



Time Correlated Single Photon Counting (TCSPC) (τ_F)

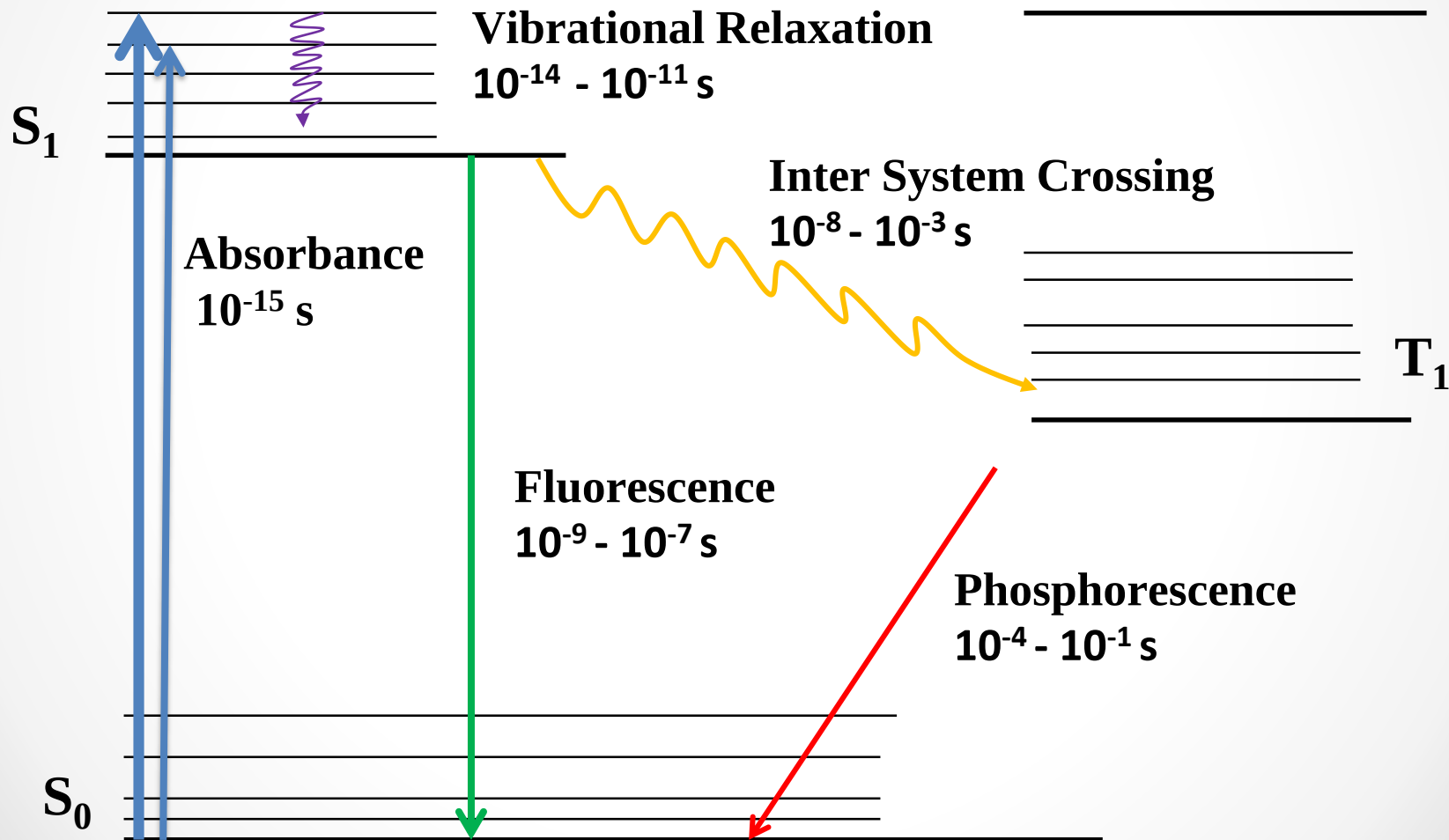
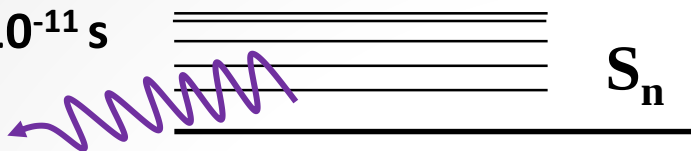
TCSPC(Fluotime 300):

