mode elements
- Acquisition
  - Collect waveforms: ~130 nsec duration in 200 psec increments
  - Step emission wavelengths: 300 – 550 nm in 1 nm increments
  - Excitation wavelengths: 280 – 305 nm in 1 nm increments
  - Collect each WTM in approximately 5 minutes

Correction of Time Domain Shifts

- Due to the laser gain profile, there is an excitation wavelength dependent shift in the time domain
  - Shift is approximately parabolic with wavelength
- Correction involves estimating shift with Raman or Rayleigh scattered excitation
  - Interpolate profiles at 50x measured data
  - Find shift values using cross-correlation
- Correct TREEM data
  - Interpolate profiles at 50x measured
  - Shift data requisite intervals
  - Down-sample data
  - Size after: 636 elements





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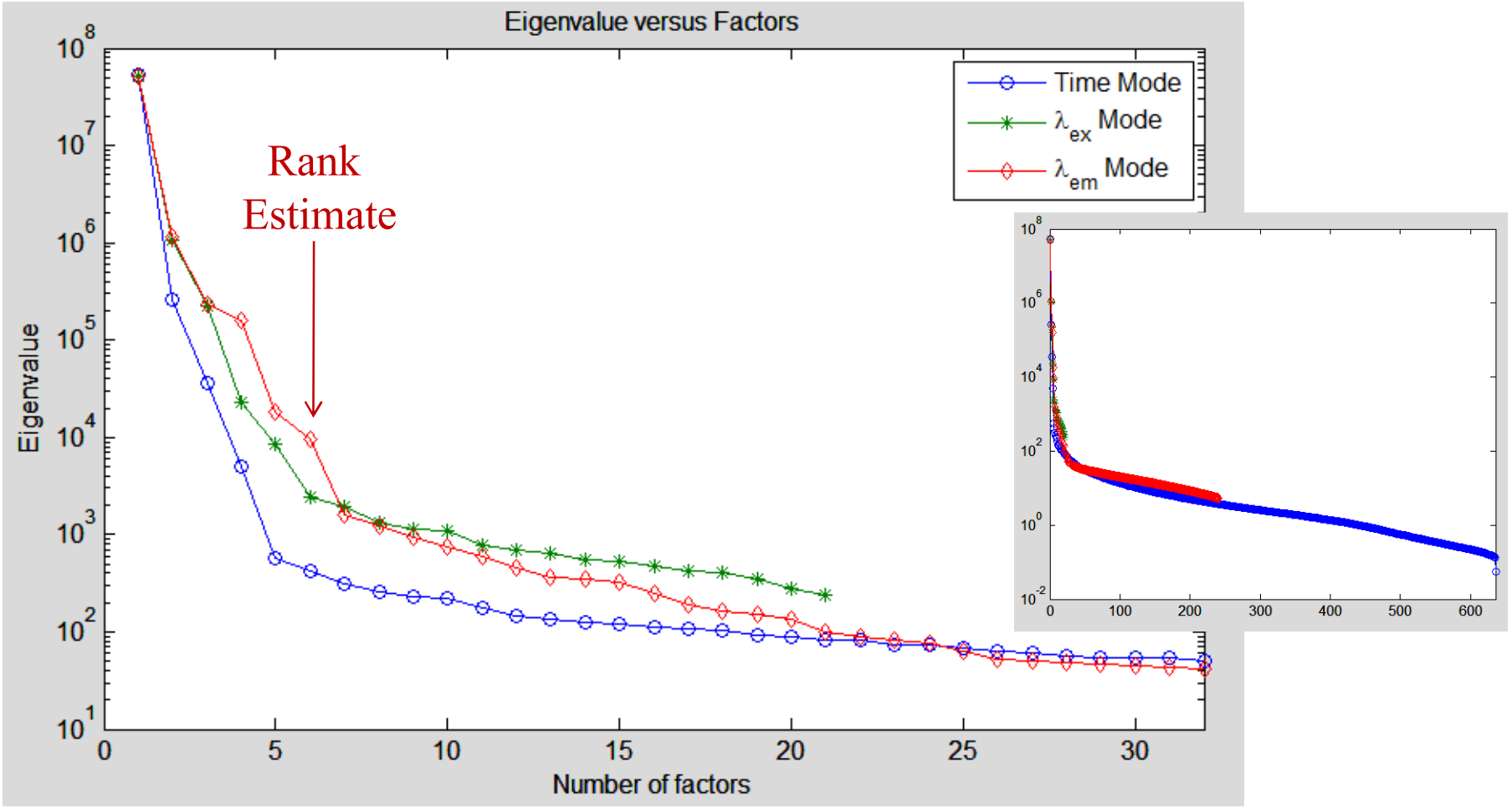
Data Analysis

