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Innovations in Fluorescence

Introduction to Fluorescence Instrumentation:

Fluorometers

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Fluorescence Instrumentation:

Hands-on Experiments

- Steady-State - PC1
- Fluorescence Lifetime

Chronos: FD

ChronosBH: TD

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Steady-State Fluorescence

PC1

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

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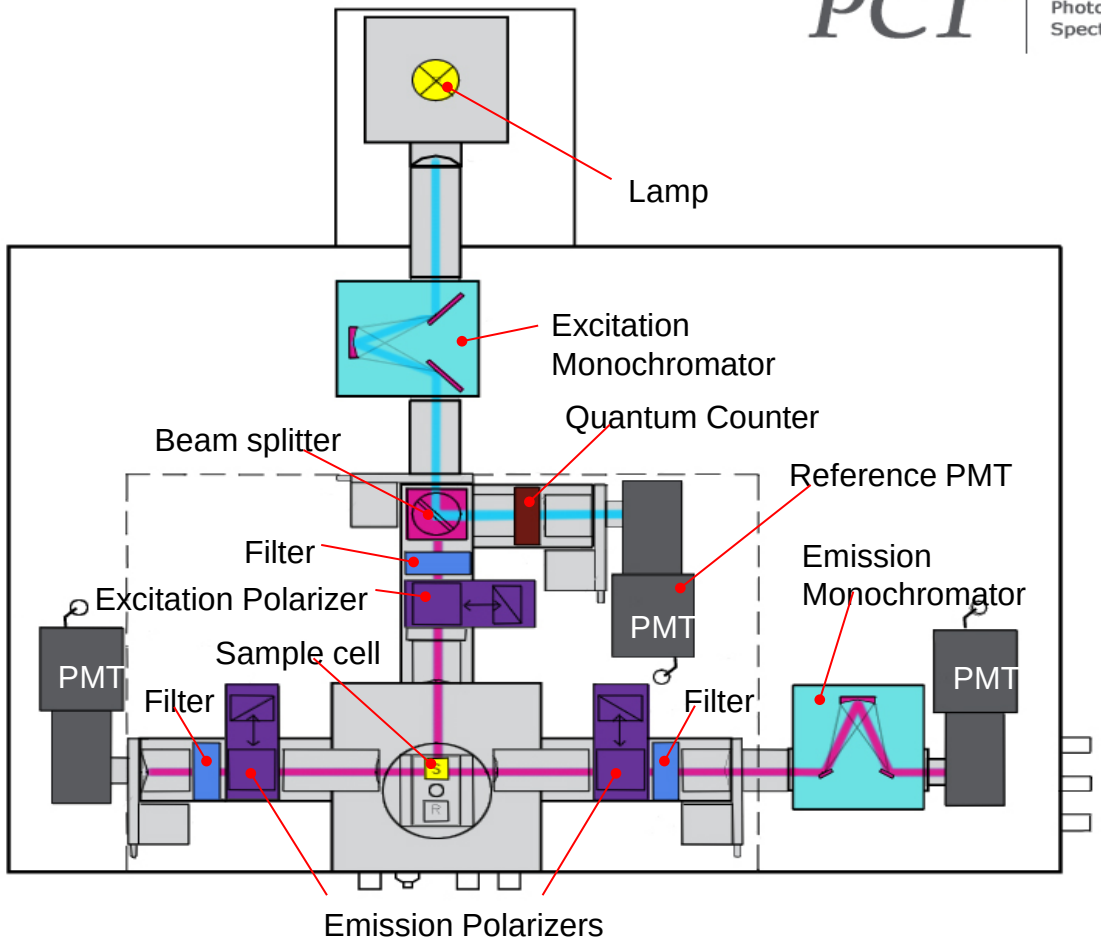
PC1™ Photon Counting Spectrofluorometer



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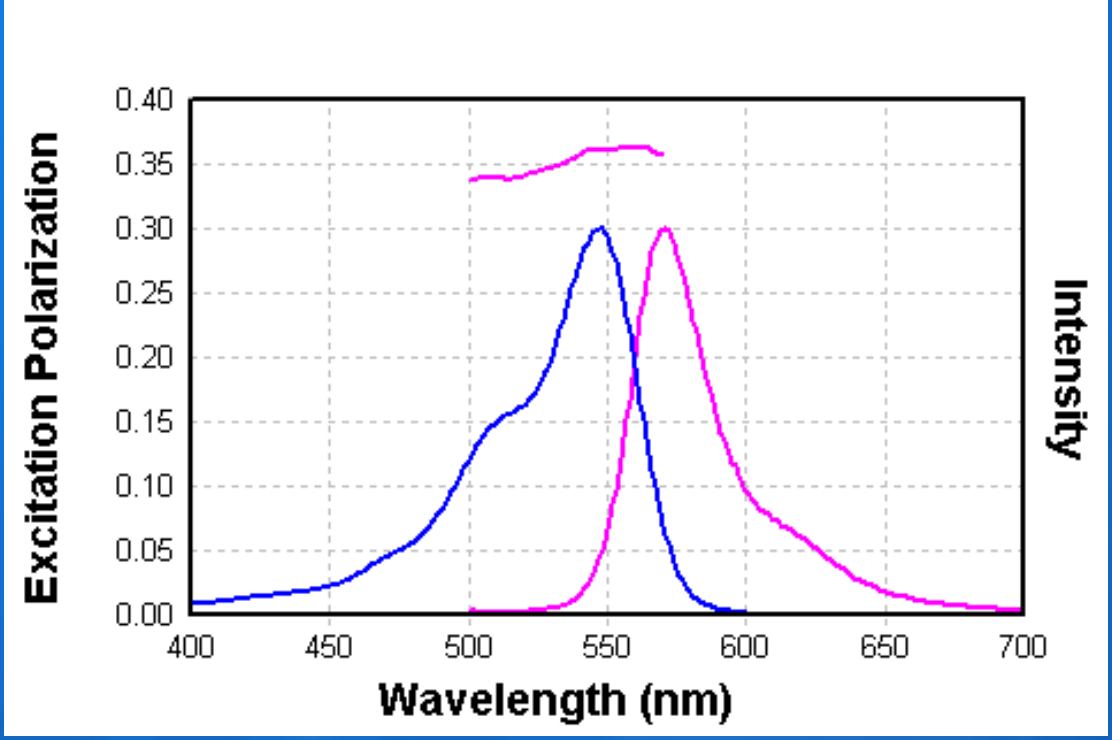
Measurement Capabilities:



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

Product-Line:

