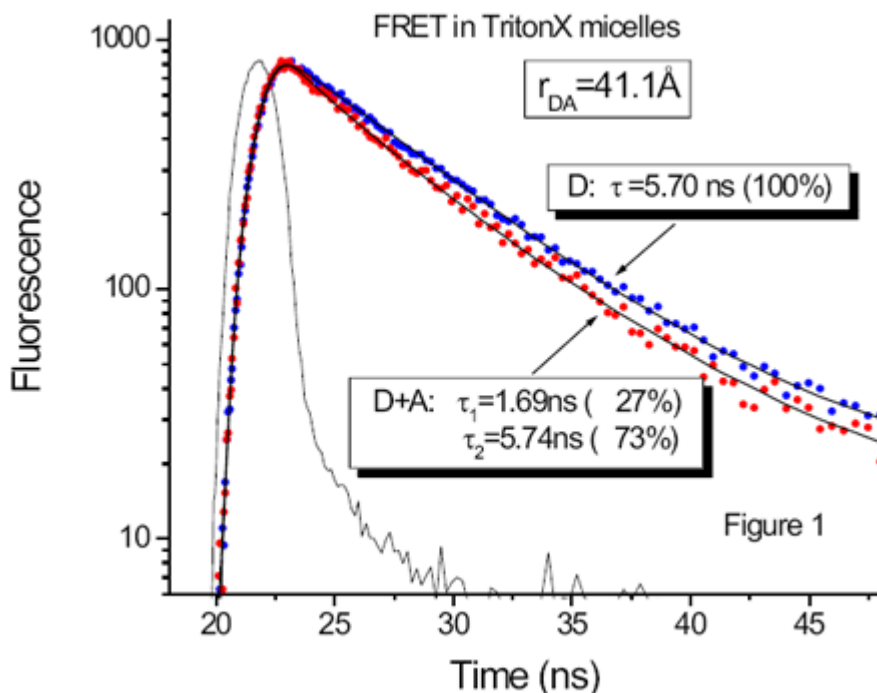




Time-Resolved FRET: Really Easy with the EasyLife LS and FeliX32 FRET Calculator!

Need to find the distance between two fluorophores in a multi-phase system, such as micelles, vesicles, liposomes, or membranes? No problem, all you need is the new, a really simple and portable, but powerful fluorescence lifetime system and the dedicated, which comes standard with the new version of FeliX32 software. Forget your steady state fluorometer, you'll never get the DA distance right with FRET when the donor and acceptor are in a multi-phase environment! As an example, consider **TritonX** micellar solution in water with **perylene** added as the **donor** and **Rhodamine B** as the **acceptor**. Perylene, an aromatic hydrocarbon, virtually insoluble in water will seek highly hydrophobic environment and will be localized exclusively inside TritonX micelles.



Its fluorescence decay as shown in Fig. 1 (trace D) is single exponential with the lifetime of 5.7 ns. The Rhodamine B is a hydrophilic molecule and will be mostly in the aqueous phase, but some fraction will also diffuse inside the micelles. When both D and A are confined to the same micelle, the energy transfer may occur. The perylene decay after the addition of Rhodamine B is shown in Fig. 1 (trace D+A). Now with the acceptor molecules added, the decay is double exponential with the short lifetime of 1.69 ns (27%) and the long lifetime of 5.74 ns (73%), the latter being the same as for the donor alone (trace D). The following conclusion can now be drawn: 27% of all micelles containing perylene also contain Rhodamine B acceptor and the FRET is quite efficient. For the

Rhodamine B molecules, which are in the aqueous phase the average distance is too great to cause FRET with the micelle-embedded perylene. To find the average distance between the D and A in a micelle, just open the FRET Calculator, enter the lifetimes of D alone (5.7ns), D in the presence of A (1.69 ns) and $R_0 = 47.5 \text{ \AA}$ (determined in a separate experiment see PTI note on FRET Calculator), click Calculate and the distance is 41.1 \AA . In addition you also get the FRET efficiency (70%) and the rate constant ($4.2 \times 10^8 \text{ s}^{-1}$).

It's that simple!

United States: 300 Birmingham Road, P.O. Box 272 Birmingham NJ 08011 Phone: 609-894-4420 Fax: 609-894-1579

Canada: 347 Consortium Court, London, Ontario, N6E 2S8 Phone: (519) 668-6920 Fax: (519) 668-8437

United Kingdom: Unit M1, Rudford Ind'l Est., Ford Rd, Ford, West Sussex BN18 0BF Phone: +44 (0) 1903 719 555 Fax: +44 (0) 1903 725 772

Germany: PhotoMed GmbH, Buero Sued, Inninger Str. 1, 82229 Seefeld Phone: +49-8152-993090 Fax: +49-8152-993098

Denmark: PhotoMed GmbH, Sondre Alle, DK-4600 Koge Phone: +45 56 66 33 86 Fax: +45 56 66 33 81

VIST US ONLINE AT WWW.PTI-NJ.COM

Copyright© 2005 Photon Technology International, Inc. All Rights Reserved. PTI is a registered trademark of Photon Technology International, Inc. Specifications are subject to change without notice. Rev. A