Limitations of Fluorescence Correlation Spectroscopy in Complex Situations

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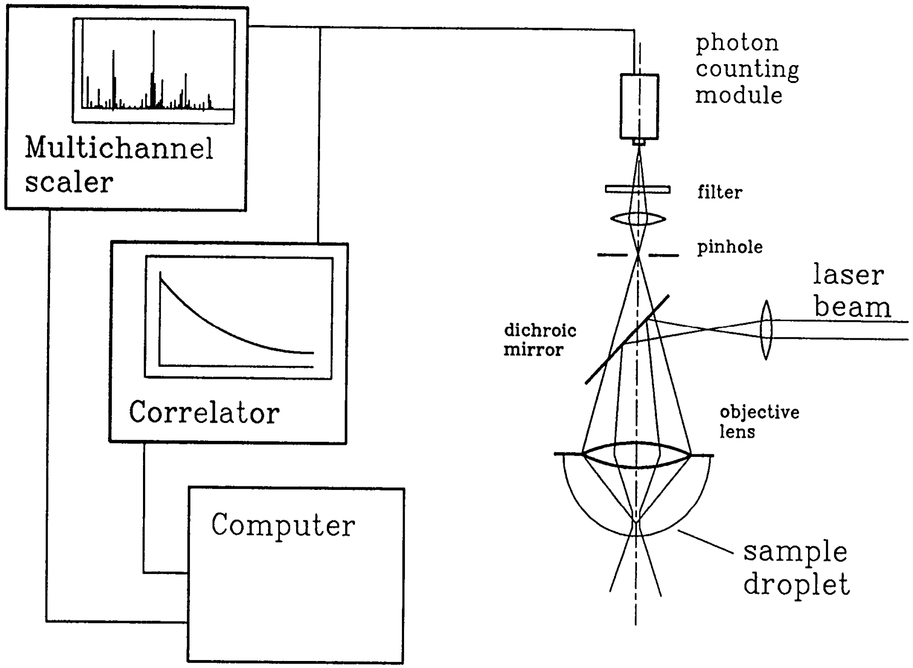
Outline

A. Introduction to FCS
B. Context of the Project
C. The Simulator
D. Conditions to Consider
E. Results
F. Conclusions
G. Future Projects

Introduction to FCS



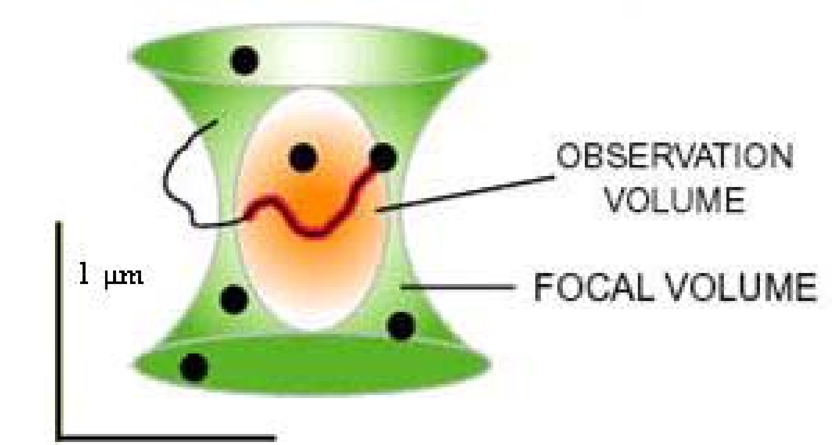


Figure 1. Standard experimental set up (Eigen and Rigler, 1994). Illustration of laser focal point on the right.