Capabilities

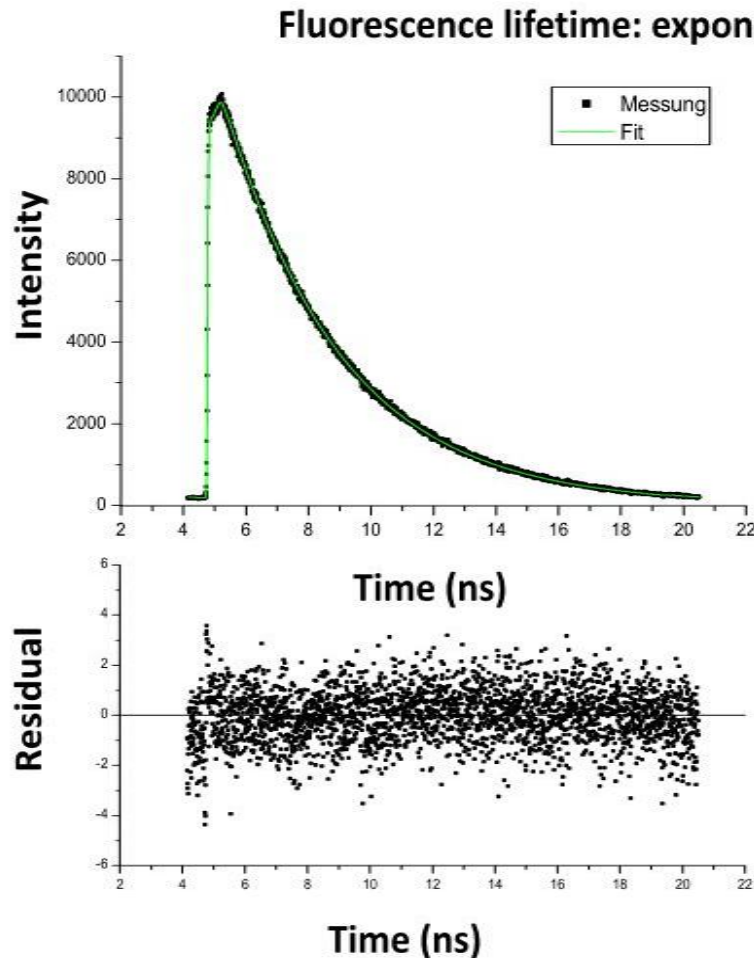
- Steady state emission (detection in the UV-Vis/ IR)
- Excitation (Xe lamp: 200 nm – 900 nm)
- Time resolved emission (UV-Vis/ IR)
- Phosphorescence measurements (singlet oxygen)
- Fluorescence upconversion
- Anisotropy
- TRES

Internal Conversion

$10^{-14} - 10^{-11} \text{ s}$



Fluorescence lifetime



Exponential curve fitting can be used to calculate the fluorescence lifetime (t_F), which is the time it takes for the intensity to fall to $1/e$ of its initial value

Why are fluorescence lifetimes important?