- **Wavelength ranges, λ_{ex}: 280-300 nm, λ_{em}: 310-550 nm**
- **PARAFAC on shift-corrected data**
  - **Use fast Tucker1 based PARAFAC algorithm**
  - **Initialize with random factors, 6 factor model**
  - **Employ fast rigorous nonnegative least squares**
  - **Data size: 636×241×21**
- **PARAFAC2**
  - **Utilize code from Bro as published1**
  - **Initialize with default, "best of 10 runs…"**
  - **Impose nonnegativity in excitation & emission modes**
  - **Data size 640×241×21**
- **Three-way Global Analysis**
  - **Use new algorithm for three-way data sets**
  - **Laser input function from laser scatter modeled from PARAFAC analysis of expanded data set**
  - **Data size: 636×241×21**
- **Computations were performed using MATLAB®**
  - **Dell Precision 690 computer, with two, dual-core, 3.2 GHz Intel® Xeon processors and 4.0 Gbyte RAM**

1. Kiers, H.A.L. TenBerge, J.M.F. Bro, R. J. Chemom., 13 (1999) 275-294.

Eigenvalue Plot



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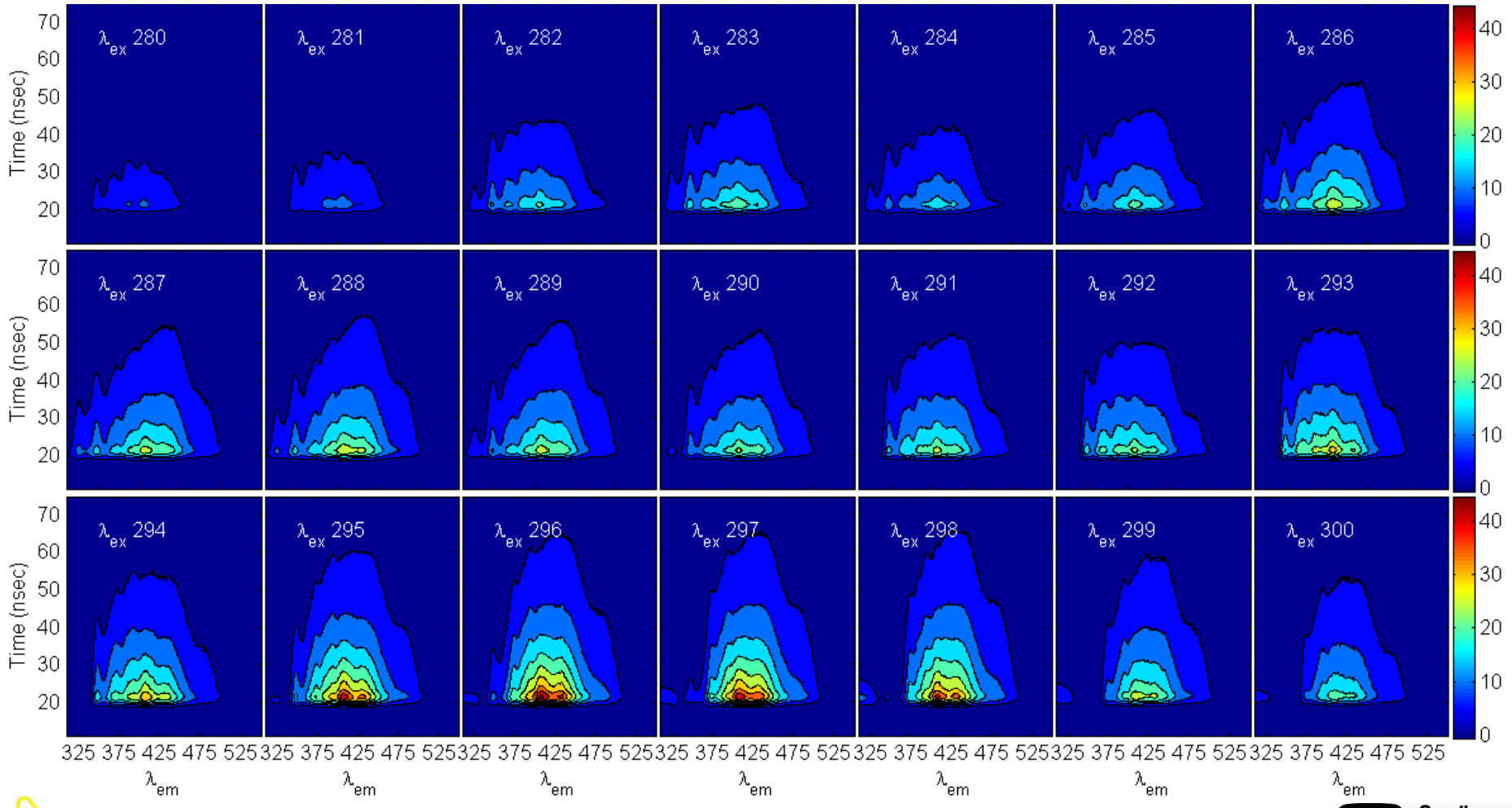
Raw TREEM Data as 21 WTMs



Time range reduced for improved comparison.

