FLUORESCENCE AND BIOMEDICAL INSTRUMENTATION

Fluorescence Lifetime Measurements of BODIPY and Alexa Dyes on ChronosFD and K2

ISS, Inc.

Introduction

ChronosFD is the first frequency-domain fluorometer that enables measurement of time-resolved data with laser diodes (LDs) and light emitting diodes (LEDs). ChronosFD offers all the benefits of a full time-resolved fluorometer but at an affordable price. ChronosFD is fully automated using Vinci, a Windows-based software and is upgradeable for steady-state measurements.

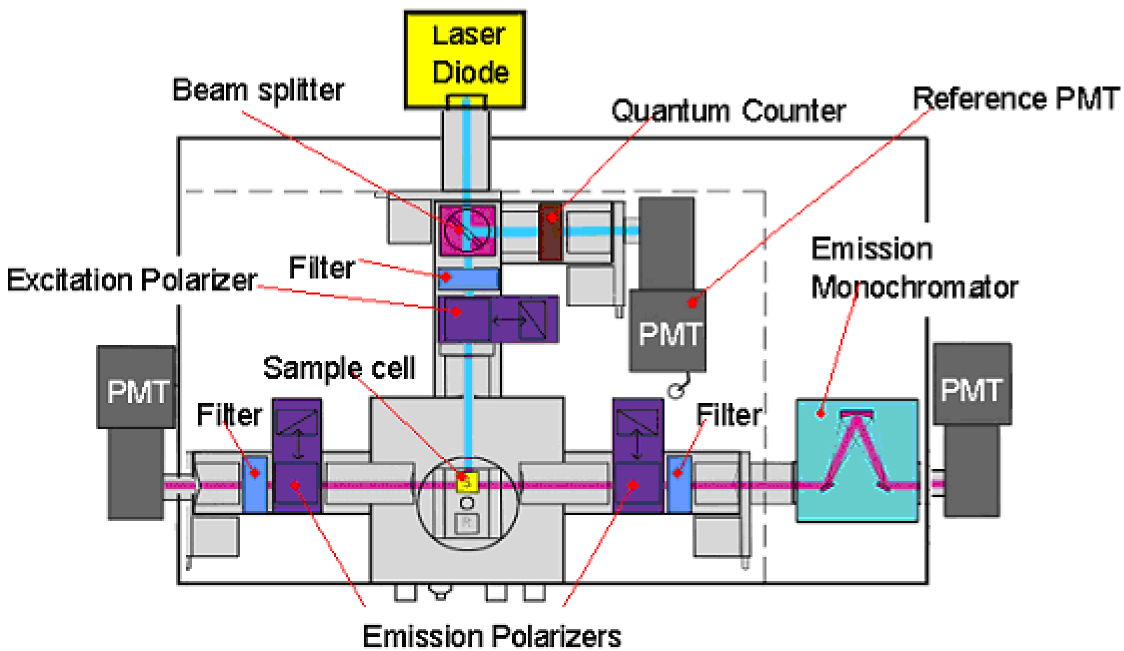

Figure 1. Schematic drawing of ChronosFD, the frequency-domain fluorometer from ISS.

With over 100 installed units all over the world, the K2 family of multifrequency cross-correlation phase and modulation fluorometers represents a solid and well-proven approach to fluorescence and phosphorescence instrumentation for the research laboratory. Its optical design and automatic instrument control are state-of-the-art for steady-state and time-resolved fluorescence measurements. Full automation and the user-friendly Vinci software package make the instrument easy to use for both the student and the scientist.

