Frequency-Domain Lifetime Measurements Using ChronosFD

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Introduction

ChronosFD is the first, frequency-domain fluorescence lifetime instrument that enables measurement of time-resolved data using laser diodes (LDs) and light emitting diodes (LEDs) as excitation sources. This powerful instrument provides all the benefits of a full lifetime fluorometer but at an affordable price. ChronosFD is fully automated using Vinci, a Windows-based software package and is upgradeable to do steady-state measurements.

In order to demonstrate the flexibility of ChronosFD we have measured several fluorescent compounds with these LED and LD light sources. Measurement of these compounds on ChronosFD is fast and easy. Switching light sources can be done in a matter of minutes. LEDs and LDs provide excellent light sources with large modulation depth - up to 100% at certain frequencies, and they can be easily modulated up to 250 MHz (some up to 500 MHz). In this context we would like to refer you to the ISS Application Note: Frequency Domain Spectroscopy Using 280nm and 300nm LEDs.

All measurements were performed on ChronosFD, the modular lifetime fluorometer from ISS.

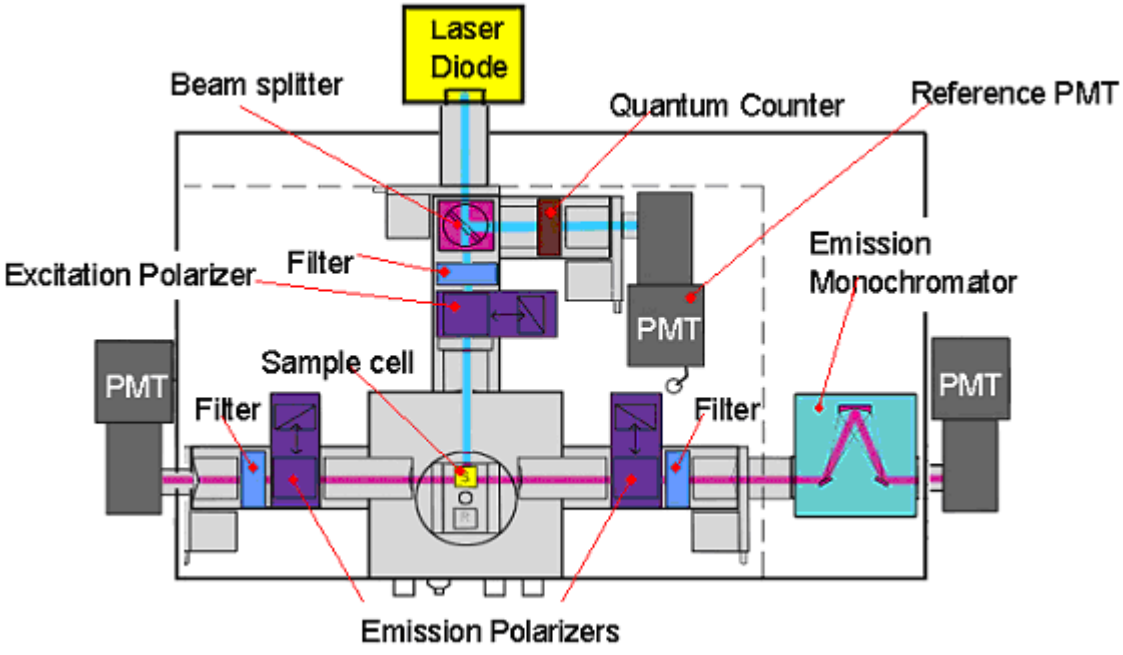

Figure 1. Schematic drawing of ChronosFD, the frequency-domain fluorescence lifetime instrument from ISS.