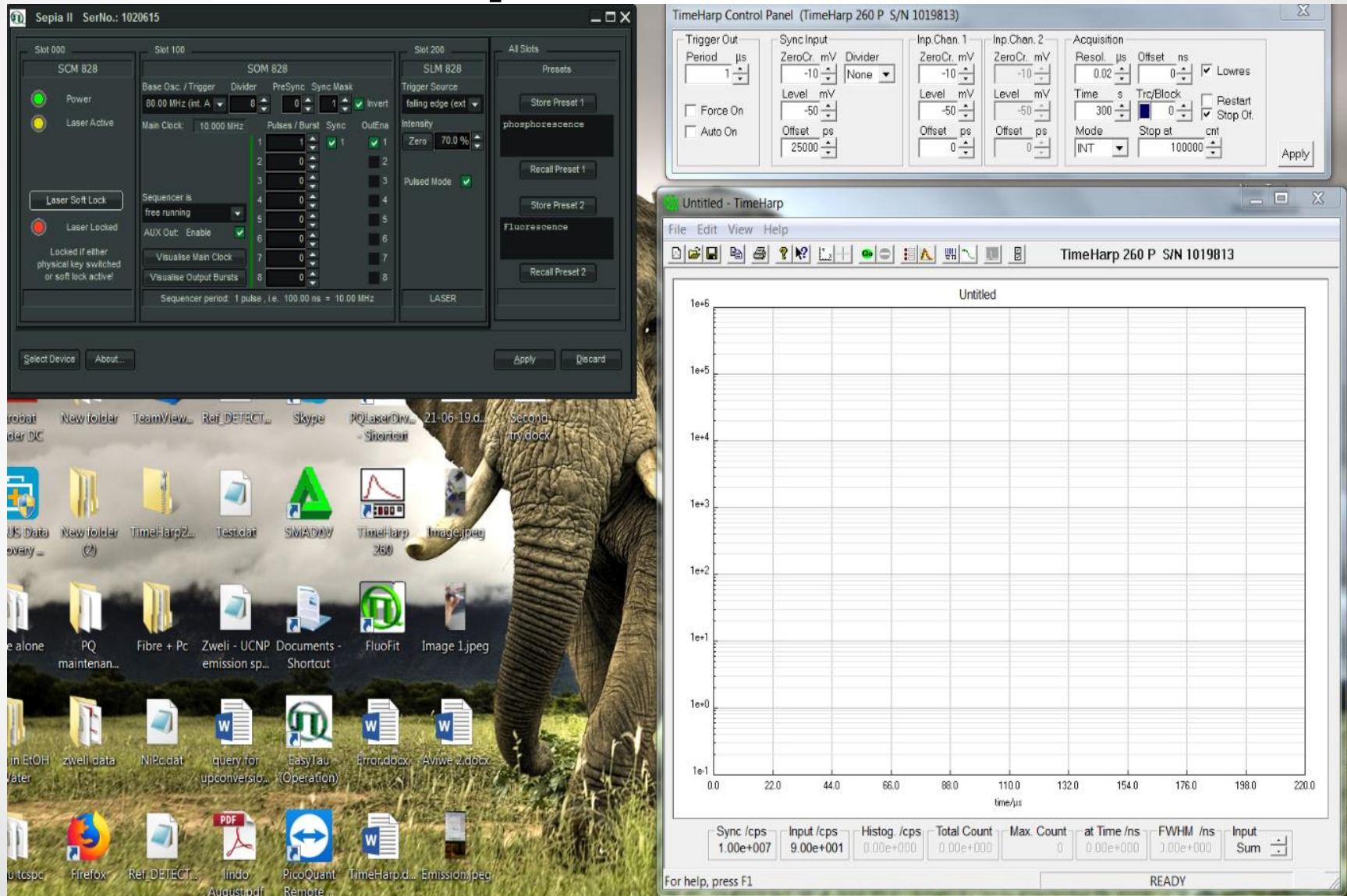
Lifetime measurements can give us information about:

- Fluorophore structure
- Fluorophore/ environmental interactions
i.e. effects of temperature, polarity,
presence of fluorescence quenchers
- Imaging - fluorescence lifetime microscopy

Phosphorescence measurements (singlet oxygen)

- Sepia PDL820 and TimeHarp (Software updates).
- This then allows use of burst mode
- Enables EasyTau to go to IR region of 1000 -1400 nm

Fig. 1: Sepia PDL820 and Time Harp software



Sample prep..

- Absorbance (0.2 to 0.3)
- Dimethylformamide (Organic solvent)
- Deuterated water

Steps to follow..

EasyTau (Measurement Mode) [Muthumuni]

File Settings Adjustment Steady-State Measurements Lifetime Measurements Analyses / Visualisations Windows Help

Customized Measurement

System status Time-resolved measurement Steady-state measurement Scripting Remote

Lamp power
Value: 0

Excitation/Detection
Signal Path: Select
Time/Channel: 1.6 μ s
EXC Polarizer [LD]: 0.0 °
EXC Attenuator: closed

EXC Monochromator
Wavelength: 250.0 nm
EXC Polarizer [Xe]: 0.0 °
EXC Shutter [Xe]: closed
EM Lens: 10.0 mm
EM Attenuator: 3.0 %
EM Polarizer: 54.7 °

