



Innovations in Fluorescence

Introduction to Fluorescence Instrumentation: Fluorometers

Fluorescence Instrumentation:

Hands-on Experiments

- Steady-State - PC1
- Fluorescence Lifetime

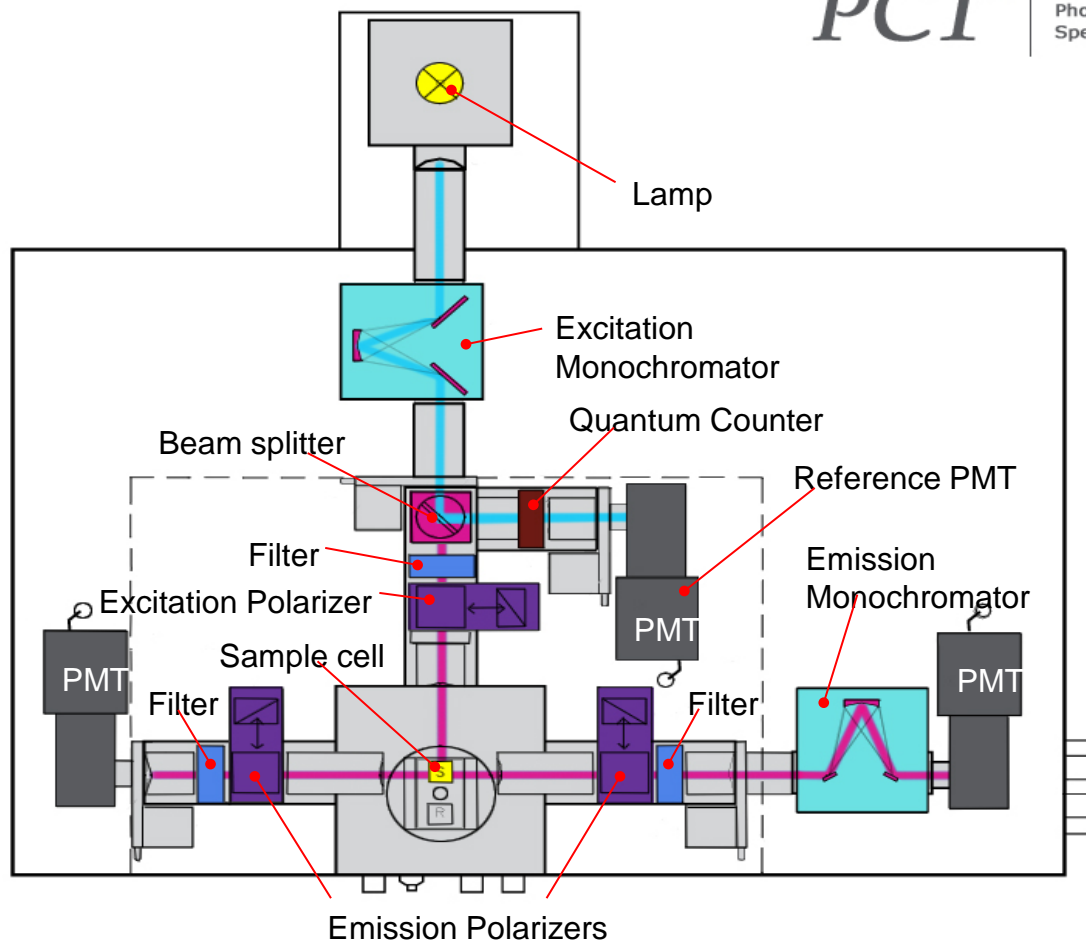
Chronos: FD

ChronosBH: TD

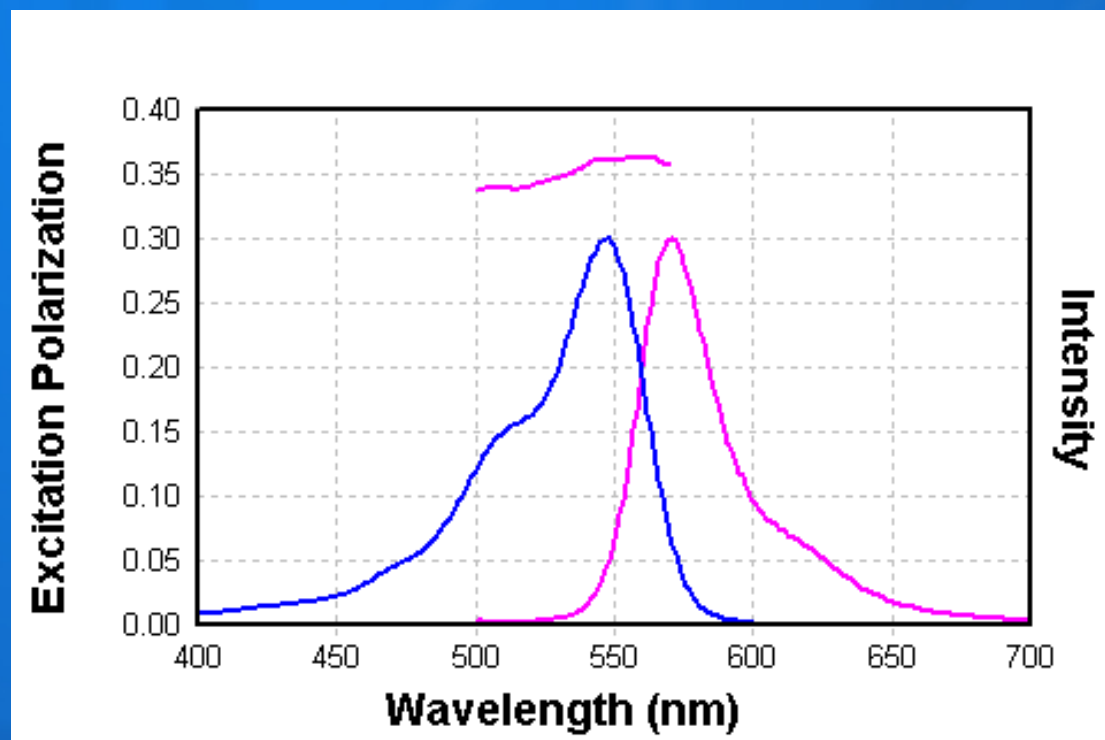
Steady-State Fluorescence PC1

- Compact Design
- T-format with Parallel Beam Geometry
- Upgradeable to Lifetime
- Fully Automated





Measurement Capabilities:



- Excitation Spectra
- Emission Spectra
- Synchronous Spectra
- Steady-State Polarization



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Product-Line:

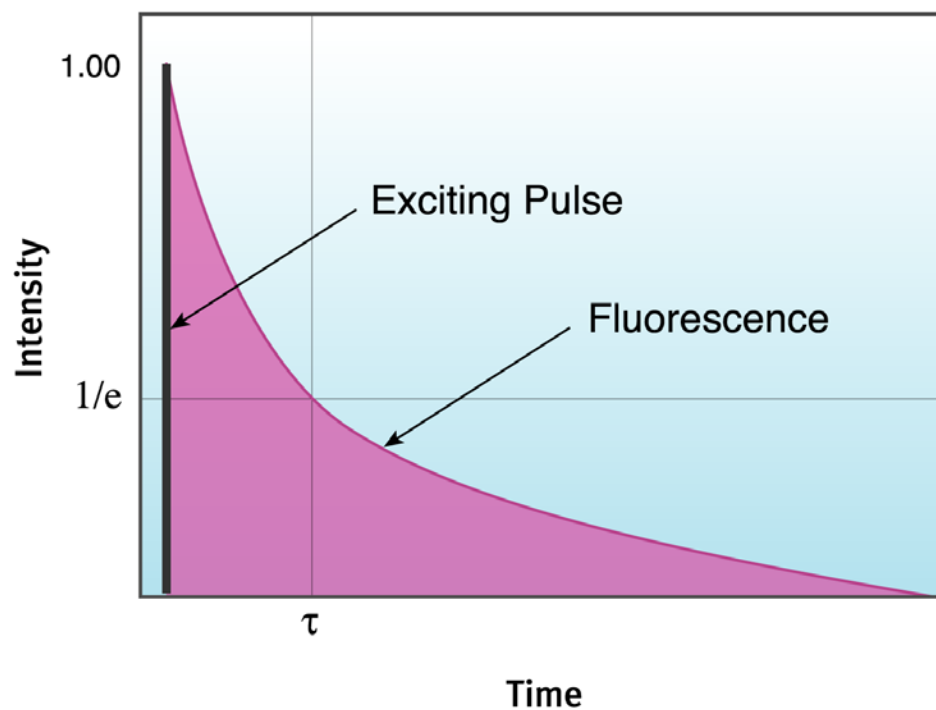
- Fluorescence Lifetime



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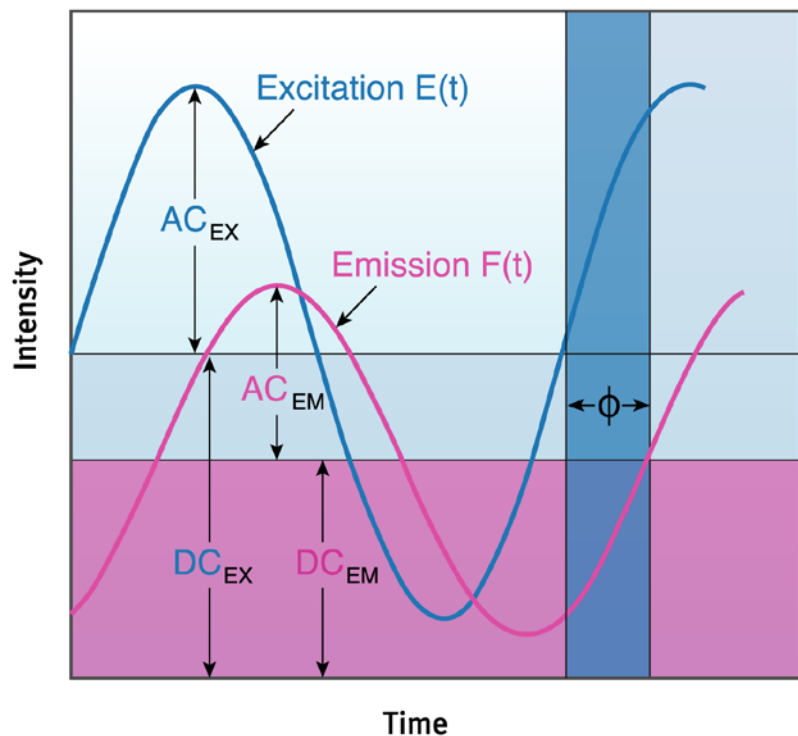
Two Ways to Measure Lifetime:

Time-Domain



$$I_t = \alpha e^{-t/\tau}$$

Frequency Domain



■ Phase Shift

$$\tan \phi = \omega \tau$$

■ Demodulation

$$M = \frac{(AC/DC)_{EM}}{(AC/DC)_{EX}}$$

$$M = \frac{1}{\sqrt{1 + (\omega \tau)^2}}$$



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Since January 2008 ISS offers both
Time-Domain and Frequency-Domain
Instrumentation

What are the main characteristics of Frequency Domain (FD)?

- In FD fluorescence lifetime is calculated from 2 measurable parameters: phase angle and modulation
- FD requires no deconvolution
- FD allows direct, one step measurements of anisotropy decays (rotational correlation times)
- FD is better in resolving short lifetime contributions as compared to TD
- FD is the method of choice for lifetime-based sensing and real-time measurements because of high sampling rates in the ms time scale

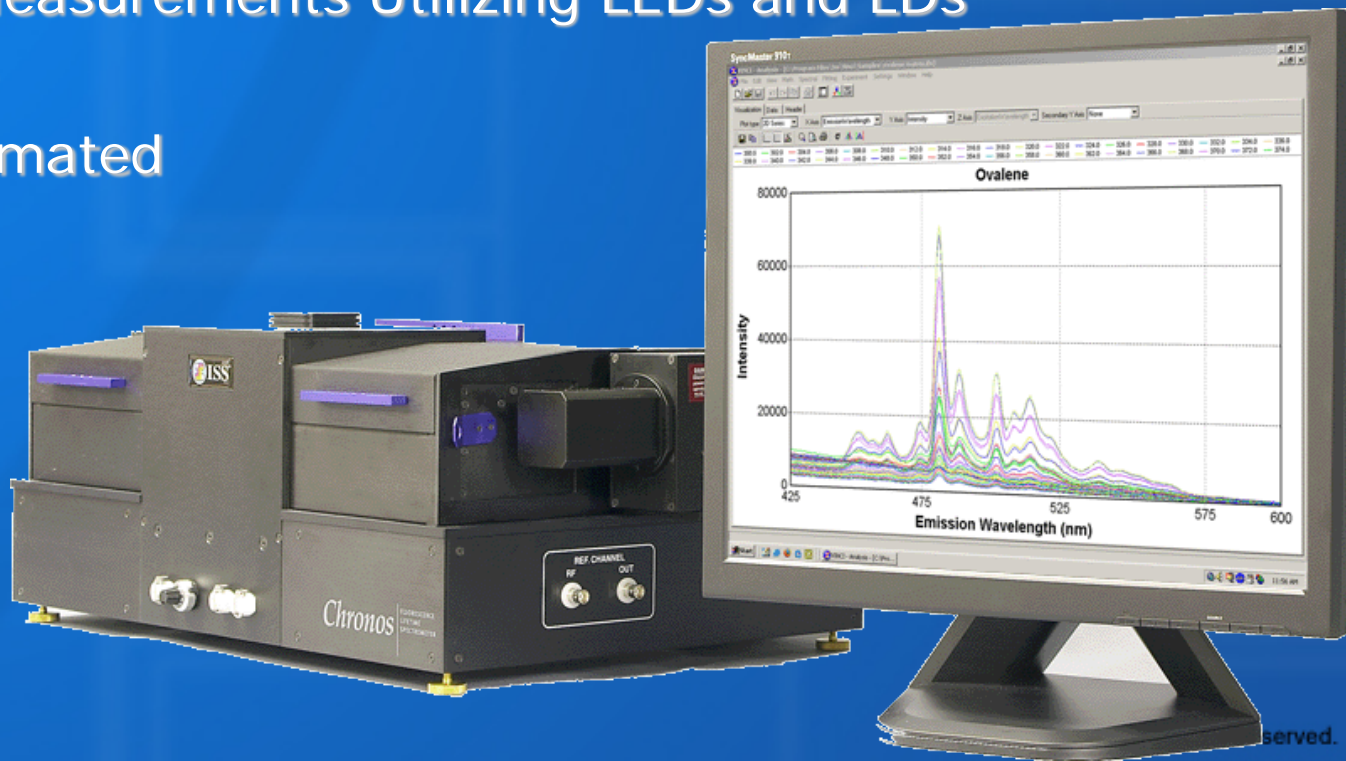
What are the main characteristics of Time Domain (TD)?