Experimental Data

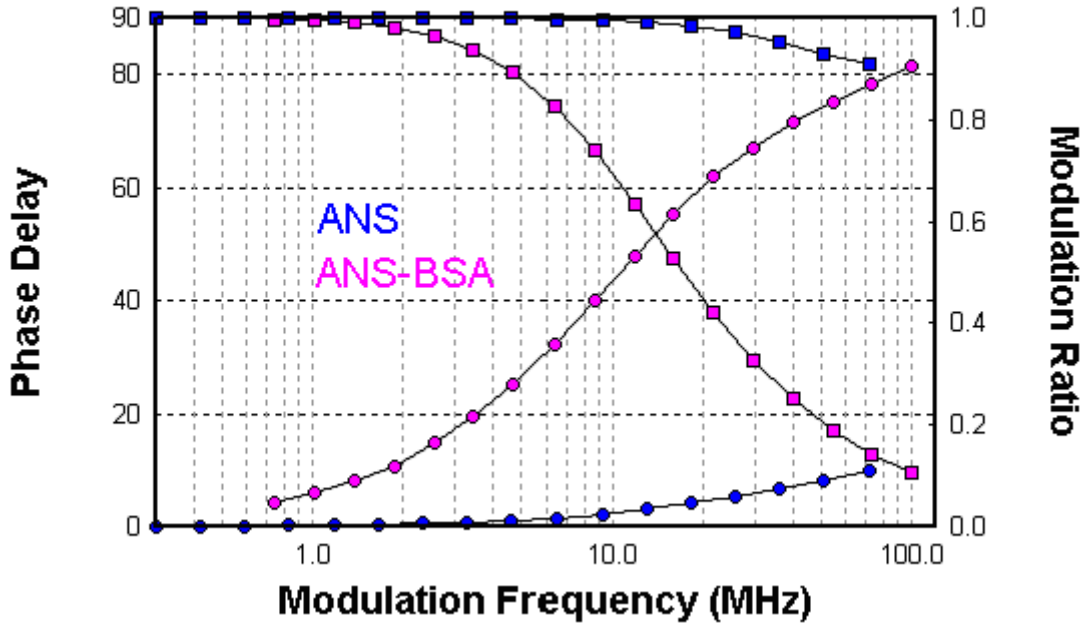

Figure 2. Frequency responses (phase and modulation) of free **ANS** in water and **ANS-BSA** in phosphate buffer pH 7.4 acquired on ChronosFD using a **370 nm LED.** The emission was collected through a high pass filter 390. The data is best fitted by a bi-exponential decay:

ANS free	τ_1 = 0.250 ns,	τ_2 = 3.55 ns,	f_1 = 0.87
ANS+**BSA** (2mg/mL)	τ_1 = 16.83 ns,	τ_2 = 3.02 ns,	f_1 = 0.83

Literature data [1]: Two components for BSA-bound **ANS**: τ_1 = 16 ns, τ_2 = 2-4 ns for DP=1/1.

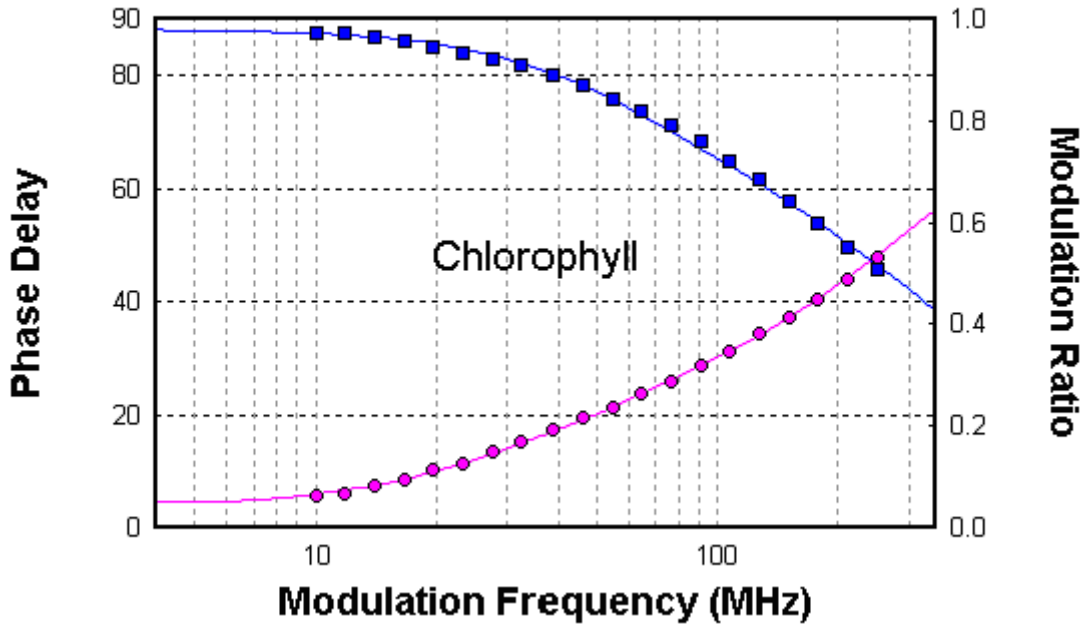

Figure 3.The plot shows the frequency responses (phase and modulation) of **Chlorophyll** (extracted from spinach leaves) acquired on ChronosFD using a **635 nm LD**. The emission was collected through a high pass filter 660. The data is best fitted by a bi-exponential decay time of 619 ps (64%) and 3.15 ns (36%), with an average lifetime of 1.52 ns.