EM Monochromator
Wavelength: 250.0 nm

IR [PMT]
Shutter State: ☐ Open ☒ Closed

UV-RED [PMT]
Shutter State: ☐ Open ☒ Closed

Excitation/Detection Time/Channel: 1.6 μ s

UV-RED [PMT] Shutter State: Closed

IR [PMT] Shutter State: Closed

EM Monochromator Wavelength: 250.0 nm

EM Polarizer Position: 54.7 °

EM Attenuator Position: 3.0 %

EM Lens Position: 10.0 mm

Lamp power Value: 0

PDL 820

EXC Polarizer [LD] Position: 0.0 °

EXC Attenuator Position: closed

EXC Shutter [Xe] Position: closed

EXC Polarizer [Xe] Position: 0.0 °

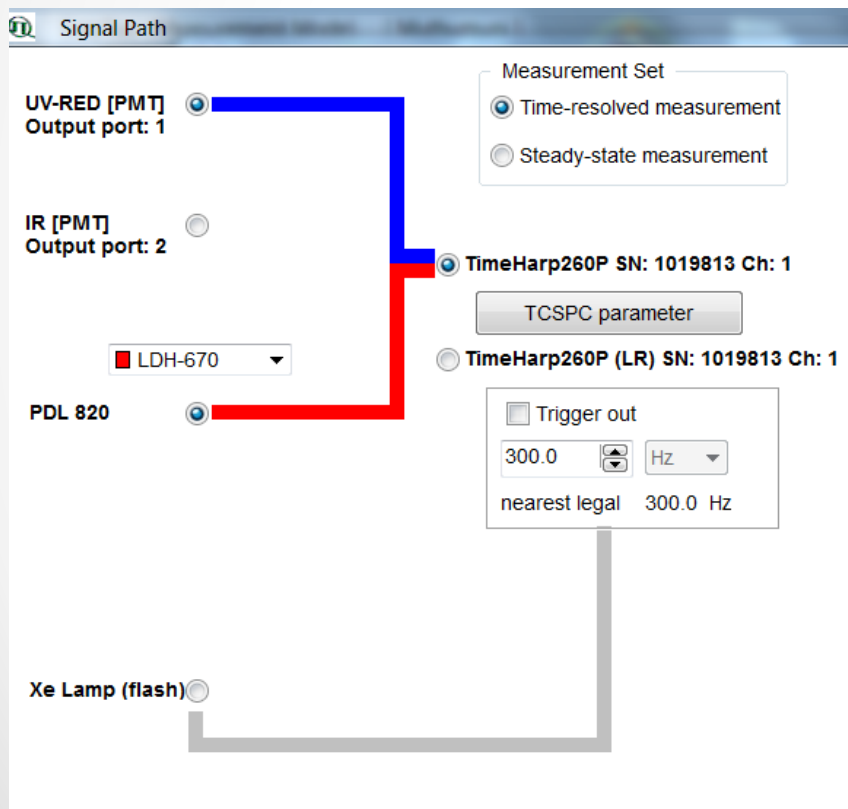
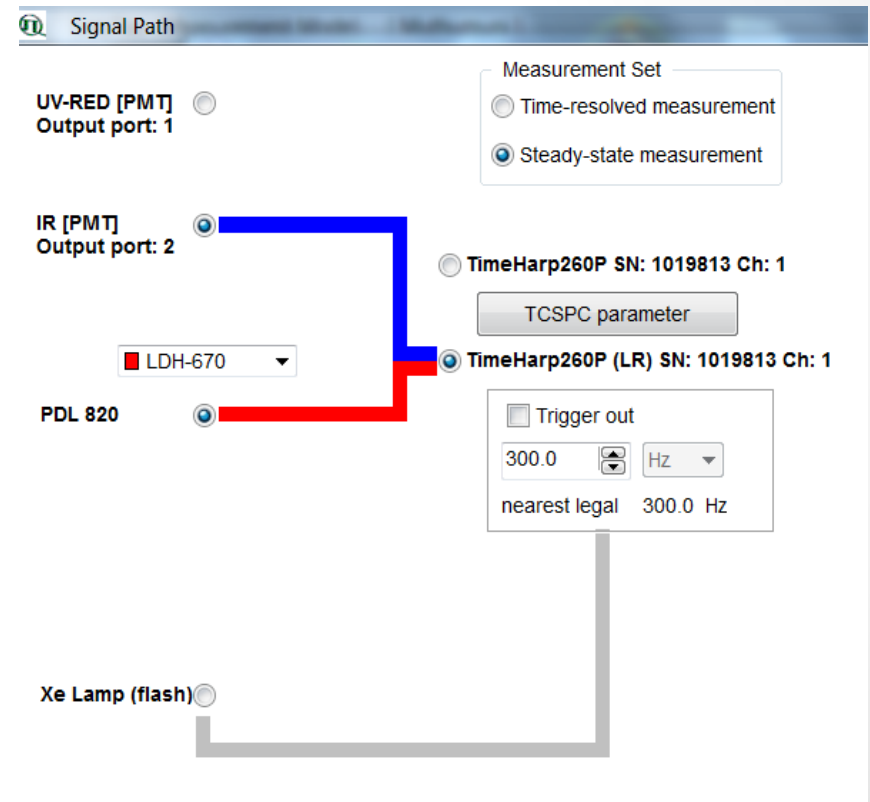
Xe Lamp

