



3-D clustering to identify multiple oligomerization states by FRET

Kim Scott

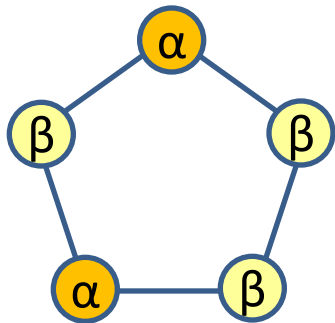
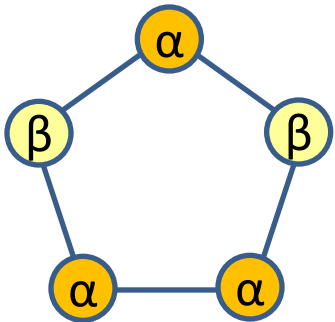
Mentor: Henry Lester

SURF Seminar Day: October 17, 2009

10 μm

Nicotinic Acetylcholine Receptors (nAChRs)

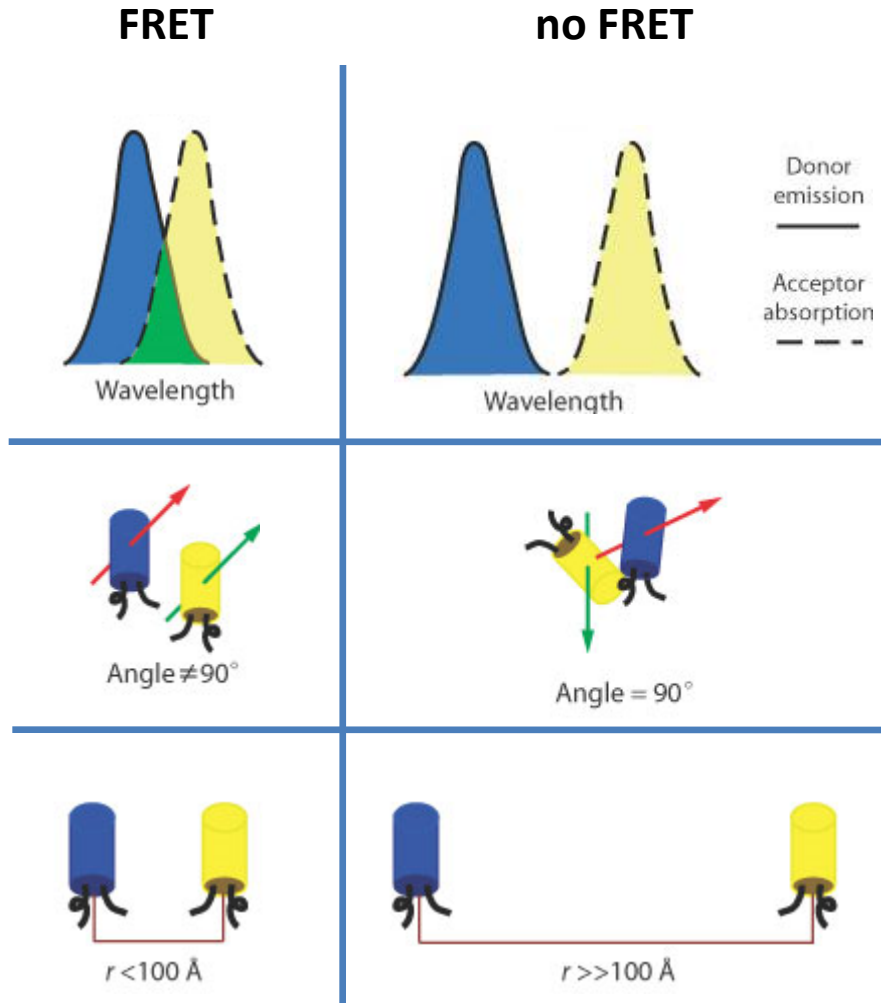
$(\alpha 4)_3(\beta 2)_2$:
EC50 $\sim 100\mu\text{M}$



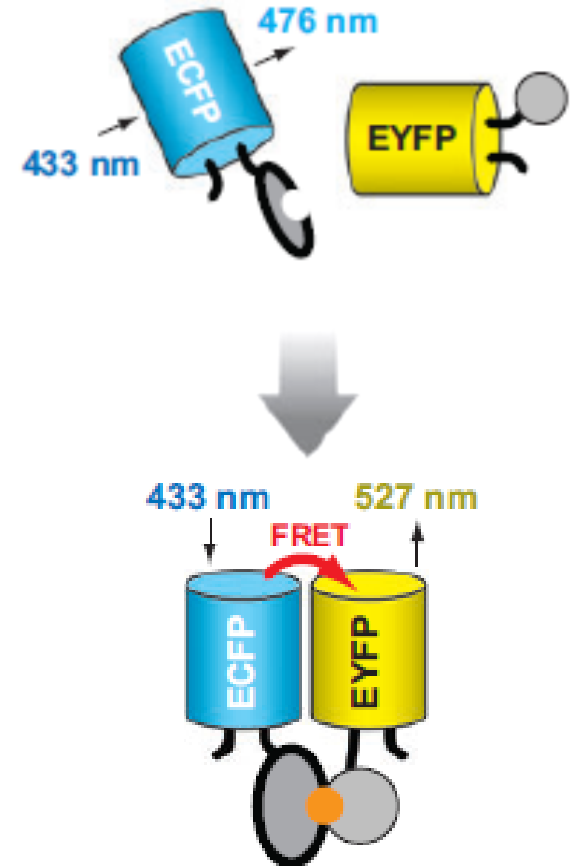
$(\alpha 4)_2(\beta 2)_3$:
100 times as sensitive!

