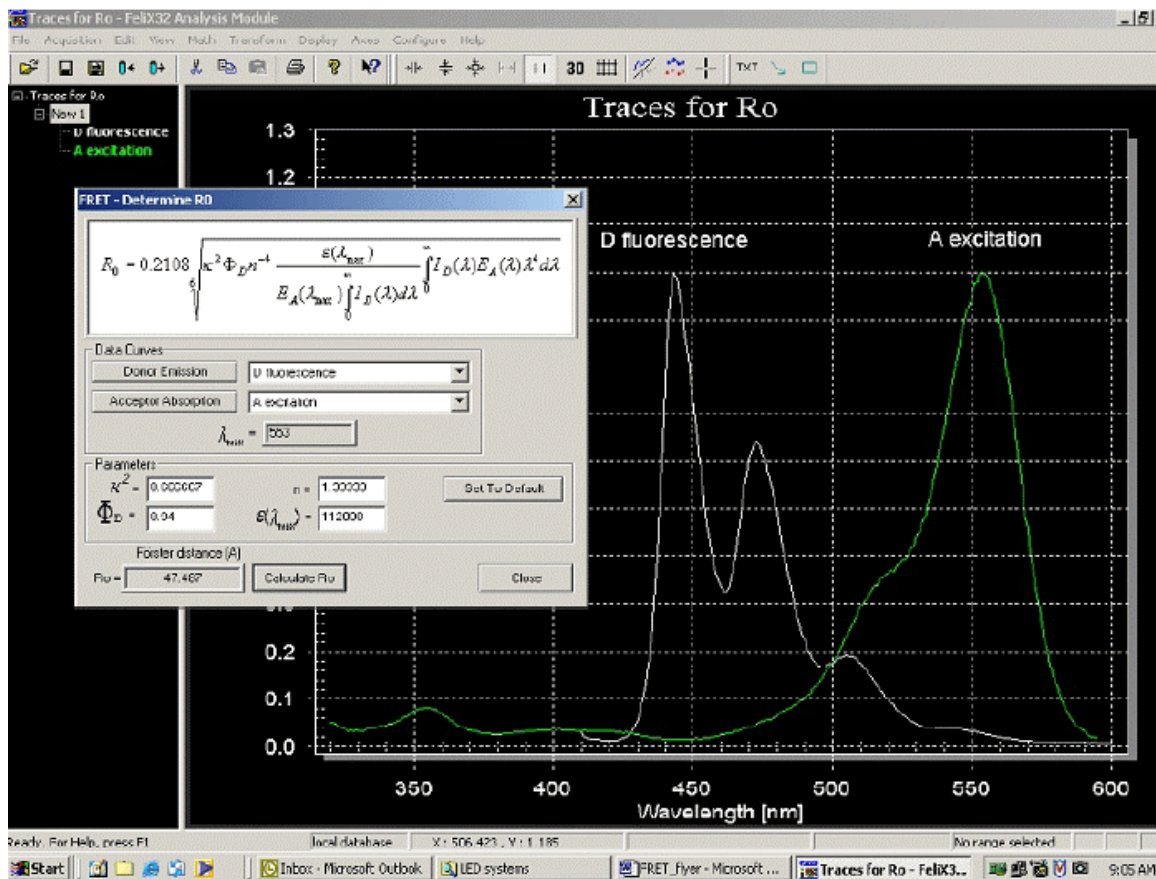




FRET Calculator from PTI: A Handy Tool for Your FRET Experiments

The **Fluorescence Resonance Energy Transfer (FRET)** technique is a very powerful tool in providing information about molecular distances, interactions in macromolecular systems, binding, diffusion, sensing etc. The FRET is essentially a molecular ruler, where distances are scaled with the Förster critical radius R_0 , which is a unique parameter for a given Donor-Acceptor (D-A) pair, defined by spectroscopic parameters of the pair and their environment. Once the R_0 is known and the FRET efficiency is determined experimentally (either from intensity or lifetime measurements), the D-A distance and the FRET rate constant can be calculated. The critical radius R_0 is crucial for any strict and quantitative application of FRET. Some R_0 values can be found in the literature, but the actual experimental conditions may change the R_0 value dramatically. For example, the tryptophan residue in various proteins can have its emission maximum ranging from ca. 310 to 370 nm and the quantum yield can vary by more than an order of magnitude, resulting in different R_0 values for D-A pairs with Trp as a donor and the same acceptor. The PTI, now included with **Felix32** software, provides an easy and convenient way of calculating all relevant FRET parameters, including R_0 . There are three easy steps to follow:



To determine R_o , open the **Determine R_o** box, provide an emission spectrum of the donor and an excitation spectrum of the acceptor (easy to measure with any of PTI's QuantaMaster steady state fluorometers!), enter the orientation factor $\hat{\epsilon}^2$, donor quantum yield \hat{O}_D (again, all you need is PTI's QuantaMaster!), refractive index n , the molar extinction coefficient \hat{a} of the acceptor and just click on **Calculate R_o** . In the case illustrated in the picture, $R_o = 47.487 \text{ \AA}$ was obtained for the Perylene-Rhodamine B pair. To determine the D-A distance and FRET efficiency from the steady state experiment, open the **Calculate FRET Parameters (steady state)** box and provide the intensities of the donor alone and the donor in the presence of acceptor. If the FRET rate constant is also required, provide the value of the donor lifetime in the absence of acceptor (all you need is PTI's TimeMaster or EasyLife system!). The intensities can either be entered manually, or read in directly from experimental curves with the data cursor, by integration or by average. Click on **Calculate** and the required FRET parameters are displayed.

To determine the FRET parameters from time-resolved experiments, open the **Calculate FRET parameters from lifetimes** box, enter the lifetimes of D alone and D in the presence of A (no problem if you have a TimeMaster or EasyLife!) and click on **Calculate**. The values of the FRET efficiency, D-A distance and FRET rate constant will be now displayed.

The FRET Calculator is part of the Felix32 software package, which comes with every QuantaMaster, TimeMaster, and EasyLife system.

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