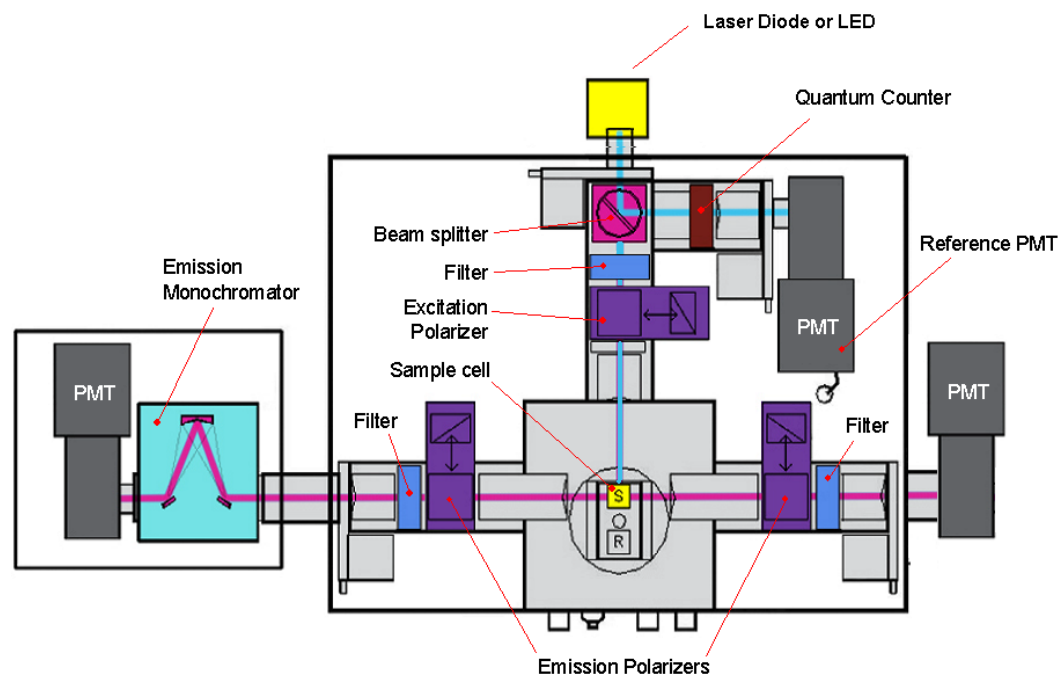
- As compared to FD, TD is a more direct way of measuring lifetime
- Unlike FD, TD requires no reference but measurement of an instrument response function (IRF)
- TD anisotropy decay measurements do require two separate measurements at each plane of polarization
- TD has a low duty cycle - approximately only one photon per every 50 flashes is measured
- TD is the preferred method for measuring low fluorescence compounds

Fluorescence Lifetime Chronos

Multi-Frequency Cross-Correlation Phase Modulation Fluorimeter

- Lifetime Measurements Utilizing LEDs and LDs
- Affordable
- Fully Automated







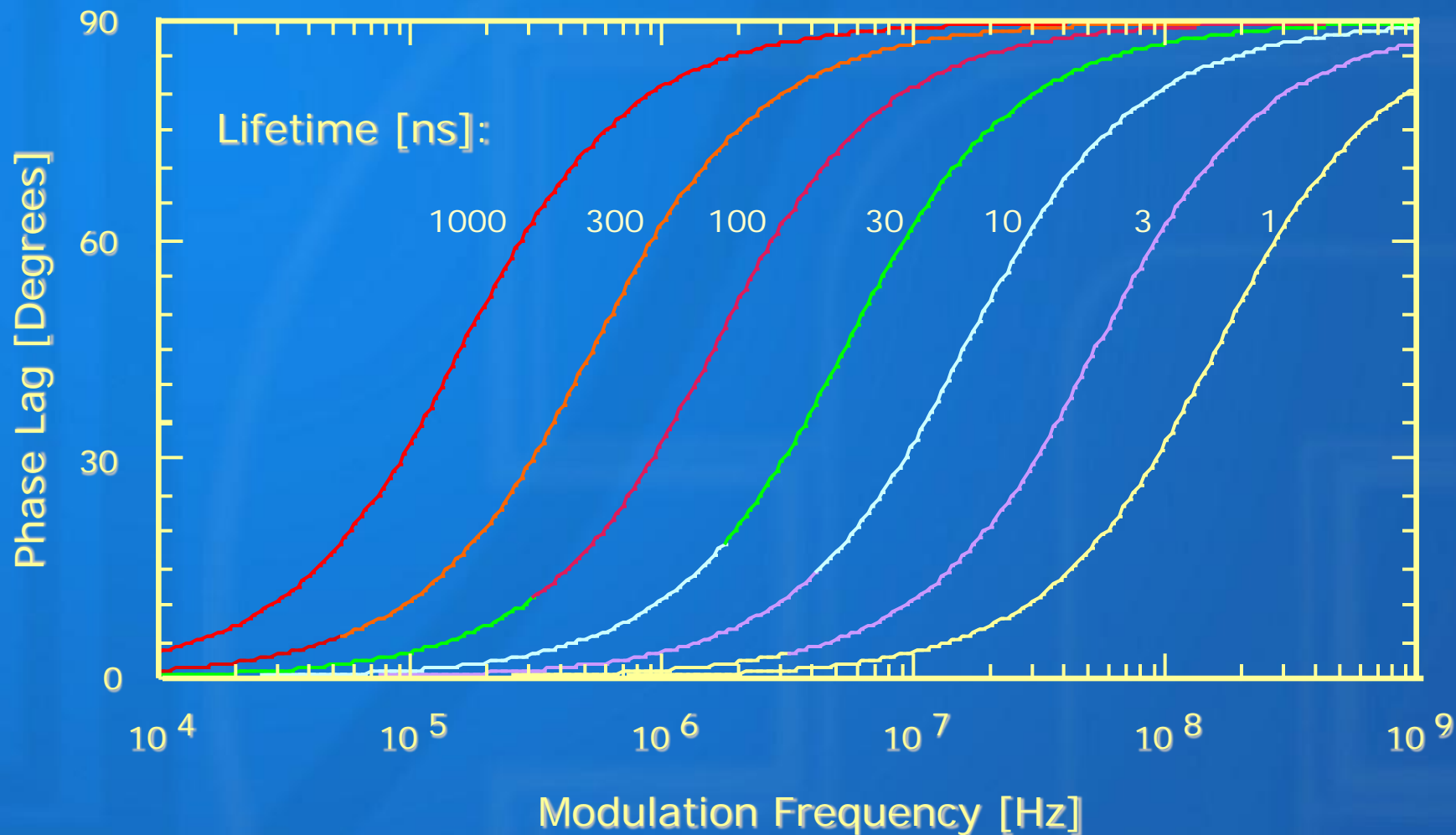
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Light Source	Lines [nm]
Lamps Mercury-Arc Xenon-Arc Tungsten-Halogen	254, 313, 366, 405, 436, 546, 5781 250–1000 350–1000
Light Emitting Diodes (LEDs)	280, 300, 370, 460, 480, 520
Diode Lasers	370, 405, 436, 470, 635, 670, 780, 830
Lasers Helium–Cadmium Argon-Ion Nd:YAG Helium–Neon Krypton Ti-Sapphire	325, 442 457, 488, 514 1064, 5322 543, 594, 633 668, 647 tunable