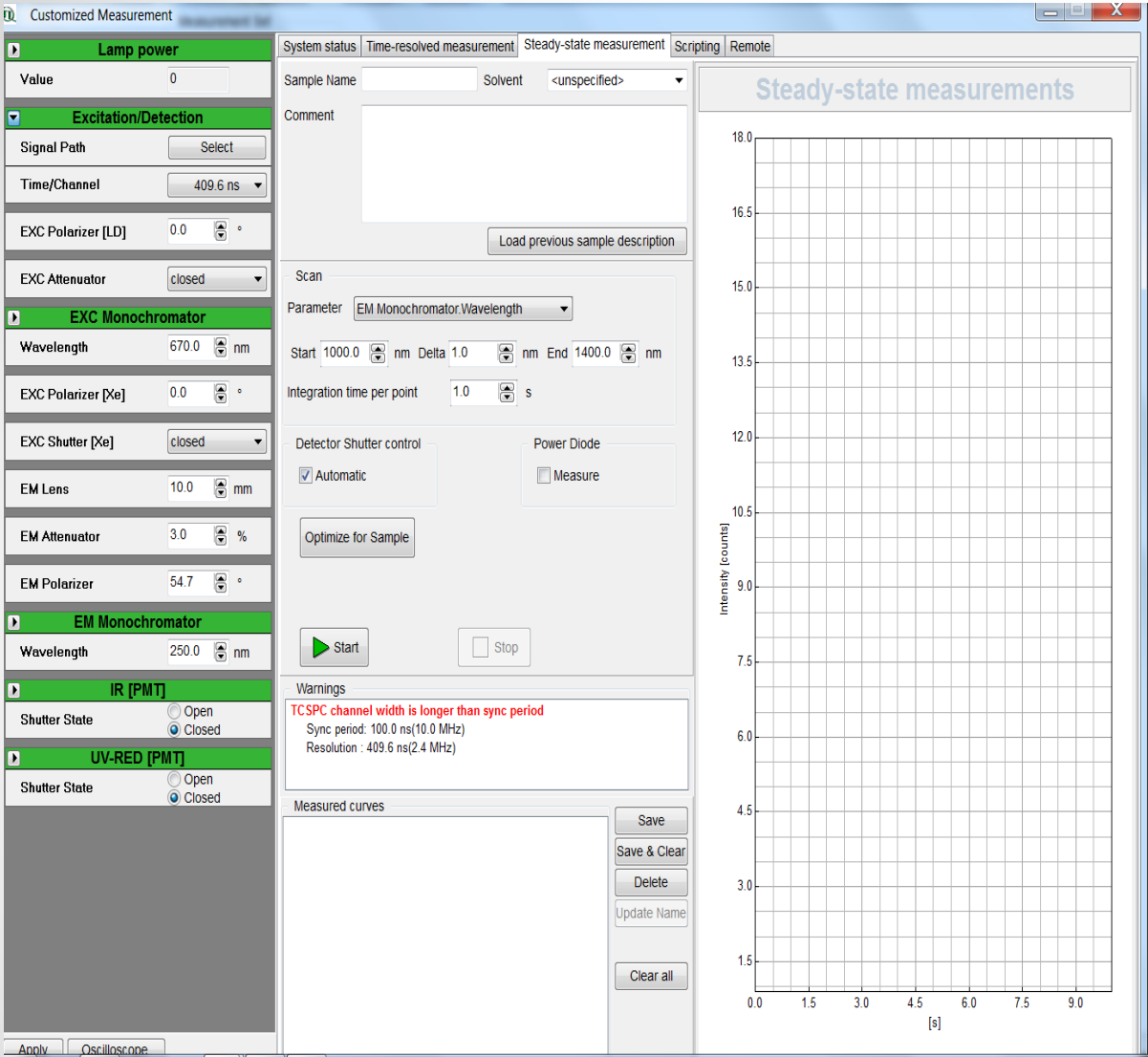
EXC Monochromator Wavelength: 250.0 nm

Apply Oscilloscope

The diagram illustrates the optical setup for time-resolved measurements. Light from the Xe Lamp passes through an EXC Monochromator (250.0 nm) and an EXC Polarizer [Xe] (0.0 °). It then encounters an EXC Shutter [Xe] (closed) and an EXC Attenuator (closed). The light path continues through an EM Lens (10.0 mm), an EM Attenuator (3.0 %), and an EM Polarizer (54.7 °). The light then enters an