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The Amazing Flexibility of Fluorescence Spectroscopy for Trilinear Modeling

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Overview

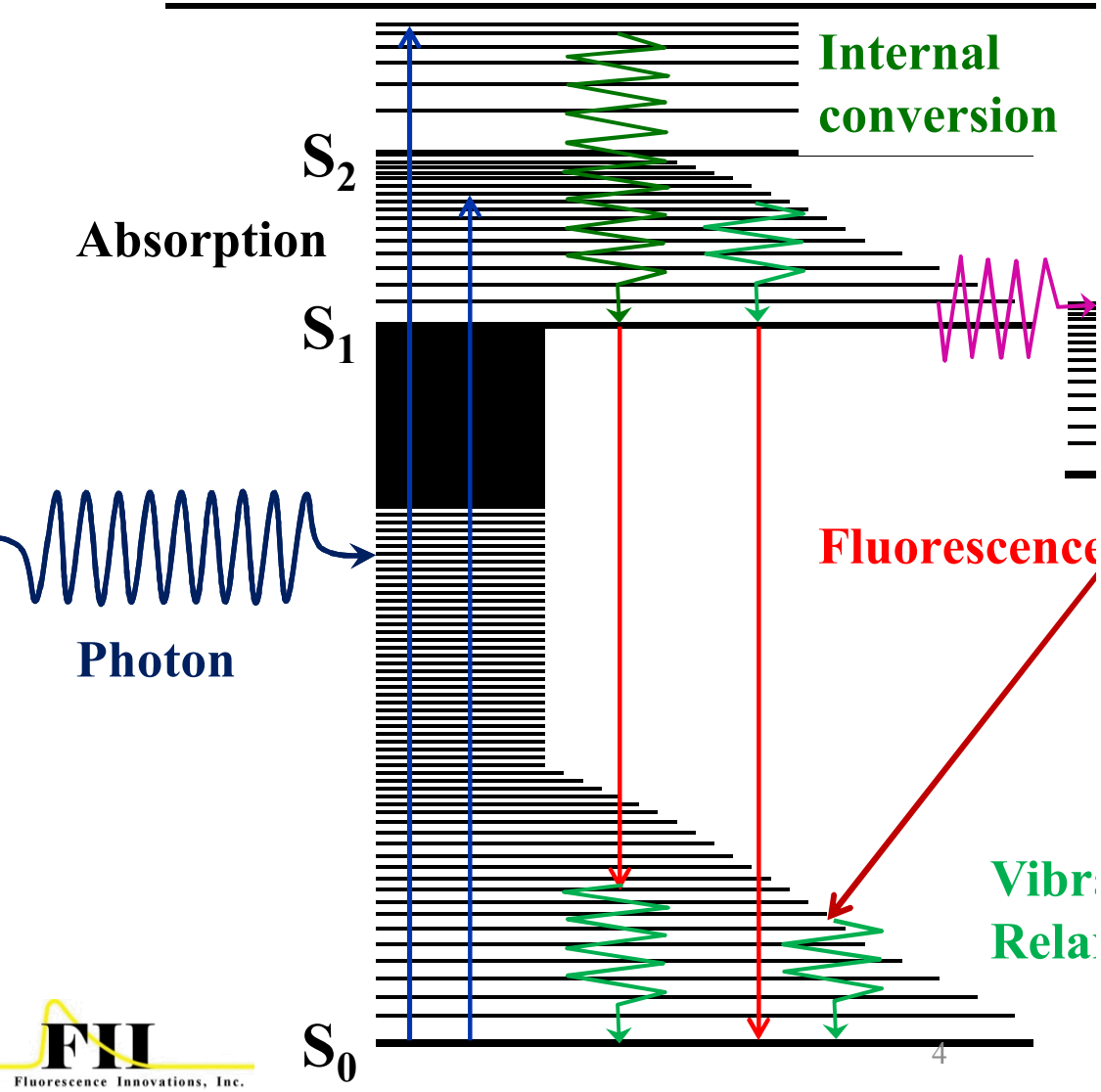
- Introduction and motivation for research
- Theory of fluorescence
- Simulations
  - Standard method of data collection
  - Novel method of data collection
- Results
- Conclusions and future directions

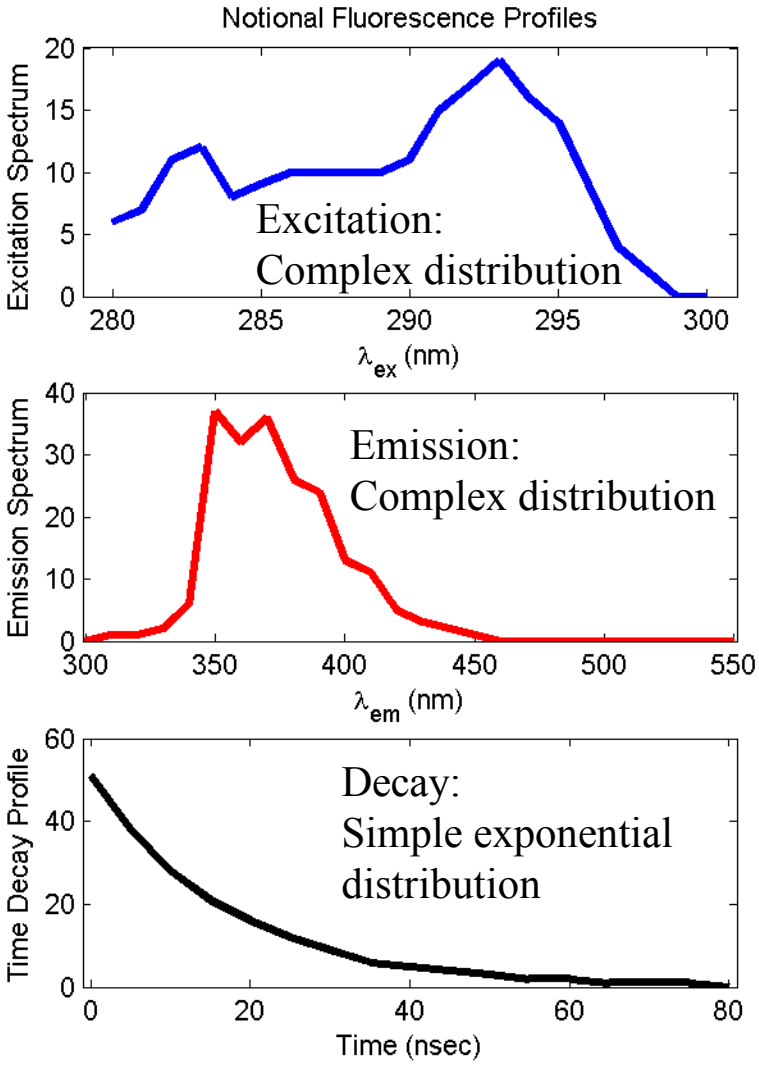
Introduction

- Drug discovery and development can be a long and costly process
  - $881M [1] to $1.2B[2]
  - 7-12 years[3,4]
- Rapid, high-throughput screening (HTS) of potential drug candidates can improve the discovery process.
  - Formerly, drug development based upon targeted chemical synthesis based on structure activity relationship[5]
  - HTS evaluates all possible drug candidates looking for activity "hits"[5]
- Molecular fluorescence measurements including emission wavelength and lifetime changes can indicate drug activity
- Multiway fluorescence measurements provide greater analytical power than single modalities

[1] G. F. Vande Woude, [Genomics in Cancer Drug Discovery and Development] Burlington : Elsevier Science, Burlington(2011).
[2] V. Litwin, [Flow Cytometry in Drug Discovery and Development] Hoboken : Wiley, Hoboken(2010).
[3] J. A. Rosier, [Global New Drug Development An Introduction] Hoboken : Wiley, Hoboken(2014).
[4] A. A. Ciociola, L. B. Cohen, P. Kulkarni et al., "How Drugs are Developed and Approved by the FDA: Current Process and Future Directions," American Journal of Gastroenterology, 109(5), 620-623 (2014).
[5] [Integrated drug discovery technologies] New York : Marcel Dekker, New York(2002).

How does fluorescence arise?
The Jablonski Diagram

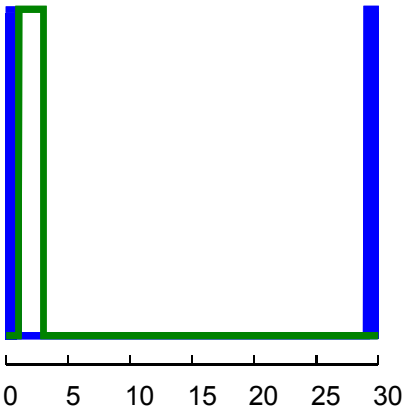




Novel trilinear data collection scheme

- Excite using a boxcar-type pulses of varying widths
  - e.g., widths of 1, 2, 5, 10 and 20 nanoseconds (nsec)
- Collect fluorescence emission with a boxcar-type window using a gated photomultiplier tube (PMT)
  - Position the gate temporally to open immediately after the excitation pulse ends
- Employ a time-gated, multi-channel (anode) PMT to allow simultaneous collection of up to 32 wavelength bins
  - Gives two-way data for each mixture sample
- Collection of multiple samples yields three-way data
- Simulation with a mixture of three chemical species
  - Anthracene (ANT)
  - 9,10 dibromoanthracene (DBA)
  - 9,10 diphenylanthracene (DPA)

Time-mode Collection Method



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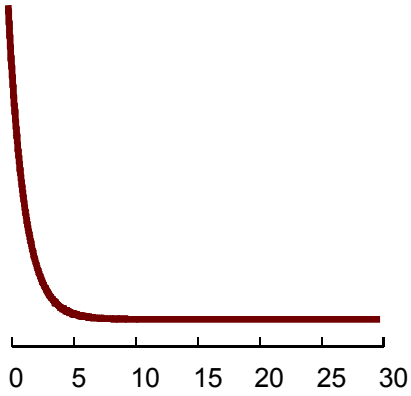

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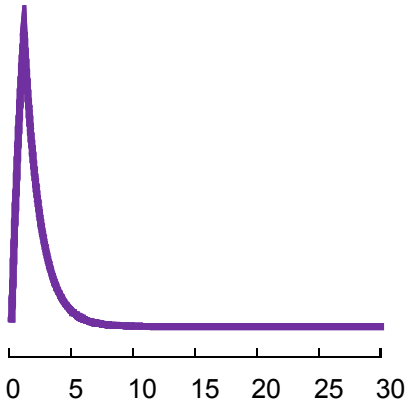

1 nsec excitation pulse

DBA
τ = 1.3 nsec

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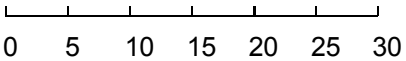

Integrate fluorescence over 2 nsec PMT gate

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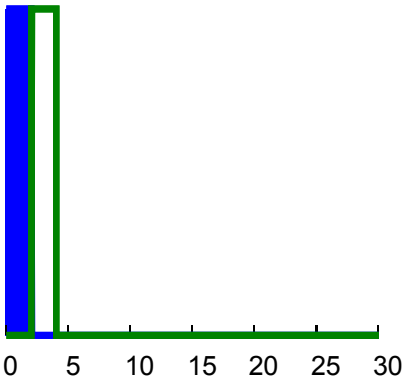
Effect of short duration excitation pulse



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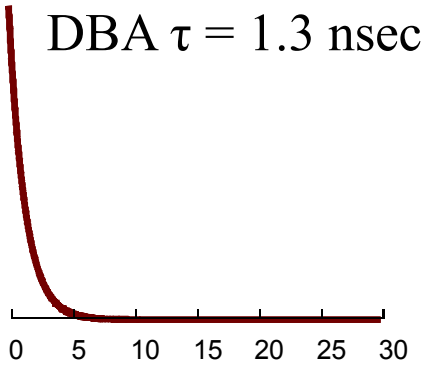

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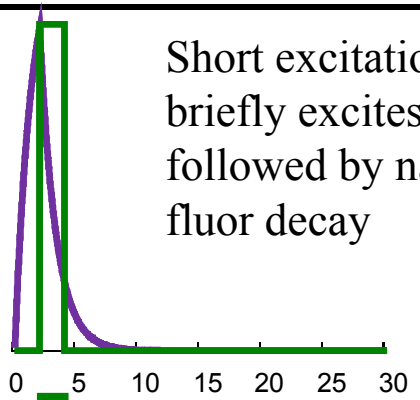

Short excitation pulse briefly excites followed by natural fluor decay

20 nsec excitation pulse
2 nsec PMT gate

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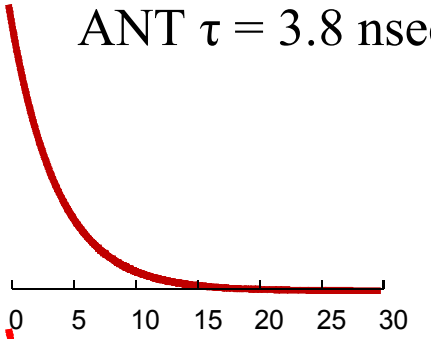

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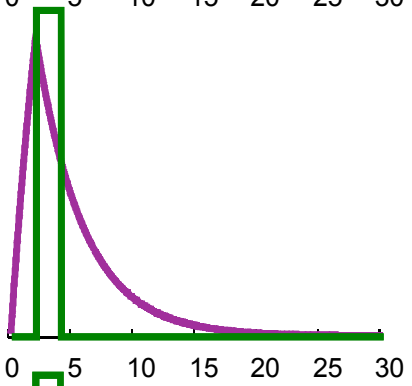

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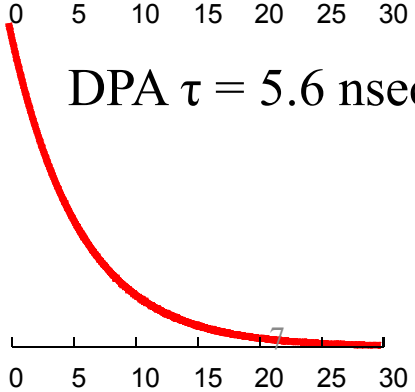

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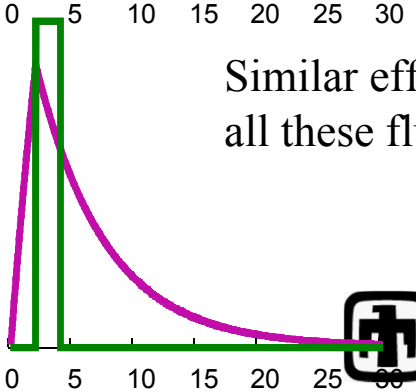
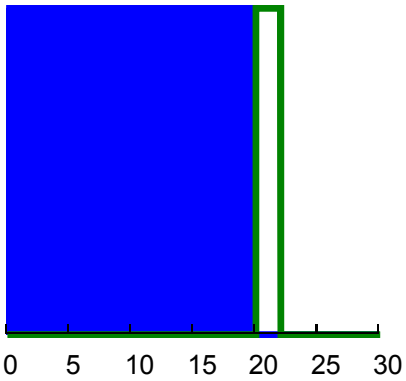


Similar effect for all these fluors

Effect of long duration excitation pulse



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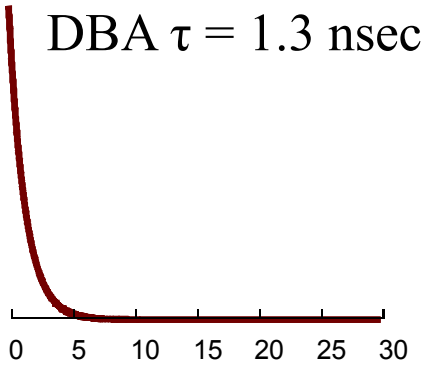

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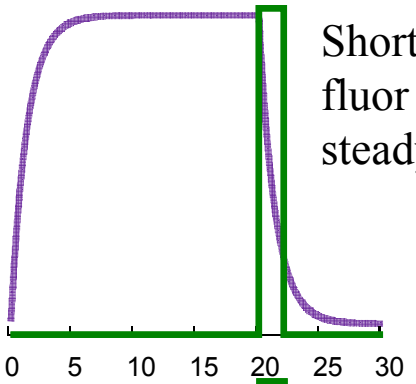