- Pentameric ion channels found throughout the brain
- Composed of a variety of possible subunits in varying stoichiometries
- Open in response to acetylcholine (naturally), nicotine (much stronger!)
- Presumed to underlie the mechanisms of nicotine addiction, tolerance, & withdrawal... plus its protective effect against Parkinson's

FRET microscopy

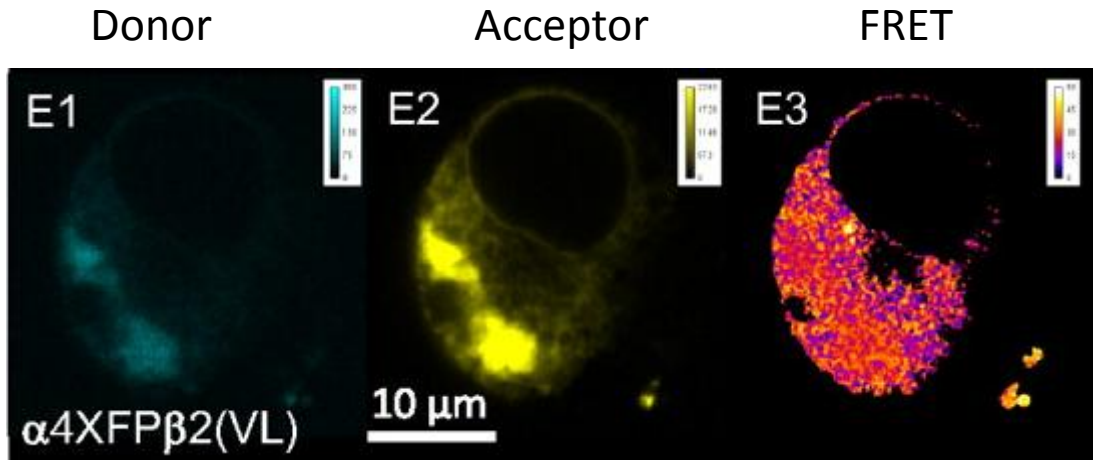


(Vogel et al., 2006)



(Wang et al., 2008)

Extending FRET to study stoichiometry



(Son et al., 2009)

Goal: to estimate the prevalence of distinct nAChR stoichiometries from the distributions of donor, acceptor, and net FRET pixel values.

Challenges:

- Multiple stoichiometries of assembled receptors, partially assembled receptors of unknown geometry, and unpaired donors and acceptors all present.
- Heterogeneous population even within single pixels
- Unknown subcellular localization of FRETing oligomers