Literature data [2]: Average lifetime for **Chlorophyll**: 1.2 ± 0.5 ns.

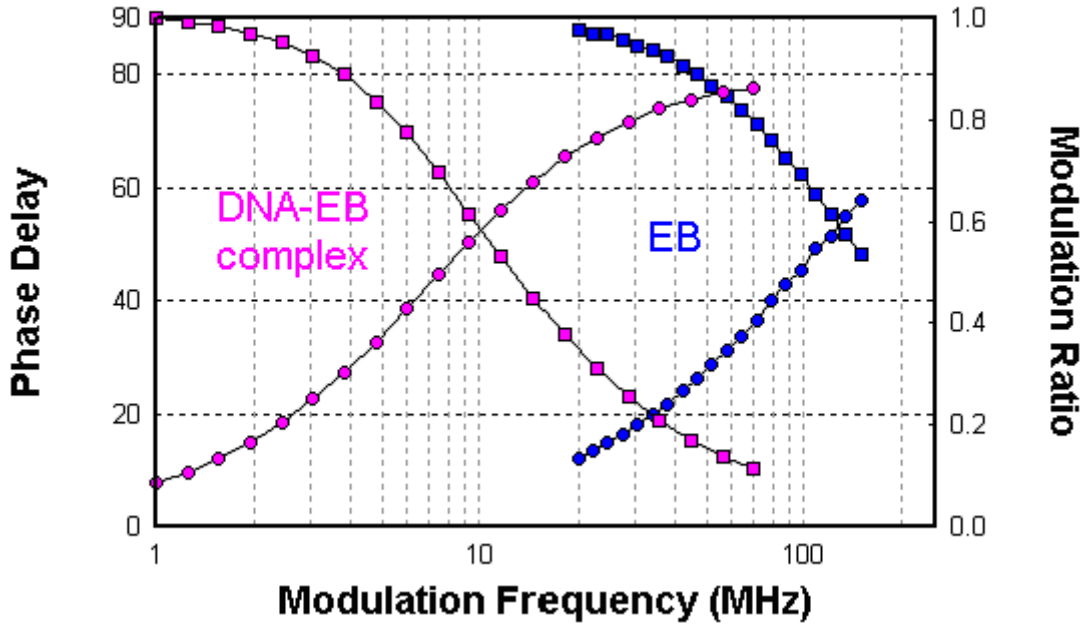

Figure 4. Frequency response curves (phase and modulation) of **Ethidium Bromide (EB)** free and in presence of calf thymus DNA (0.1 mg/mL) acquired on ChronosFD™ using a **471 nm LD**. The emission was collected through a high pass filter KV520. For the EB-DNA-complex the data is best fitted by two decay

times, 21.86 ns (98%) and 1.93 ns (2%) with an average lifetime of 21.4 ns. The free Ethidium Bromide is best fitted by a single decay time of 1.68 ns.

Literature data [3]: Average lifetime (**EB** bound to **DNA**)**:** 23 ns.

Literature data [4]: Lifetime of free **EB**: 1.7 ns (single exponential).