Short lifetime fluor reaches steady state

20 nsec excitation pulse
2 nsec PMT gate

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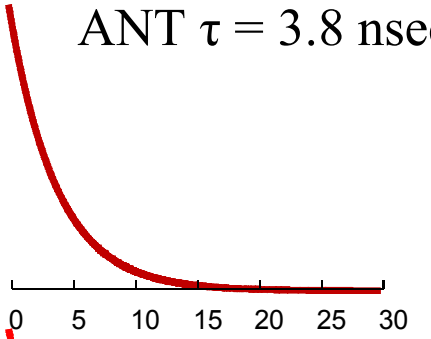

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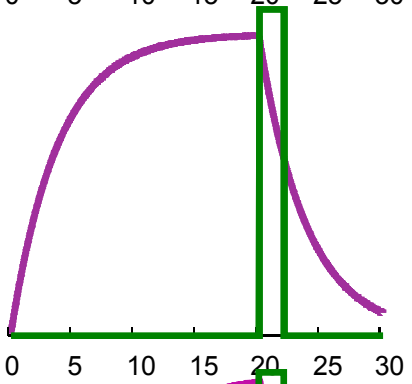

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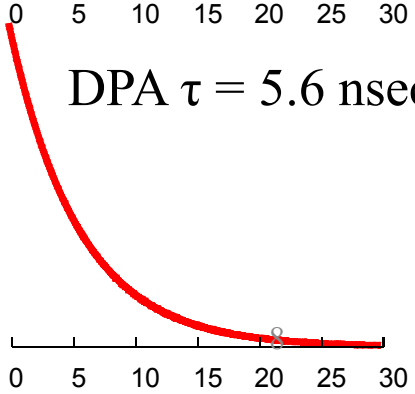

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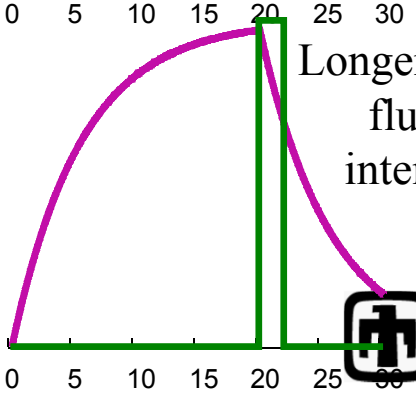
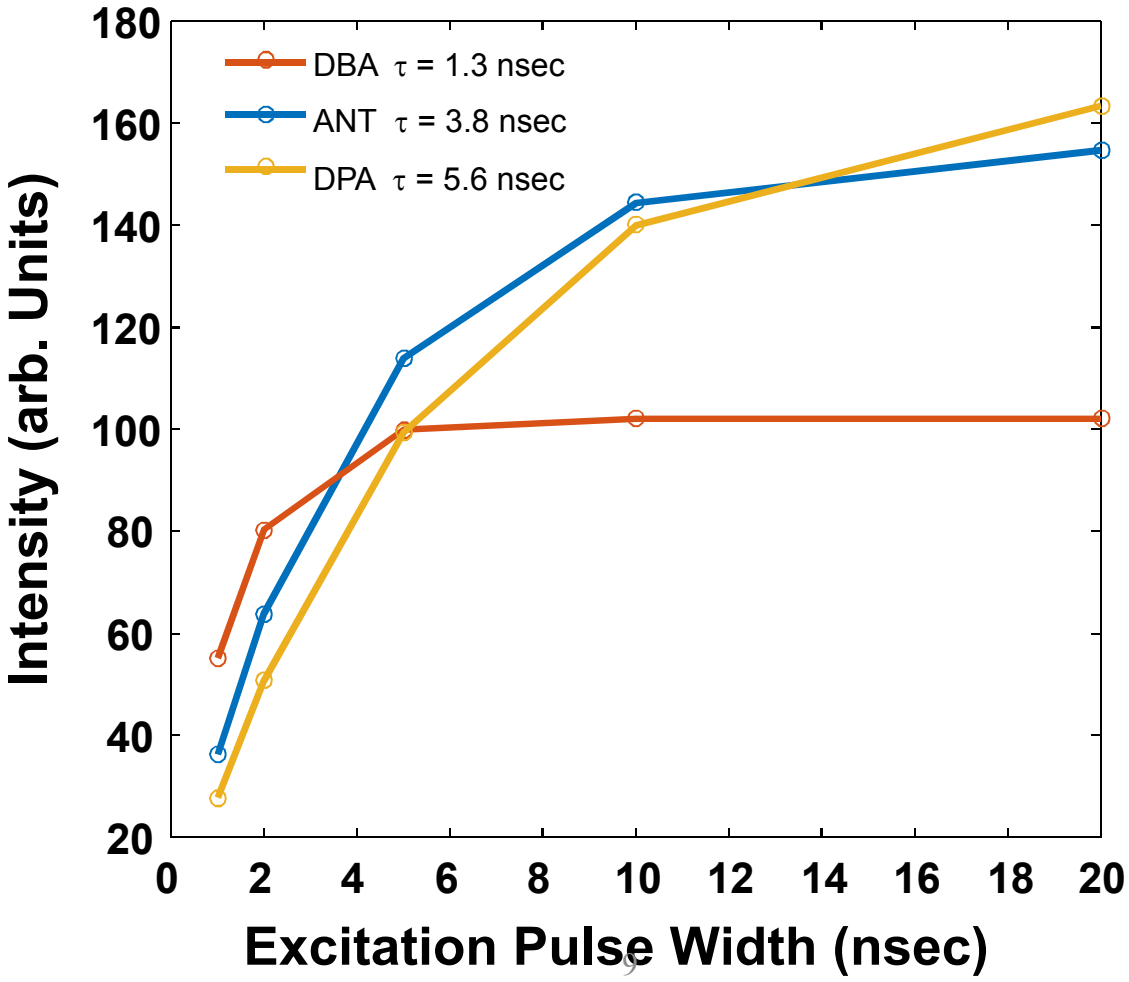


Longer lifetime fluor shows intensity still growing

Temporal Mode Notional Factors



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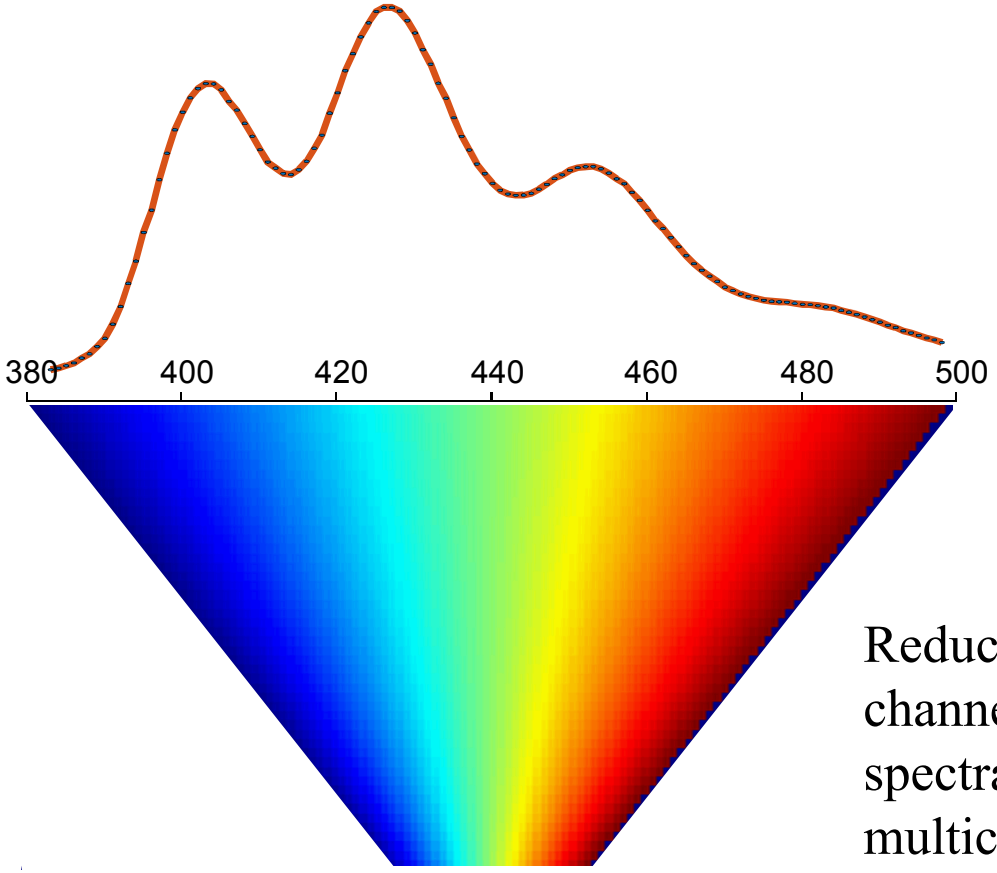
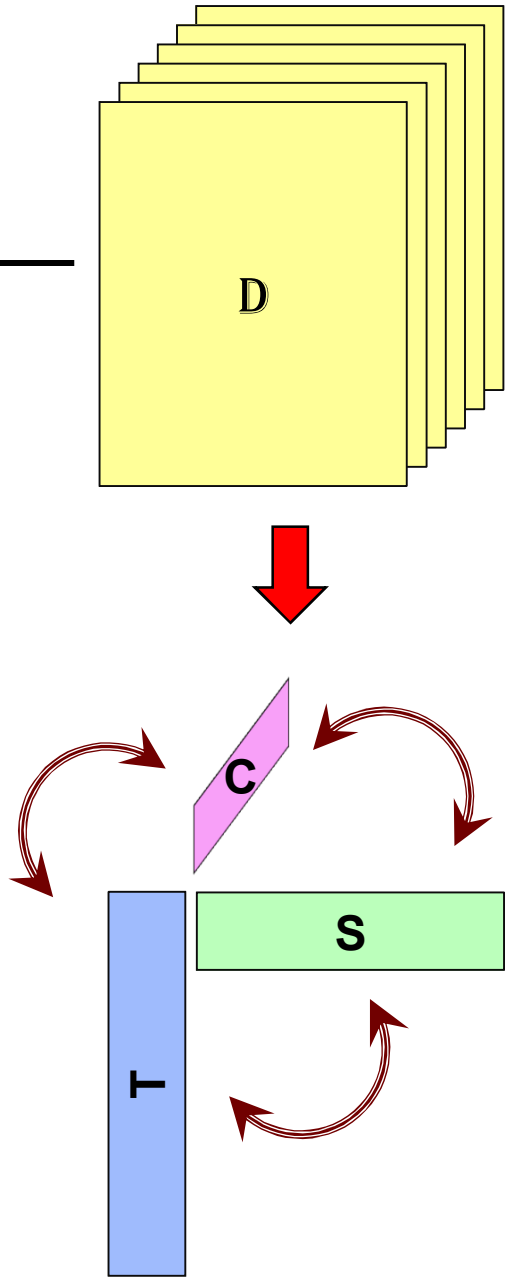
DBA Spectral Factor Compression



Reduce from 116 channels to 29 to simulate spectral binning with multichannel PMT

PARAFAC (Conceptual)

- Objective
  - Least squares model of $\mathbb{D} = \otimes\{T,S,C\}) + \mathbb{E}$
  - T matrix of time-mode decay profiles
  - S matrix of emission mode profiles
  - C matrix of excitation mode profiles
- Decomposition of $\mathbb{D}$
  - Initialize with some guesses for T and C
  - Using estimates for T and C, solve for S
  - $S = (T^T T * C^T C)^{\ddagger} (T \odot C)\ {}_{CT}D_S$
  - Using estimates for S and T, solve for C
  - $C = (S^T S * T^T T)^{\ddagger} (S \odot T)\ {}_{TS}D_C$
  - Using estimates for C and S, solve for T
  - $T = (C^T C * S^T S)^{\ddagger} (C \odot S)\ {}_{SC}D_T$
- Iterate until convergence



Simulated Standard Time Domain Data

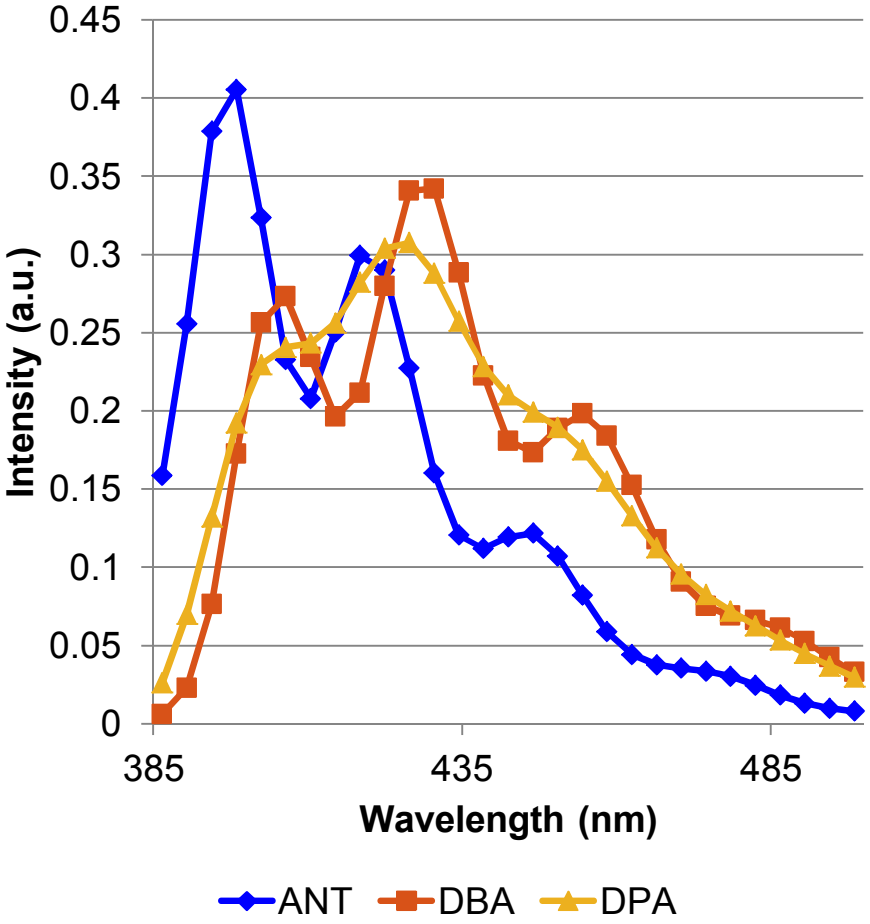
- Time domain from 0-30 nsec at 0.2 nsec intervals
  - Exponential decay based of lifetime
- Use 4x wavelength data compression
  - Reduce from 116 increments to 29
  - Makes comparable to pulse method
- Form triple product with same spectral and concentration pures
- Scale maximum values of 10,000 counts before adding noise
- Add “Poisson-like” noise using percentage of root maximum signal
  - E.g., Root of 10,000 counts is 100 or 1%. 1% is root of max signal times values drawn from standard normal distribution
  - Three levels 1%, 0.5% and 0.2%
- PARAFAC conditions
  - First 1000 iterations impose rigorous nonnegativity
  - Convergence at 10^{-8} RMSE difference between iterations
  - Random starts for 10 trials