Current NFRET histogram analysis

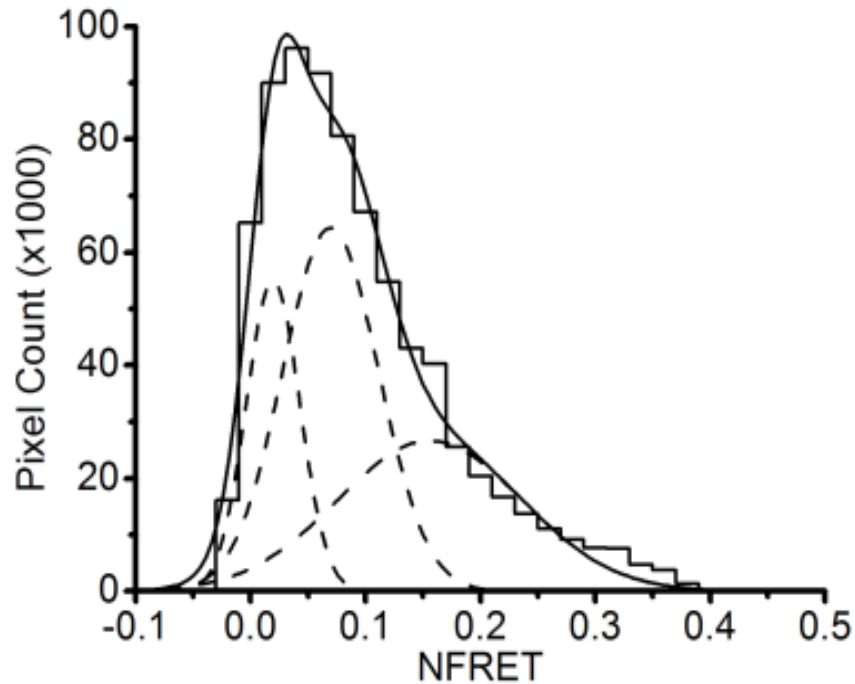
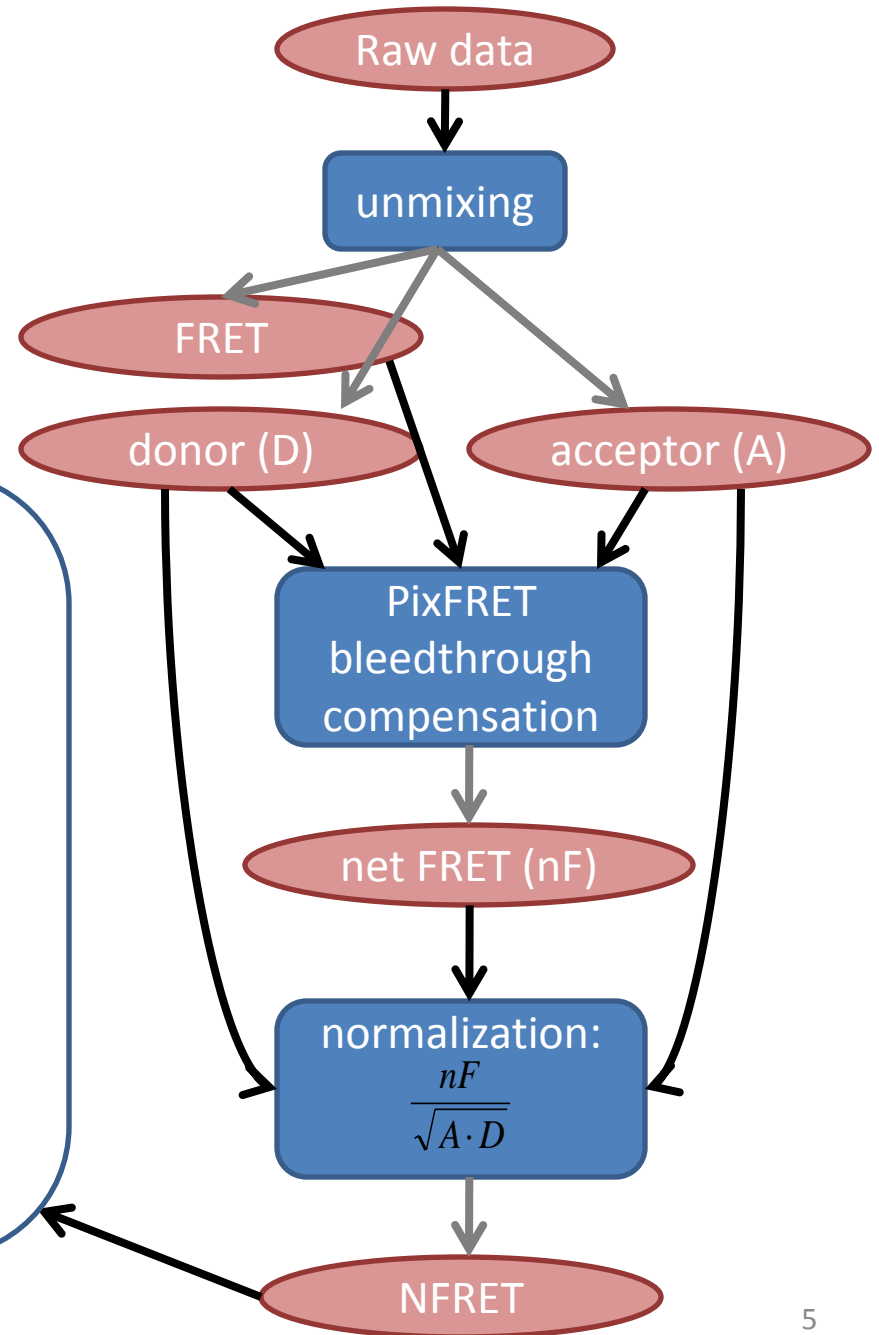