- Fluorescence Lifetime

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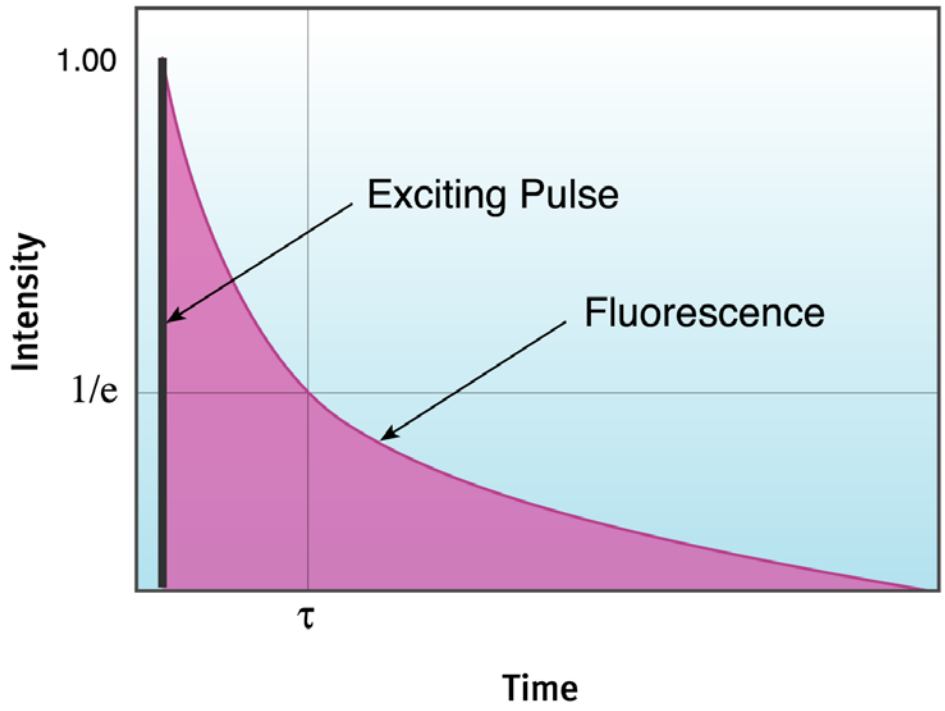
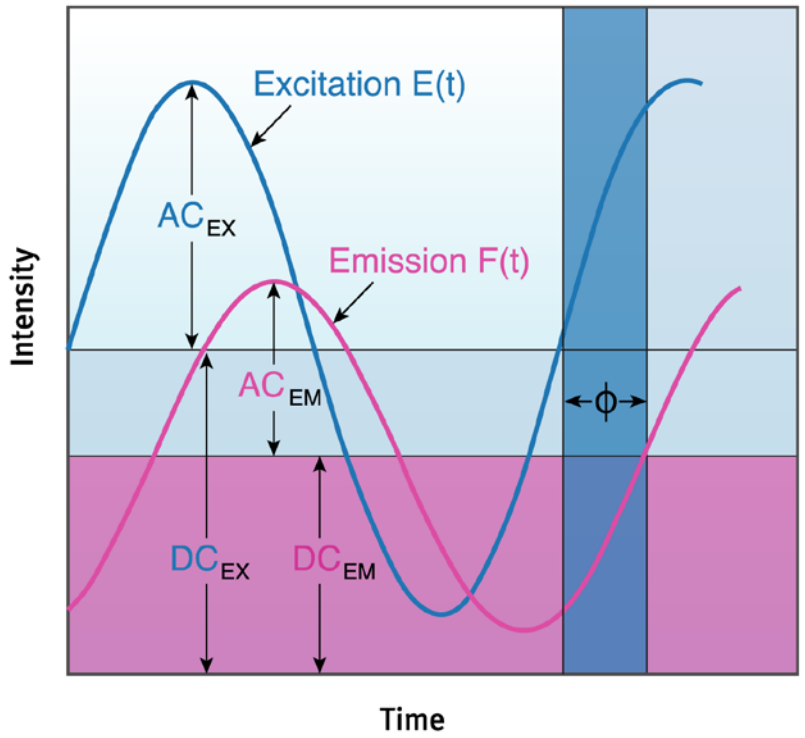
Two Ways to Measure Lifetime:

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

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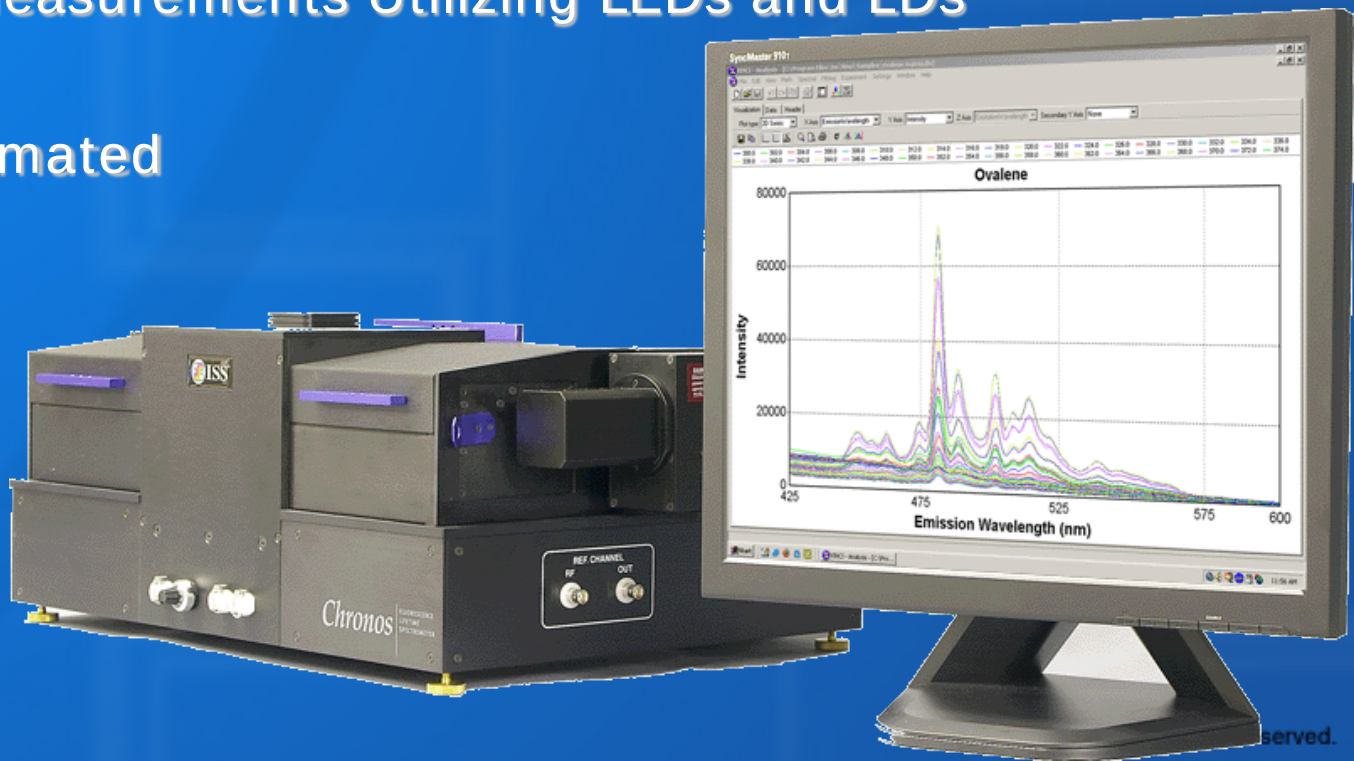
Fluorescence Lifetime