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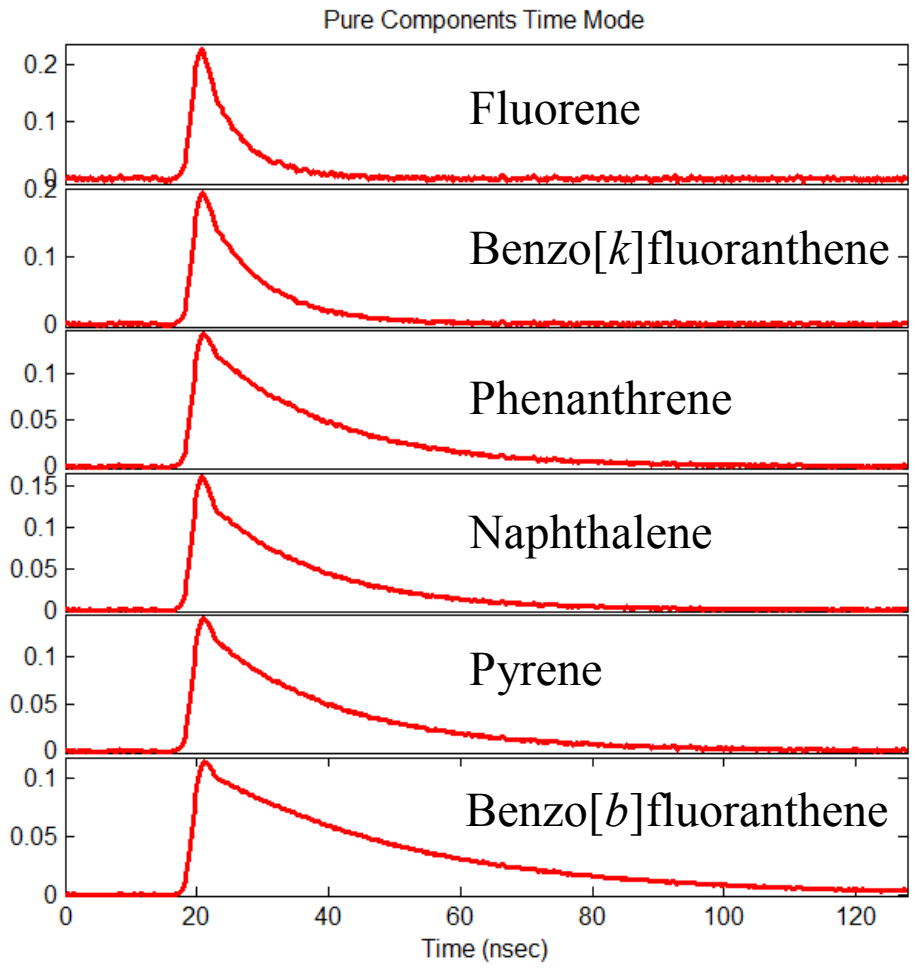