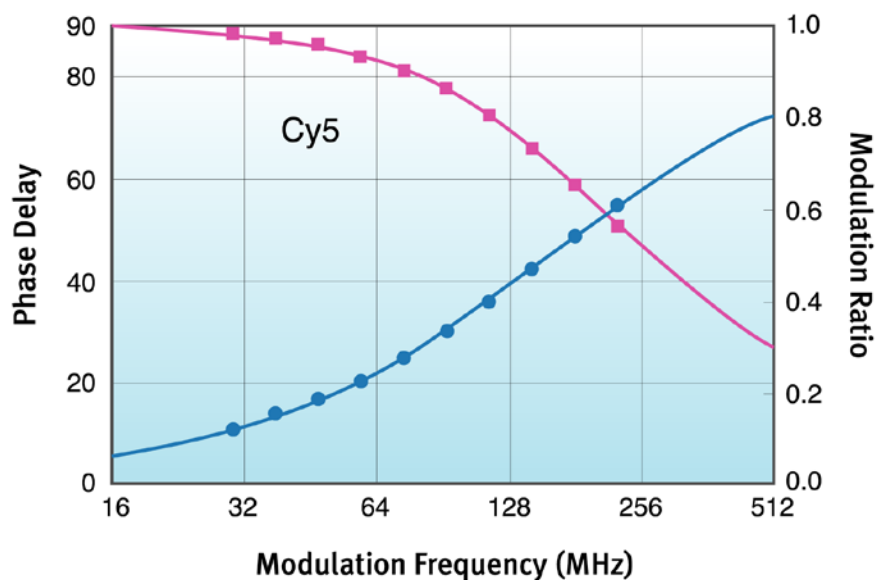


ISS[®]

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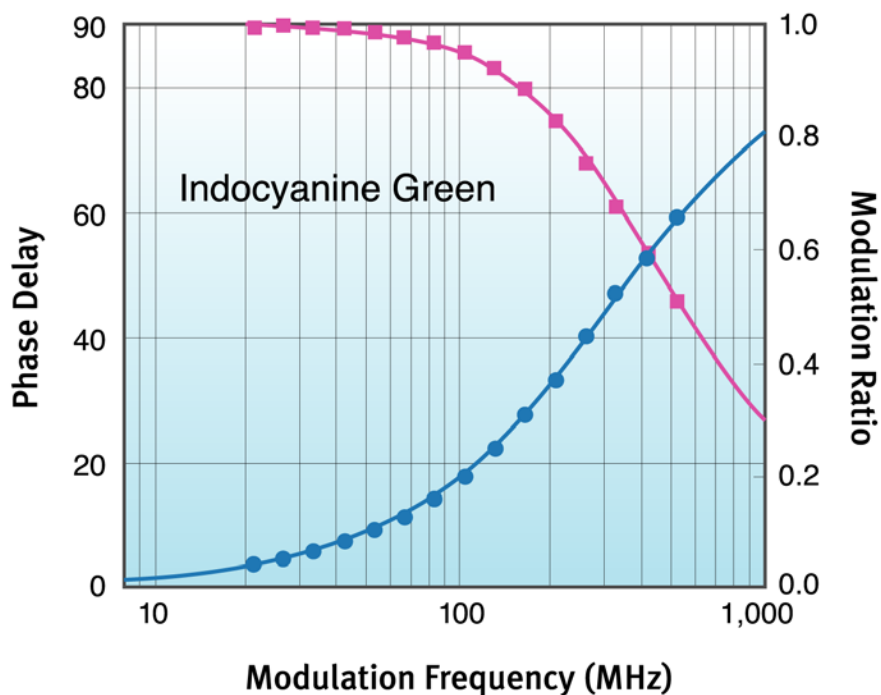


Fluorescence Lifetime of Probes and Labels



- λ_{Ex} : 645-nm Diode Laser
Em: 675-nm LP
- $\tau = 1.01$ ns (PB 7.4)

Fluorescence Lifetime of Probes and Labels



- λ_{Ex} : 786-nm Diode Laser
Em: 830-nm LP
- $\tau = 0.56$ ns (water)

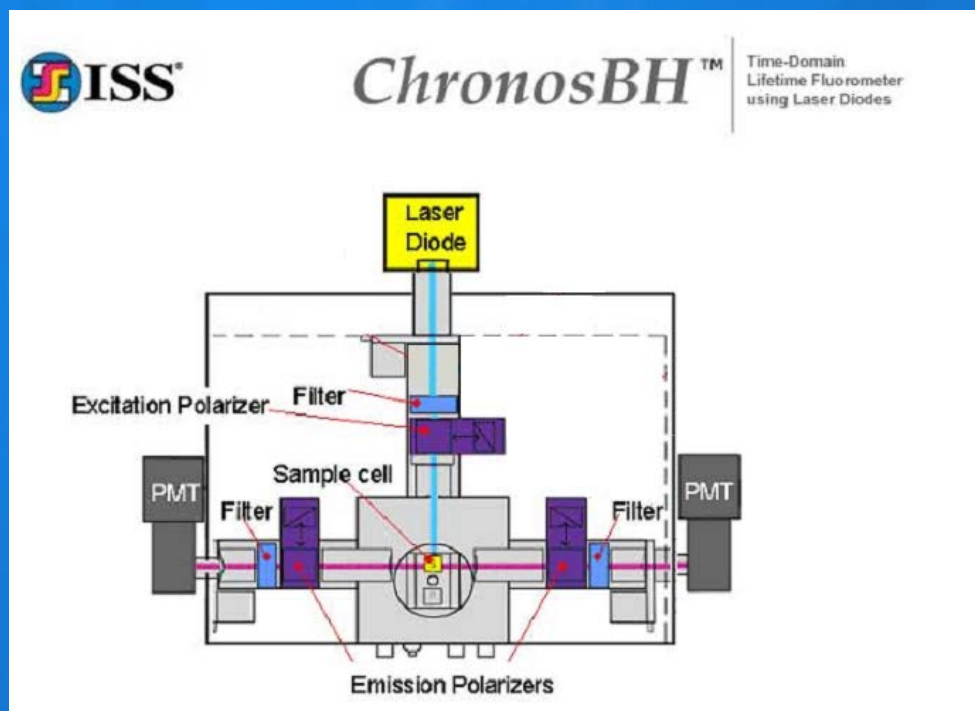
Fluorescence Lifetime **ChronosBH**

Time-Correlated-Single-Photon-Counting Fluorometer

- Lifetime Measurements Utilizing Lasers and LDs
- Fully Automated
- Intensity- and Anisotropy Decay Measurements in a Few Sec