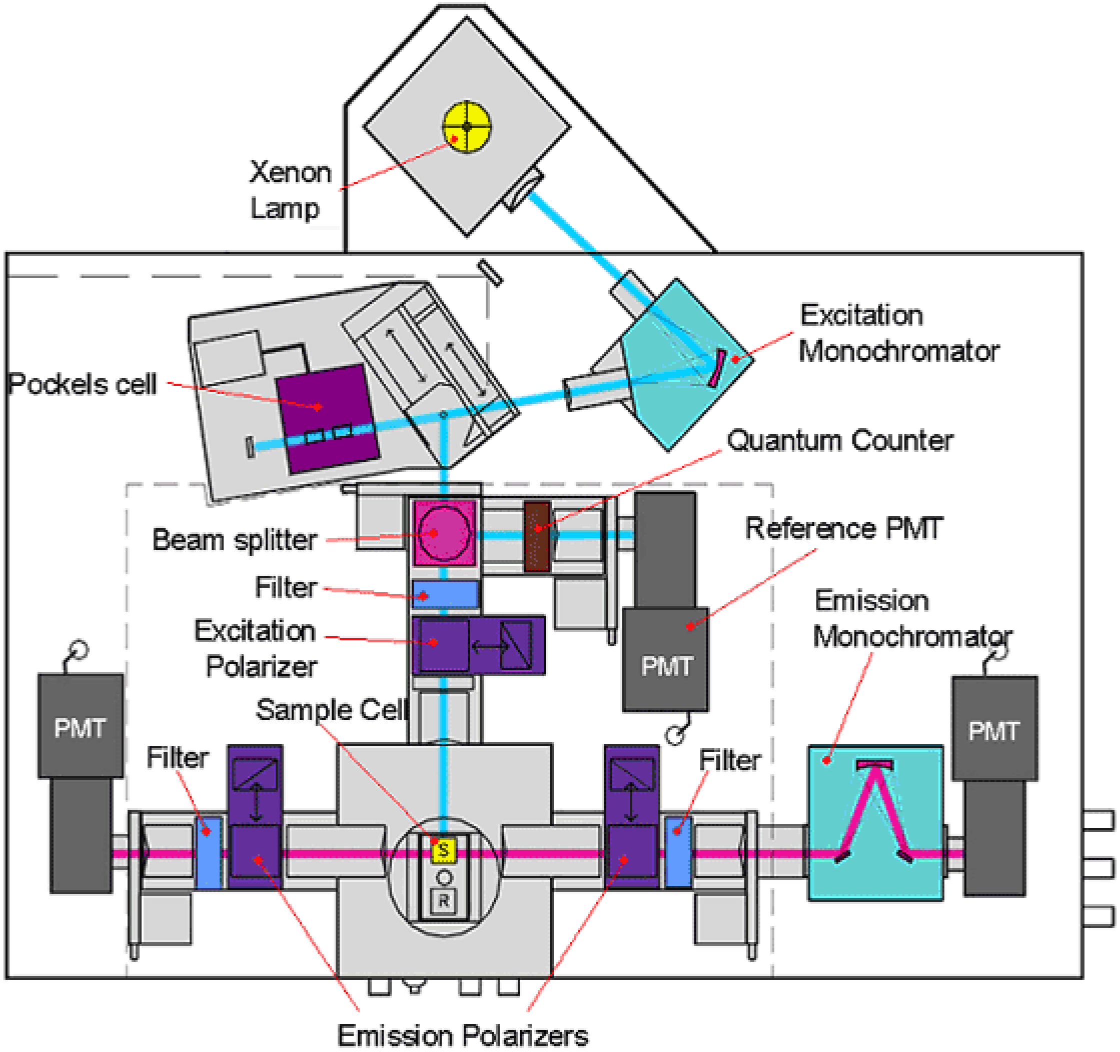

Figure 2. Schematic drawing of K2, the steady-state and time-resolved fluorometer from ISS.

K2 utilizes a Pockels Cell for light modulation and is capable of measuring lifetimes as low as a few hundred picoseconds using a conventional Xenon lamp (see measurement example below).

Lifetime measurements on ChronosFD are fast and easy. Switching light sources can be done in 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 280-nm and 300-nm LEDs.

For a demonstration of the capabilities of ChronosFD and K2 we have acquired lifetime measurements on a series of BODIPY and Alexa dyes.