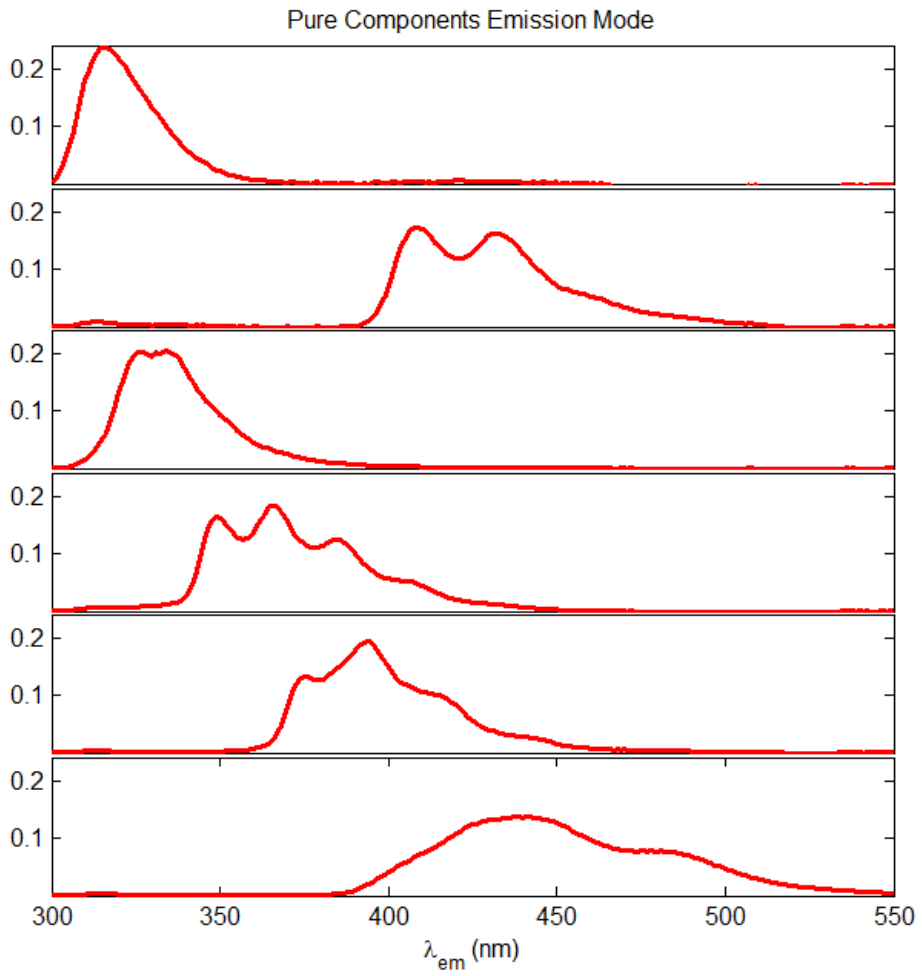
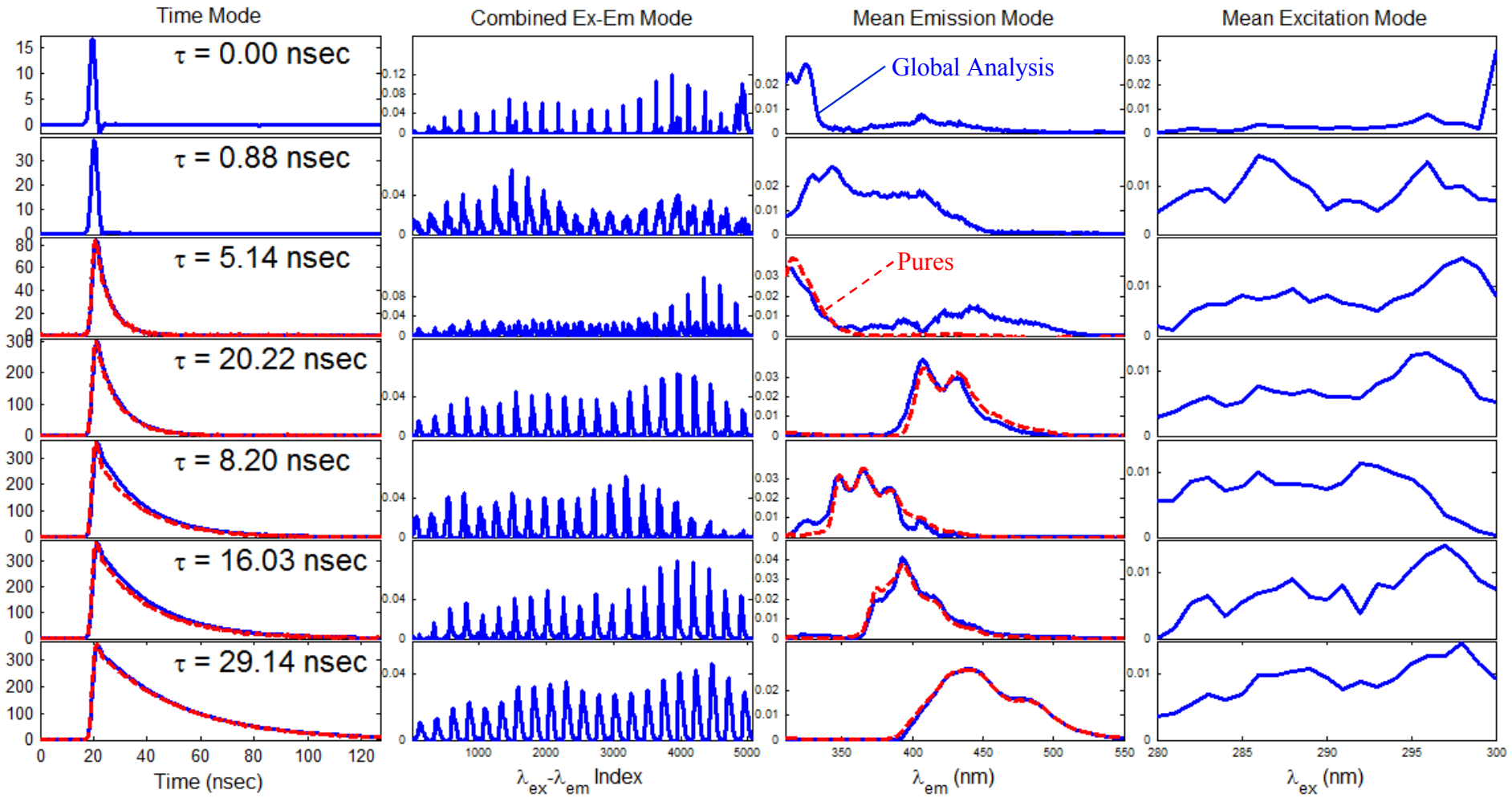
Pure Component Time and Emission Modes