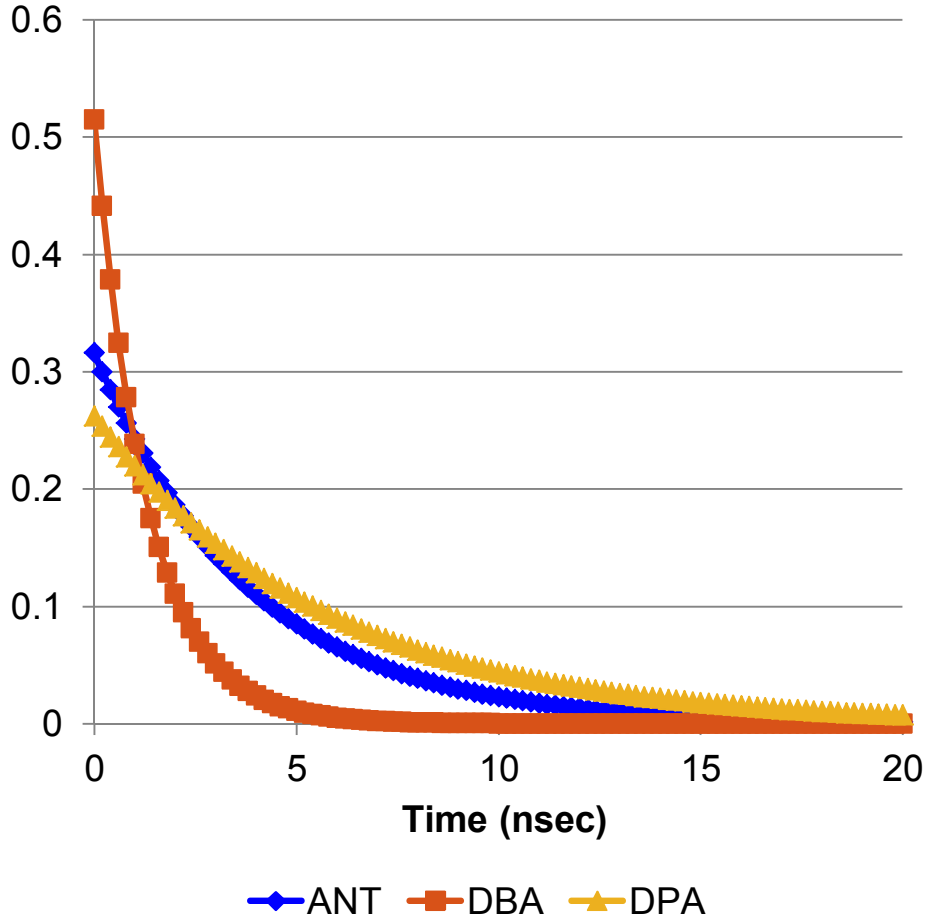
Design Concentrations

- Create additive design concentration matrix
- Simulates progressive additions to a pure solvent
- Provides high sample-to-sample intensity contrast
- Used same design for both

Design Concentrations		
ANT	DBA	DPA
100	100	0
200	100	100
300	300	200
400	500	400
500	500	500

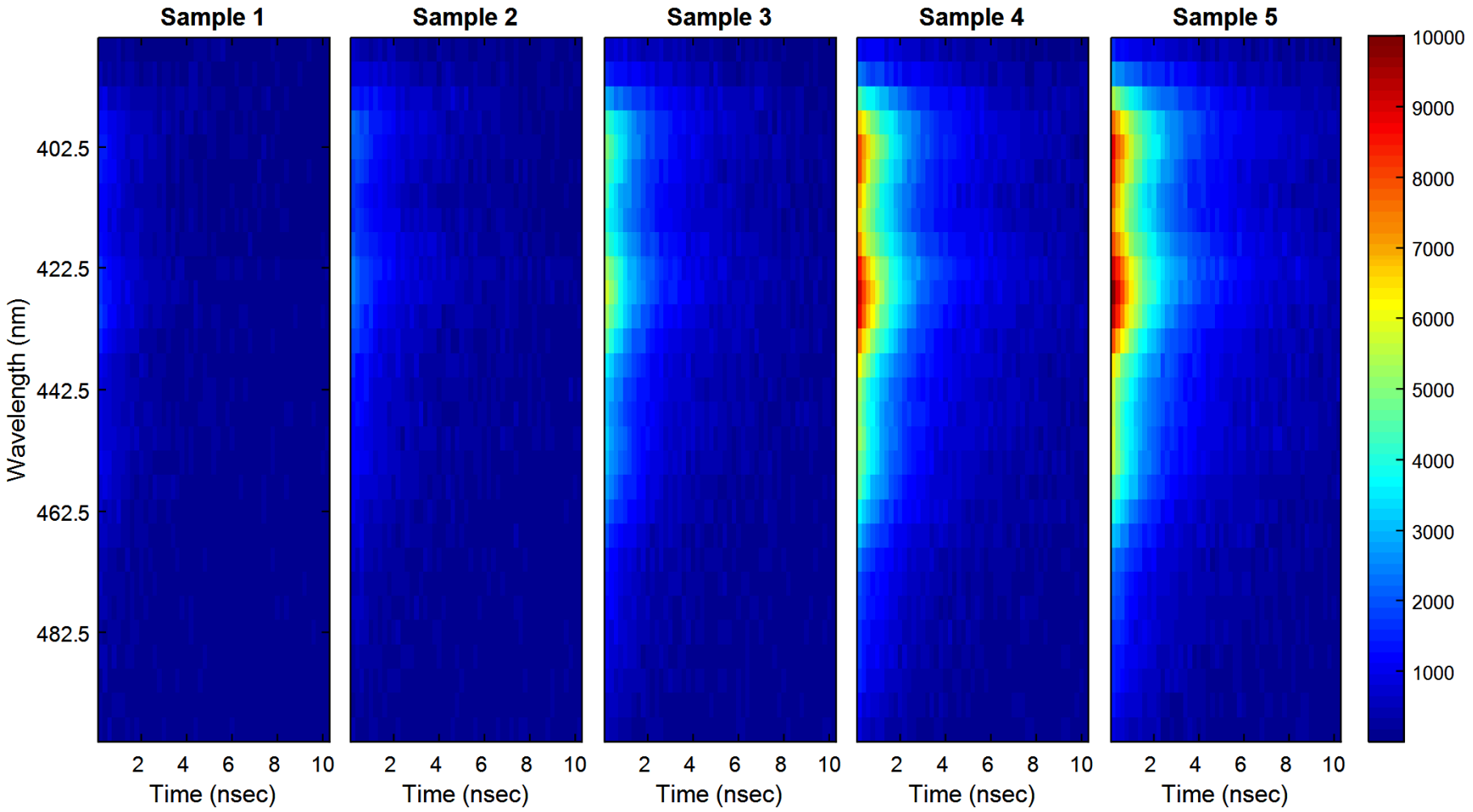
Pure Spectral and Time Decay Components



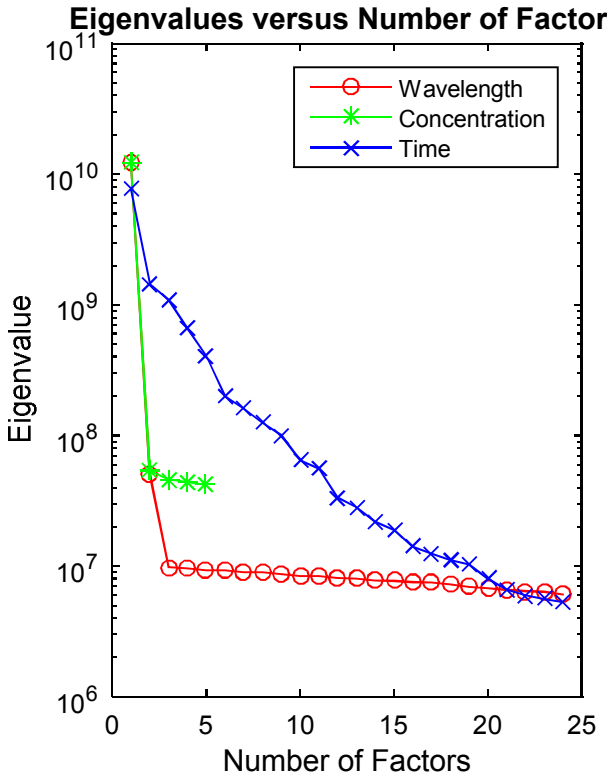


Simulated Data

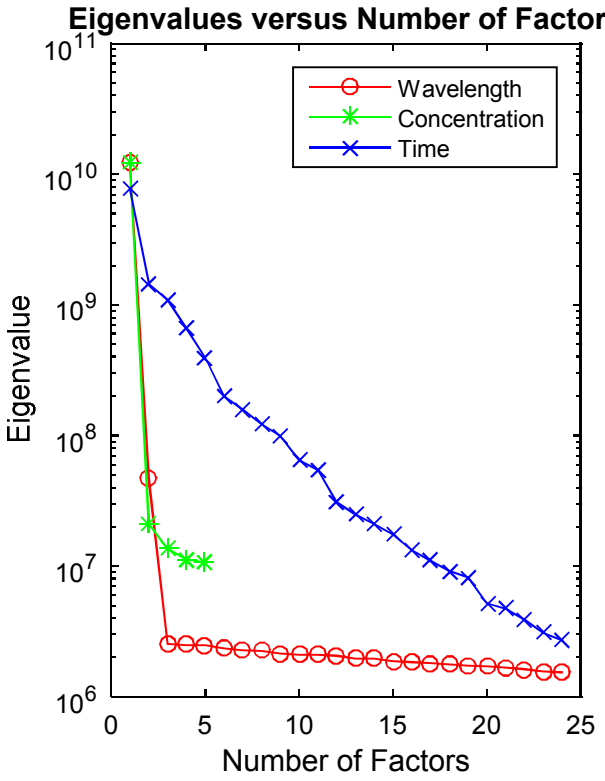
Trilinear Fluorescence Data with 1% of Data Noise



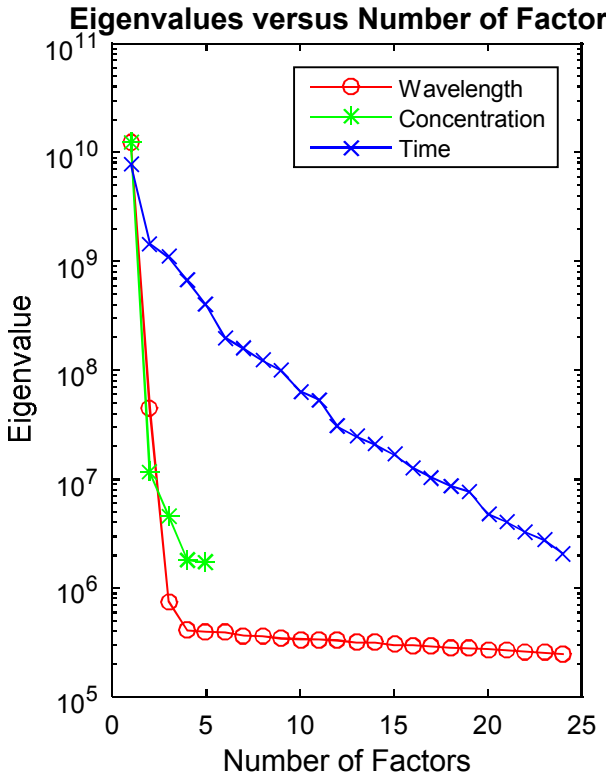
Eigenvalues Traditional Data



1% Noise

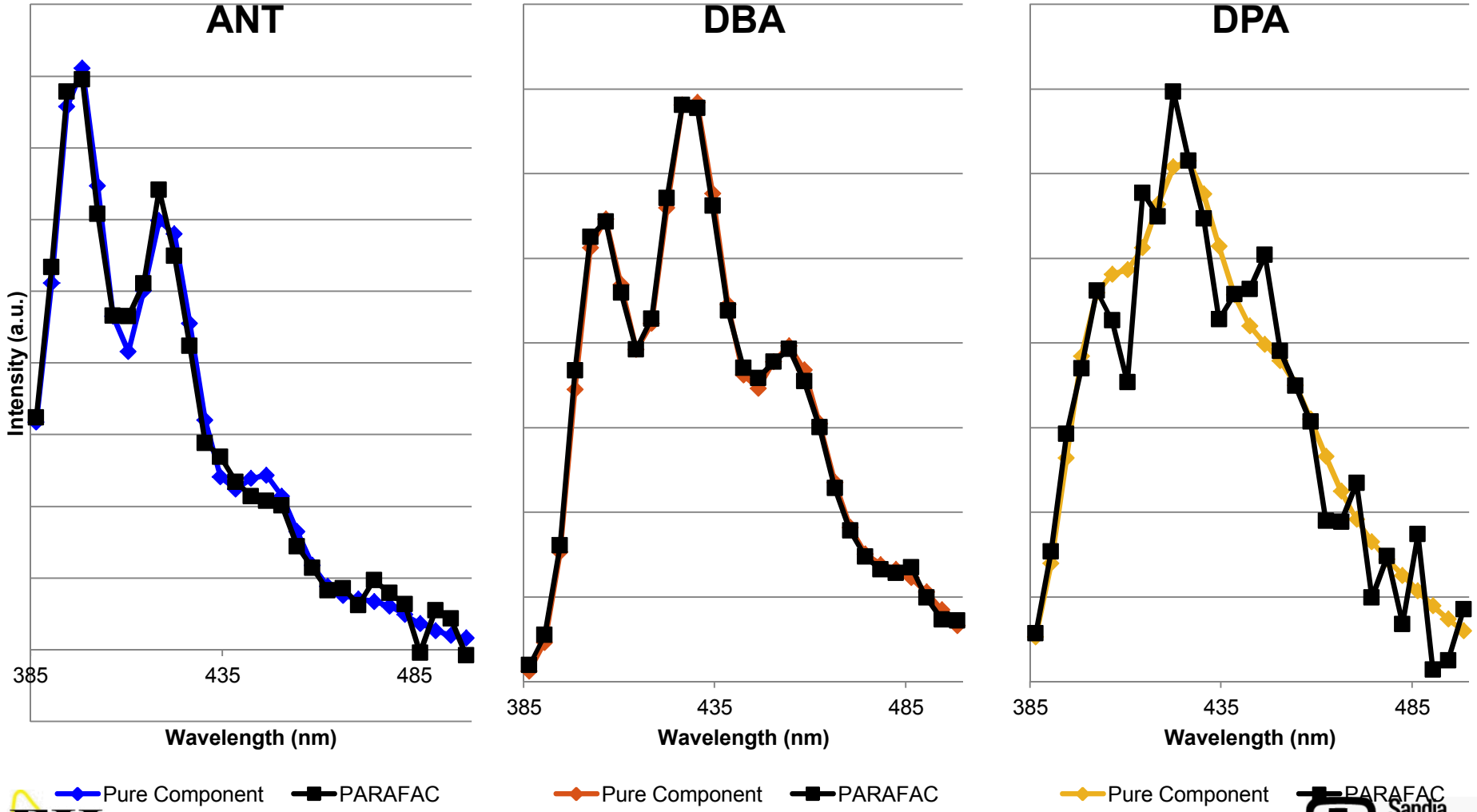


0.5% Noise



0.2% Noise

Wavelength-mode Factors (1% noise)