Schematic Drawing



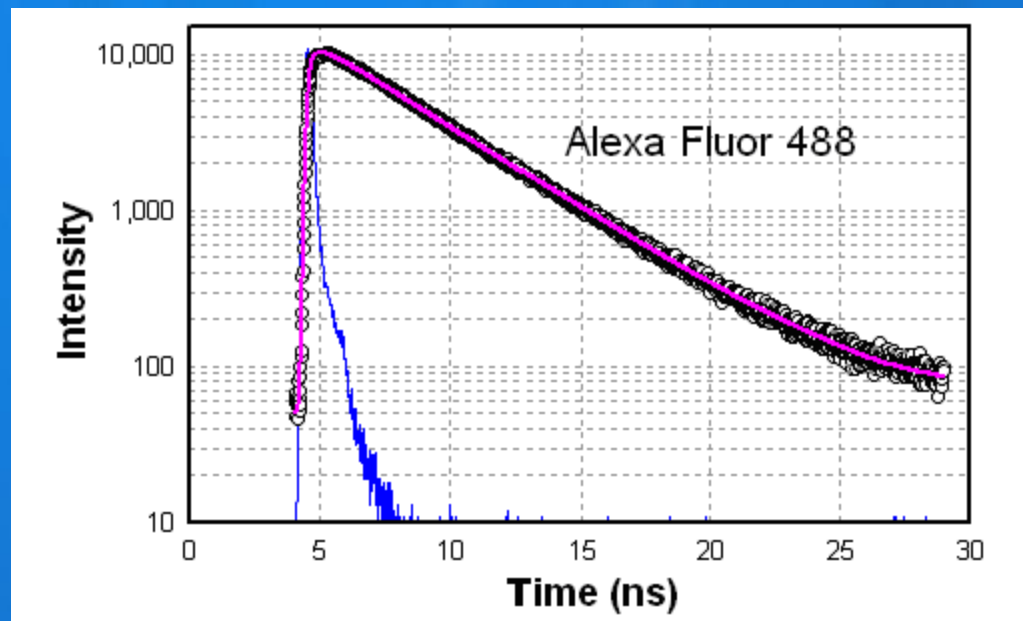
PDL-445 nm pulsed DL with 20, 50, and 80 MHz repetition rates

Detector: PMC-100

4096 time bins

Average measurement time ~ few sec

Fluorescence Lifetime of Probes and Labels

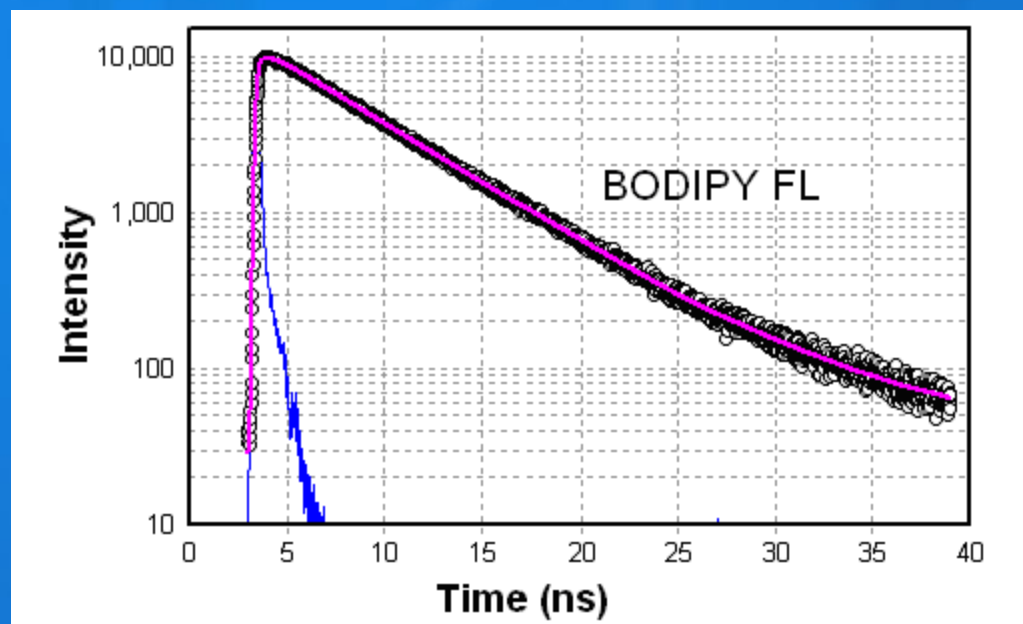


λ_{Ex} : 447-nm Diode Laser

Em: 505-nm LP

$\tau = 4.05$ ns (water)

Fluorescence Lifetime of Probes and Labels



λ_{Ex} : 447-nm Diode Laser

Em: KV 505 LP

$\tau = 5.66$ ns (water)



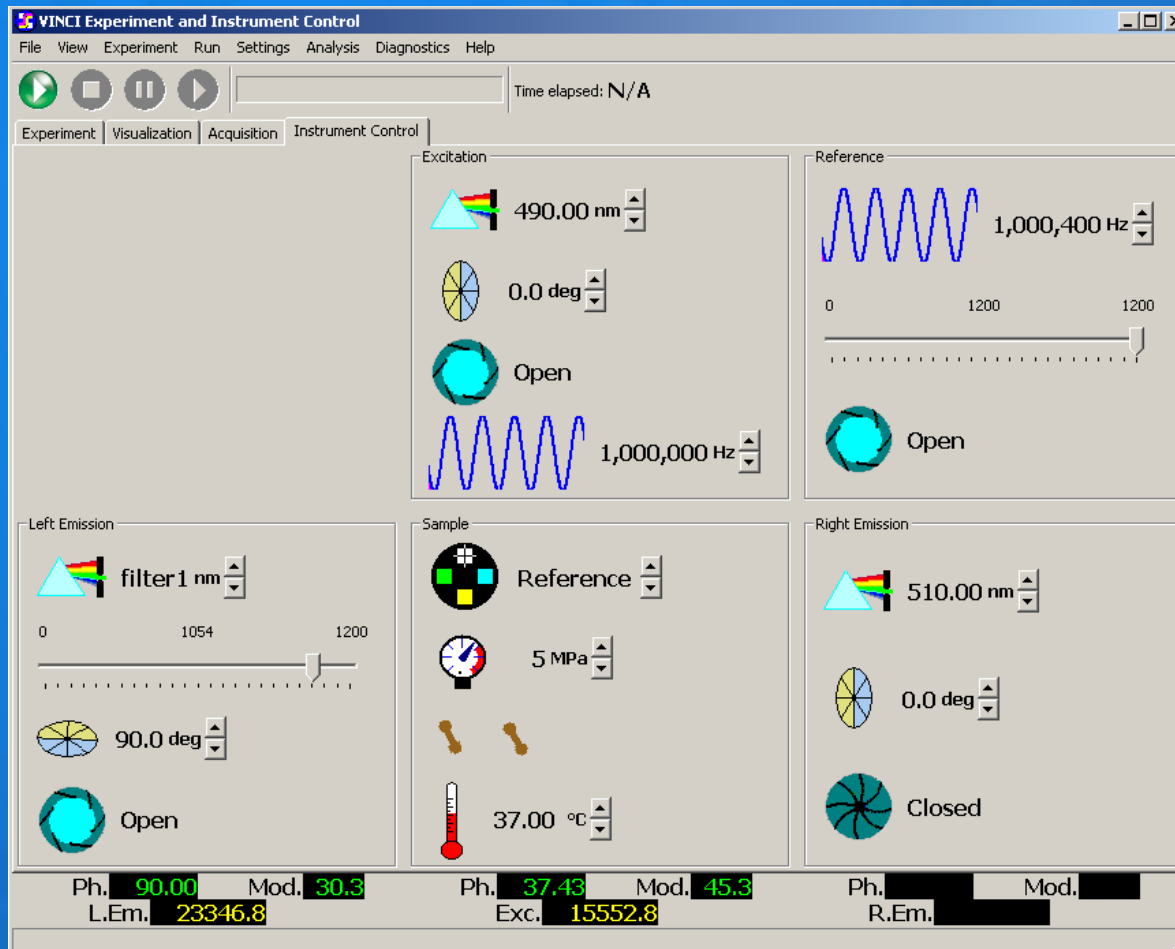
ISS[®]