EM Monochromator (250.0 nm). The detection system includes an IR [PMT] and a UV-RED [PMT], both with their shutters closed. The setup is controlled via EasyTau software, which shows the current state of all components and allows for real-time adjustments.

Conti...

 τ_F  Φ_{Δ} 



Emission icon

Instructions

Insert sample and define sample and measurement parameters!

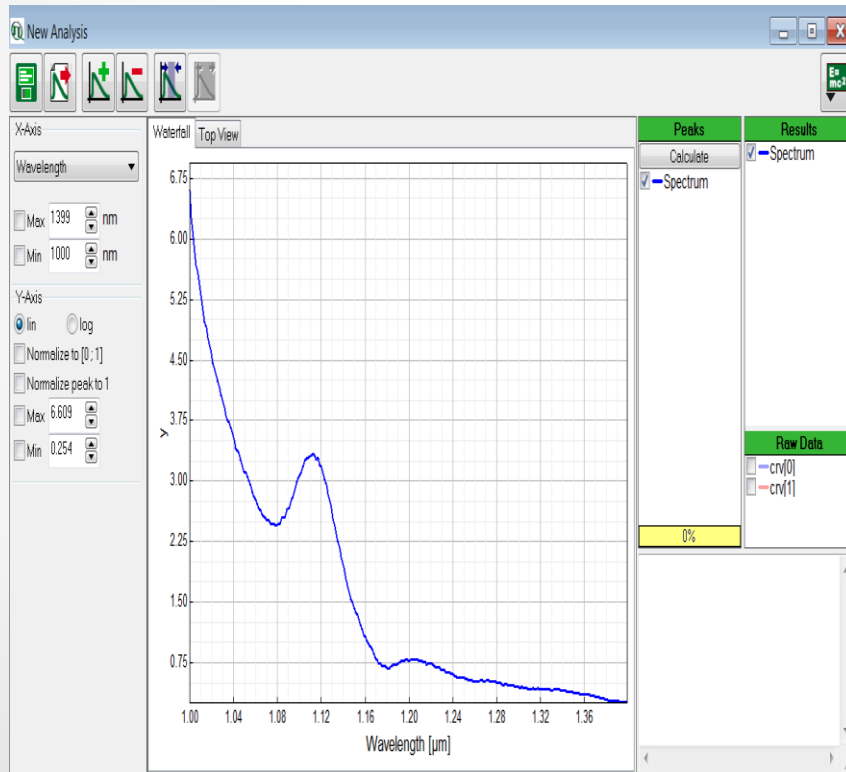
User: Temperature: Control: Off 20 °C Current: -- °C Stirrer: State: Off Speed: 0