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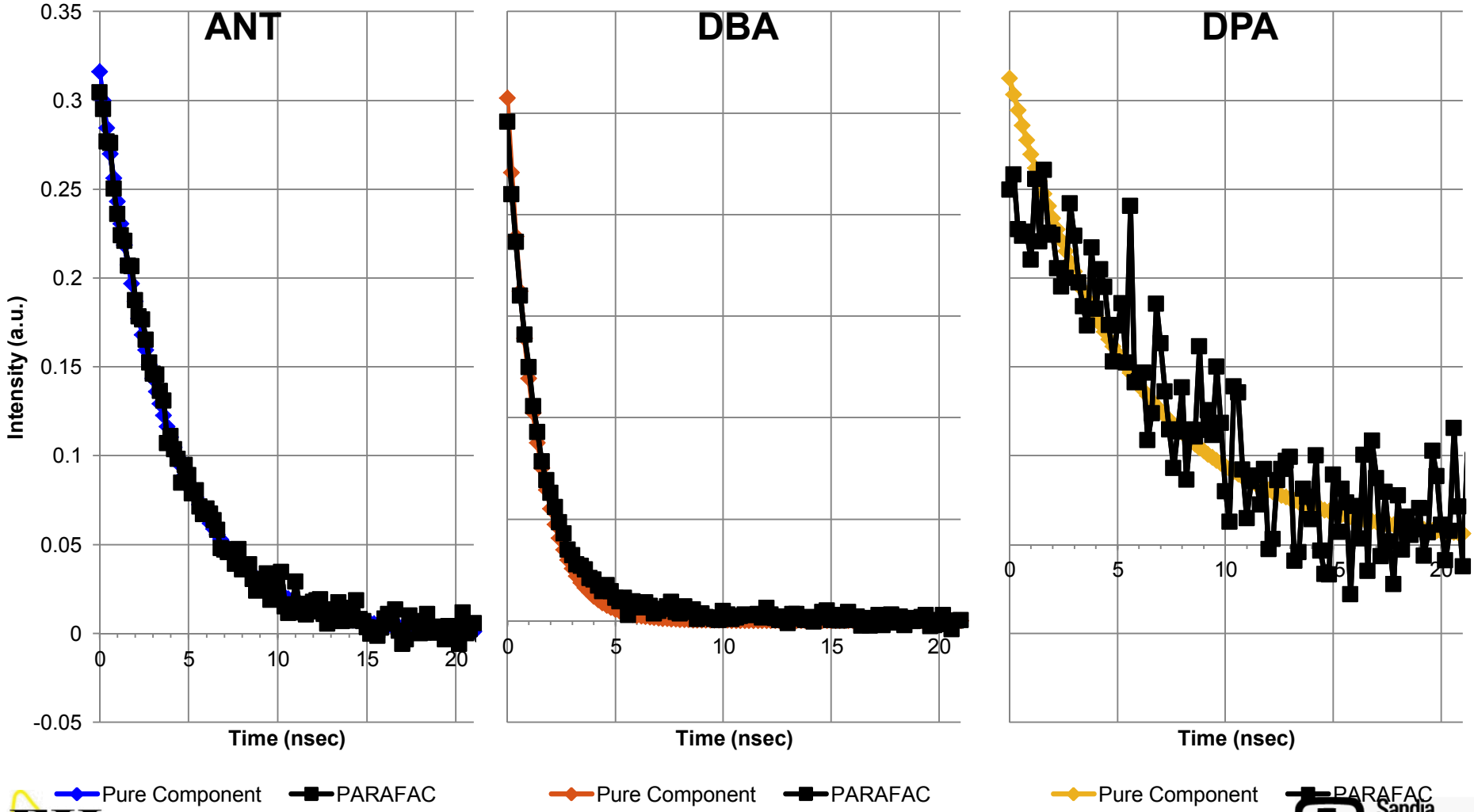
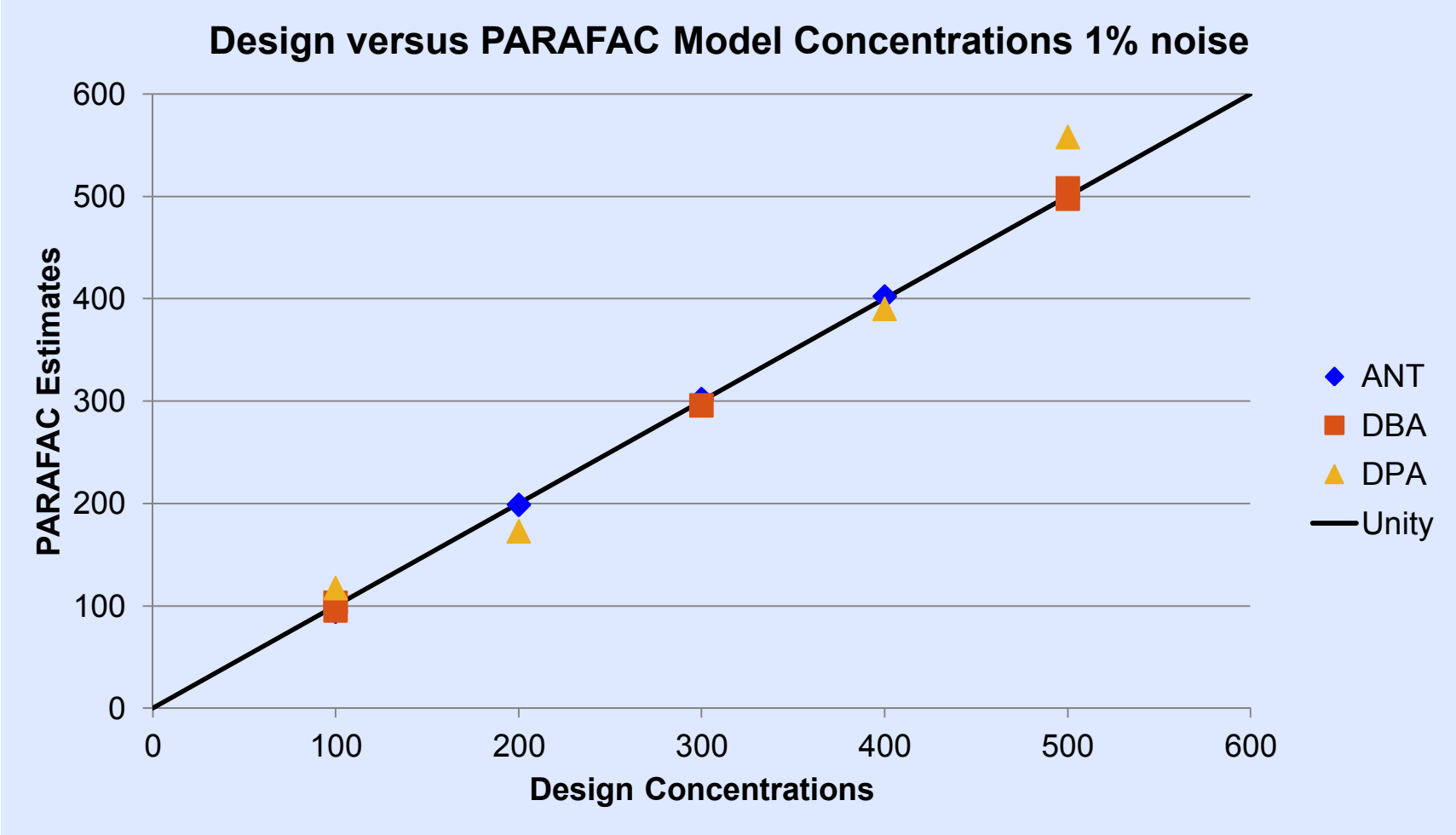
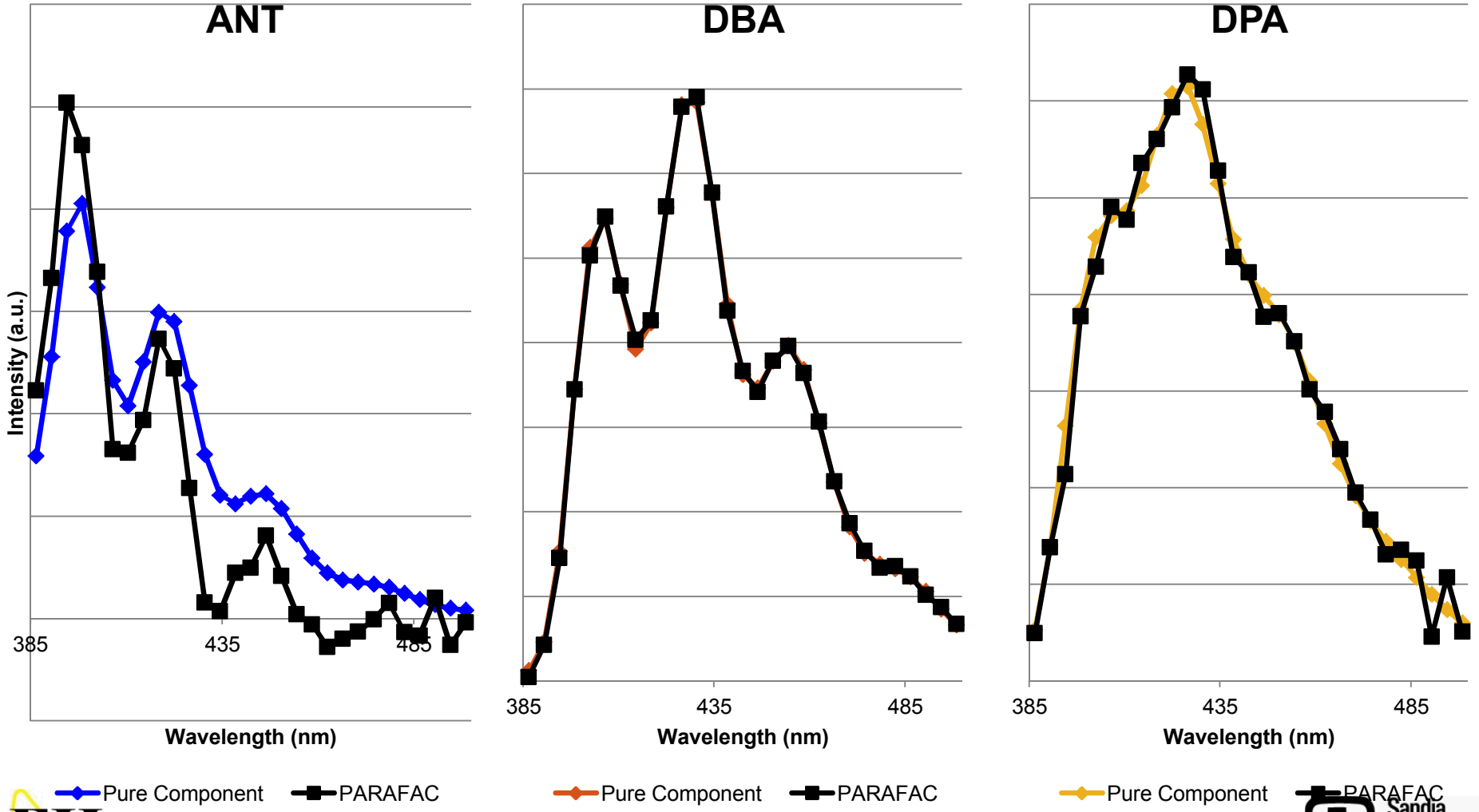
Temporal-mode Factors (1% noise)



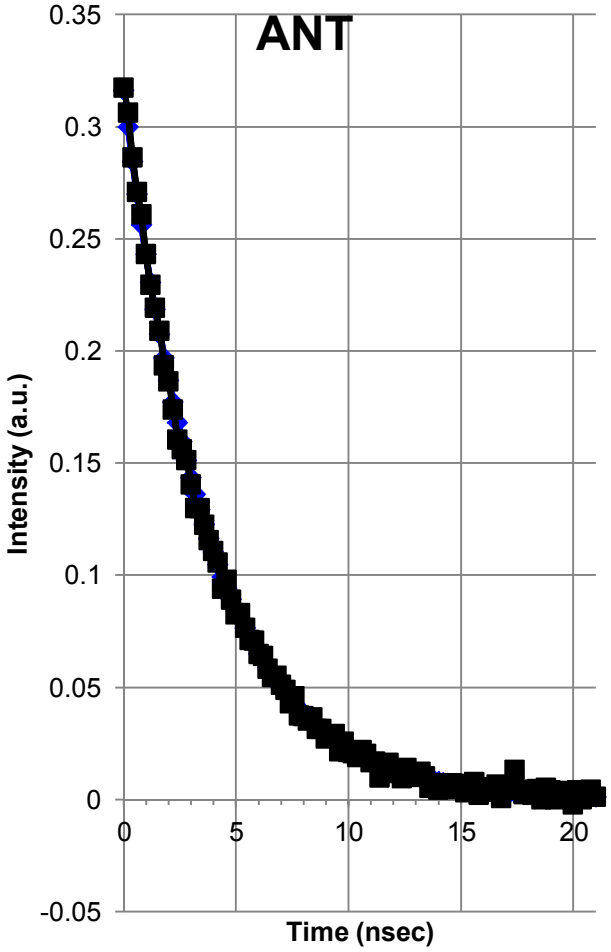
Concentration-mode Factors (1% noise)

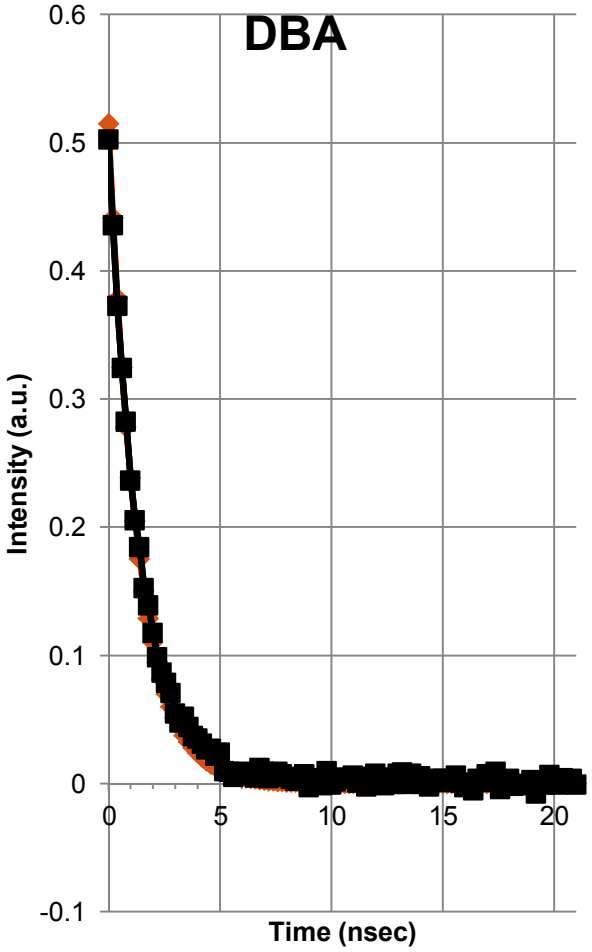


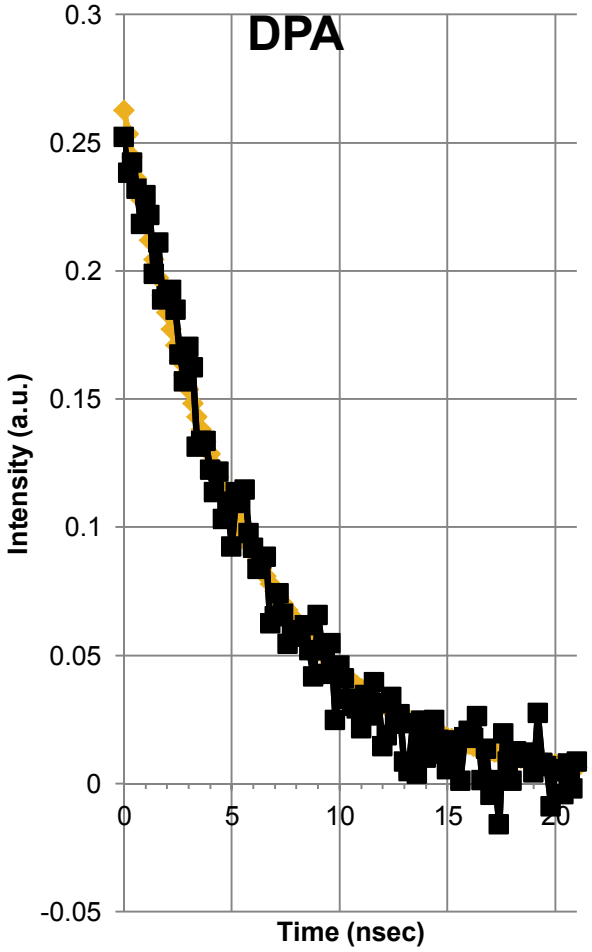
Wavelength-mode Factors (0.5% noise)