Innovations in Fluorescence

Vinci - Multidimensional Fluorescence Spectroscopy

- Full Remote Instrument Control
- Automated Data Acquisition
- Data Analysis

Vinci - Remote Instrument Control



All instrument components are remotely accessible

Virtual layout same as instrument layout



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Acquisition Set Up

VINCI Experiment and Instrument Control

File View Experiment Run Settings Analysis Diagnostics Help

Time elapsed: 139,7

Experiment Visualization Acquisition Instrument Control

Acquisition Control

Mode: ☐ Photon Counting ☒ Analog

Format: ☒ L ☐ T

Side: ☒ Left ☐ Right

☒ Time-Resolved

Cross-Correlation at: 400 Hz

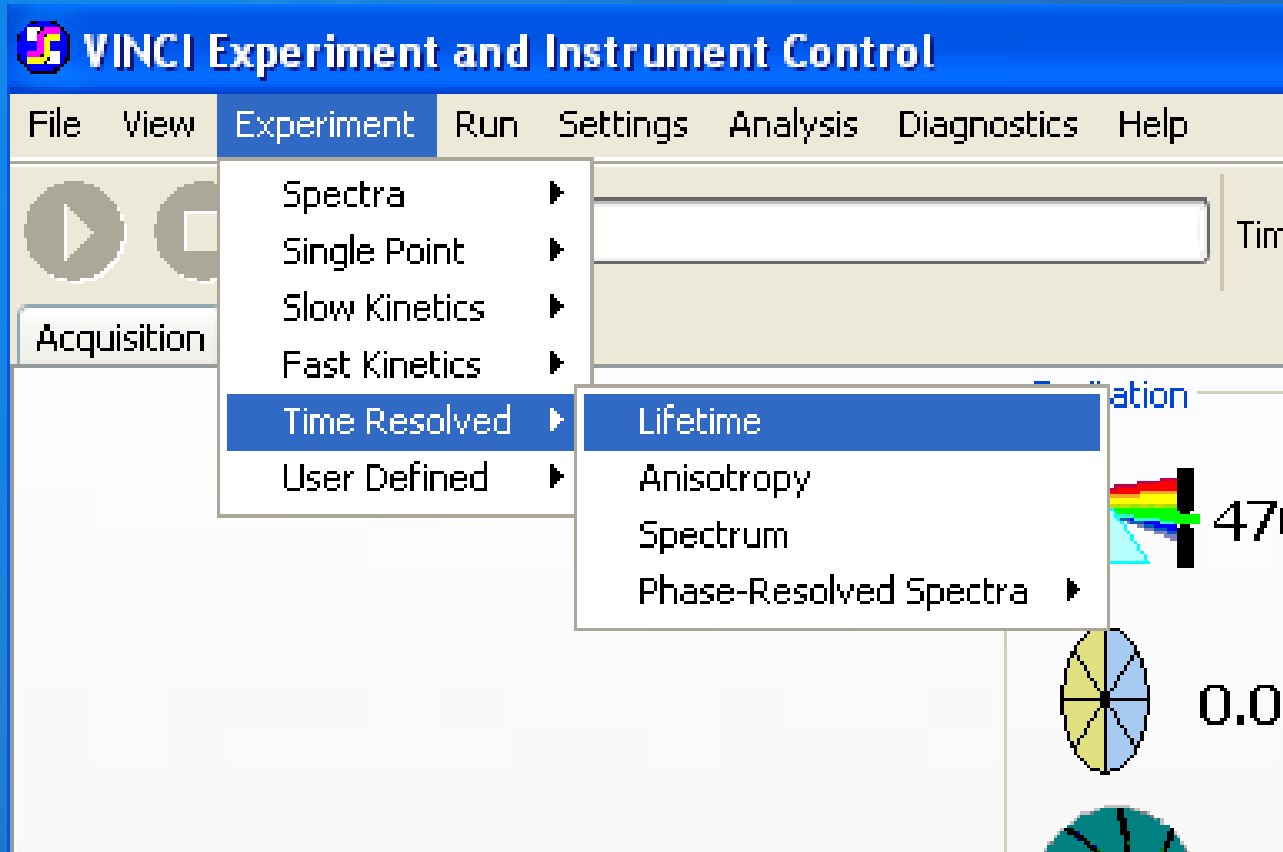
Dual Channel Analog Acquisition

Left Emission Gain 1 Overflow 32000

Excitation Gain 1 Overflow 32000

Ph. 91.40 Mod. 30.6 Ph. 36.59 Mod. 45.8 Ph. R.Em. Mod.

L.Em. 23310.8 Exc. 15550.9





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Experimental Parameters

VINCI Experiment and Instrument Control

File View Experiment Run Settings Analysis Diagnostics Help

Time elapsed: N/A

Experiment Visualization Acquisition Instrument Control

Title: Lifetime

Comment:

Measurement: Phase-Mod

Signal Averaging

Max Iterations: 100 Max ESE (%): 0.2

☐ Stop experiment if ESE not met

Reference

Lifetime: 0.000 ns

Position: Reference

☐ Emission Wavelength

☐ Match Excitation

Advanced

☐ Blank Sample

☐ Fast Scan

☐ Ratio with excitation

☐ Stop stirrers during measurement

☐ Minimal sample exposure to light

Re-estimate Dark every: 30 minutes

Variable Parameters

Modulation Frequency

Start: 0.009 Stop: 600.000 Unit: MHz

Frequencies: 10 Log ☒ Custom ☐ Preview

Fixed Parameters

Excitation Wavelength (nm)