Chronos

Multi-Frequency Cross-Correlation Phase Modulation Fluorimeter

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



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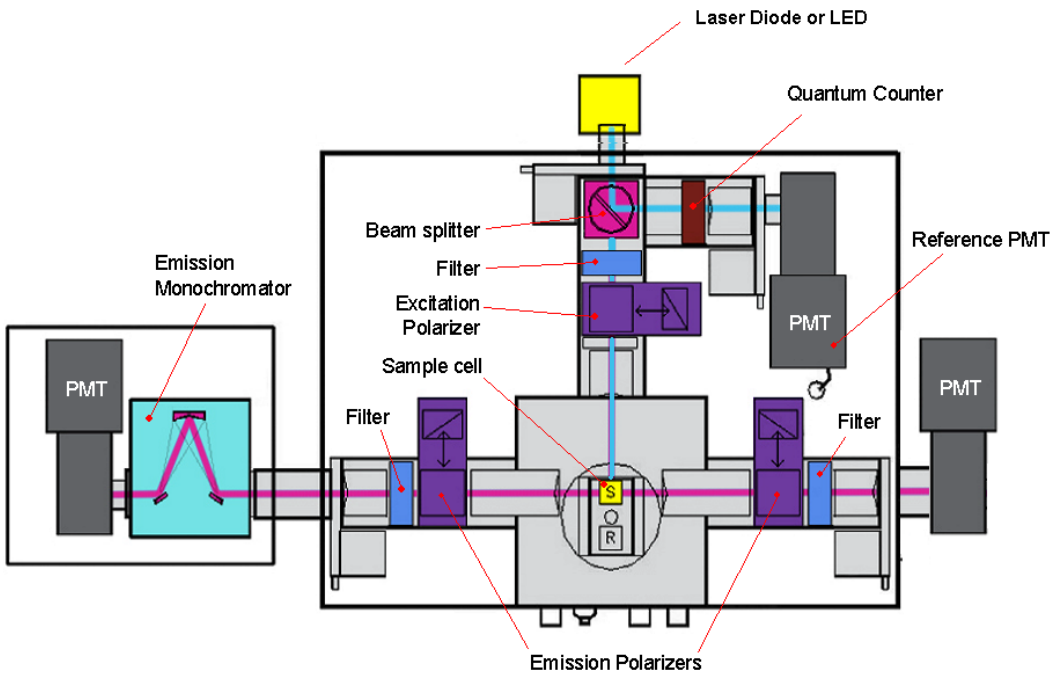
Chronos™

Modular Steady-State and Lifetime Fluorometer using Laser Diodes, LEDs and Mode-Locked Lasers



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

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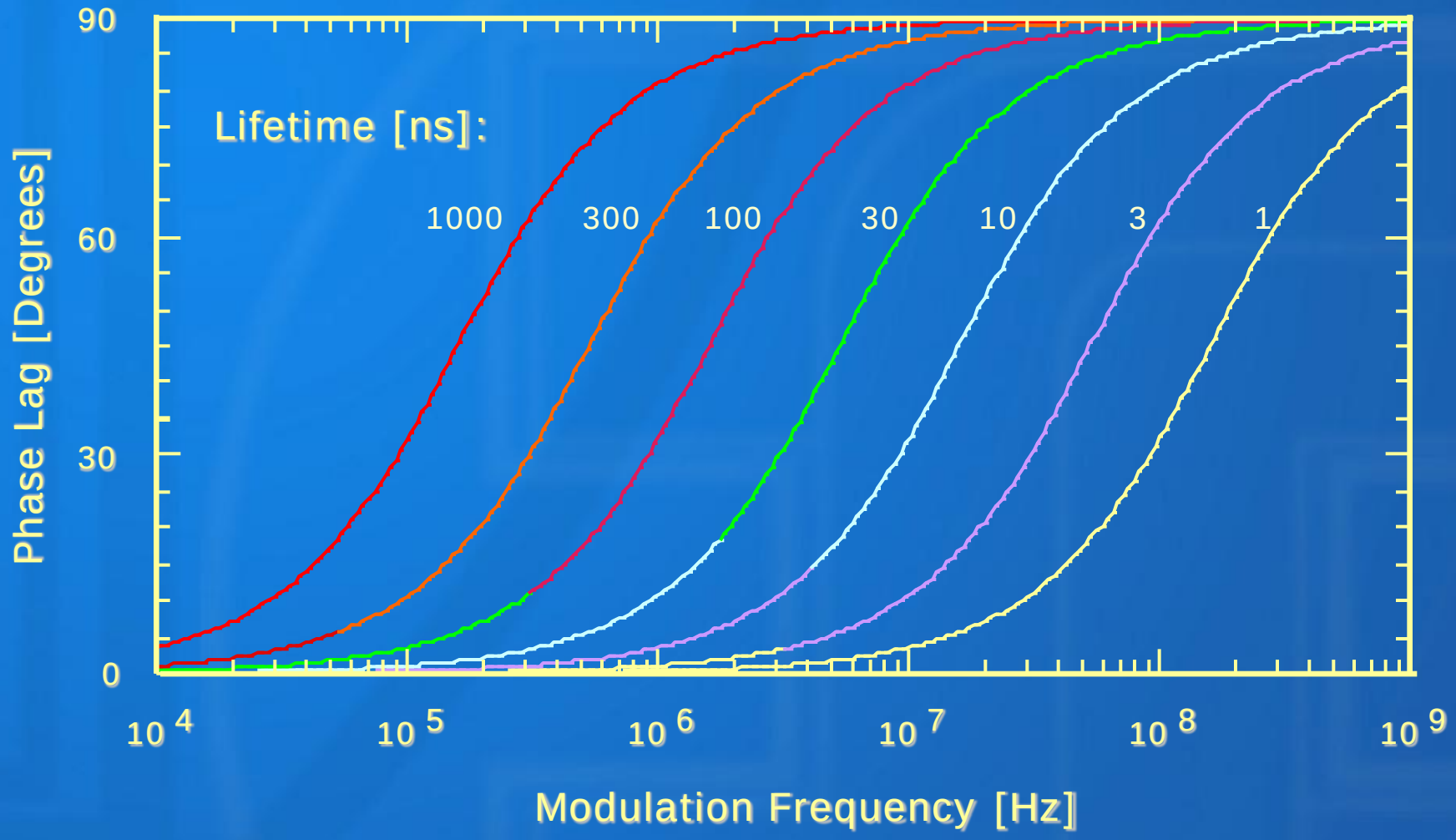
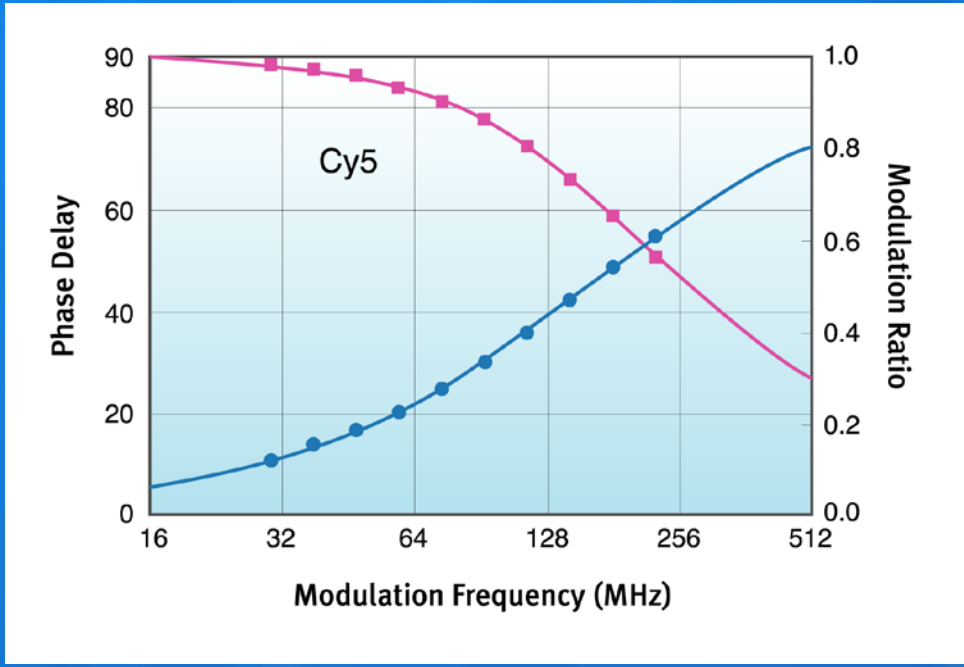
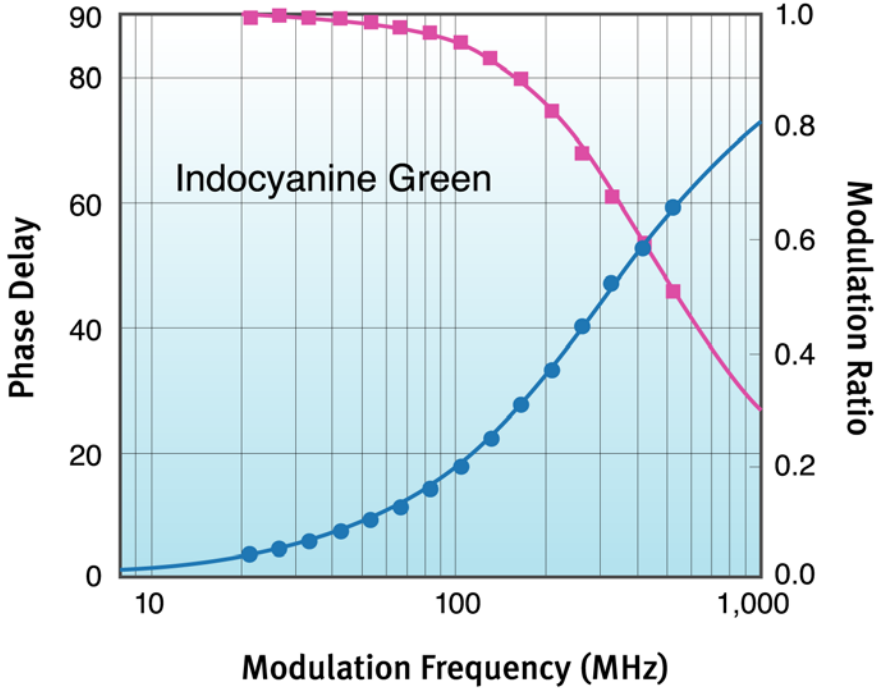