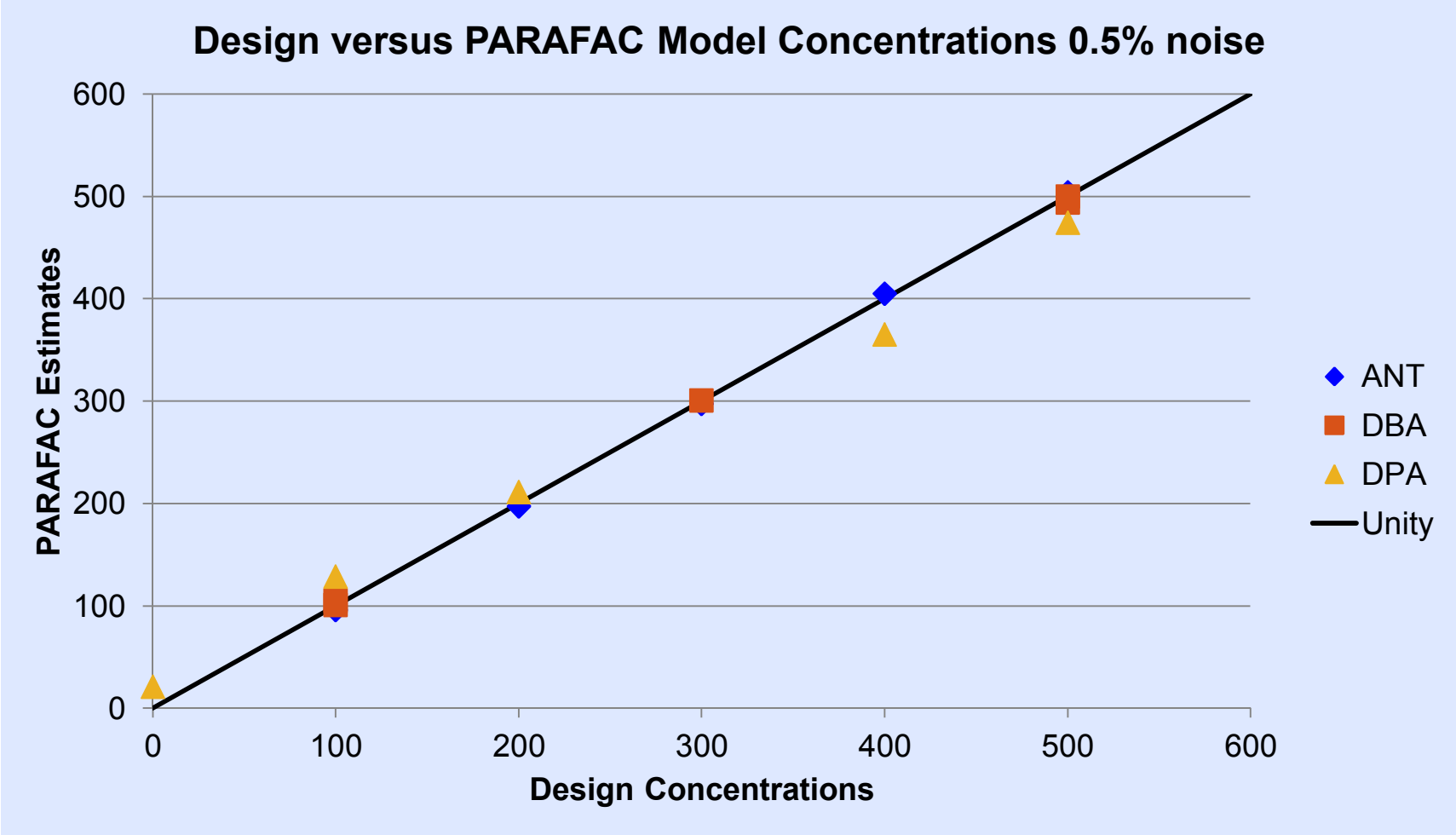
Temporal-mode Factors (0.5% noise)



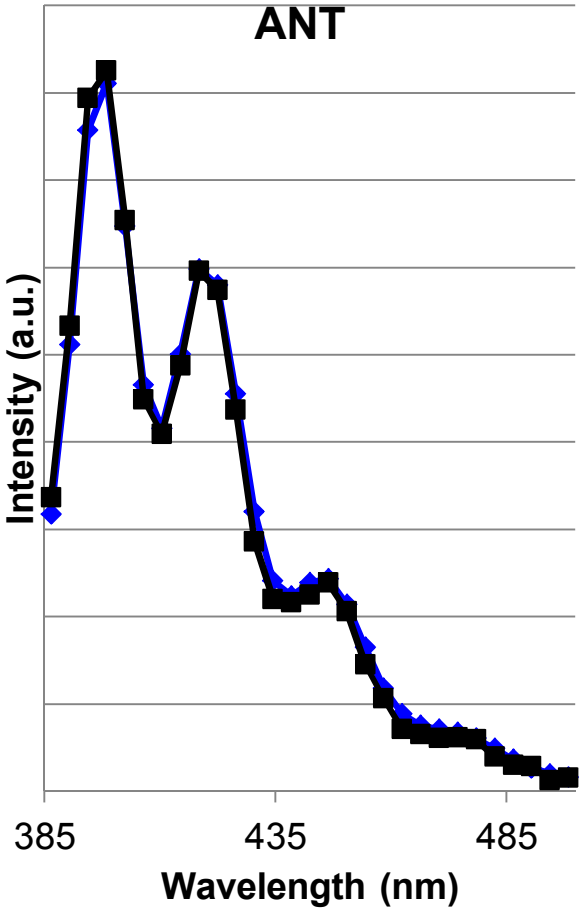


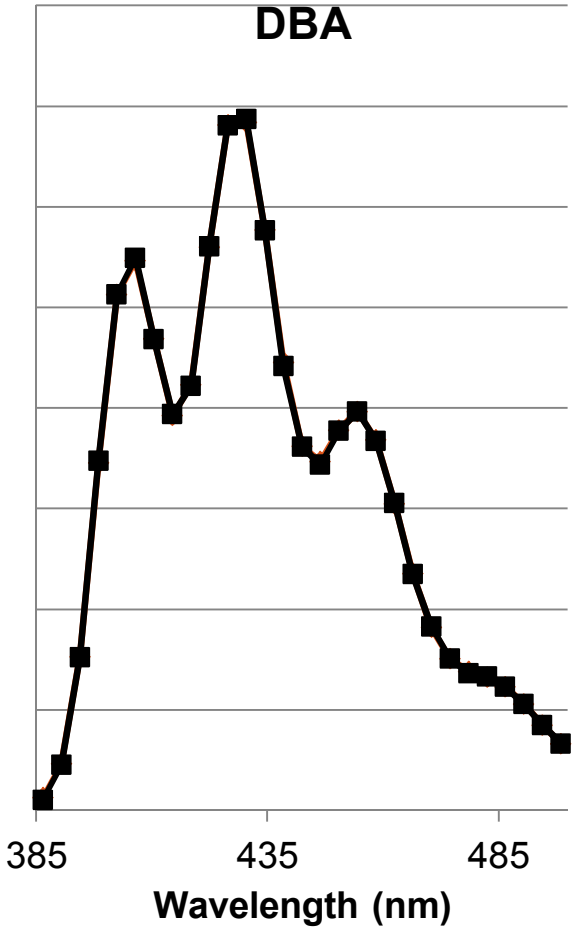


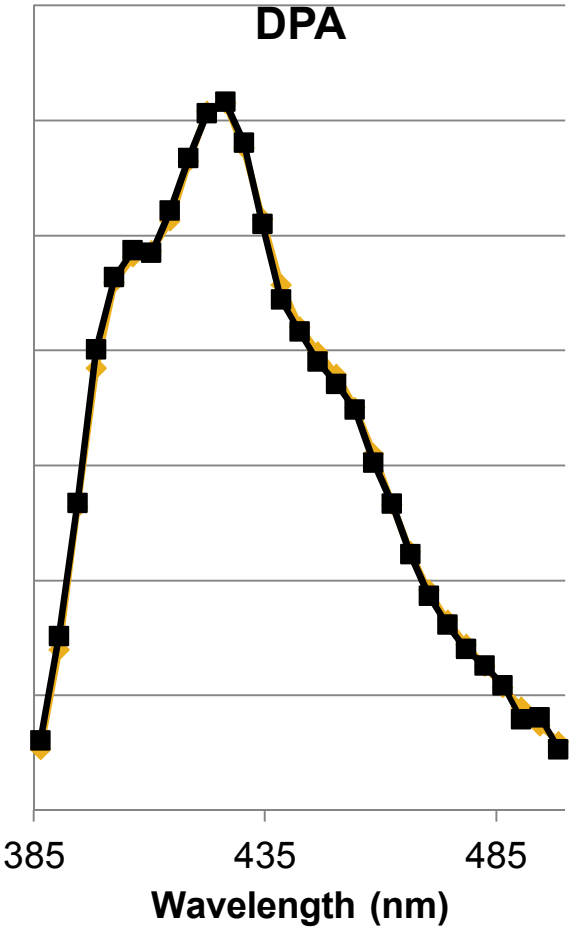
Concentration-mode Factors (0.5% noise)



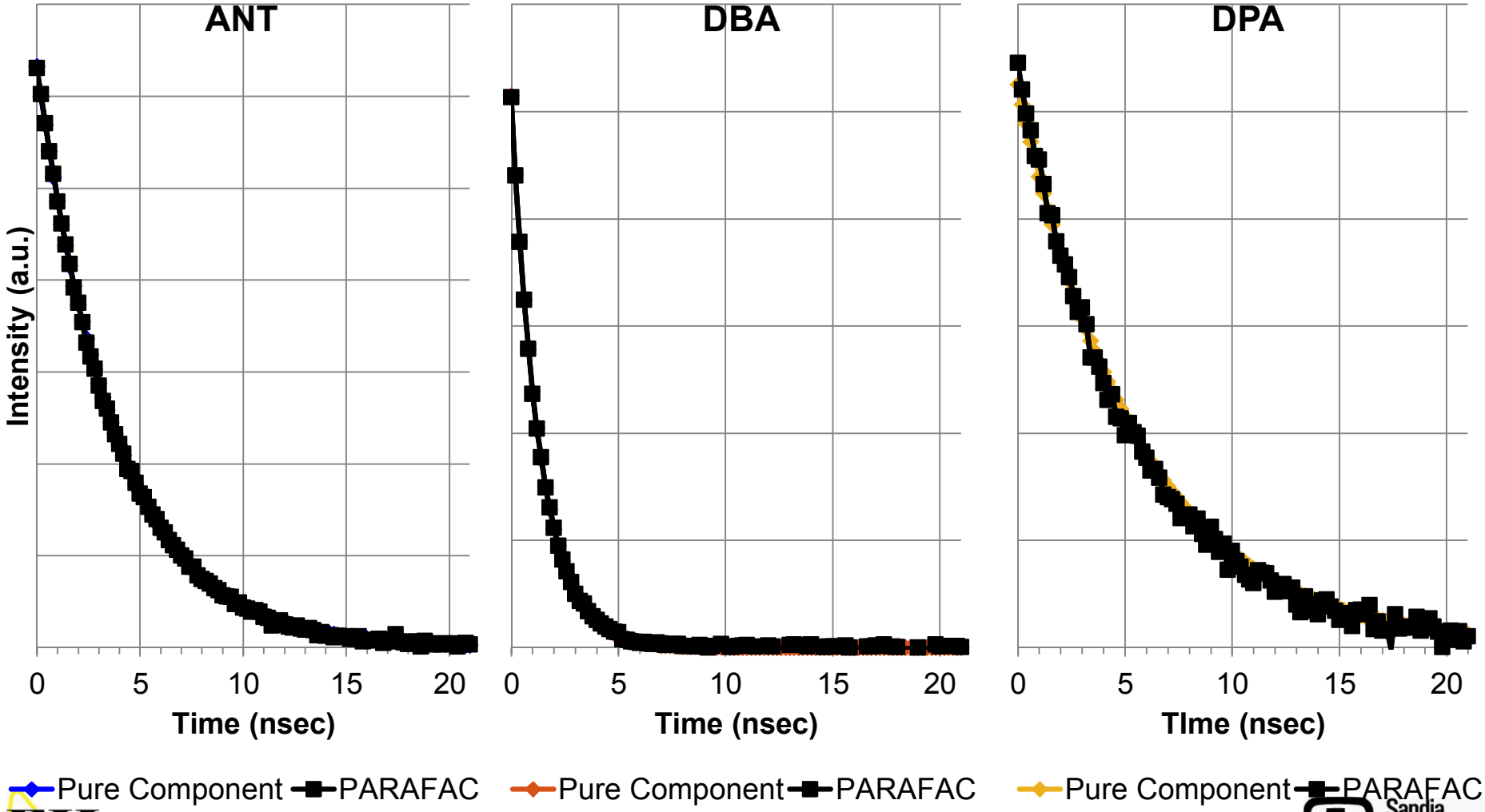
Wavelength-mode Factors (0.2% noise)







Temporal-mode Factors (0.2% noise)



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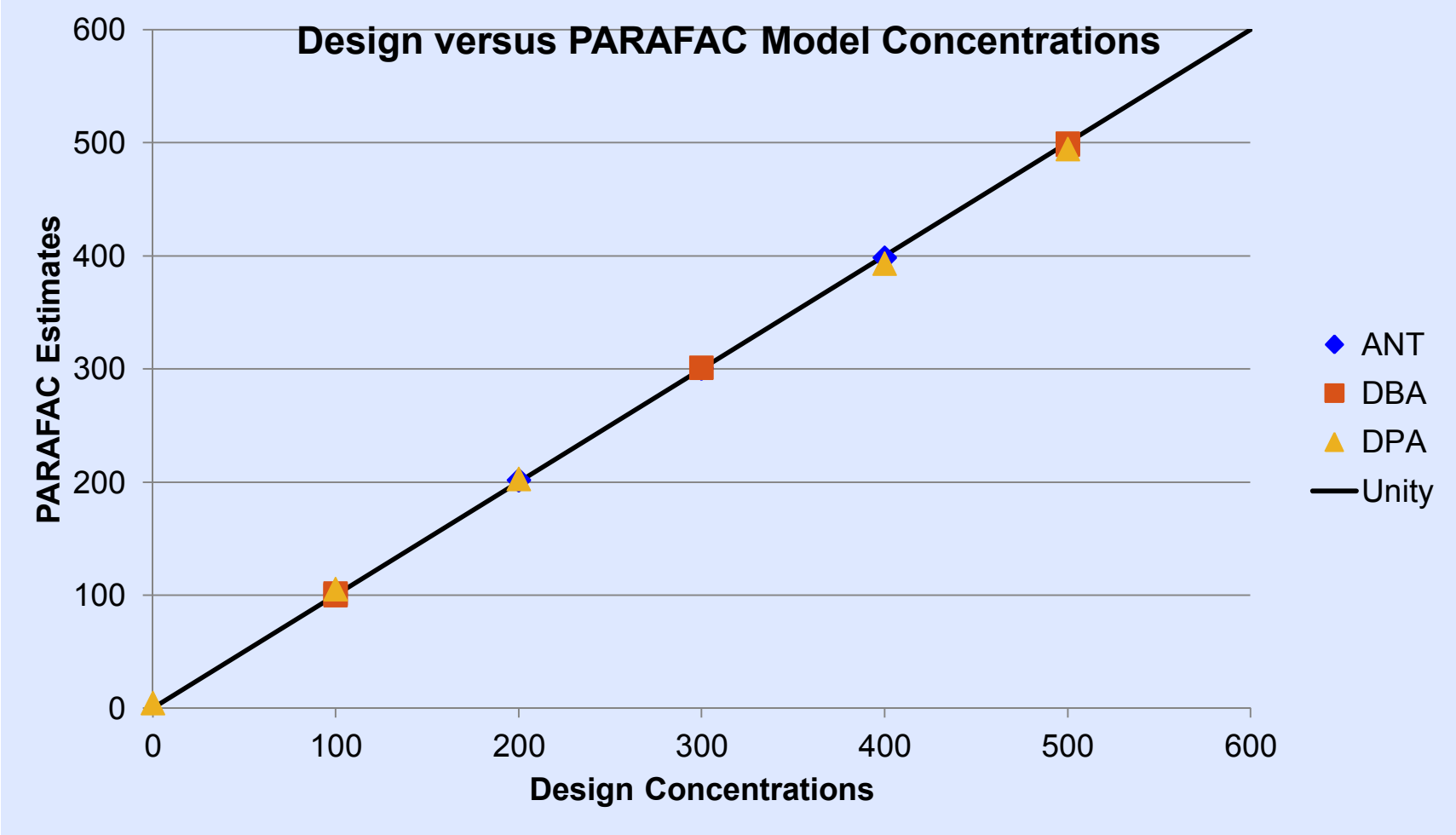
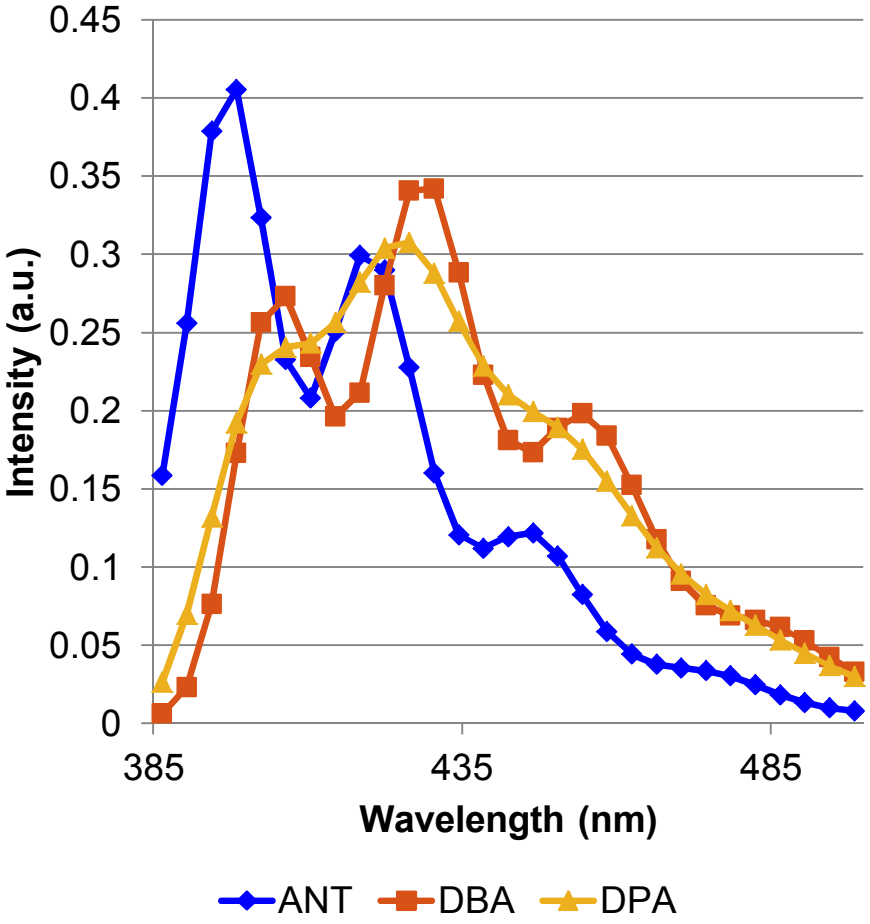
Concentration-mode Factors (0.2% noise)