Fig 8H, Moss et al., submitted to JGP



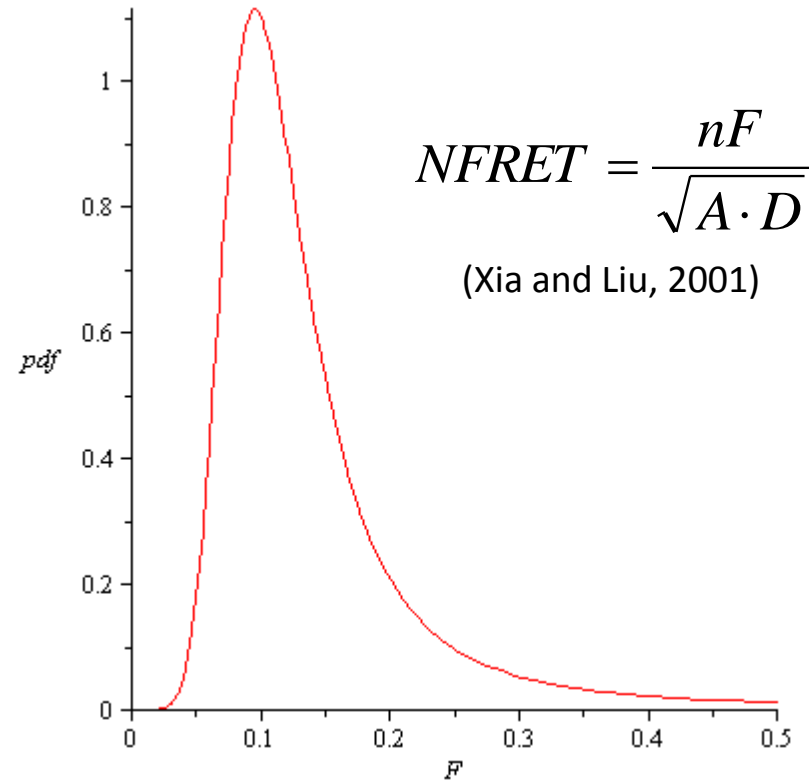
Dangers of fitting NFRET histograms

Single oligomer NFRET values with

$$nF \sim N(1, 0.2)$$

$$A \sim N(10, 4)$$

$$D \sim N(10, 4)$$



1) NFRET distribution from a single oligomer with varying nF , A , and D measurements is skew.

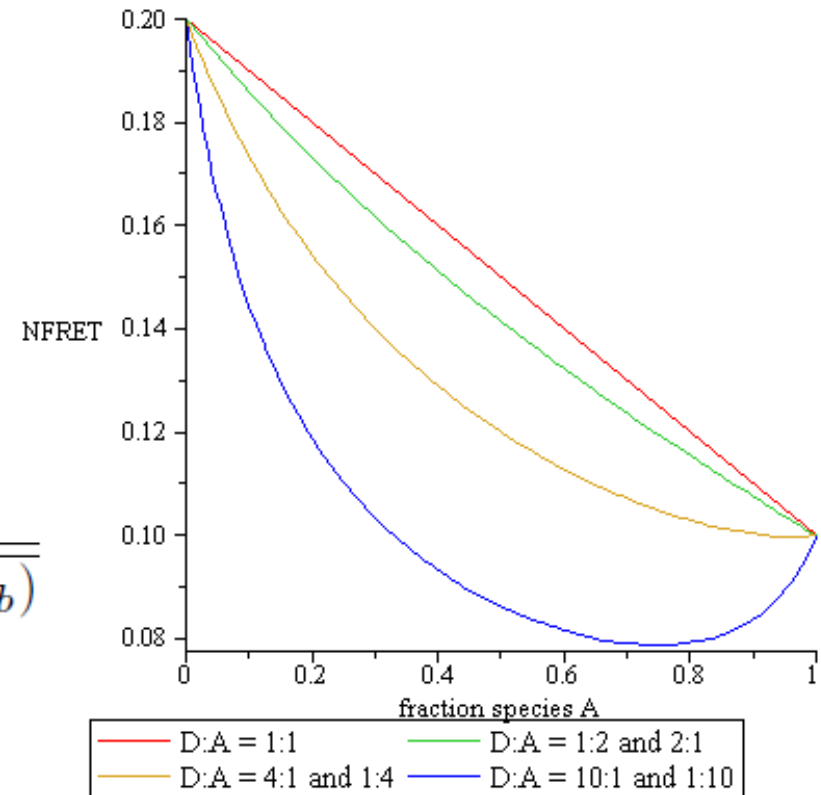
Dangers of fitting NFRET histograms

$$NFRET = \frac{nF}{\sqrt{A \cdot D}}$$

(Xia and Liu, 2001)

Given that a fraction f of the total FRETing constructs are of type A, the NFRET value