Fluorescence Lifetime of Probes and Labels



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

Fluorescence Lifetime of Probes and Labels



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

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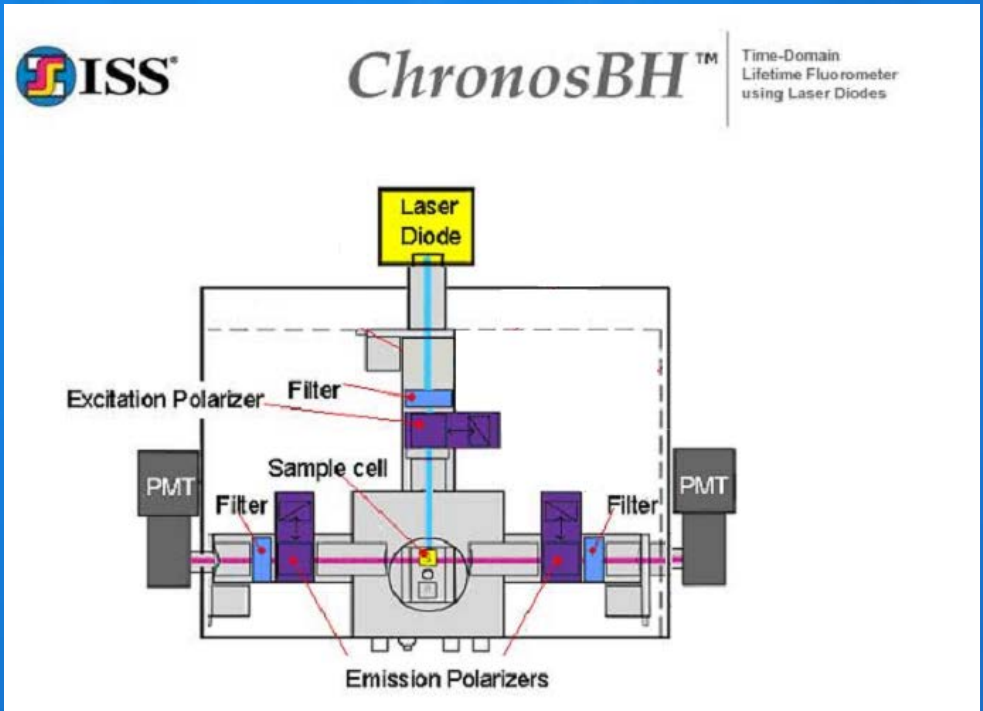
Fluorescence Lifetime

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



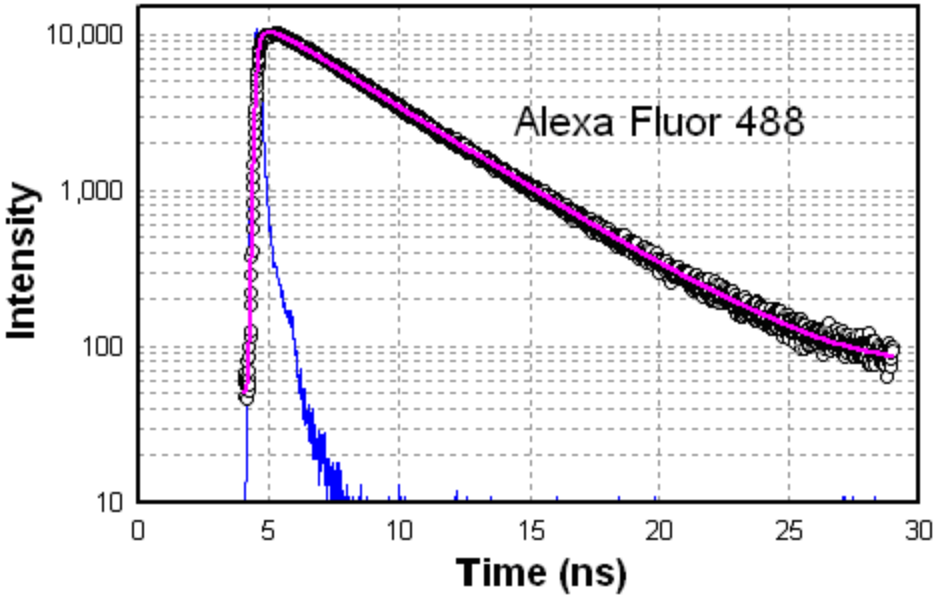
PDL-445 nm pulsed DL with 20, 50, and 80 MHz repetition rates

Detector: PMC-100

4096 time bins

Average measurement time ~ few sec

Fluorescence Lifetime of Probes and Labels

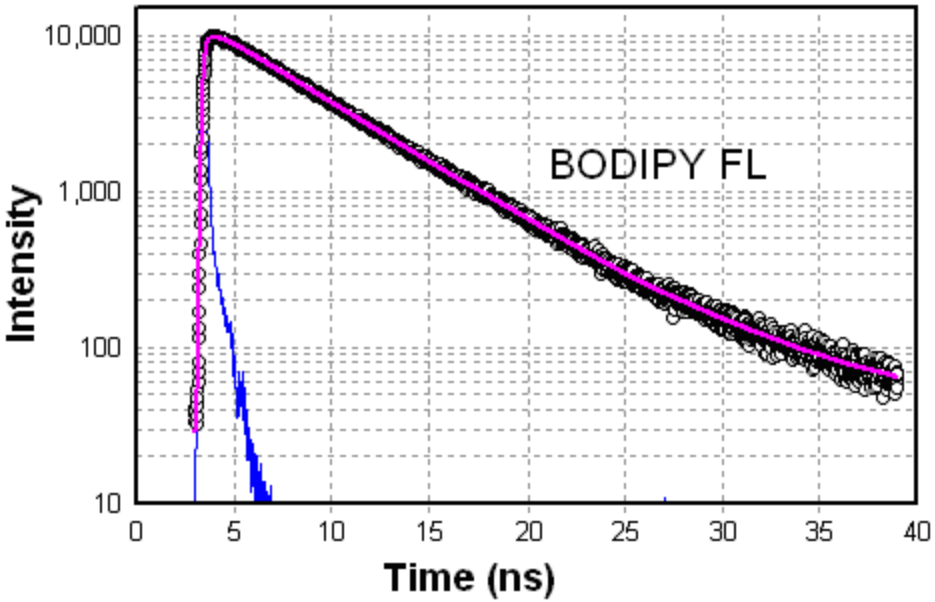


λ_{Ex}: 447-nm Diode Laser

Em: 505-nm LP

τ = 4.05 ns (water)