Analytical Model: Eq. 1

$$G(\tau) = \frac{\gamma}{N}\left(\frac{\alpha_1^2 a g_{3d1}(\tau) + \alpha_2^2 (1-a) g_{3d2}(\tau)}{[\alpha_1 a + \alpha_2 (1-a)]^2}\right) + DC$$

$$g_{3di}(\tau) = \left(1 + \frac{\tau}{\tau_{di}}\right)^{-1}\left(1 + \frac{\tau}{\kappa^2 \tau_{di}}\right)^{-1/2}$$

Context of the Project

- Limitations of FCS:
  - Type of system being analyzed
  - Shape of the observation volume
  - The analytical model
- Literature:
  - Meseth *et al.* (1999)
    - Measurement accuracy of diffusion, concentration, and mole fraction
- Goal:
  - Characterize trends in the resolution limits of FCS
  - Explore novel methods of analysis

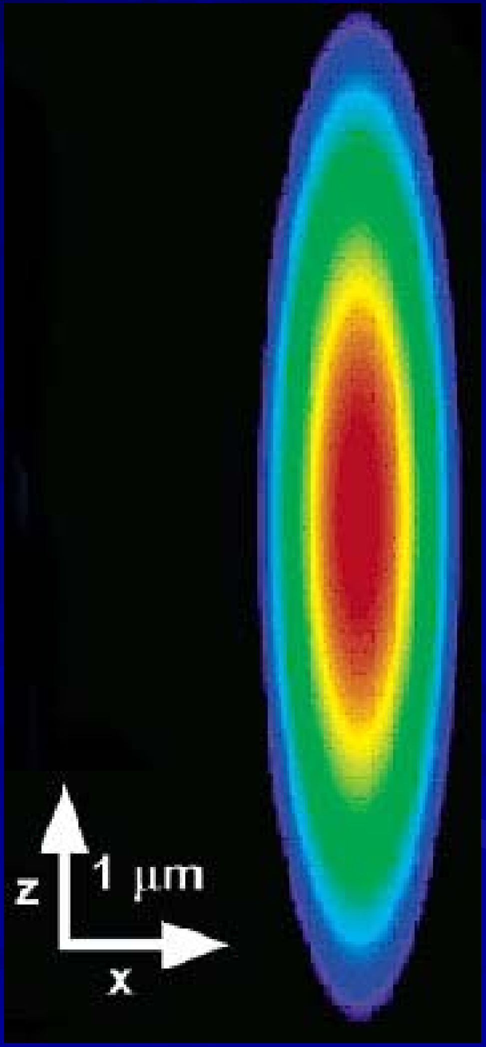
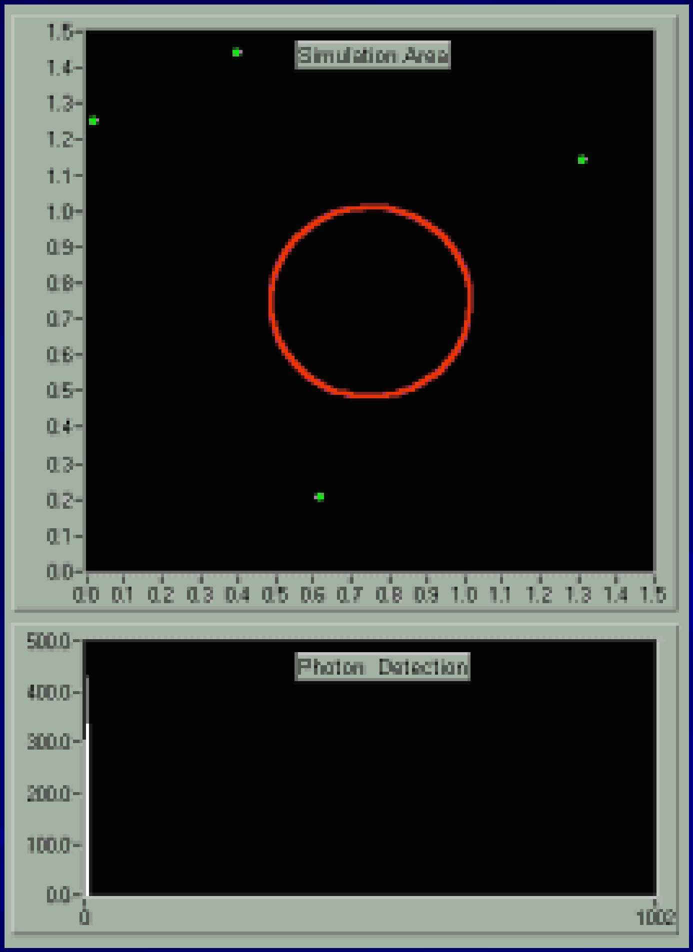
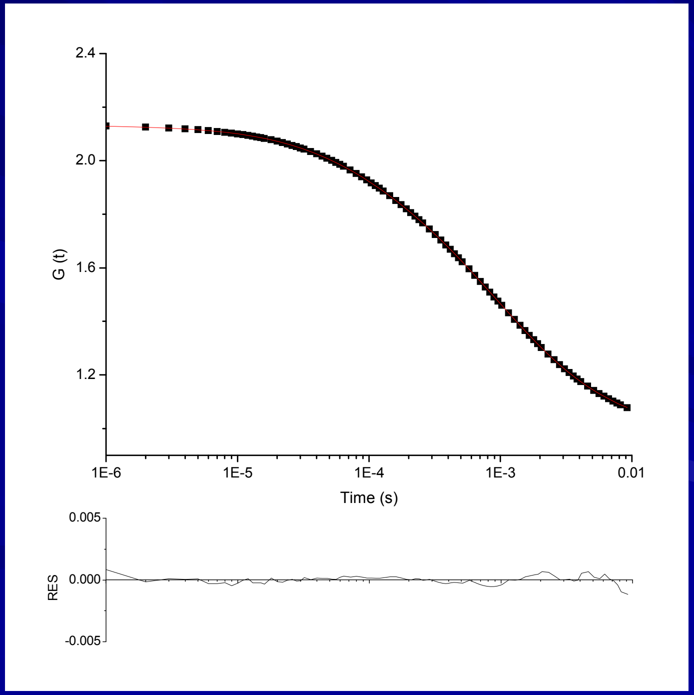