Experimental Data

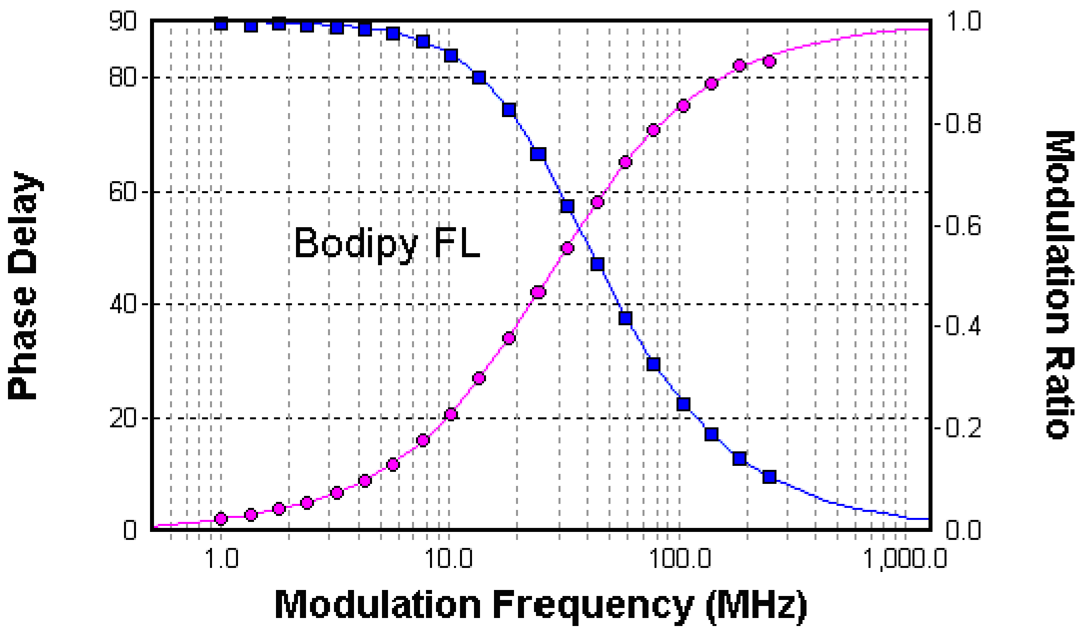

Figure 3. Frequency responses (phase and modulation) of BodipyFL in water acquired on ChronosFD using a 471-nm laser diode. The emission was collected through a high pass filter 520KV. The data is best fitted with a single exponential decay time of 5.87 ns (x^2 = 0.97).