Six Factor + Raman Global Analysis Model



Nonnegativity in combined excitation-emission mode.
Computational time: 156 seconds
Blue: Global Analysis, Red: Pure Components

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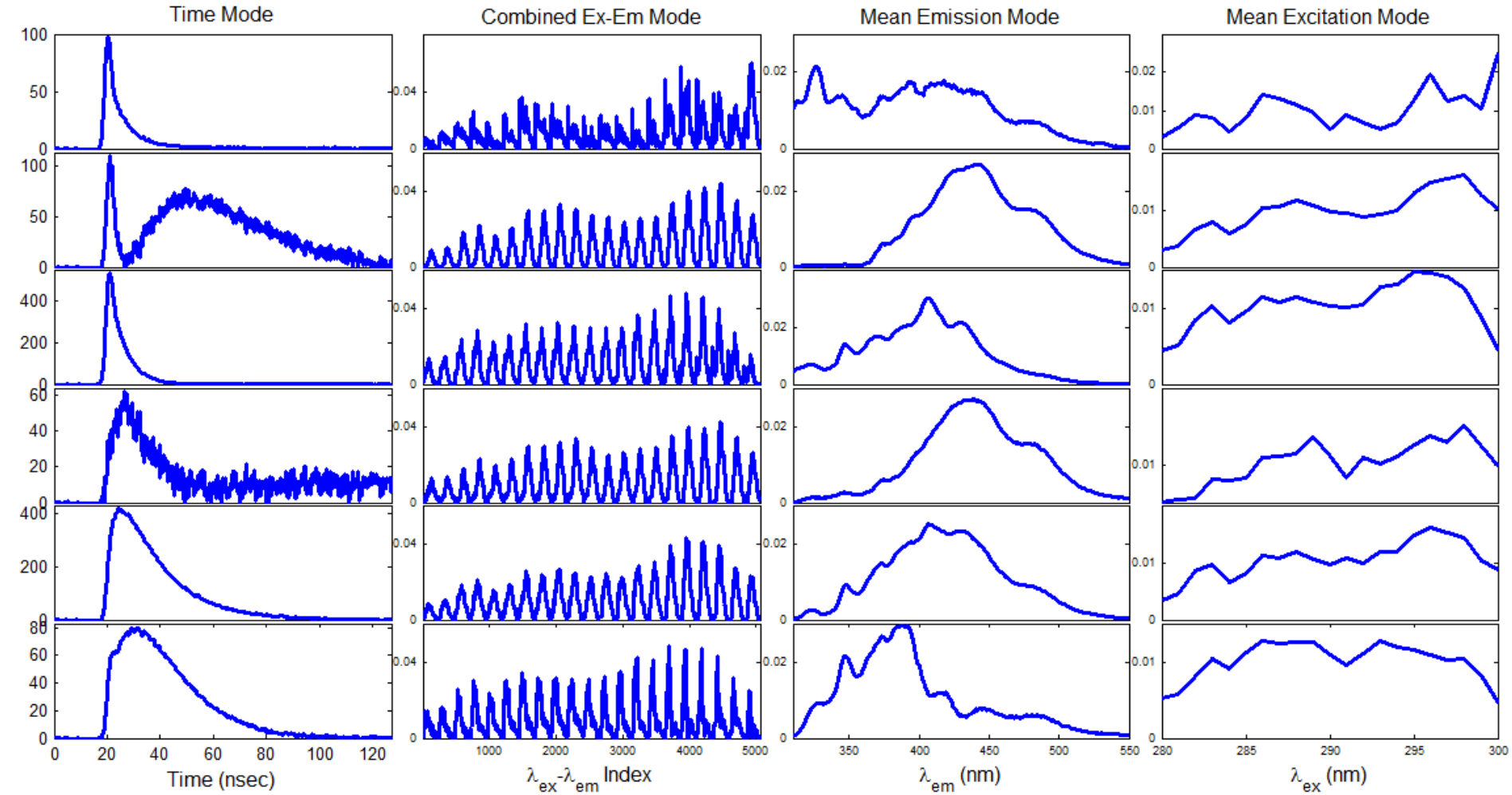
Six Factor + MCR Model



Nonnegativity in time and combined excitation-emission mode.
Computational time: 687 seconds