Figure 2. 0.448 fL Ideal Gaussian Beam Profile (Culbertson *et al.* 2007)

The Simulator

- Single Molecule Diffusion Simulator (SMDS)
- Python Scripts → Simulation Core
- Parallelized Processing
- Random Walk
- Generates synthetic autocorrelation curve
- Fitting using analytical model





Conditions to Consider

- Simulation Conditions:
  - Two-Component Diffusion (Diffusion coefficients fixed)
    - Different Intensity Ratios (dim fast or slow component)
    - Different Mole Fractions (ranging from 0.01 – 0.99)
  - Non-Ideal Beam Profile (Two Crossed Beams)
- Analytical Model:
  - Free and Fixed Parameters (D_f)
- Results comparison:
  - Diffusion coefficient of the slow component (D_s)
  - Mole fraction of the fast component (a_f)
  - Total concentration (C_{total})

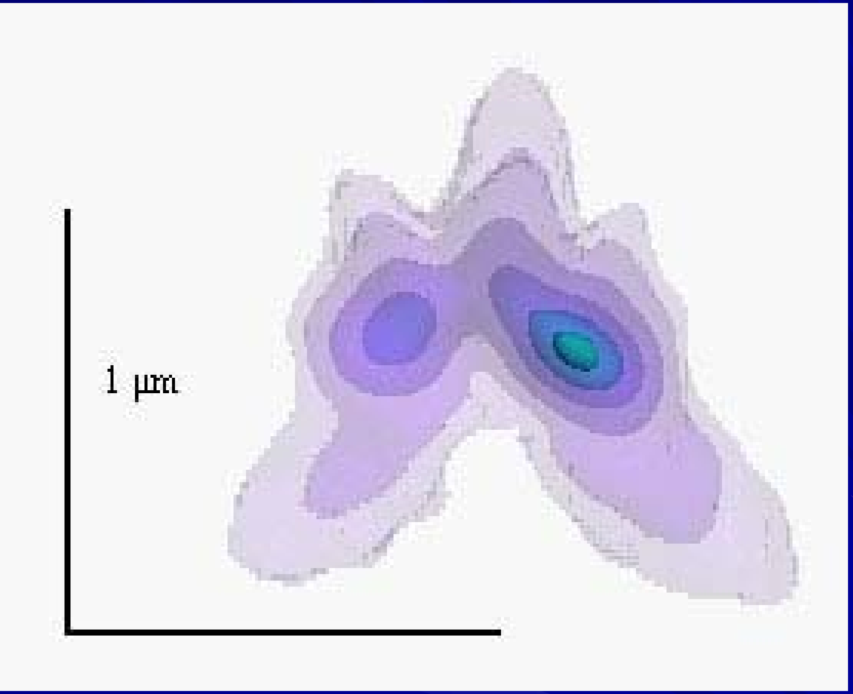
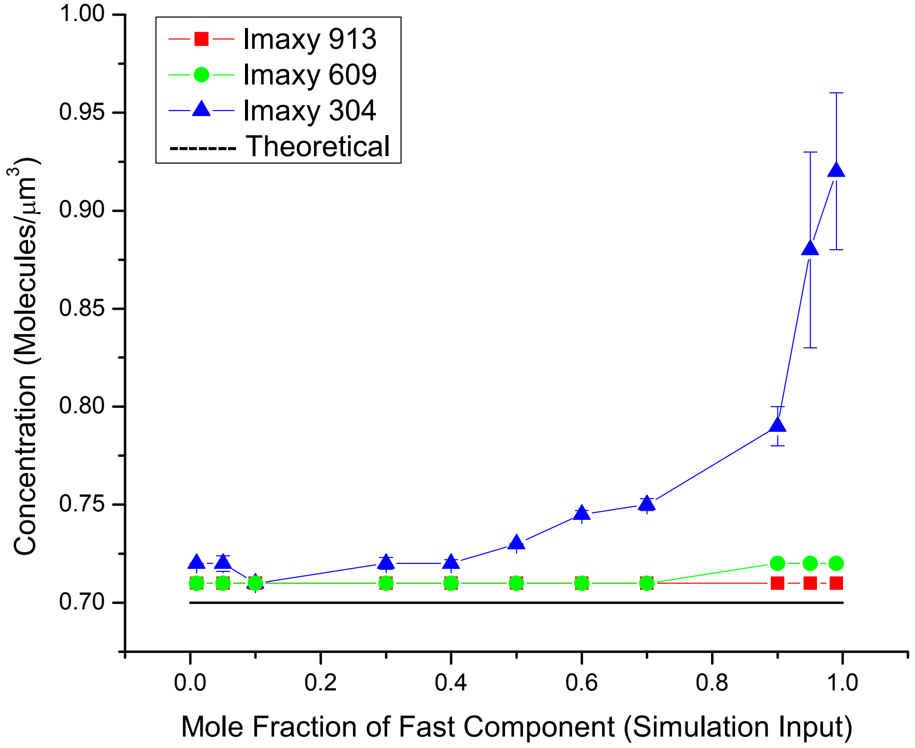