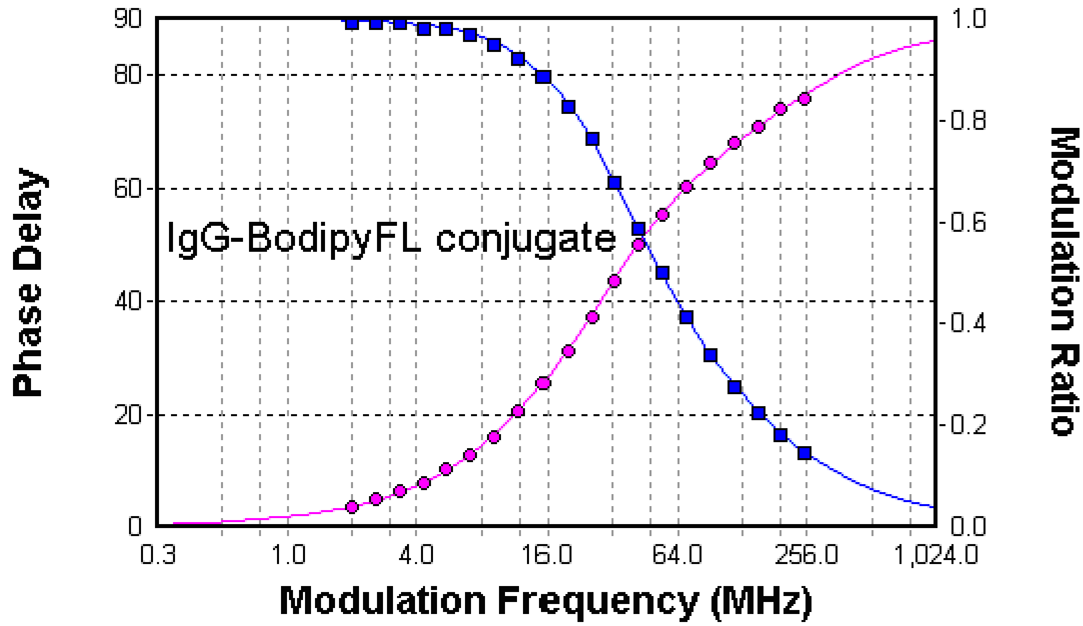

Figure 4. Frequency responses (phase and modulation) of IgG-BodipyFL conjugate in water acquired on ChronosFD using a 471-nm laser diode. The emission was collected through a 520KV high pass filter. The data is best fitted with a bi-exponential decay time of 10% - 1.3 ns, 90% - 5.5 ns, with an average lifetime of 5.08 ns (= 1.35).

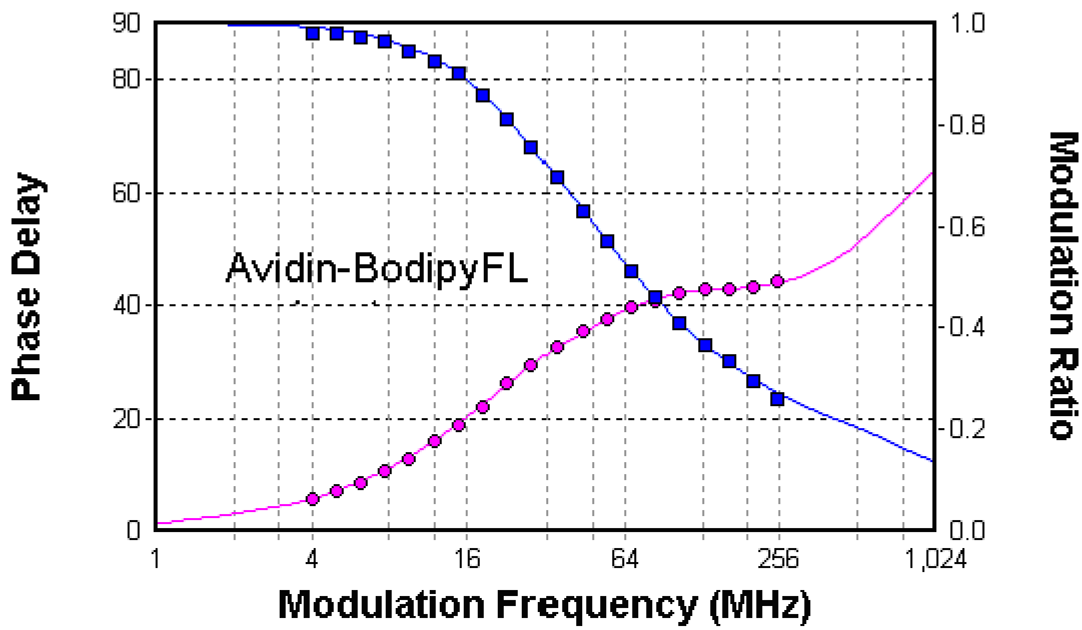

Figure 5. Frequency responses (phase and modulation) of Avidin-BodipyFL conjugate in water acquired on ChronosFD using a 471-nm laser diode. The emission was collected through a 520KV high pass filter. The data is best fitted with a tri-exponential decay time of 19% - 244 ps, 22% - 2.02 ns and 59% - 6.04 ns, with an average lifetime of 4.05 ns (x^2 = 1.43).