Fluorescence Lifetime of Probes and Labels



λ_{Ex}: 447-nm Diode Laser

Em: KV 505 LP

τ = 5.66 ns (water)

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Vinci - Multidimensional Fluorescence Spectroscopy

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

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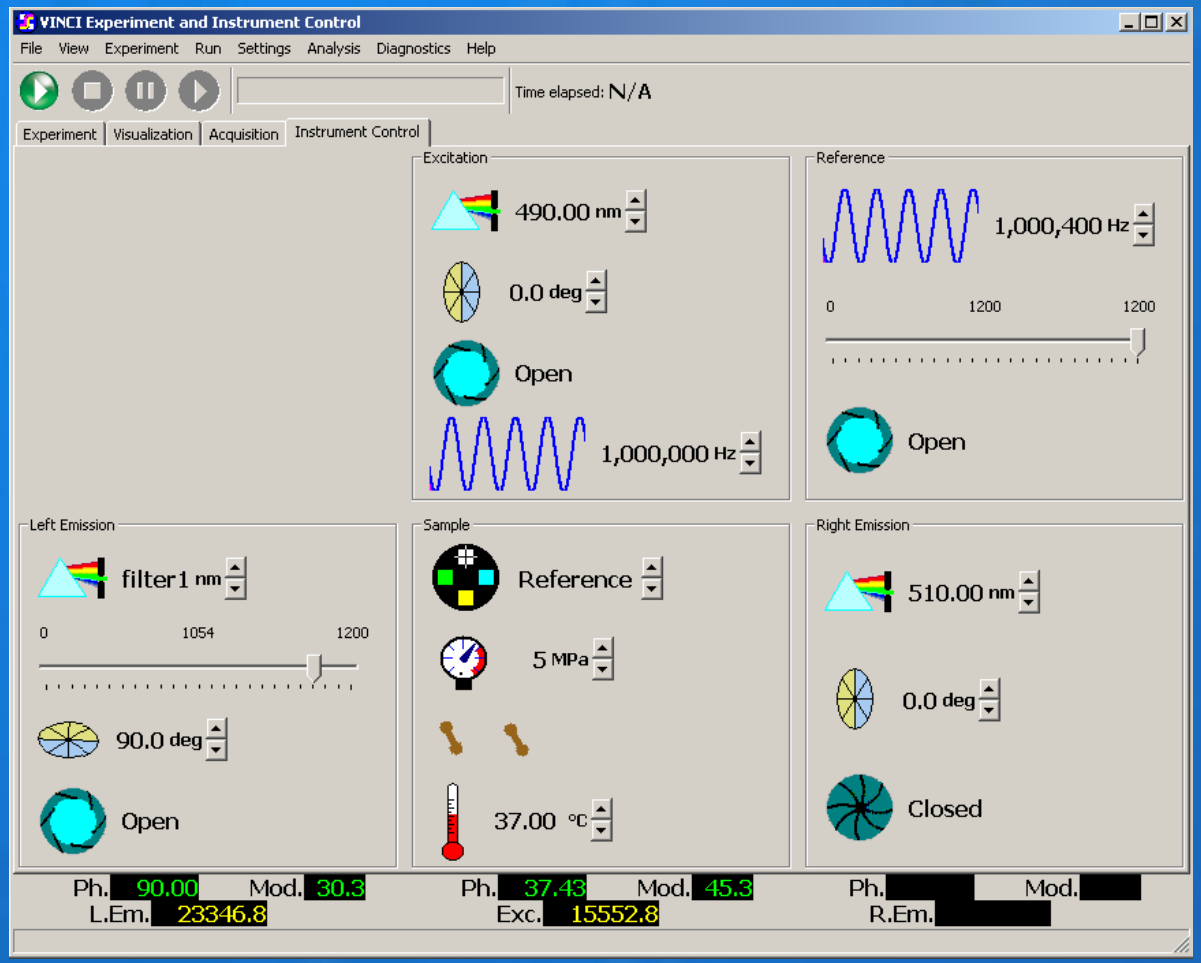
Vinci - Remote Instrument Control



All instrument components are remotely accessible

Virtual layout same as instrument layout

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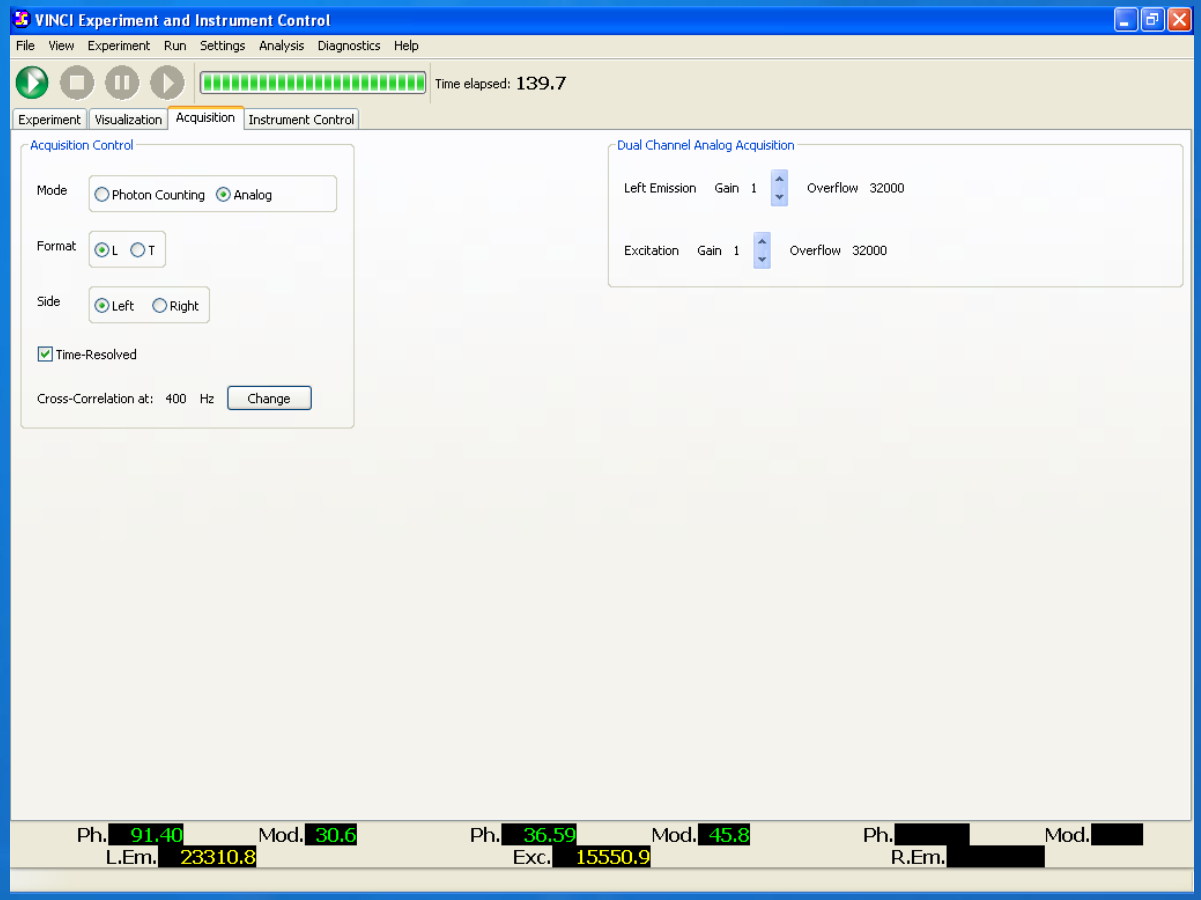
Acquisition Set Up