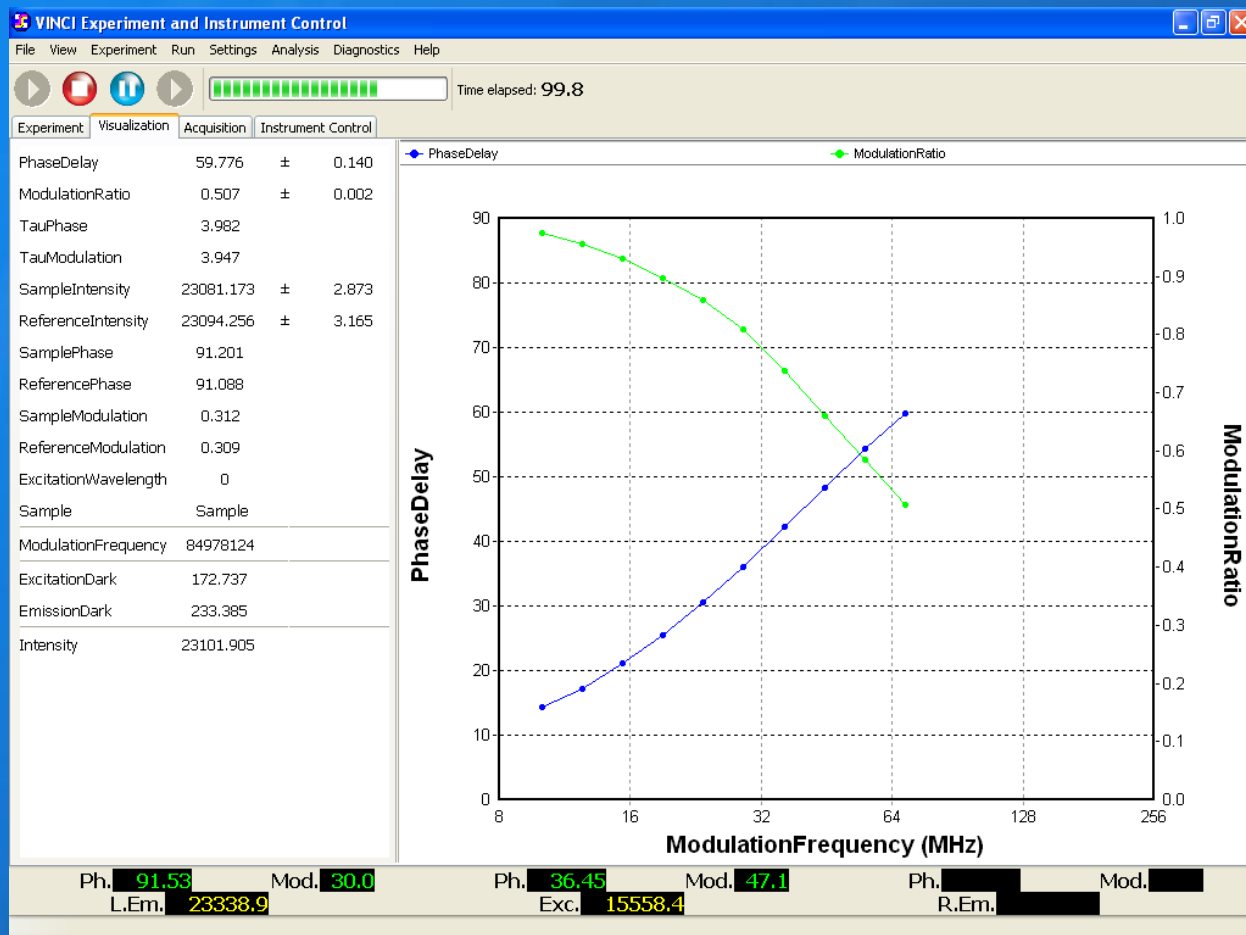
Value: 0.00

Sample

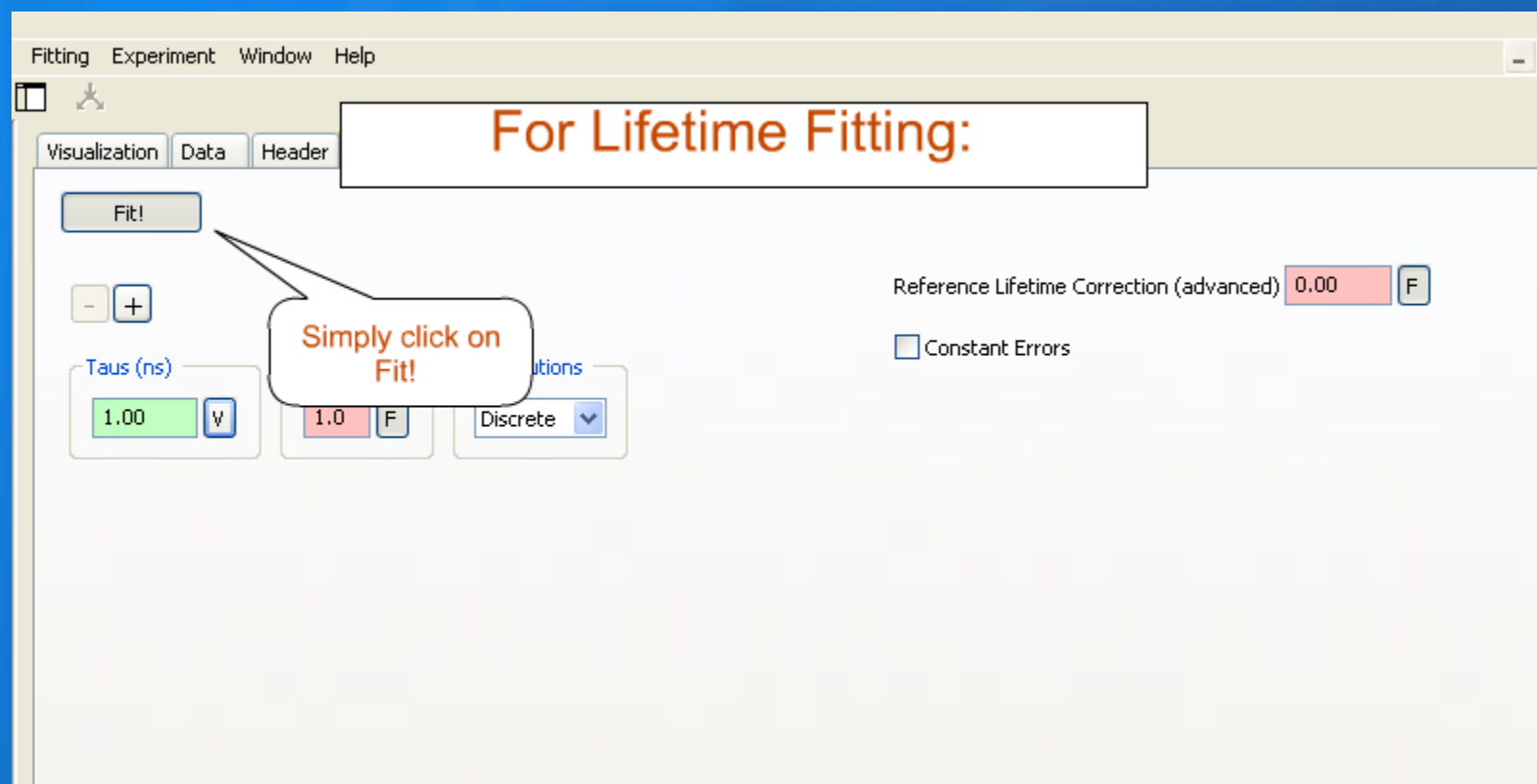
Position: Sample

Ph. 90.29 Mod. 31.1 Ph. 37.69 Mod. 47.1 Ph. R.Em. L.Em. 23314.6 Exc. 15554.6

Measurement

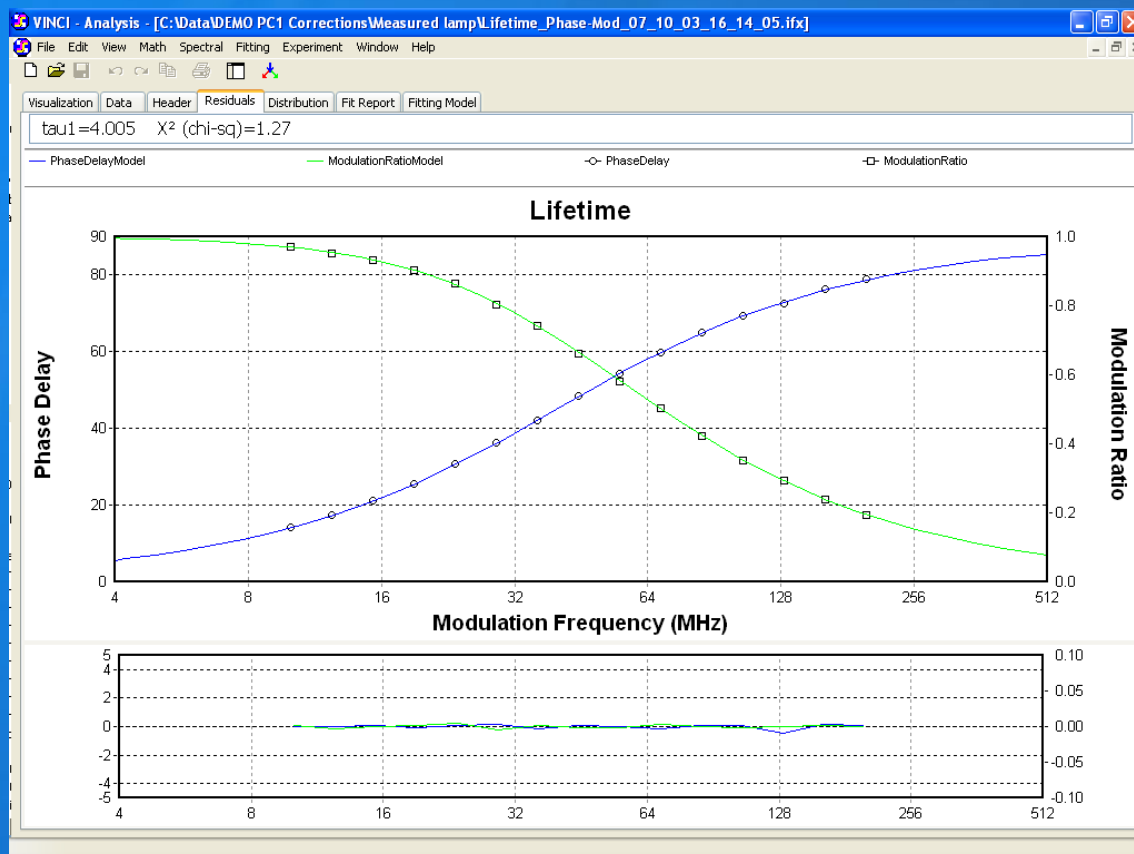


Fitting

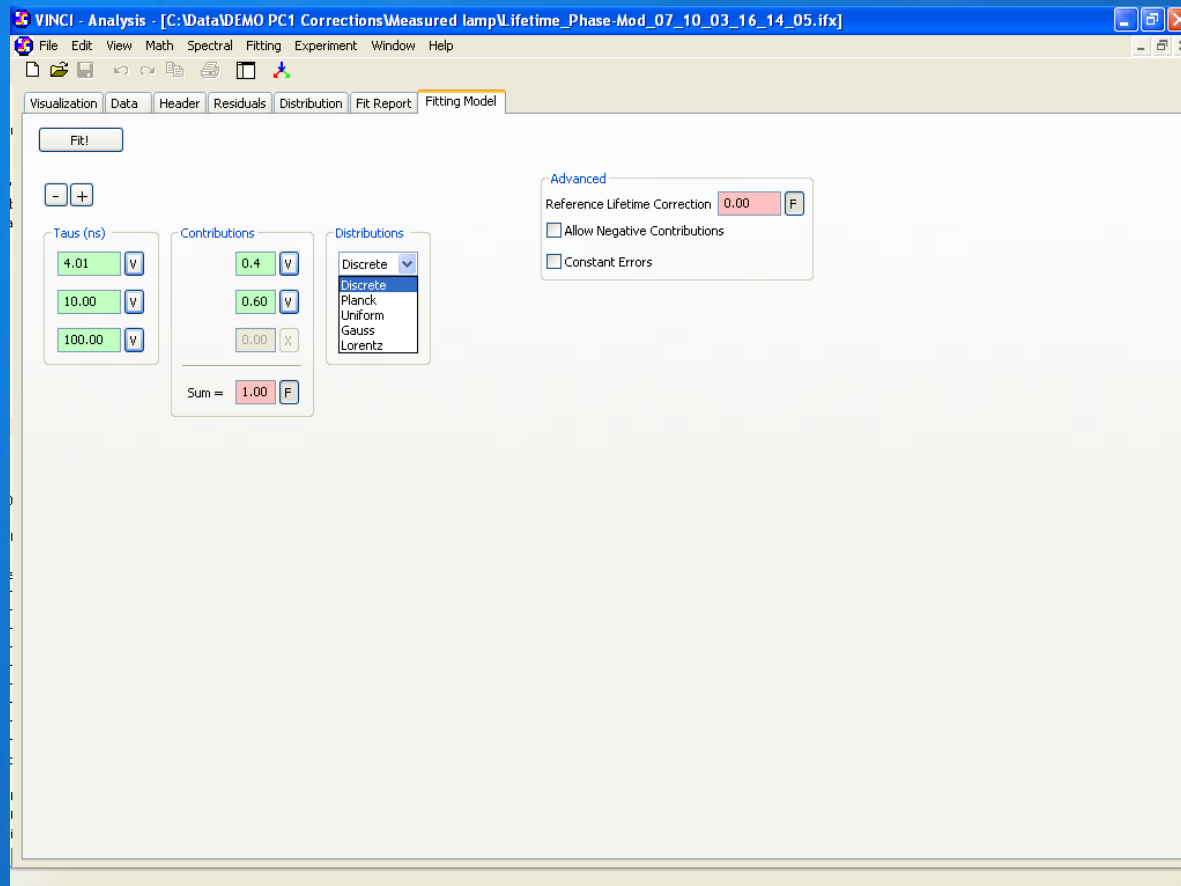


The screenshot shows the 'Fitting' window of the ISS software. The menu bar includes 'Fitting', 'Experiment', 'Window', and 'Help'. Below the menu bar are three tabs: 'Visualization', 'Data', and 'Header'. A large white box with orange text 'For Lifetime Fitting:' is positioned over the 'Data' tab. In the 'Data' tab, there is a 'Fit!' button. A callout box points to this button with the text 'Simply click on Fit!'. Below the 'Fit!' button are several controls: a minus and plus button, a 'Taus (ns)' input field with a value of '1.00' and a unit button 'V', a '1.0' input field with a unit button 'F', and a 'Discrete' dropdown menu. To the right of these controls, there is a 'Reference Lifetime Correction (advanced)' input field with a value of '0.00' and a unit button 'F', and a checkbox labeled 'Constant Errors'.

Fitting Results



Fitting Models:





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Vinci-Multidimensional Fluorescence Spectroscopy

No Need to Export Data to Excel or Origin

Vinci – Produces Publication-ready

Plots and Figures



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Thank You!