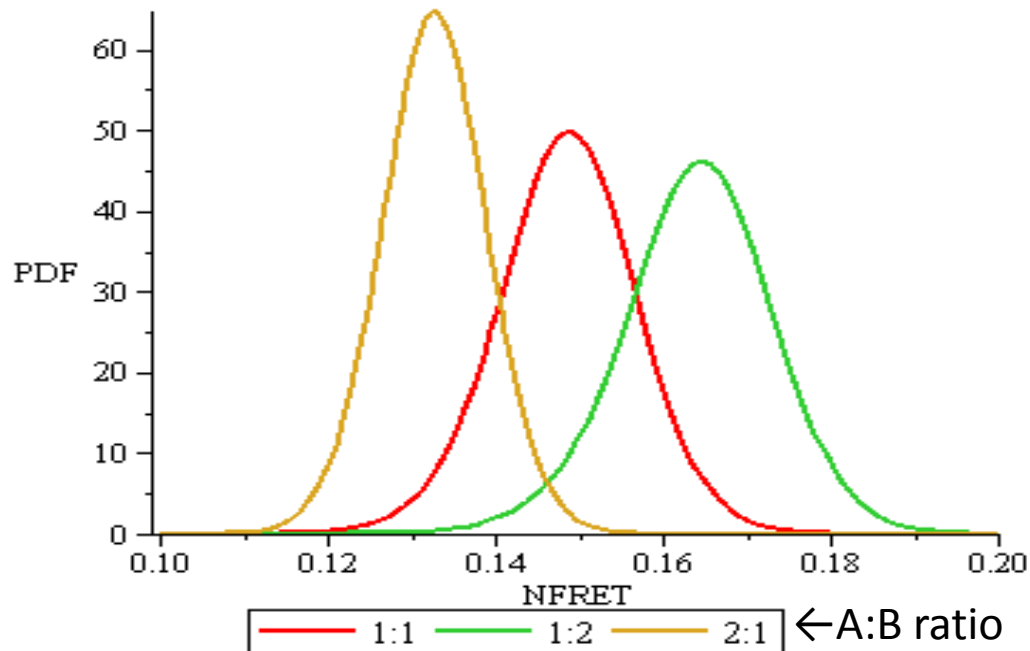
$$T(f) = \frac{f nF_a + (1 - f) nF_b}{\sqrt{(f A_a + (1 - f) A_b) (f D_a + (1 - f) D_b)}}$$



2) NFRET from multiple species combines nonlinearly (sometimes non-monotonically)

Dangers of fitting NFRET histograms

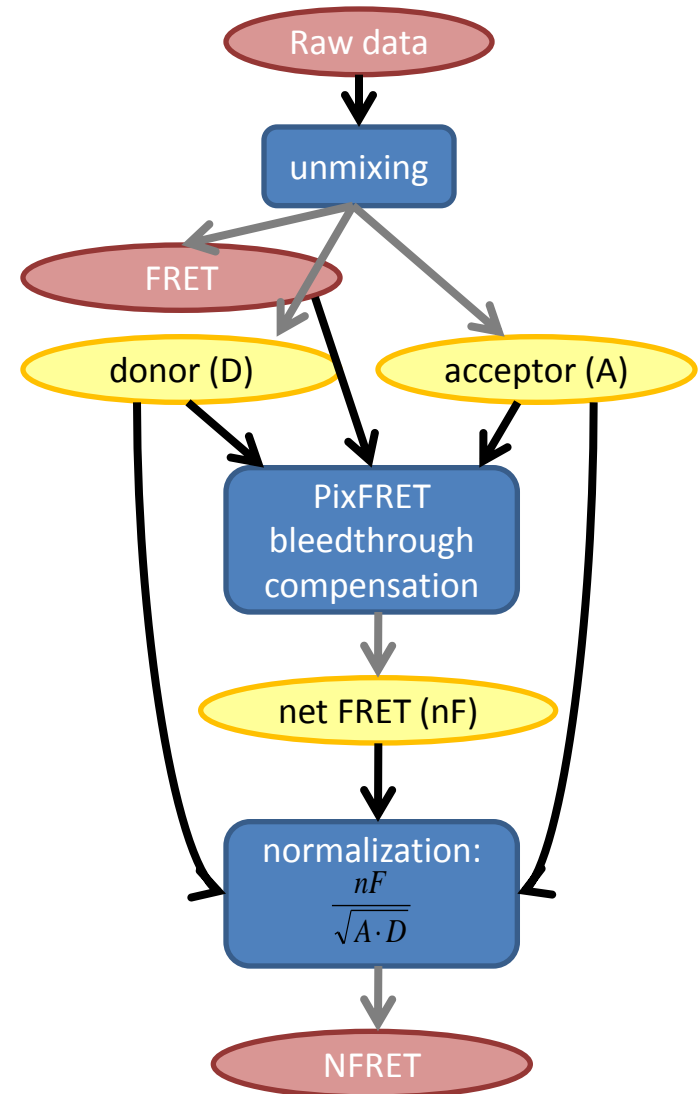
Two independently normally-distributed species with fixed nF , A , and D values per oligomer:



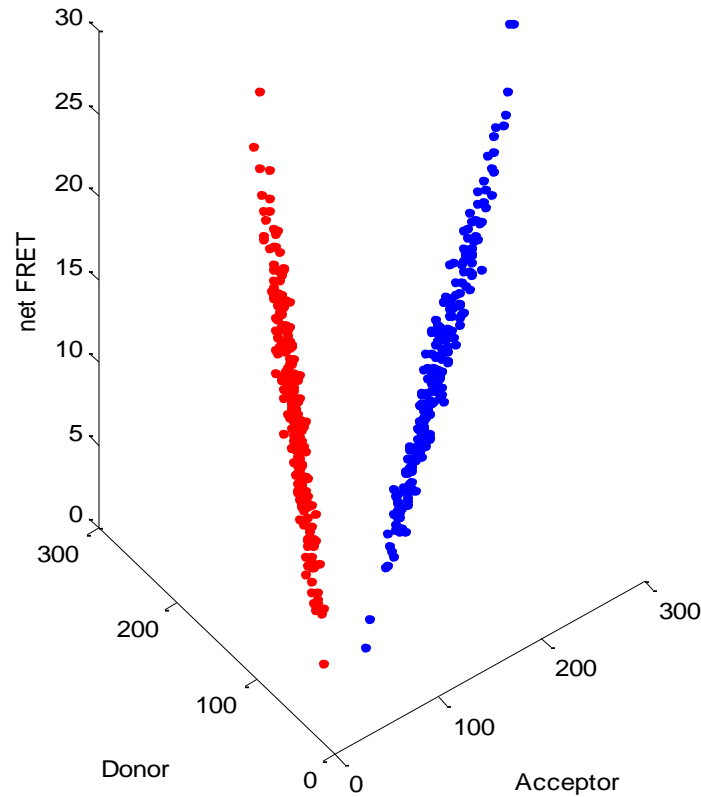
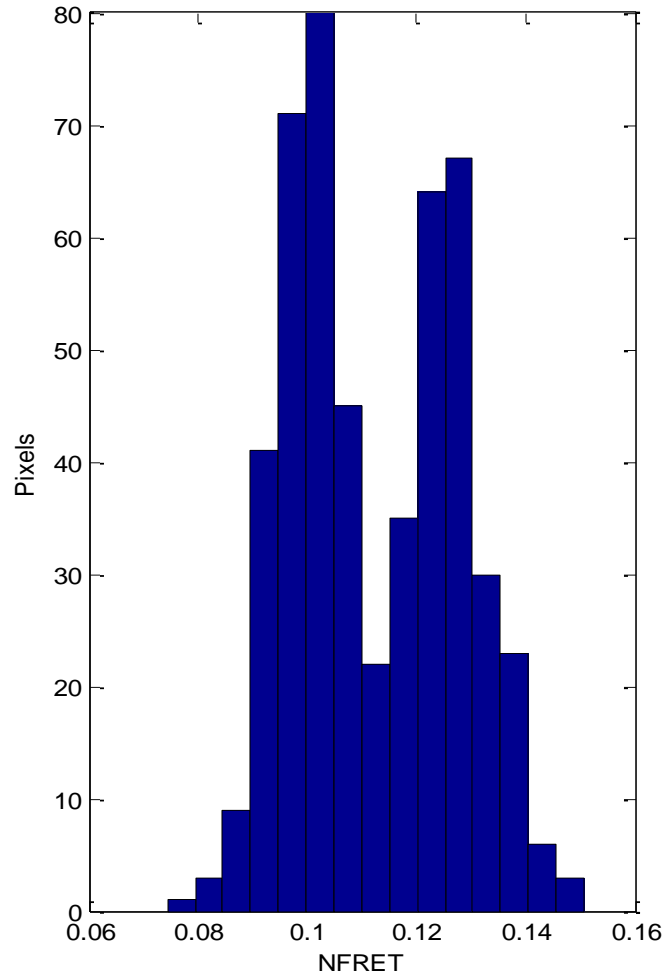
3) Even “ideal” situations (with no variation in nF , A , and D) give skew distributions of NFRET values.

The case for direct clustering instead

- Why collapse 3D information to 1D unnecessarily?
- Clustering automatically assigns pixels to populations.
- Deals more readily with unpaired fluorescence.



