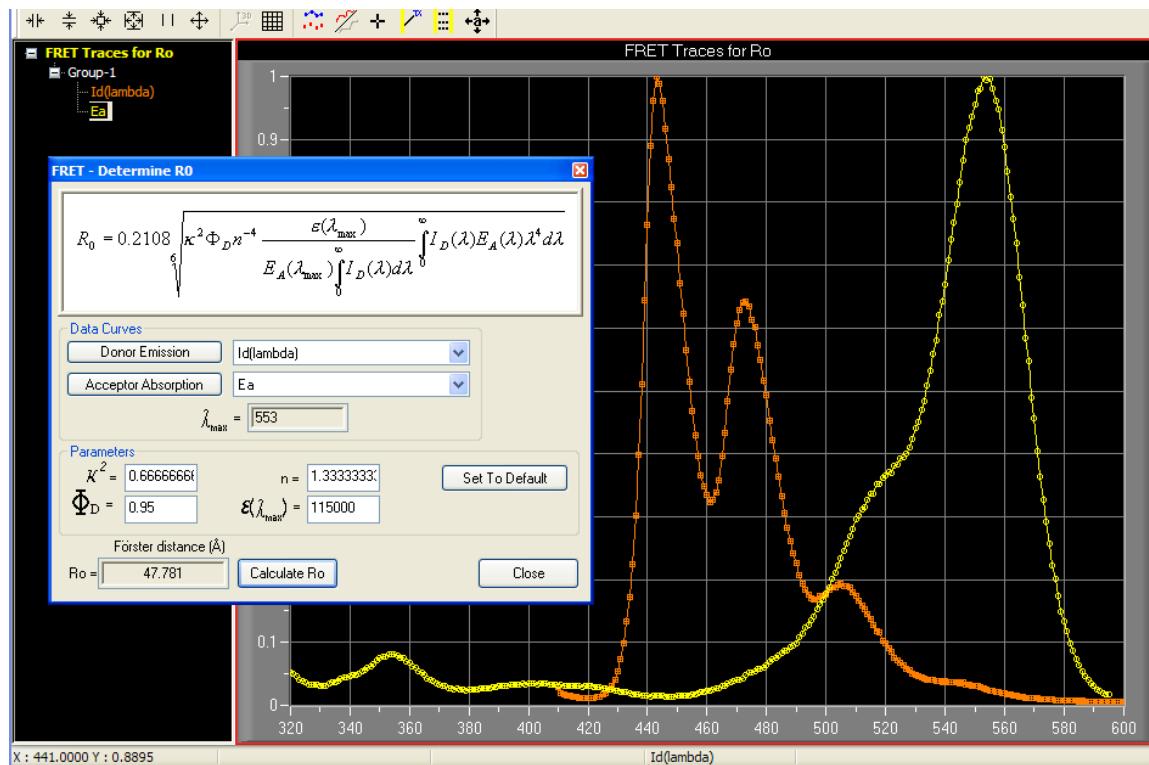
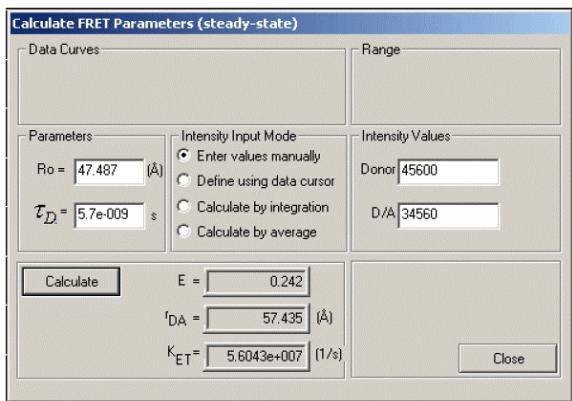




## THE FRET Calculator: A Handy Tool for Your FRET Experiments

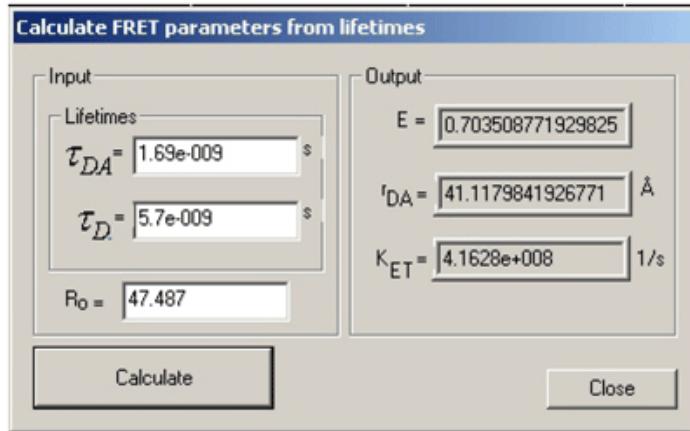
The **Fluorescence Resonance Energy Transfer (FRET)** technique is a very powerful tool in providing information about molecular distances, interactions in macromolecular systems, binding, diffusion, sensing etc. FRET is essentially a molecular ruler, where distances are scaled with the Förster critical radius  $R_o$ , 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  $R_o$  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  $R_o$  is crucial for any strict and quantitative application of FRET. Some  $R_o$  values can be found in the literature, but the actual experimental conditions may change the  $R$  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  $R_o$  values for D-A pairs with Trp as a donor and the same acceptor. The FRET Calculator, now included with **EasyLife** software, provides an easy and convenient way of calculating all relevant FRET parameters, including  $R_o$ . 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  $\kappa^2$ , donor quantum yield  $\Phi_D$ , refractive index  $n$ , the molar extinction coefficient  $\epsilon$  of the acceptor and just click on **Calculate  $R_o$** . In the case illustrated in the picture,  $R_o = 47.781 \text{ \AA}$  was obtained for the Perylene-Rhodamine D-A pair.

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 an 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 EasyLife's software package.

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