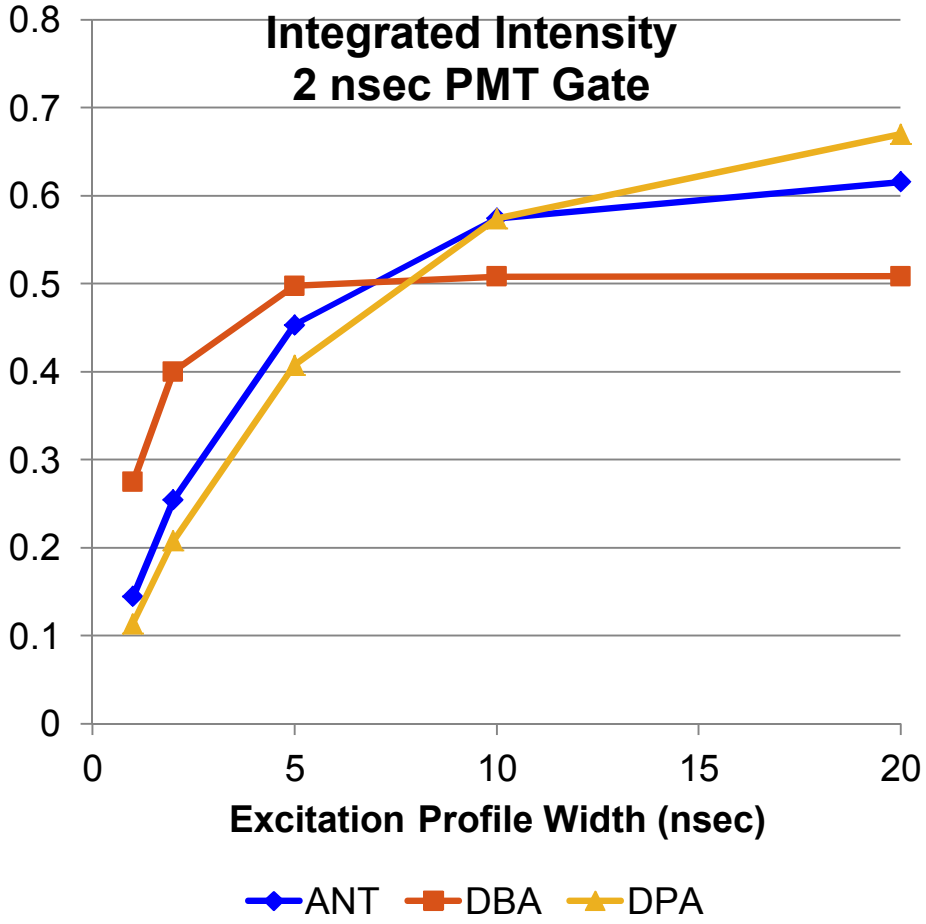


Simulation Conditions for Pulse Method

- Create variable temporal pulse width excitations
  - Use timescale of 250 nsec with 0.01 nsec steps
  - Scale decays by integrated intensity
  - 1, 2, 5, 10 and 20 nsec pulse widths
  - Use 2 nsec PMT gates for all excitations
- Use 4x wavelength data compression
  - Reduce from 116 increments to 29
  - Approximate 32 channel PMT
- Scale maximum values of 10,000 counts before adding noise
- Add “Poisson-like” noise using percentage of root maximum signal
  - Identical to standard method data
- PARAFAC conditions
  - All iterations impose rigorous nonnegativity
  - Convergence at 10^{-8} RMSE difference between iterations
  - Random starts for 10 trials

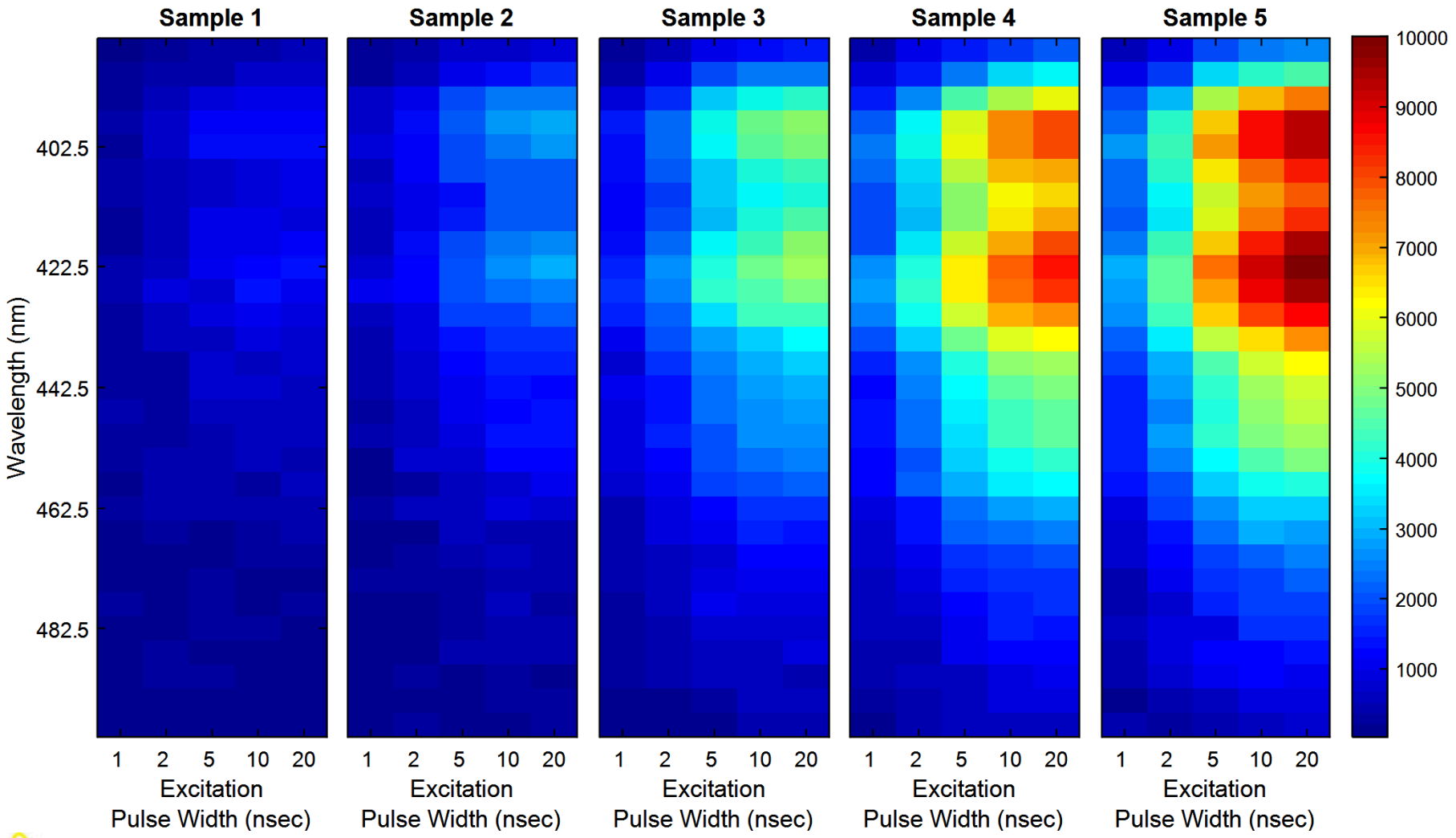
Pure Spectral and Temporal Components





Simulated Data

Trilinear Fluorescence Data with 1% of Max Noise



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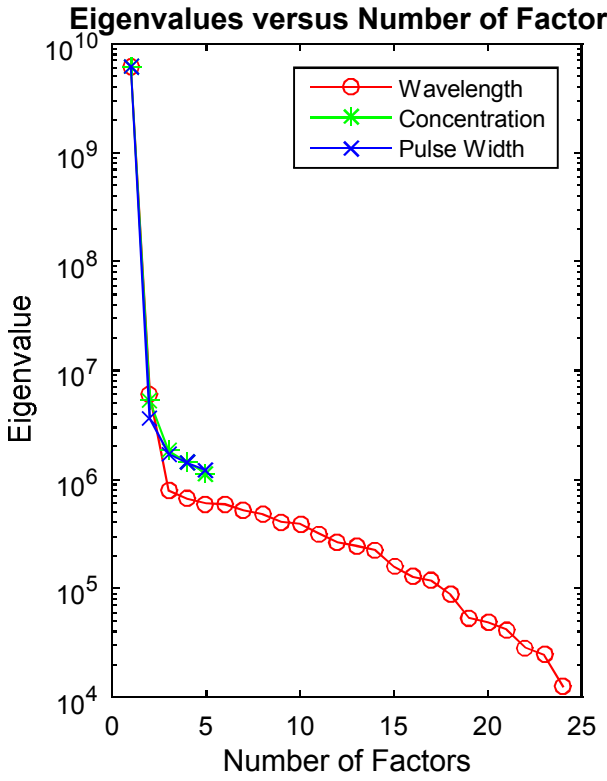
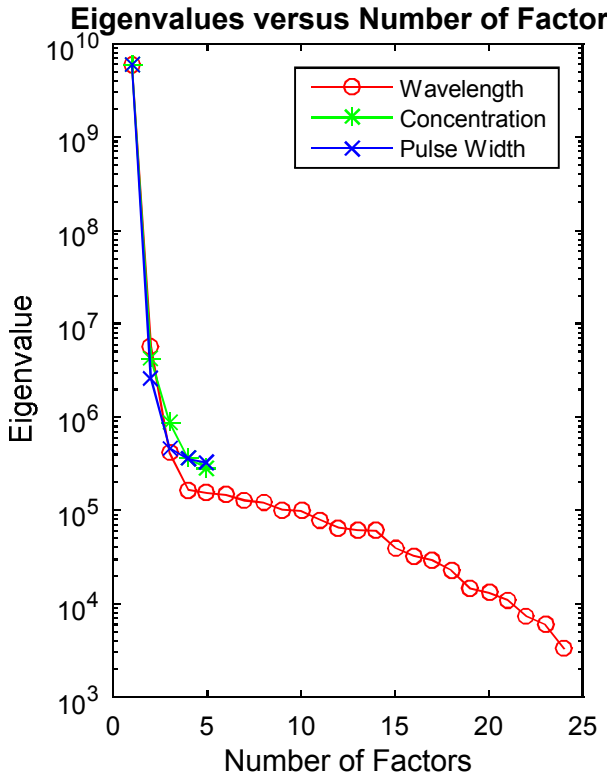
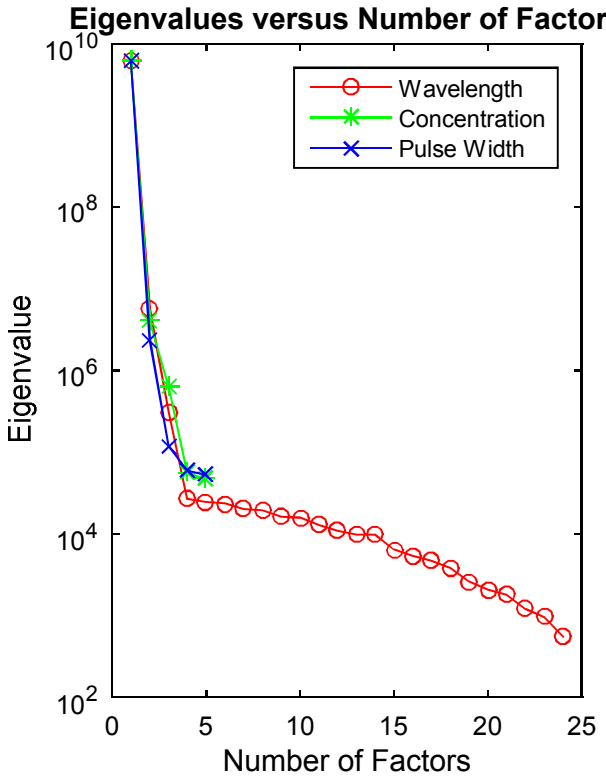
Eigenvalues for Pulsed Data