Excitation: ☒ LDH-67 Detection: Range: 300-1600 nm

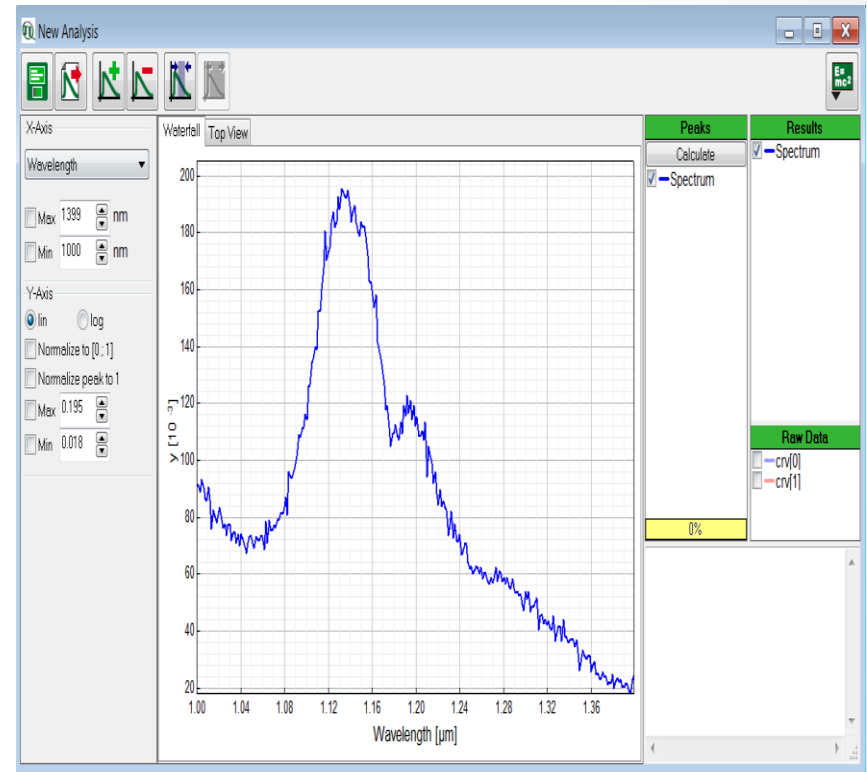
	Start	Step size	Stop
670.0 nm	1000 nm	1.5 nm	1400 nm
1.8505 eV	1.2398 eV		0.8856 eV
14925 cm ⁻¹	10000 cm ⁻¹	2 Step 7143 cm ⁻¹	