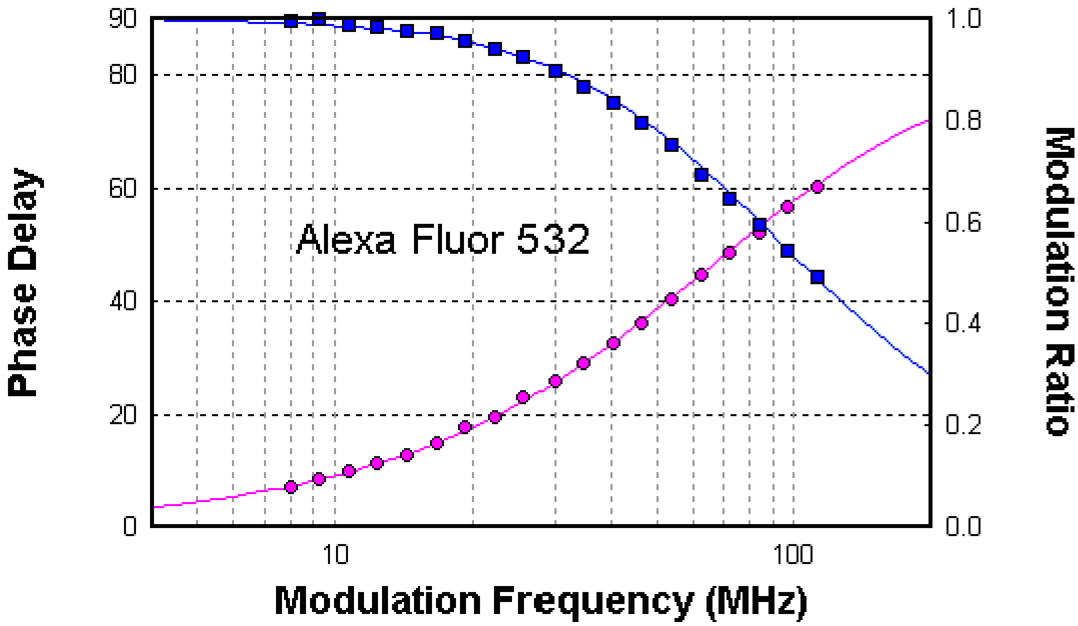

Figure 6. Frequency responses (phase and modulation) of Alexa 532 in water acquired on K2 using a Xenon lamp. The excitation wavelength was 470-nm. The emission was collected through a 545/65 band pass filter. The data is best fitted with a single exponential decay time of 2.53 ns (x^2 = 1.04).

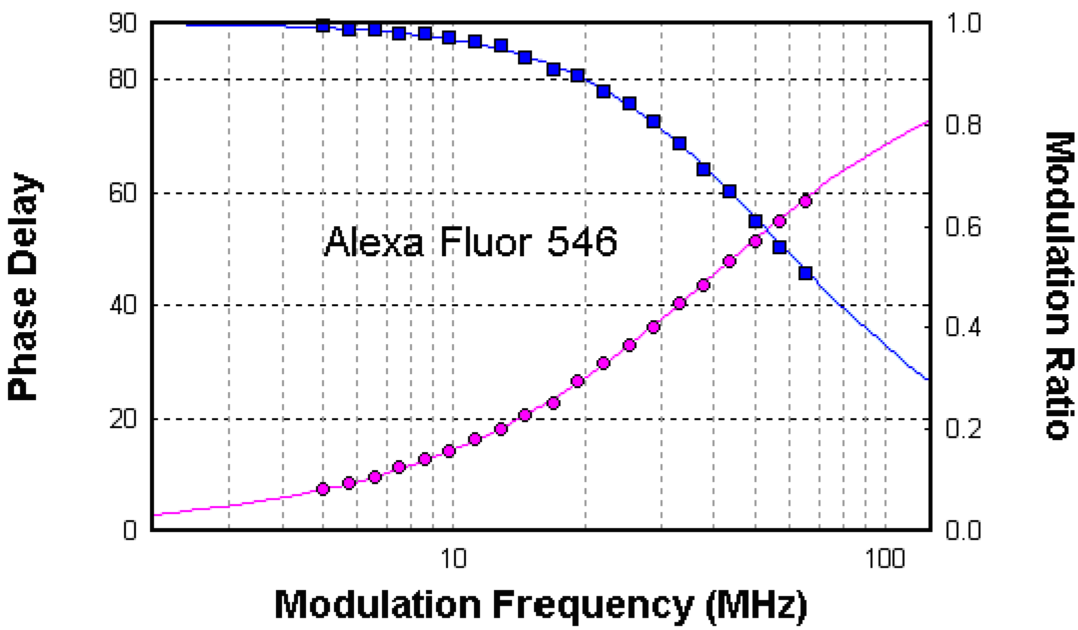

Figure 7. Frequency responses (phase and modulation) of Alexa 546 in water acquired on K2 using a Xenon lamp. The excitation wavelength was 485-nm with a 1mm slit size. The emission was collected through a 545/65 band pass filter. The data is best fitted with a single exponential decay time of 4.06 ns (x^2 = 0.98).