1% Noise

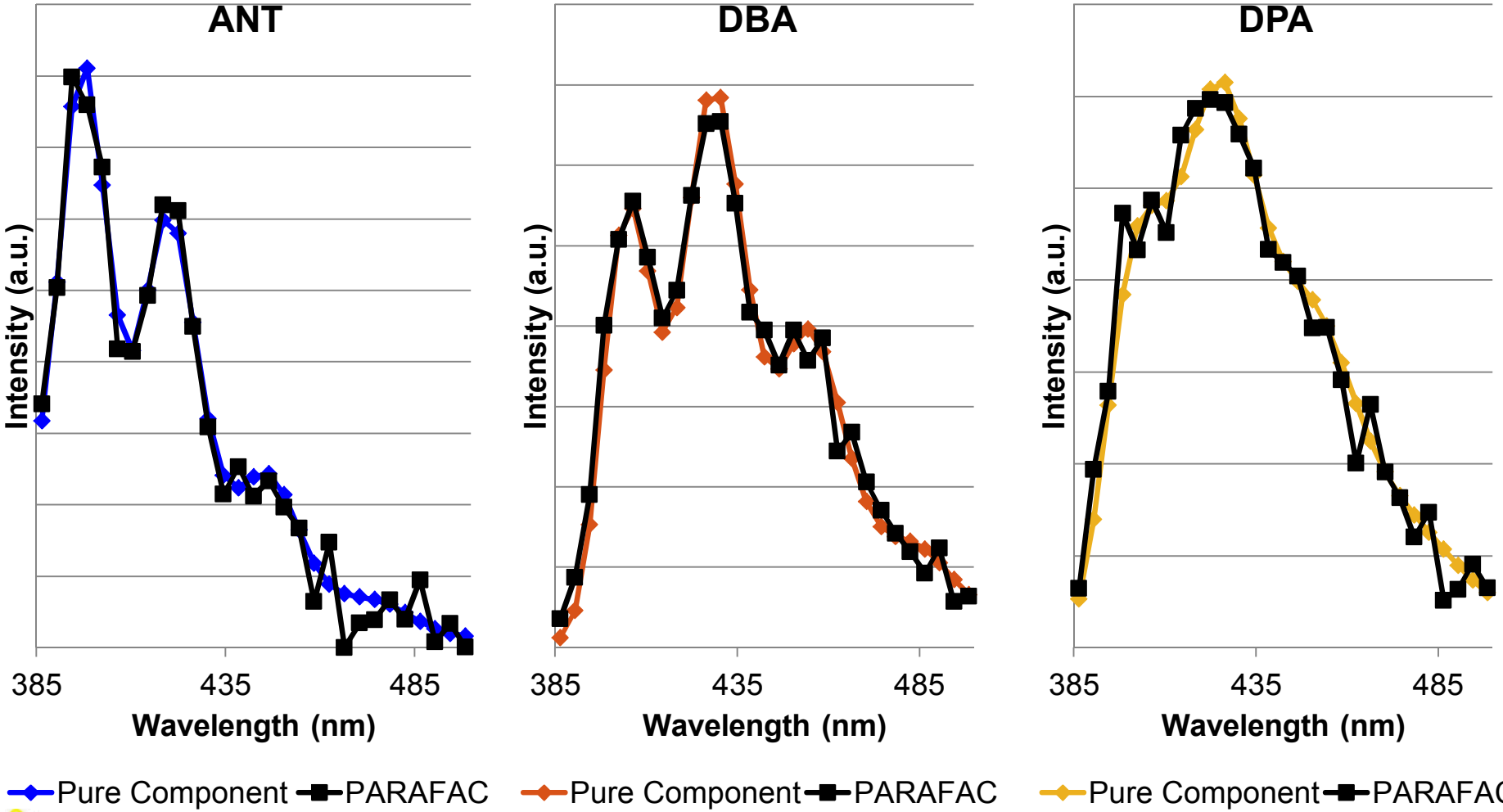


0.5% Noise

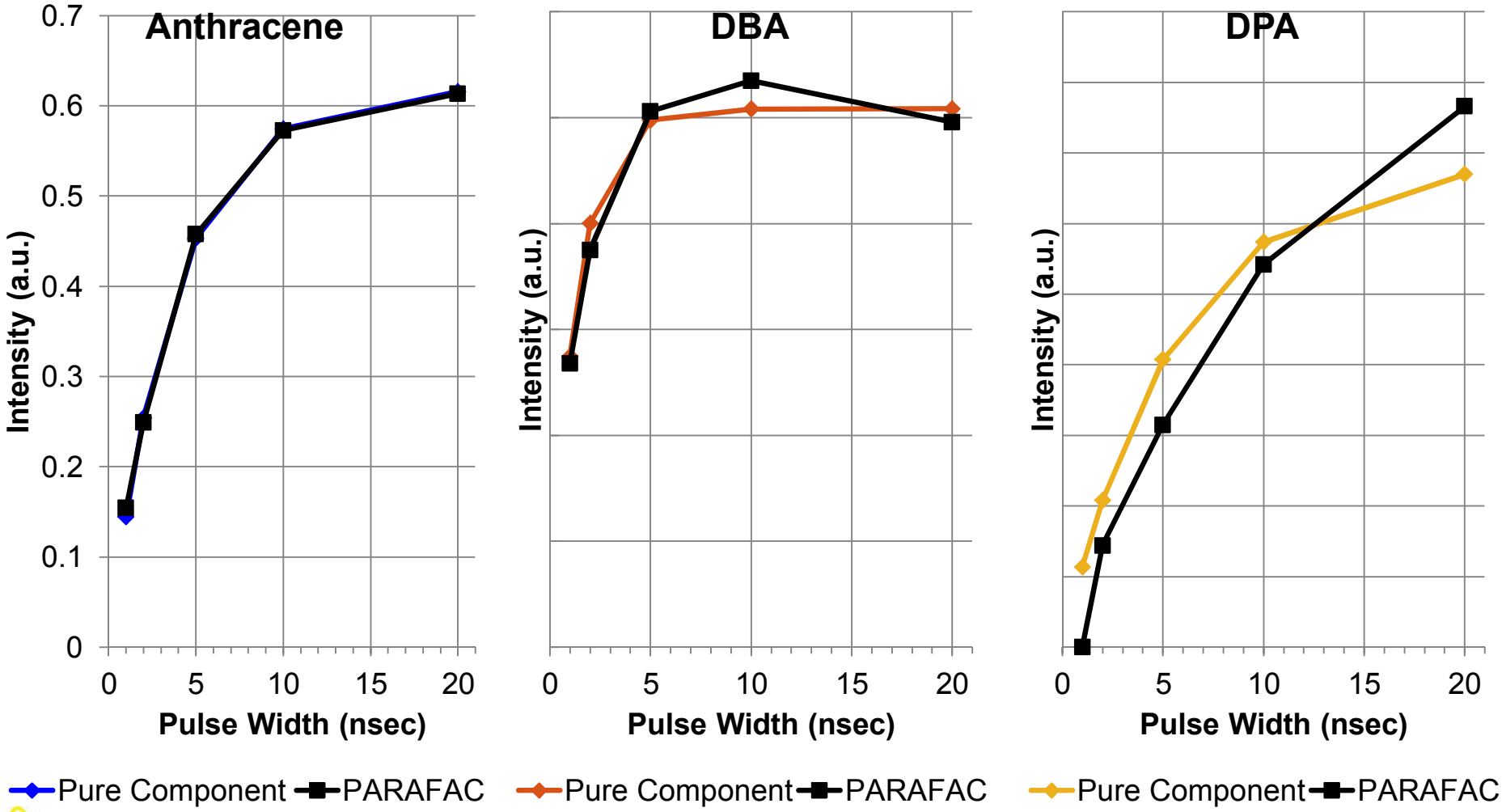


0.2% Noise

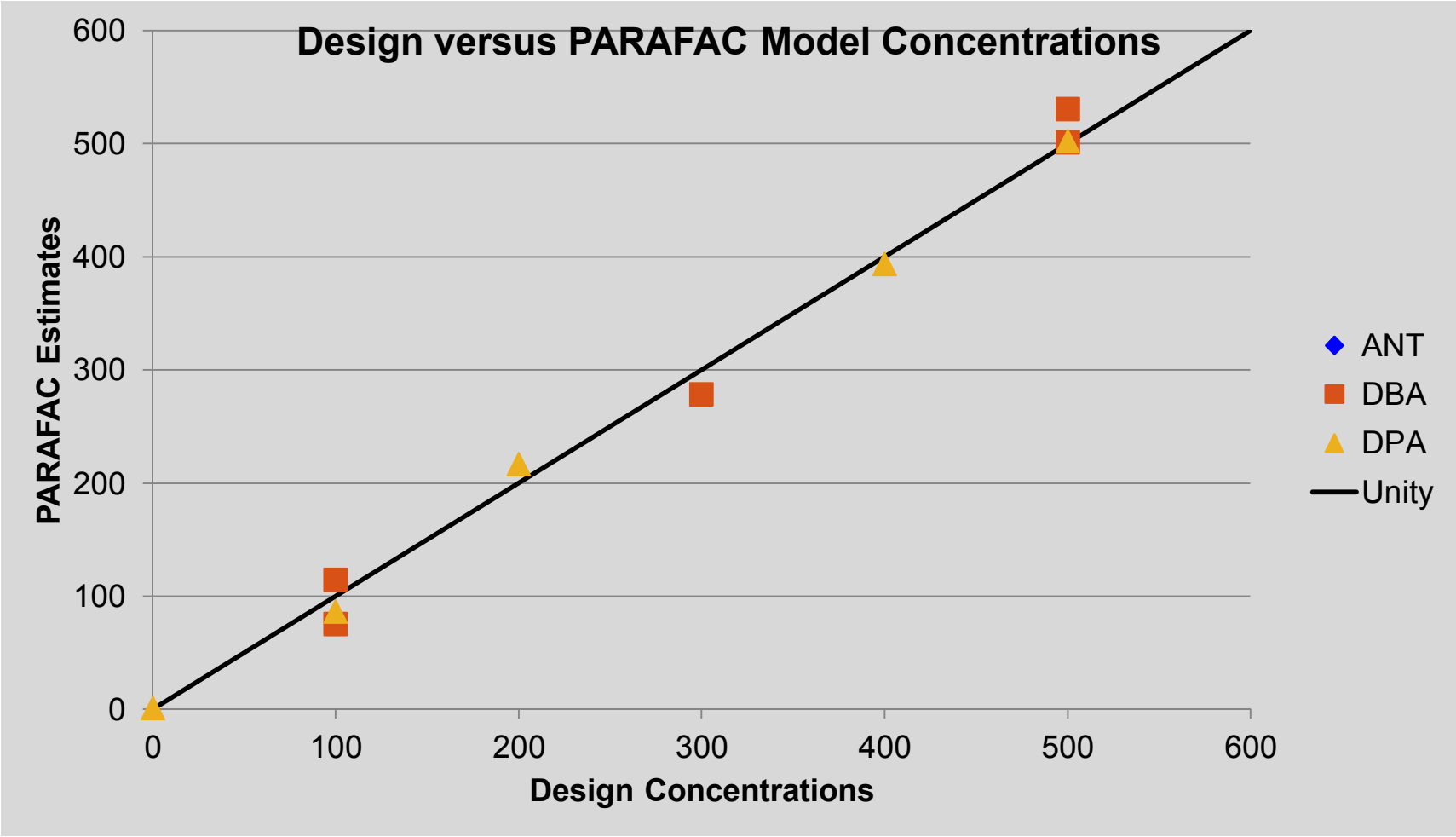
Wavelength-mode Factors (1% noise)



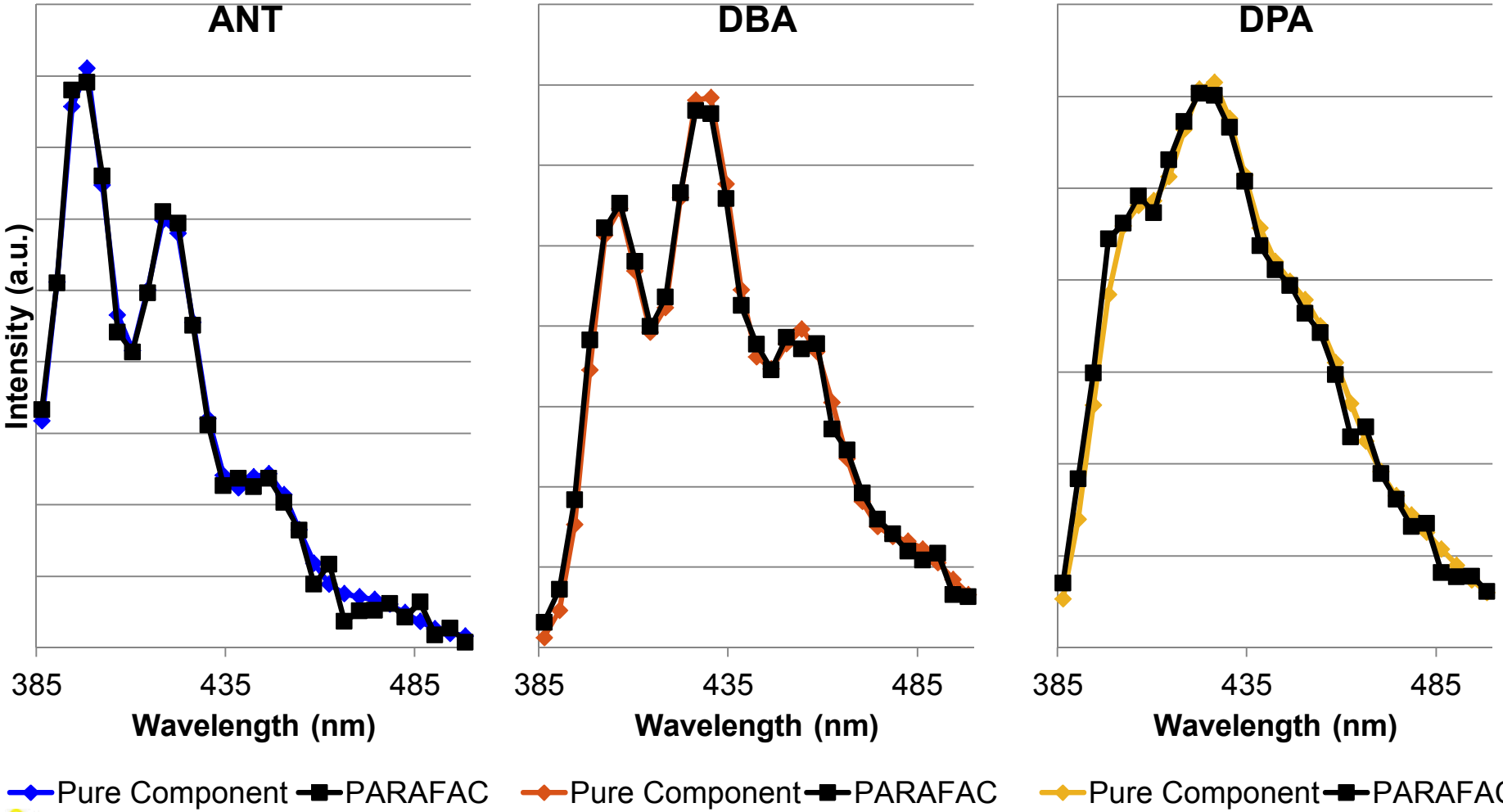
Temporal-mode Factors (1% noise)



Concentration-mode Factors (1% noise)



Wavelength-mode Factors (0.5% noise)