Two pure population model



Species A, mean NFRET 0.1:

$$nF \sim N(1, 0.2)$$

$$A \sim N(7, 0.7)$$

$$D \sim N(14, 1.4)$$

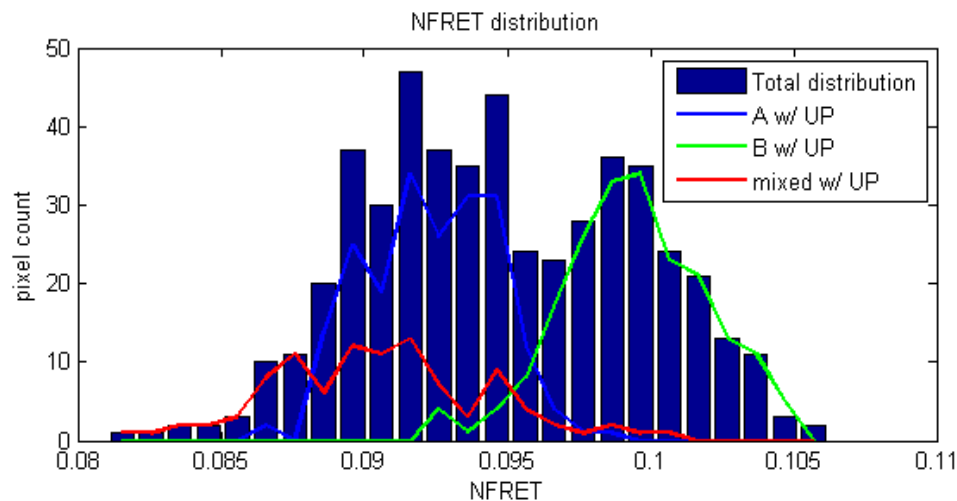
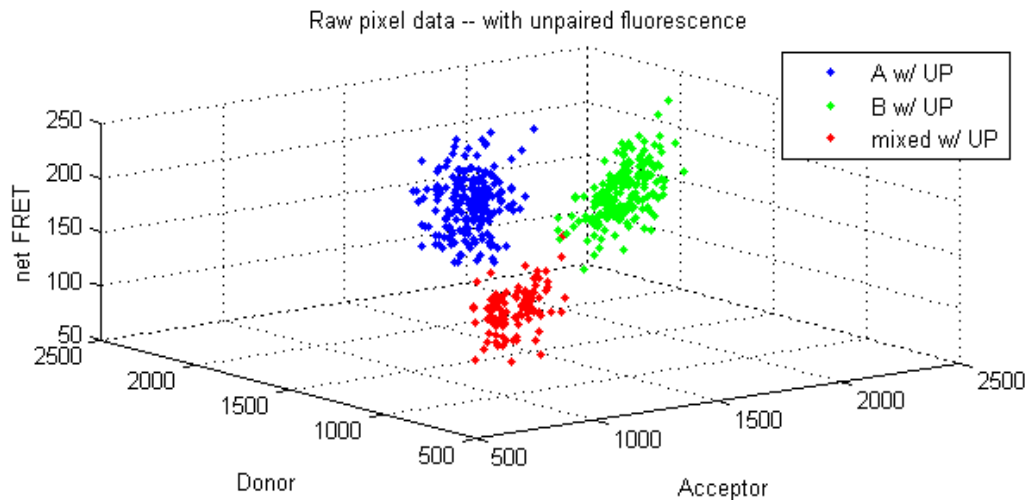
Species B, mean NFRET 0.125:

$$nF \sim N(1.25, .25)$$

$$A \sim N(14, 1.4)$$

$$D \sim N(7, 0.7)$$

Segments with unpaired donor/acceptor



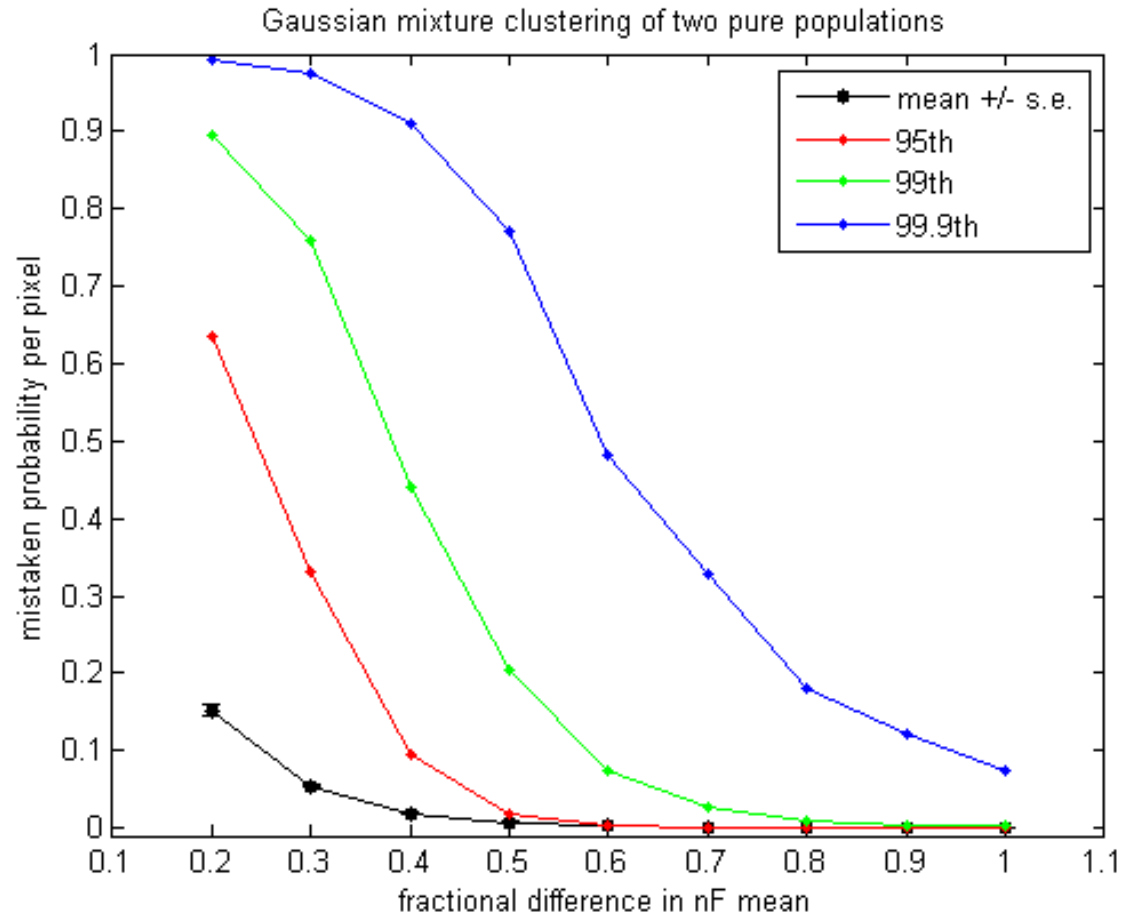
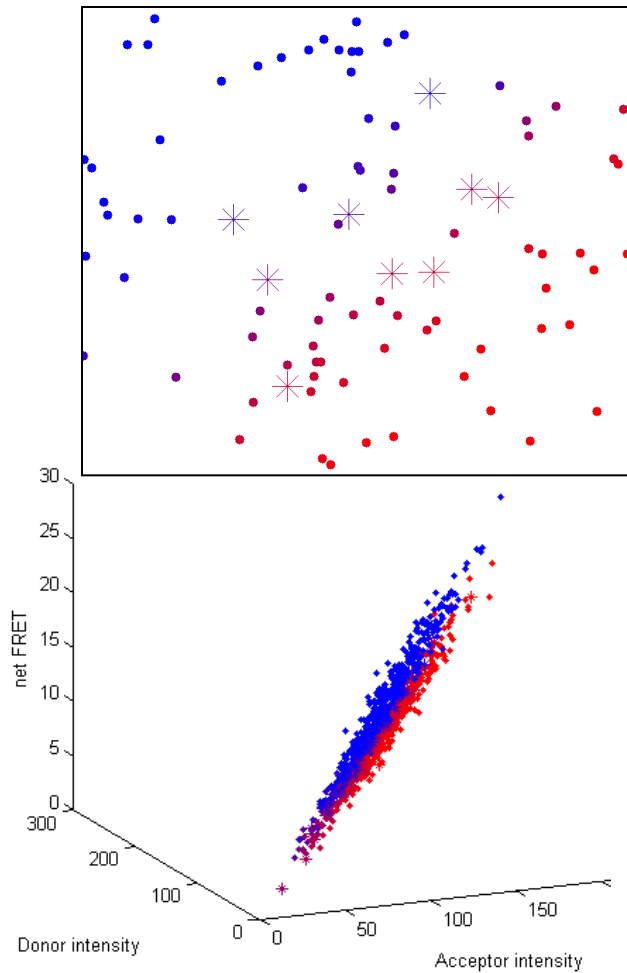
- Same species A and B, concentrations [100 50 50] and [50 100 50]
- Total unpaired concentration [25 25 25]
- Unpaired fluorophores have same properties as lesser of donor & acceptor in FRETing species

Choice of clustering algorithm

- Projective k-means
 - Clusters points along lines (representing varying concentrations of a single ratio of species, plus unpaired fluorescence)
 - Doesn't split high- and low-concentration regions
- **Gaussian mixture (GM) model**
 - Fits points to a set of Gaussian clusters
 - Doesn't ignore concentration
 - May be more robust to impure "segmentation"

Both easily extended to probabilistic clustering.

Performance of GM clustering



25 images each, 2000 pixels per image. 20% uncertainty in nF, 10% in A and D
Average concentration 10 oligomers (small) in both populations.

Performance of GM clustering

- **Accurately and reproducibly** clusters pixels from pure-population and segmented models, even with unpaired fluorescence
- Consistently **identifies the number of clusters** using Bayesian information criterion (introduces a parameter penalty to avoid overfitting)

Next steps

- Next focus is on clustering real data from two experiments: with three and one putative populations of nAChRs
- Use of membrane-specific and non-FRETing distributions to calibrate expected clusters
- Modeling varied transfection ratios and matching clusters across cells

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