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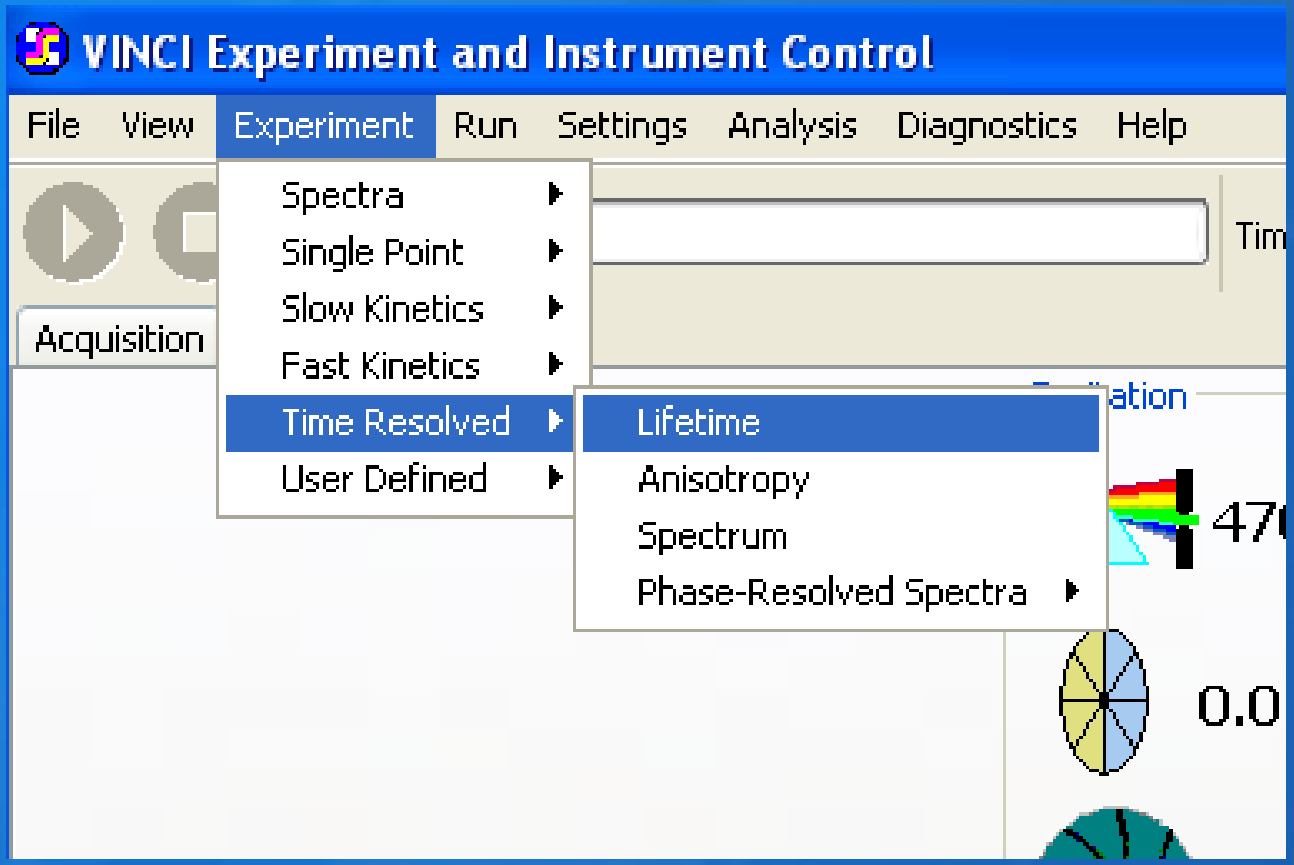