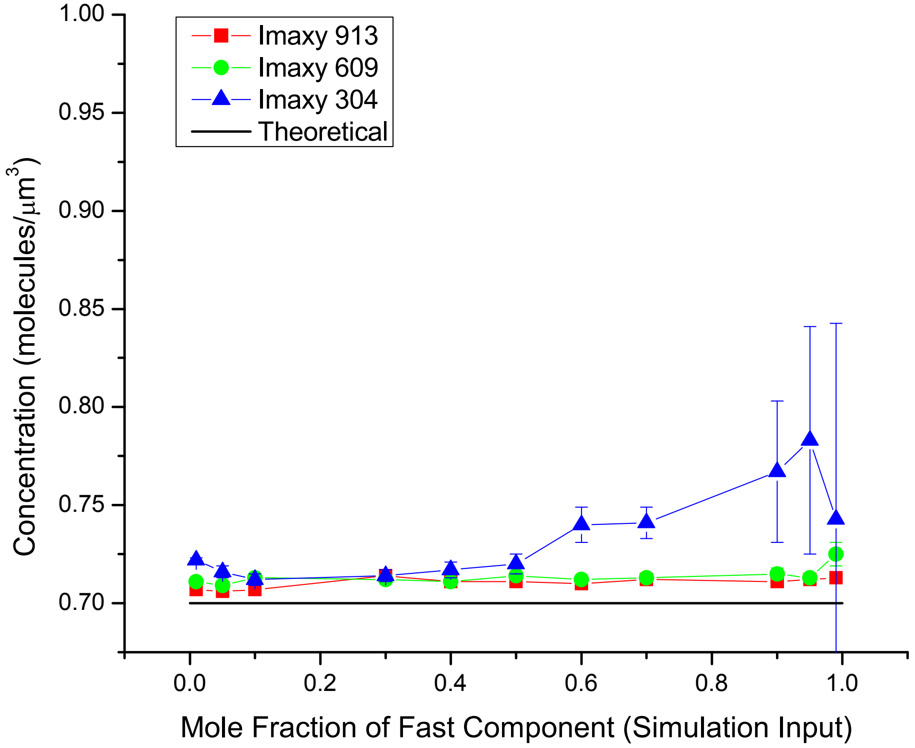
Figure 4. 0.469 fL crossed beam profile.