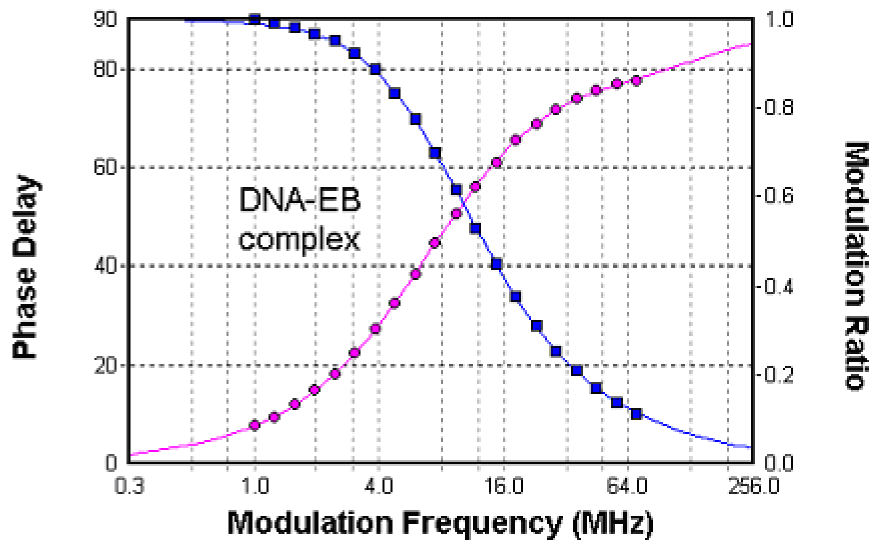

Figure 8. Frequency responses (phase and modulation) of Ethidium Bromide (EB) in presence of calf thymus DNA (0.1 mg/mL) acquired on ChronosFD using a laser diode excitation at 471-nm. The emission was collected through a high pass filter KV520. The data is best fitted with a mean bi-exponential decay time of 21.4 ns.

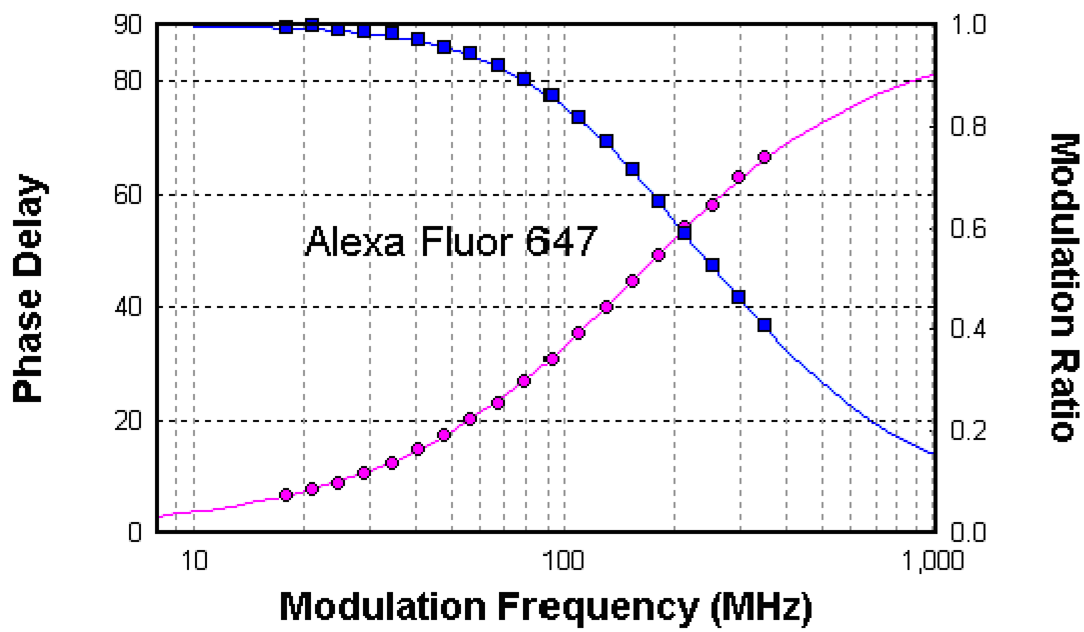

Figure 9. Frequency responses (phase and modulation) of Alexa 647 in water acquired on ChronosFD using a 635-nm laser diode. The emission was collected through a 660HP high pass filter. The data is best fitted with a single exponential decay time of 1.03 ns (x^2 = 1.05).