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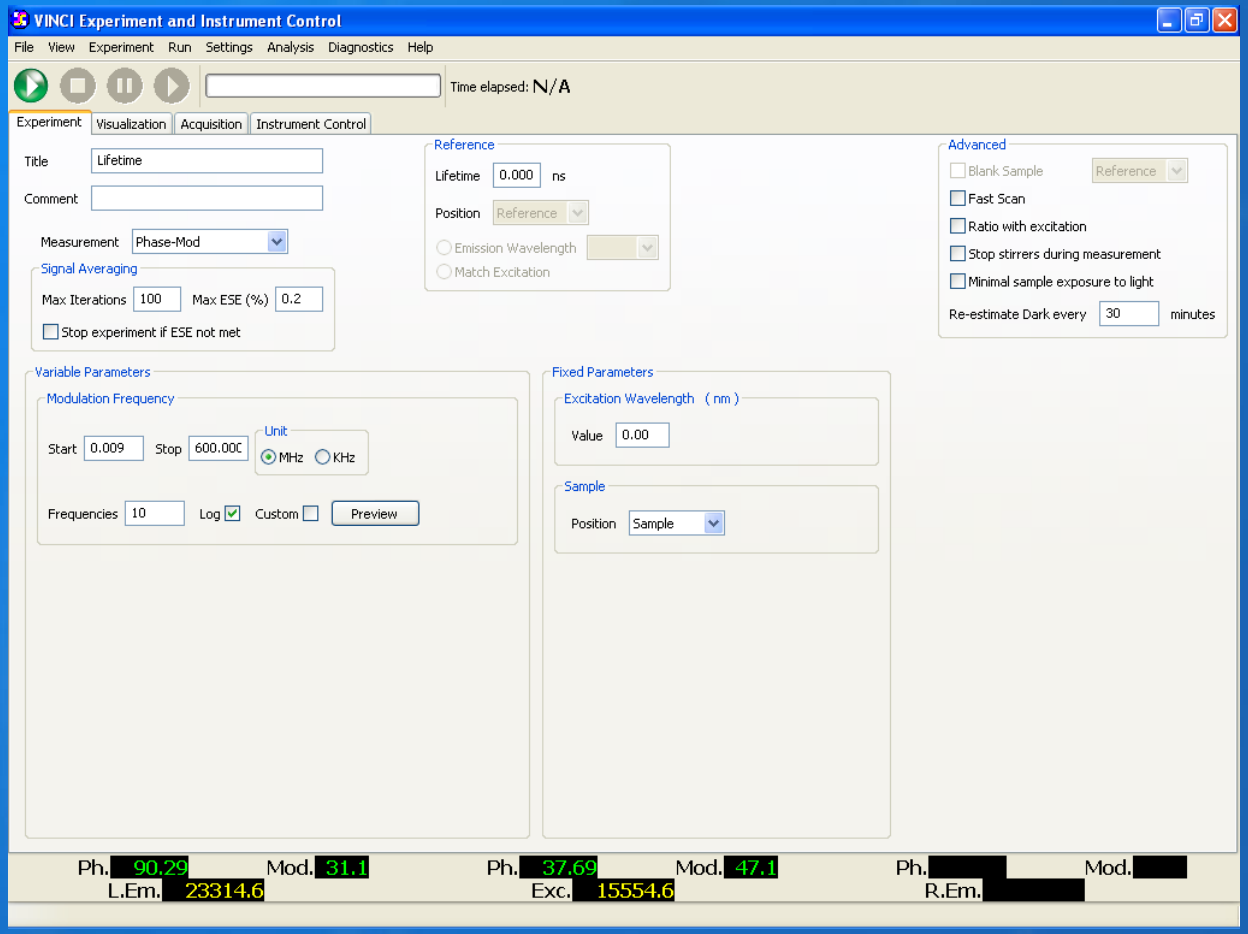
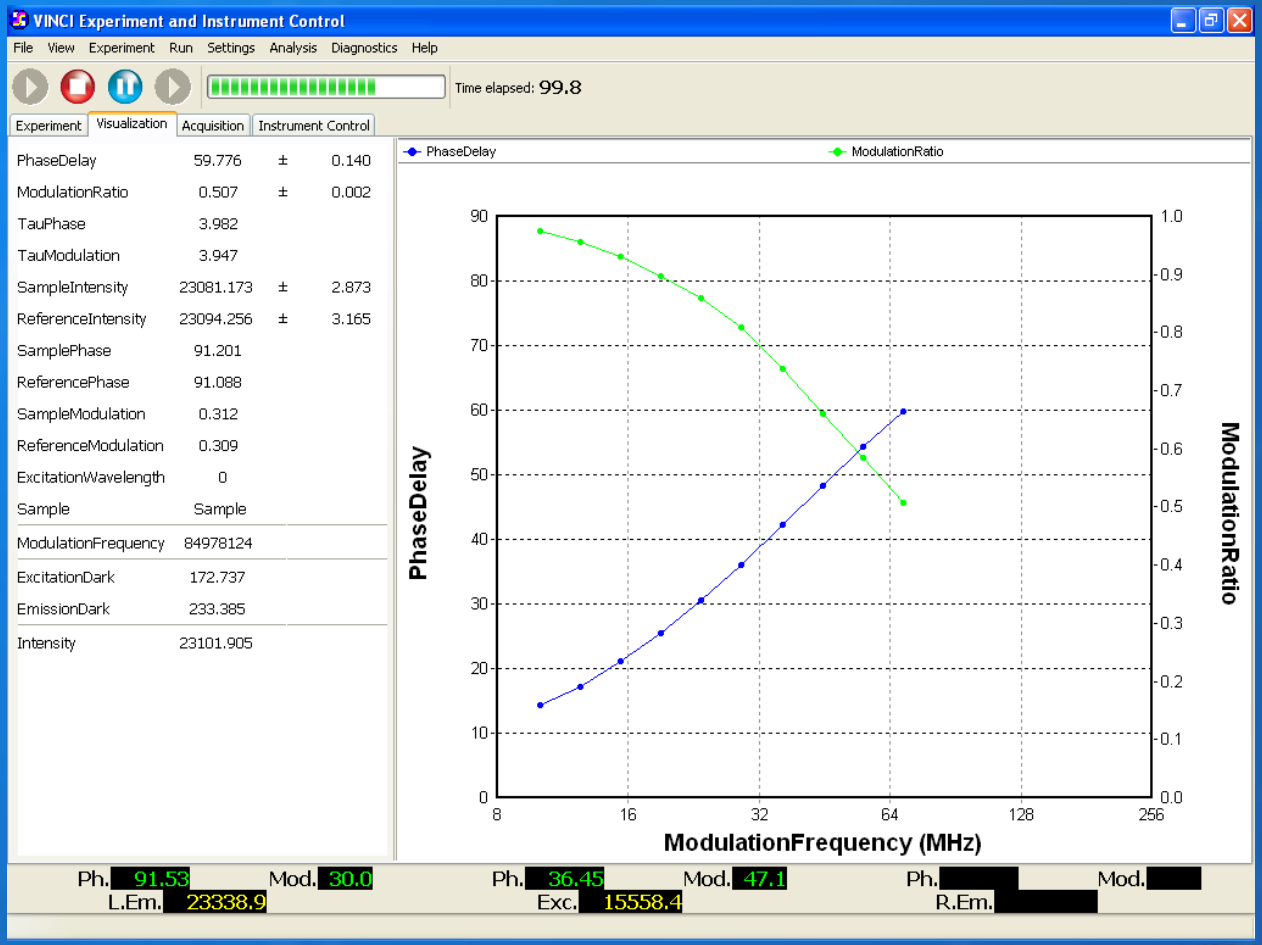
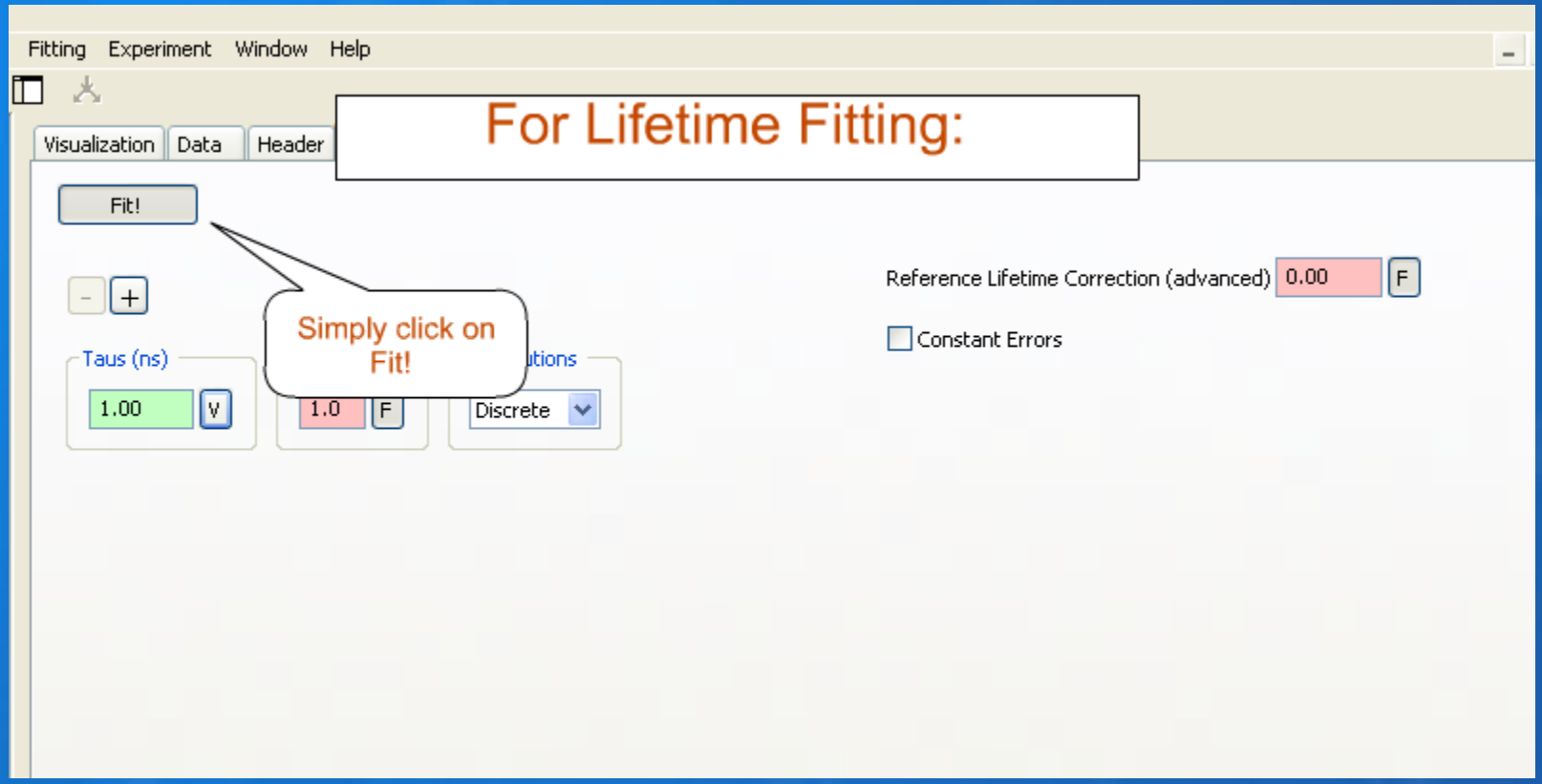
Experimental Parameters