Results

Trends in Total Concentration Due to Different Fitting Routines

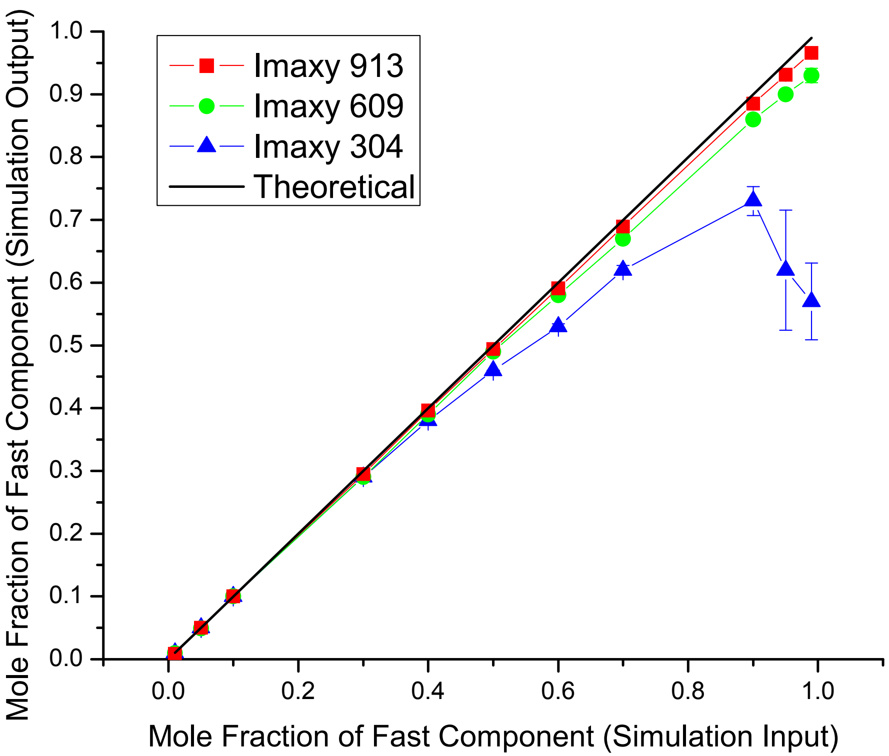


Fixed tdx Parameter

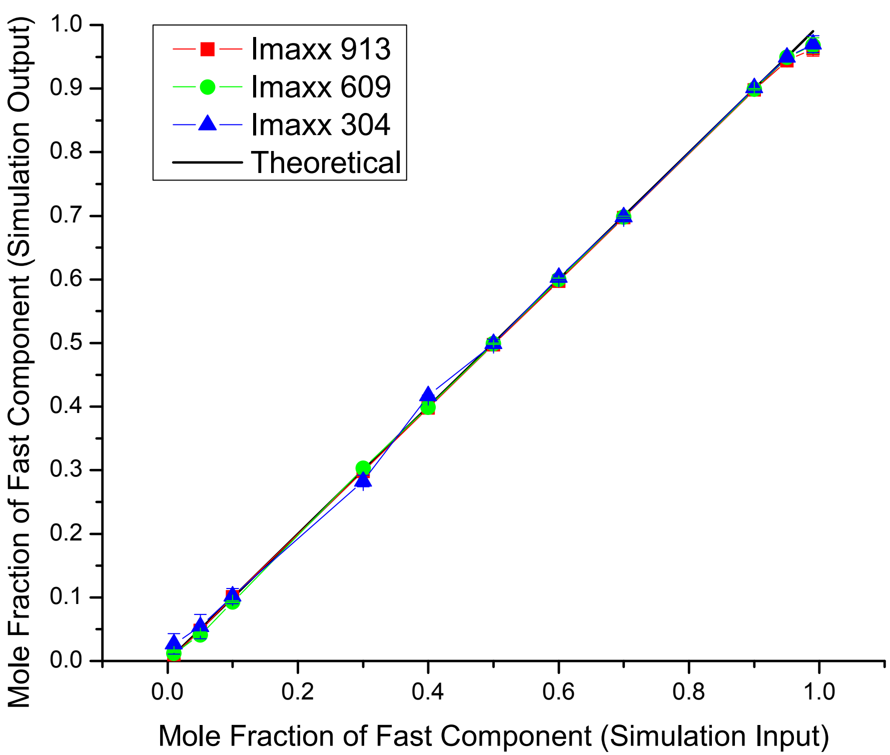


Free tdx Parameter

Trends in Mole Fraction of Fast Component Due to Different Simulation Conditions

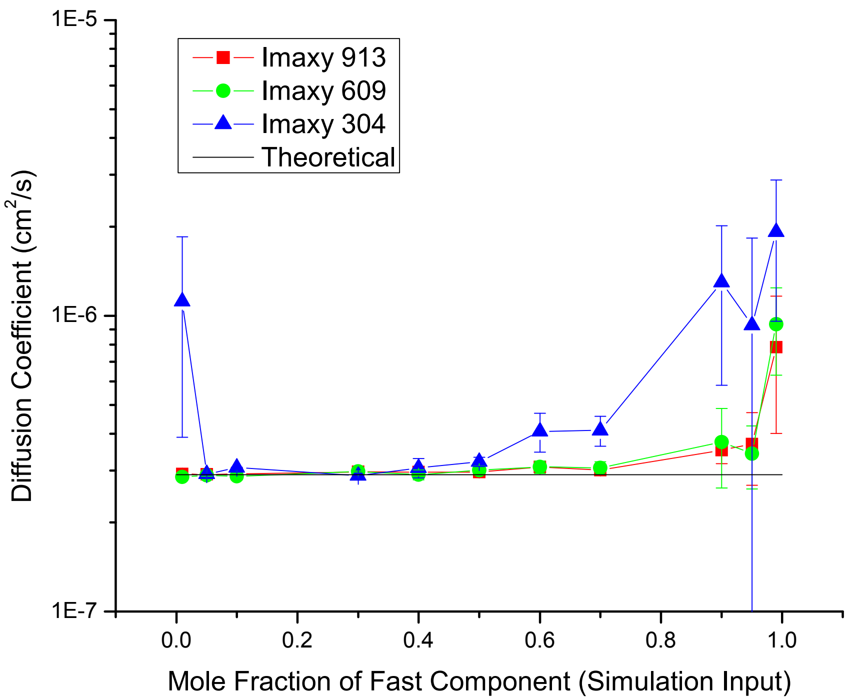


Dim Slow Component

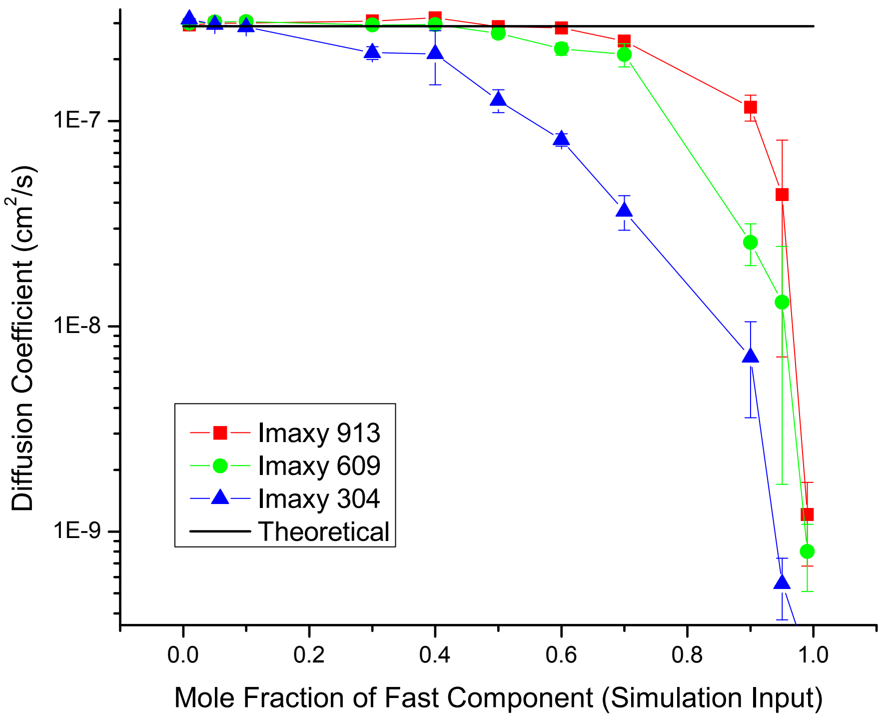


Dim Fast Component

Trends in Diffusion Coefficient Due to Changes in Volume Shape



Ideal Gaussian Beam Profile



Crossed Beam Profile

Results Summary

Table 1. Effects of Different Intensity Ratios (α_2)