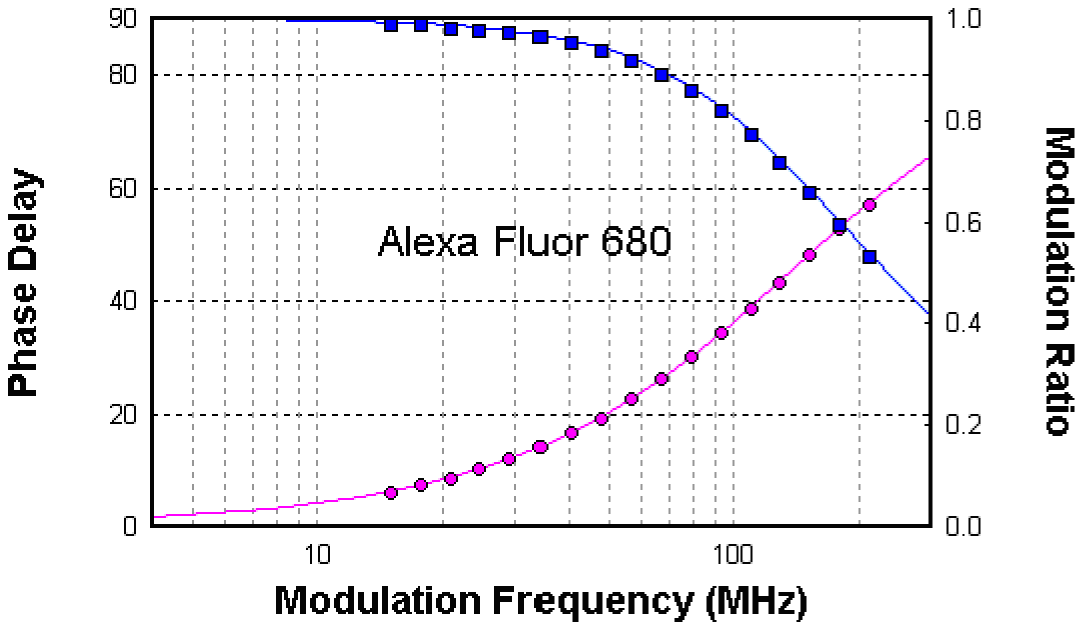

Figure 10. Frequency responses (phase and modulation) of Alexa 680 in water acquired on ChronosFD using a 635-nm laser diode. The emission was collected through a 660HP high pass filter. The data is best fitted with a single exponential decay time of 1.17 ns (x^2 = 1.72).