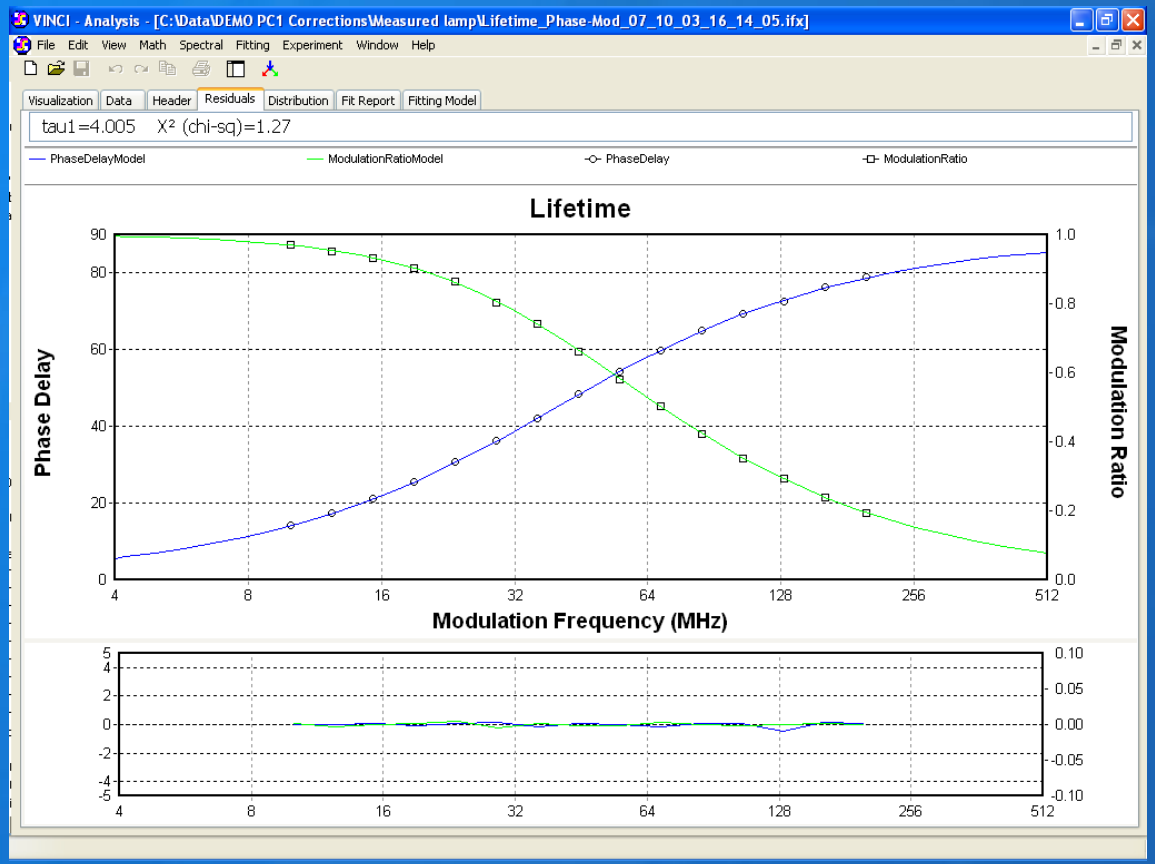
Measurement



Fitting



Fitting Results



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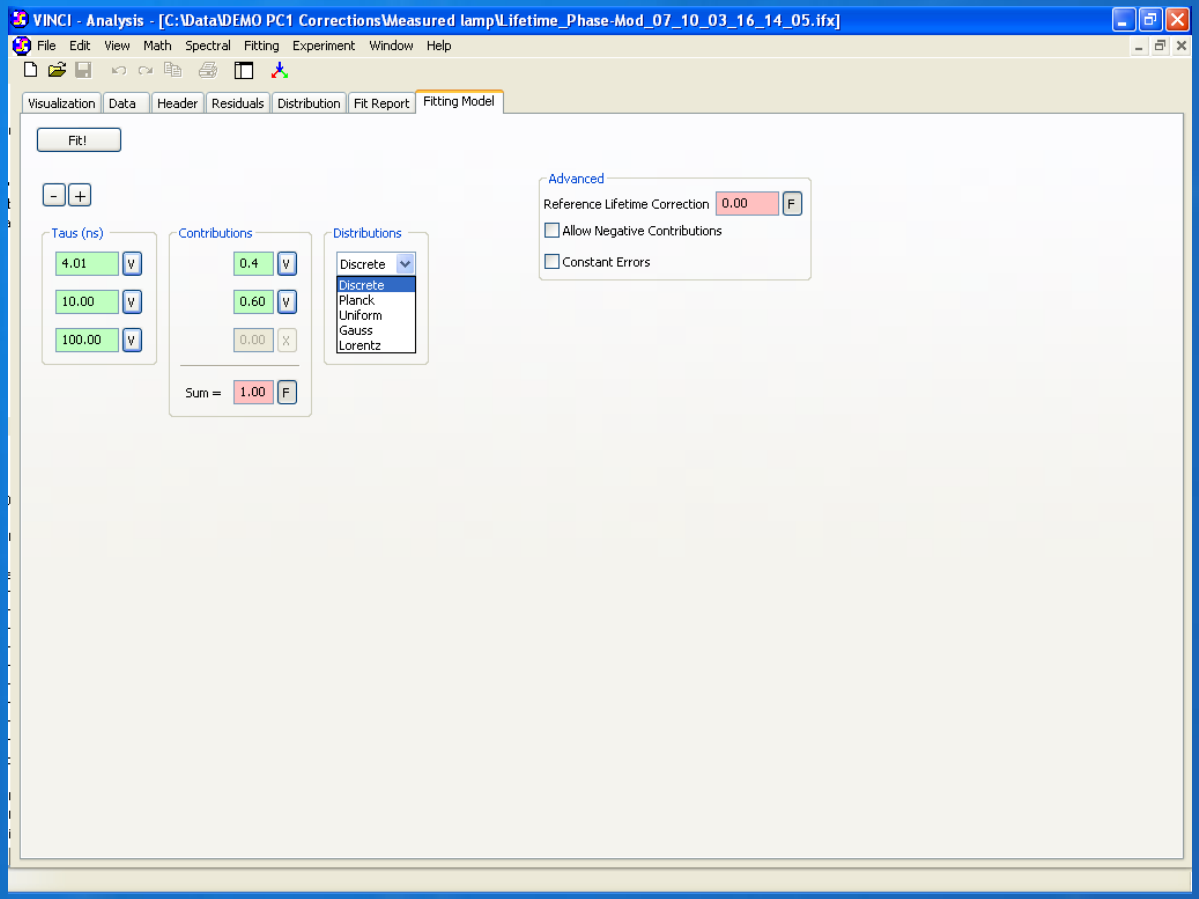
Fitting Models:



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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!