		Range of Error over a			
		Ideal Gaussian			Non-Ideal (Crossed Beams)
		Dim Slow		Dim Fast	Dim Slow
Parameter	Value of α_2	Fixed D_f	Free D_f	Fixed D_f	Free D_f
D_s	0.25	1-590%	1-562%	0-13%	2-100%
(cm^2/s)	0.5	0-248%	0-223%	0-32%	1-100%
	0.75	0-166%	0-170%	0-76%	0-100%
a_f	0.25	0-43%	1-70%	0-170%	1-75%
	0.5	0-6%	0-20%	0-20%	1-60%
	0.75	0-10%	1-20%	0-4%	0-30
C	0.25	1-31%	2-12%	0-4%	30-63%
(Molec/μm^3)	0.5	1-3%	1-4%	1-2%	27-35%
	0.75	1-2%	1-2%	1-2%	21-35%

•All D_f values had an error of less than ~1%

Conclusion

- Using an ideal Gaussian beam profile, the analytical model is more robust than expected.
  - The model properly functions when diffusion is a free parameter
  - Mole fractions can be resolved with reasonable accuracy in a range of conditions
  - Concentration measurements have greater limitations that reported
- The non-ideal beam profile, if unadjusted for, severally limits the application of FCS.
- There are clear limitations in FCS that require new methods of analysis to overcome.

Future Projects

- Completing trials on combinations of conditions
  - Variable diffusion coefficients
- Publication writing
- Applying NFCS analysis

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