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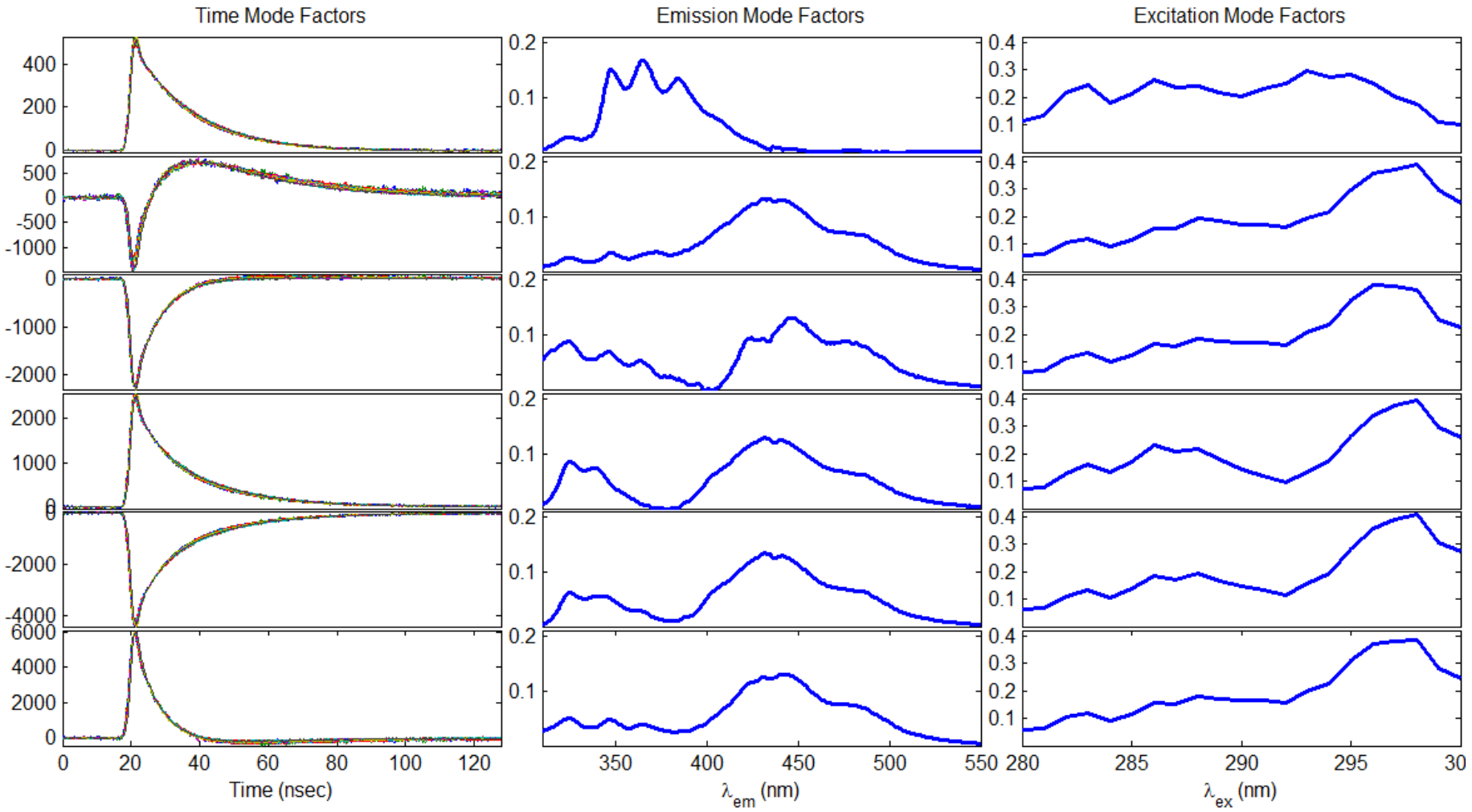
Six Factor PARAFAC2 Model



Nonnegativity in emission and excitation modes.
Computation time: 2182 seconds

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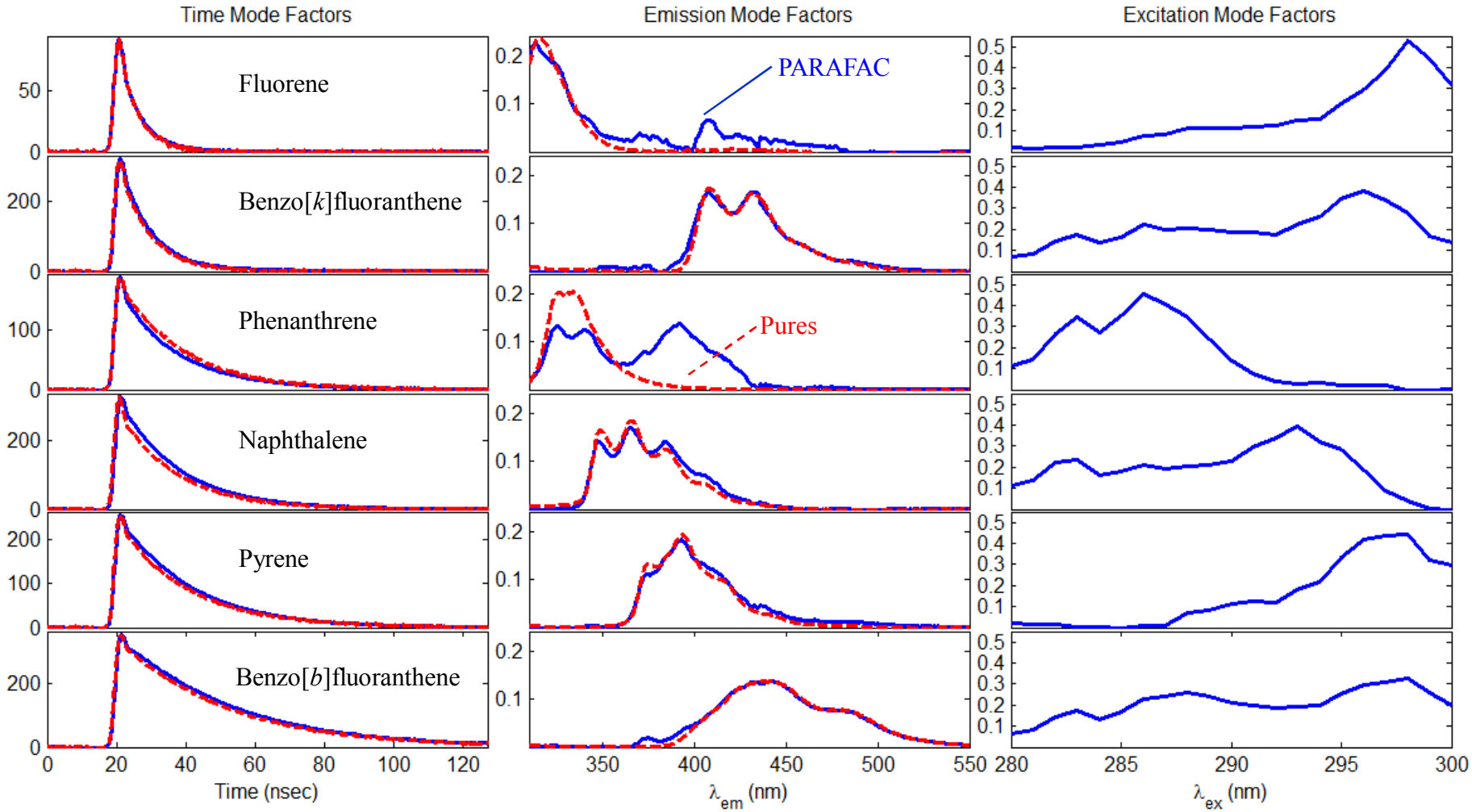
Six Factor Fast-PARAFAC Model