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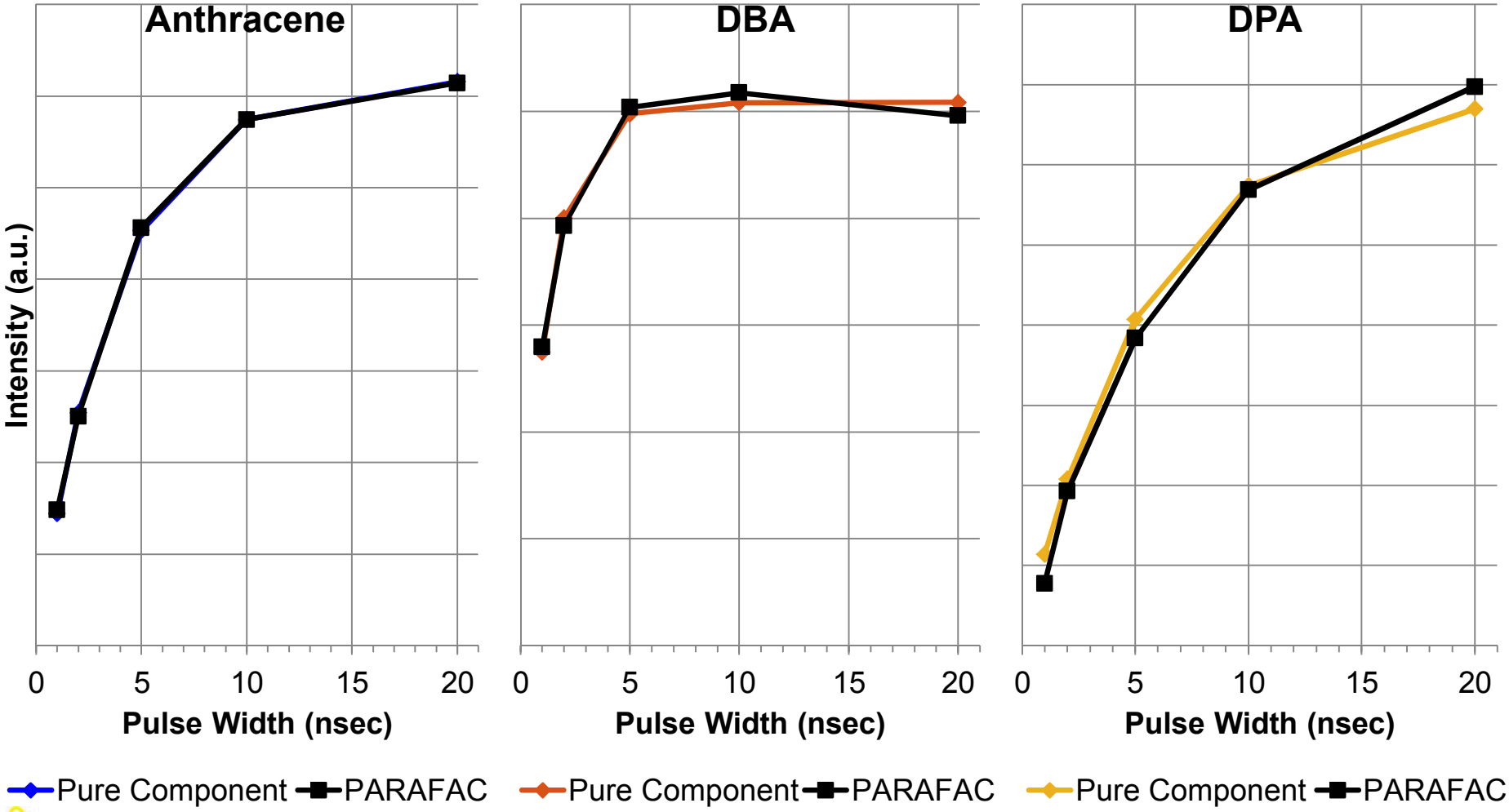
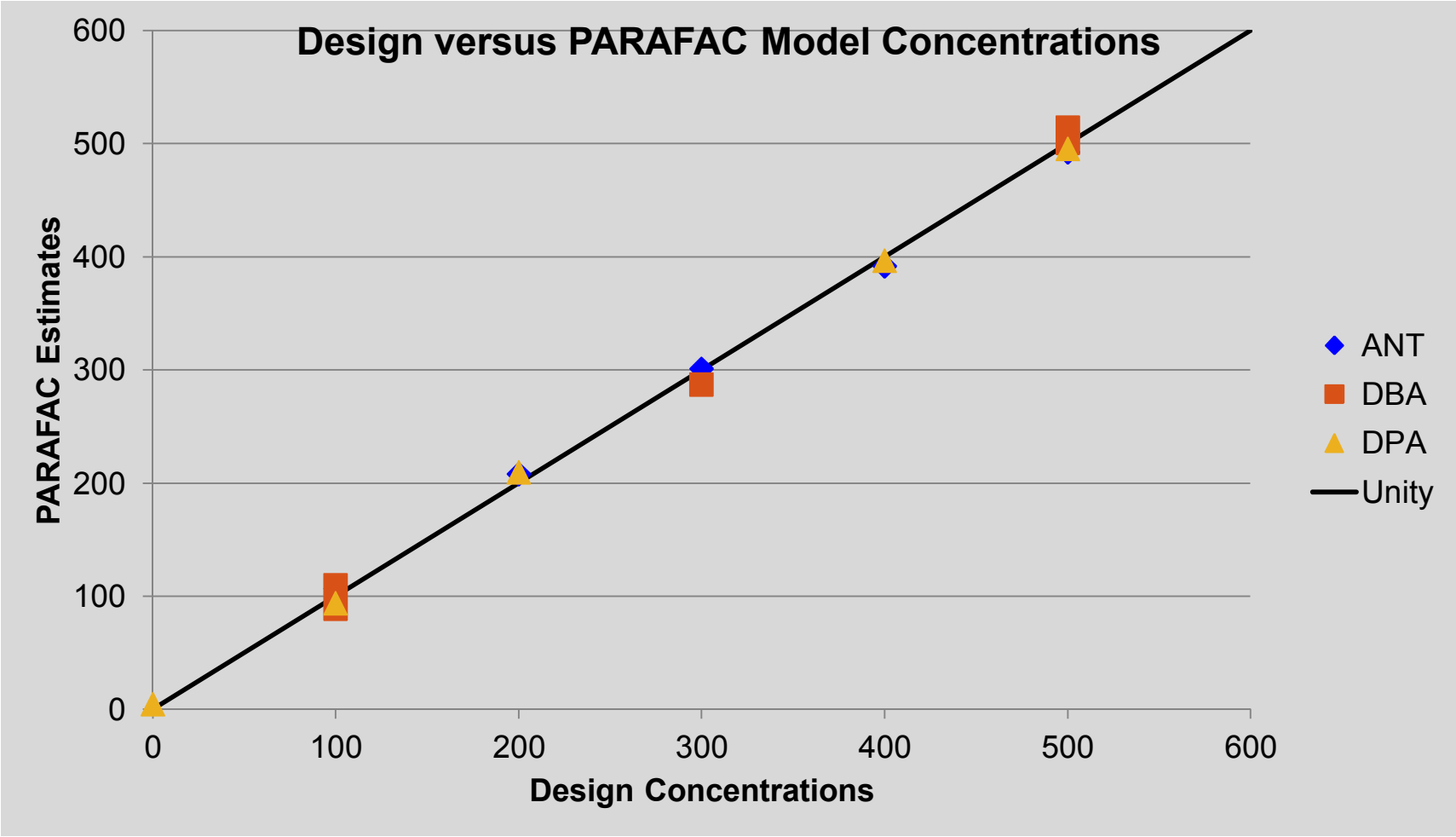
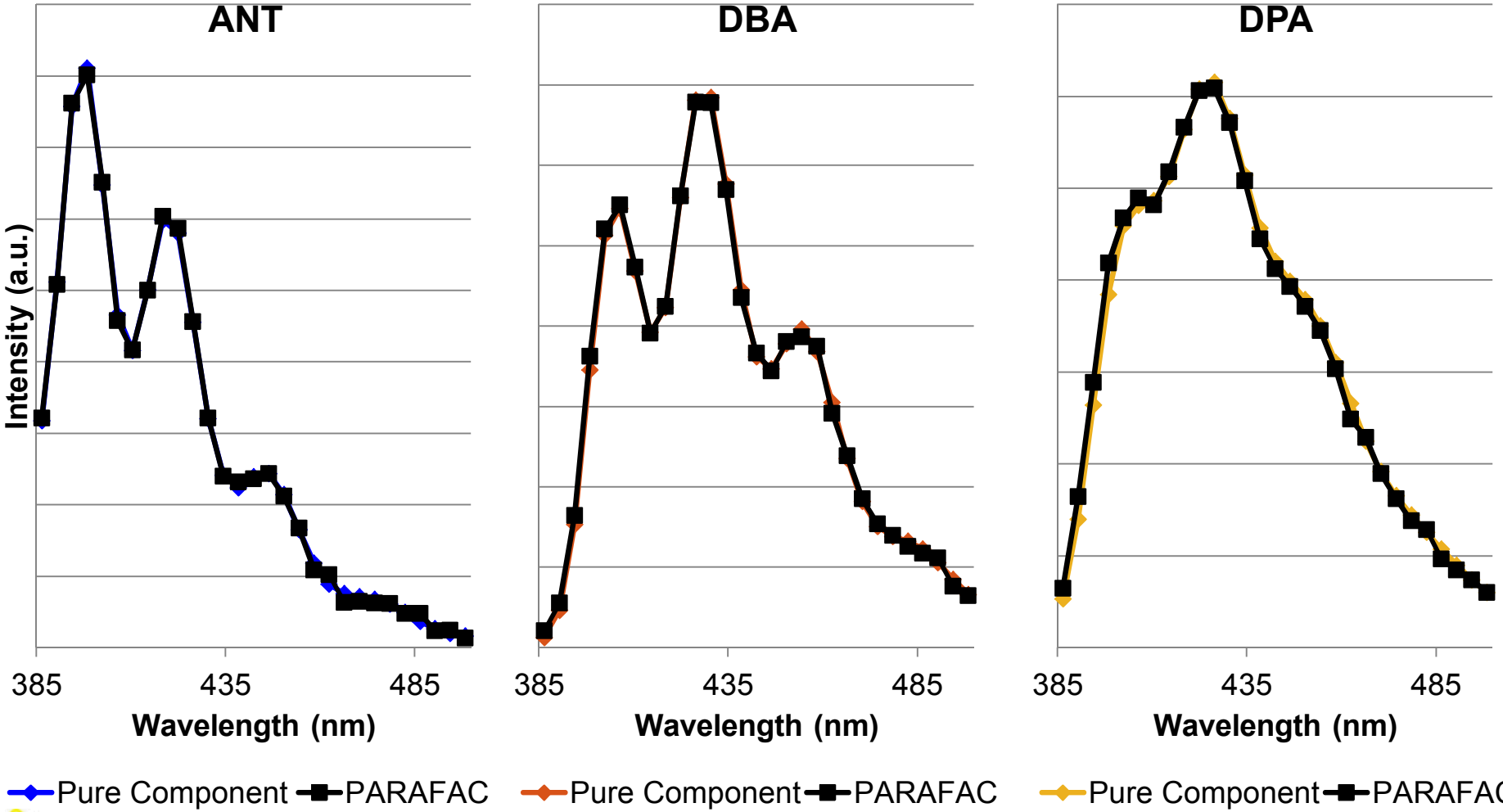
Temporal-mode Factors (0.5% noise)



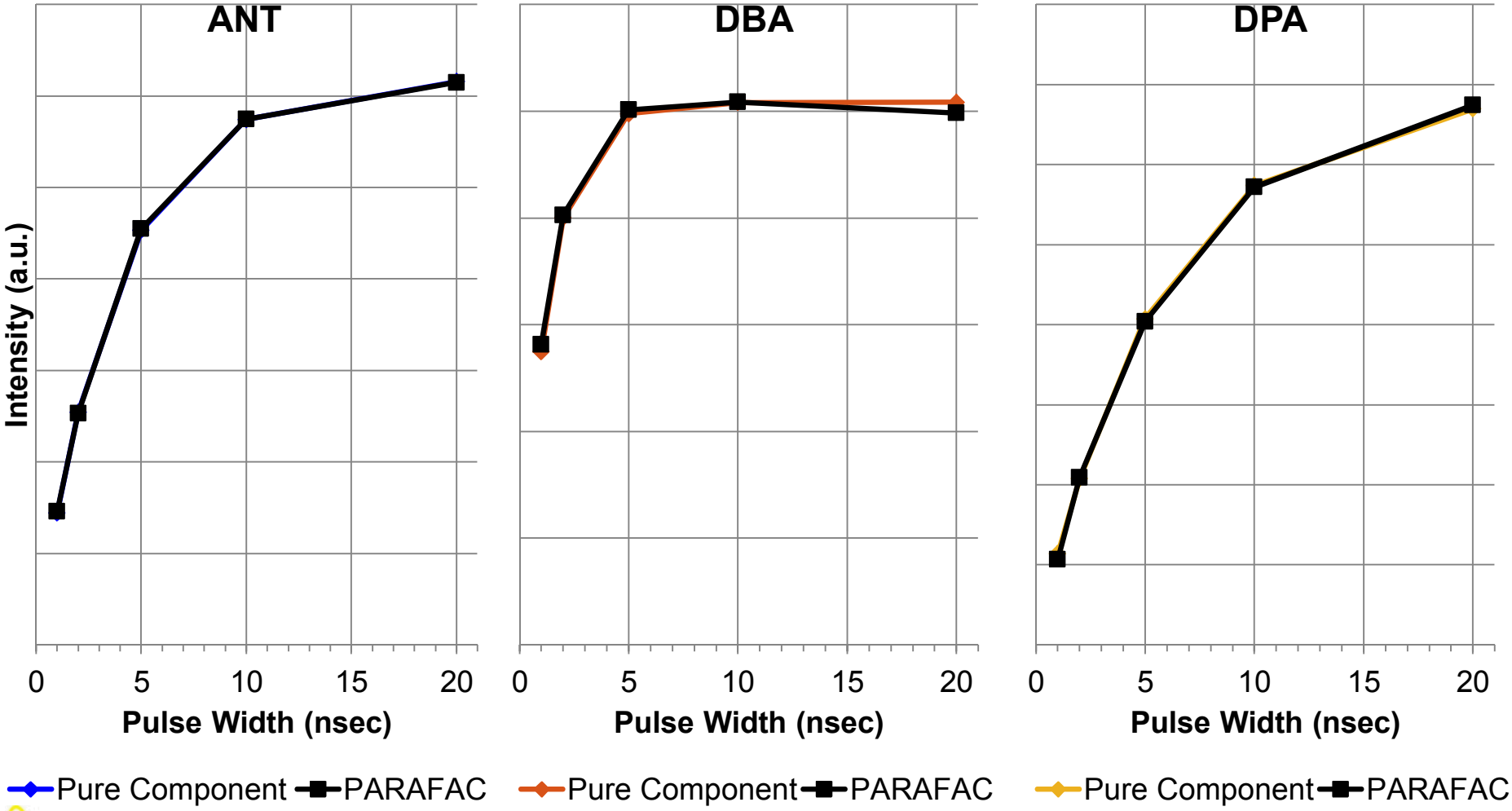
Concentration-mode Factors (0.5% noise)



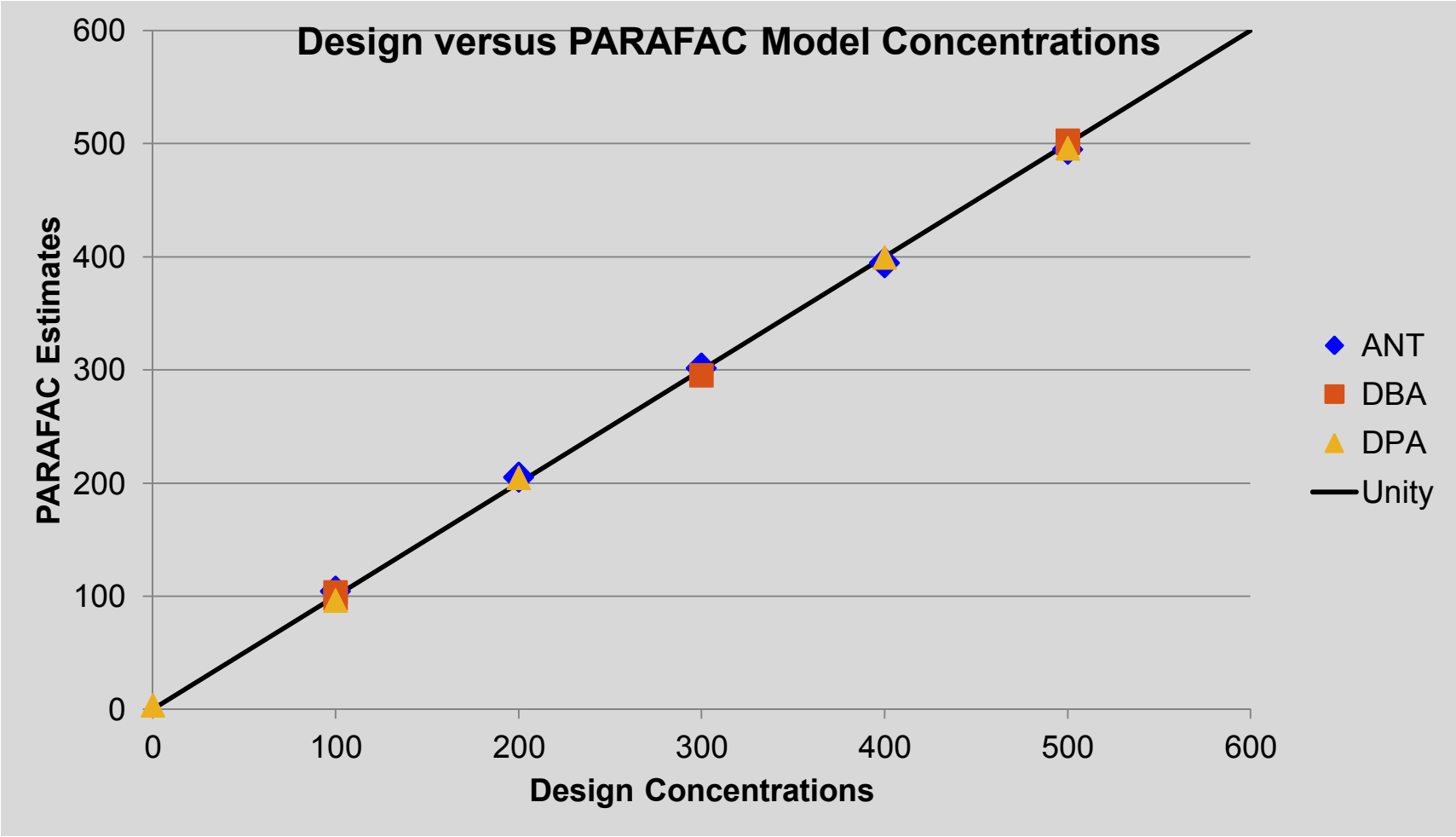
Wavelength-mode Factors (0.2% noise)



Temporal-mode Factors (0.2% noise)



Concentration-mode Factors (0.2% noise)



Results and Conclusions

- New data acquisition method theoretically produces excellent data quality for multivariate analysis
- New method takes advantage of rise-time phenomenon as well as exponential decay for exploiting physics of fluorescence
- Excitation pulse methods performs as well as or better than traditional waveform capture method when analyzed with PARAFAC

Future Work

- Explore effects of narrow and long pulse widths on factor analysis of various mixtures
- Evaluate performance of method while varying PMT gate width and displacement from excitation pulse end
- Conduct experiments on samples including simple mixtures and drug discovery probes
  - ANS (8-Anilinonaphthalene-1-sulfonic acid)

Acknowledgements

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