Near-Infrared phosphorescence spectrum

Zn Standard



Sample



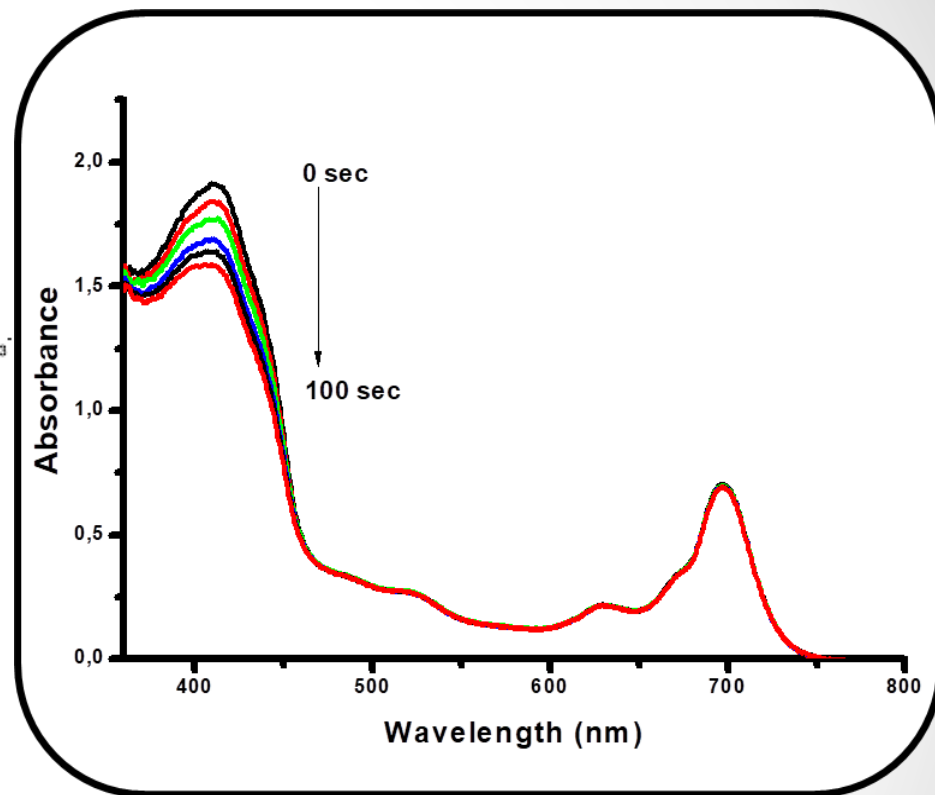
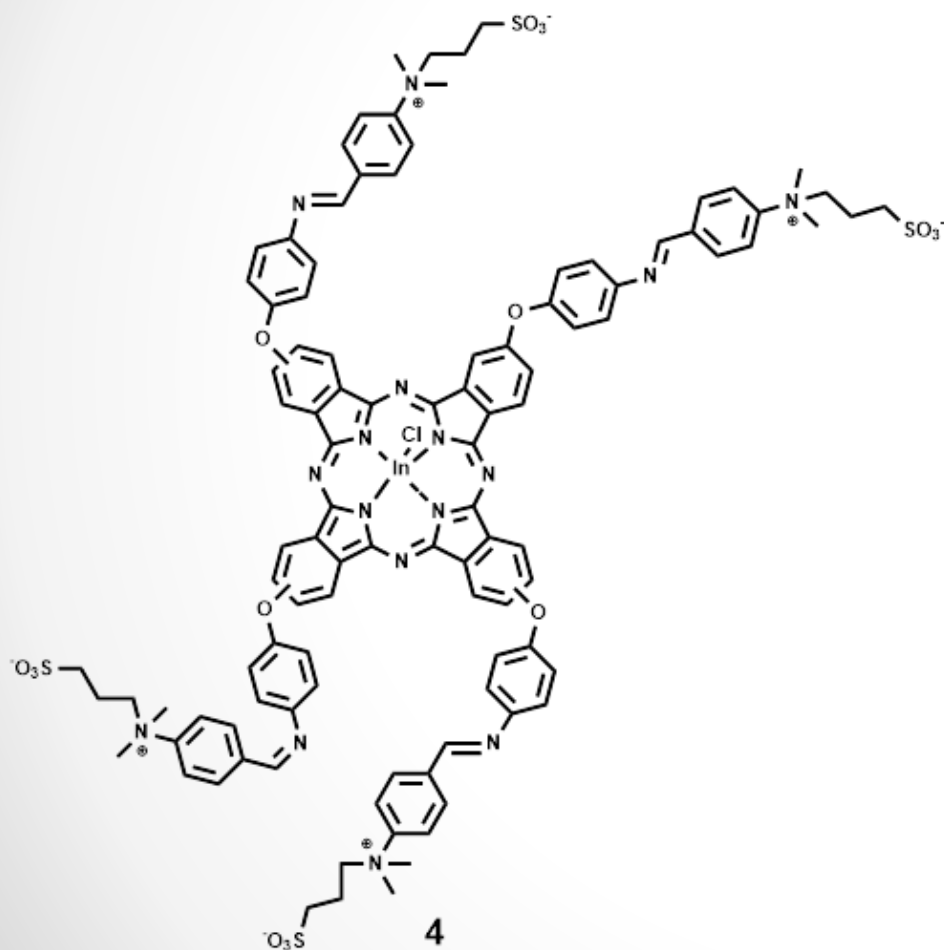
phosphorescence spectrum was plotted as a function of the fraction
of absorbed light ($1 - 10^{-A}$)

Calculation

- The slope of these plots (r_R and r_S , respectively) are proportional to the corresponding Φ_{Δ}^R and Φ_{Δ}^S
- The absorption needs to be taken into concentration as the samples and standard were both excited at 670 nm. The Equation was multiplied by the absorption factor: $A_{\text{abs}}^R / A_{\text{abs}}^S$

$$\Phi_{\Delta}^S = \Phi_{\Delta}^R \frac{r_S}{r_R}$$

Results



Previous calculated value is 0.61
The calculated value using TCSPC is 0.58