Nonnegativity in all three modes.
Computation time: 14 seconds
Blue: Fast PARAFAC, Red: Pure Components

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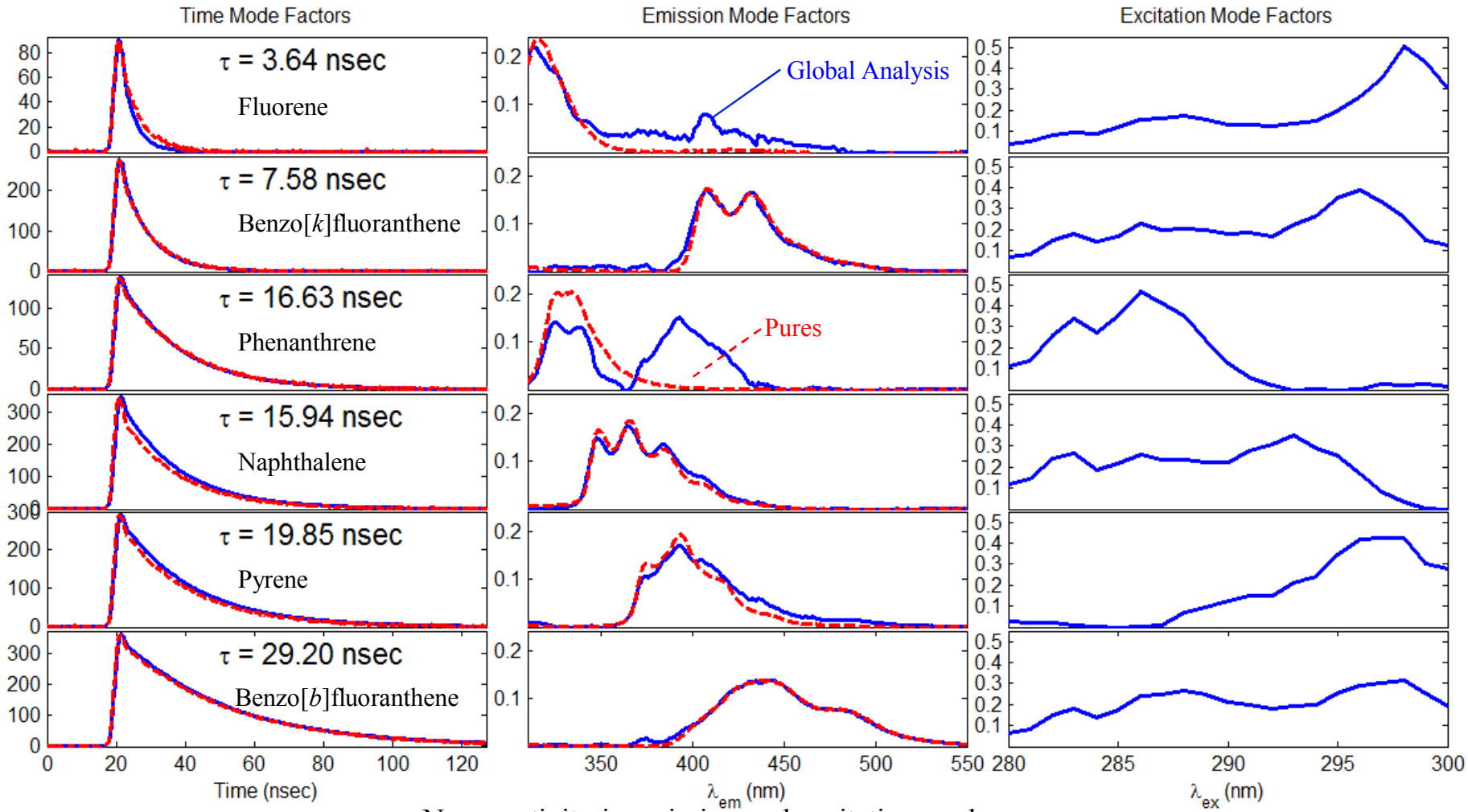
Six Factor 3-way Global Analysis Model