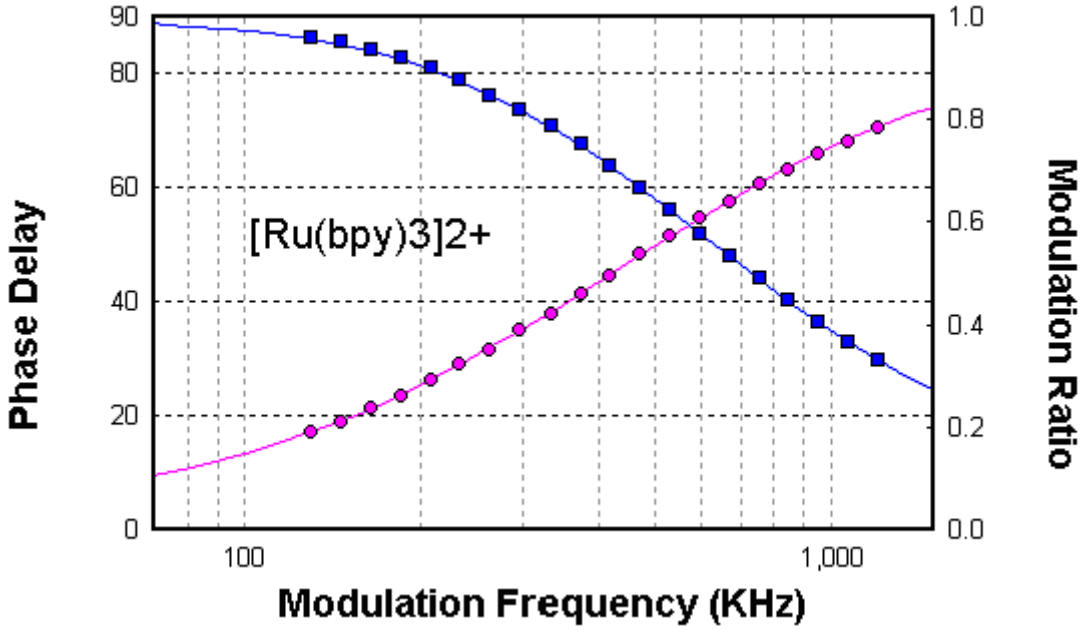

Figure 5. Frequency response curves (phase and modulation) of **$[Ru(bpy)_3]Cl_2$** (**T ris[2,2'-bipyridyl]Ruthenium(II) chloride**) in water acquired on ChronosFD using a **471 nm LD**. The emission was collected through a high pass filter 520KV. The data is best fitted by a single exponential decay time of 377 ns (air).

Literature data [5]: lifetime = 367 ns (single exponential, air).

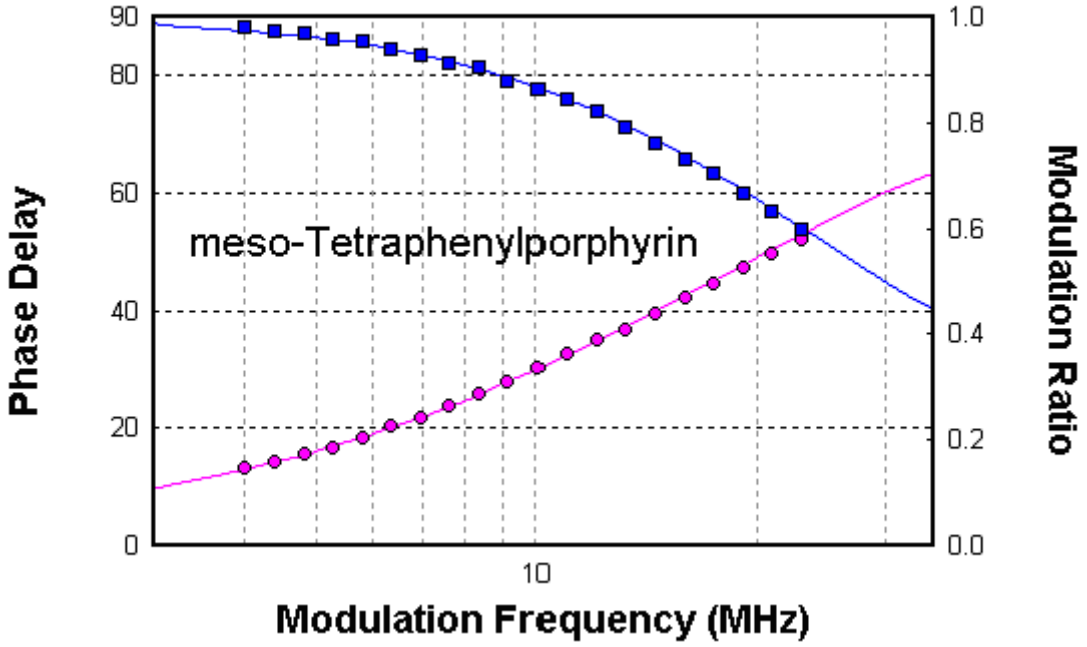

Figure 6. The plot shows the frequency response curve (phase and modulation) of **Meso-tetraphenylporphyrin** in toluene acquired on ChronosFD using **473 nm LD**. The emission was collected through a high pass filter 625 KV. The data is best fitted by a single exponential decay time of 9.16 ns.

Literature data [6]: 9.32 ns (single exponential) in toluene.

References:

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