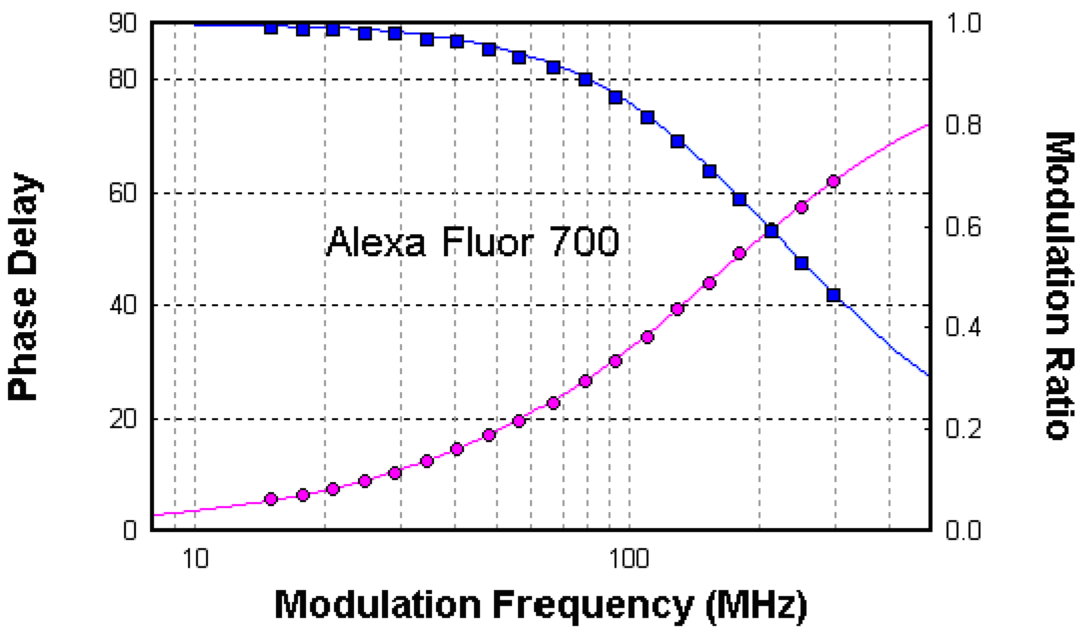

Figure 11. Frequency responses (phase and modulation) of Alexa 700 in water acquired on ChronosFD using a 635-nm laser diode. The emission was collected through a 660HP high pass filter. The data is best fitted with a single exponential decay time of 1.01 ns (x^2 = 1.43).

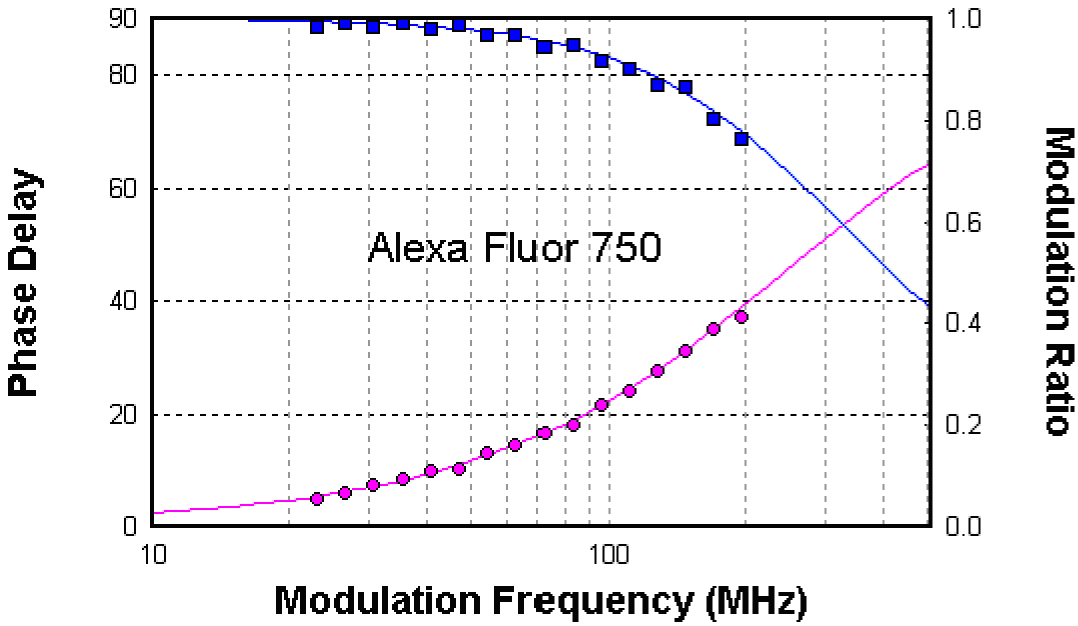

Figure 12. Frequency responses (phase and modulation) of Alexa 750 in water acquired on K2 using a Xenon lamp. The excitation wavelength was 700-nm (slit size: 1mm). The emission was collected through a 710HP high pass filter. The data is best fitted with a single exponential decay time of 657 ps (x^2 = 1.28).

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