Nonnegativity in emission and excitation modes.
Computational time: 155 seconds
Blue: 3-way Global Analysis, Red: Pure Components

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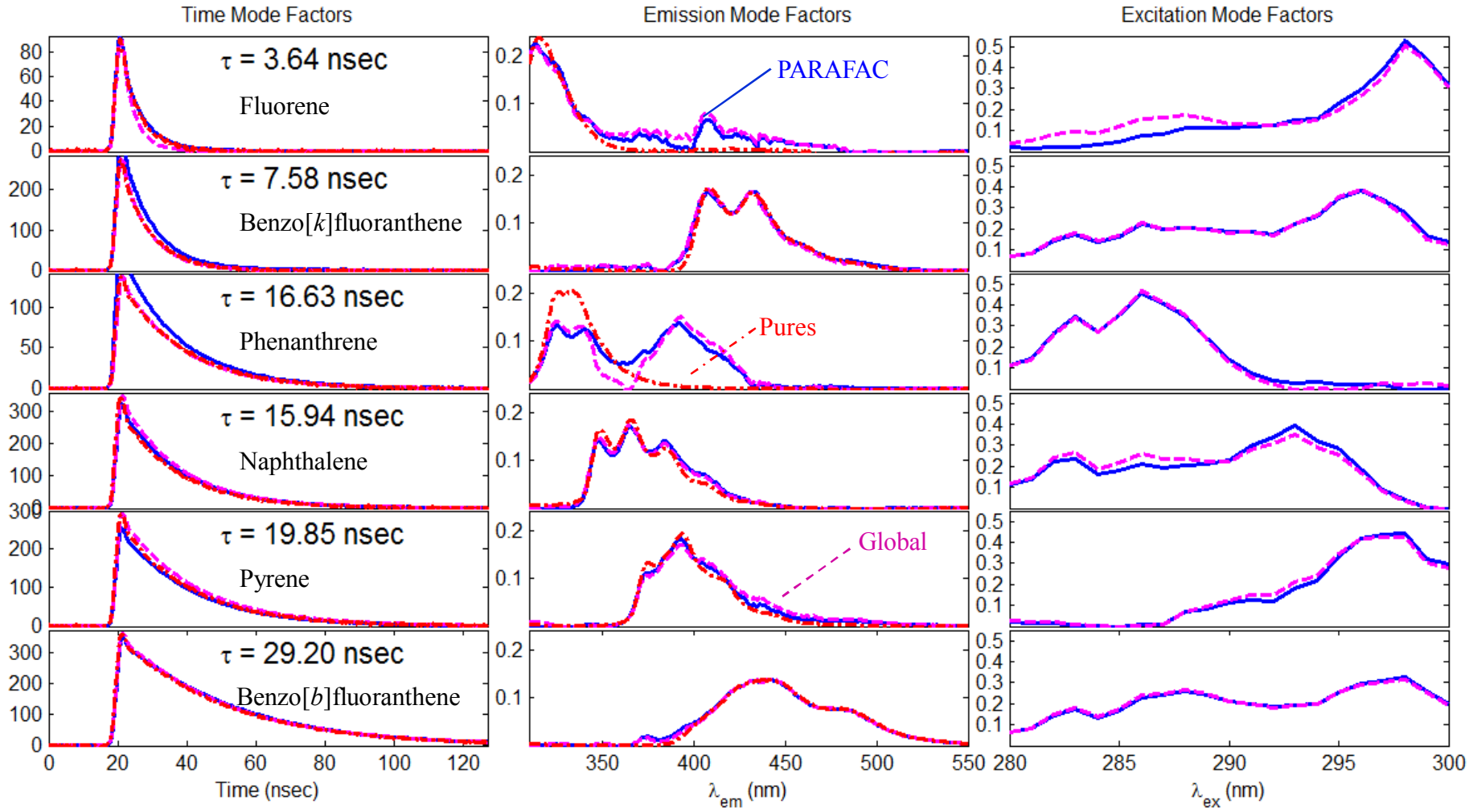
Compare PARAFAC with 3-way Global



Blue: Fast PARAFAC, Magenta: 3-way Global Analysis, Red: Pures

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Results and Conclusions

- High temporal and spectral overlaps pose significant challenges for factor analysis
- Two-mode analysis methods used here, MCR and global analysis, yield poor results on these data
- PARAFAC2 is appropriate for time-shifted data, but inability to impose nonnegativity in time domain results in poor factor resolution
- Fast PARAFAC with imposed nonnegativity renders reasonable pure component factors for most species
  - Time shifting algorithm appears to make a suitable correction for follow-on analysis
  - Predicted pure component emission spectra and decay curves are in good agreement with single species solution data.
- Three-way global analysis also produces impressive results
  - Again, predicted pure component emission spectra and decay curves are in good agreement with single species solution data.

Future Work

- Attempt collection of data to include additional excitation mode data at finer intervals
- Investigate methods to impose nonnegativity for all modes in PARAFAC2
- Improved algorithms for performance of three-way global analysis
  - Collect laser inputs for each excitation wavelength to obviate need to shift each WTM
  - Optimize algorithm to reduce computational burden

Acknowledgements

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