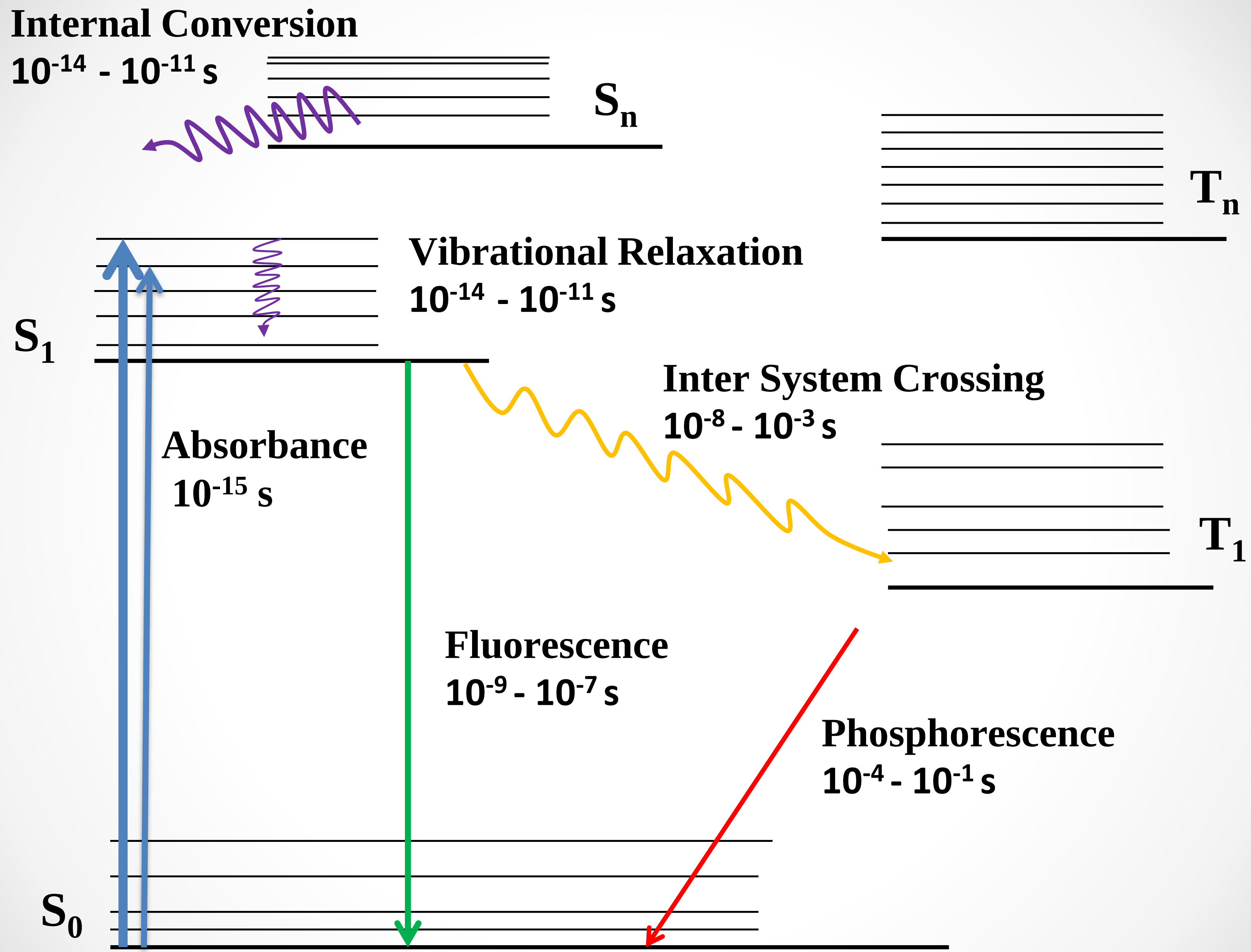
Time Correlated Single Photon Counting (TCSPC) (τ_F)

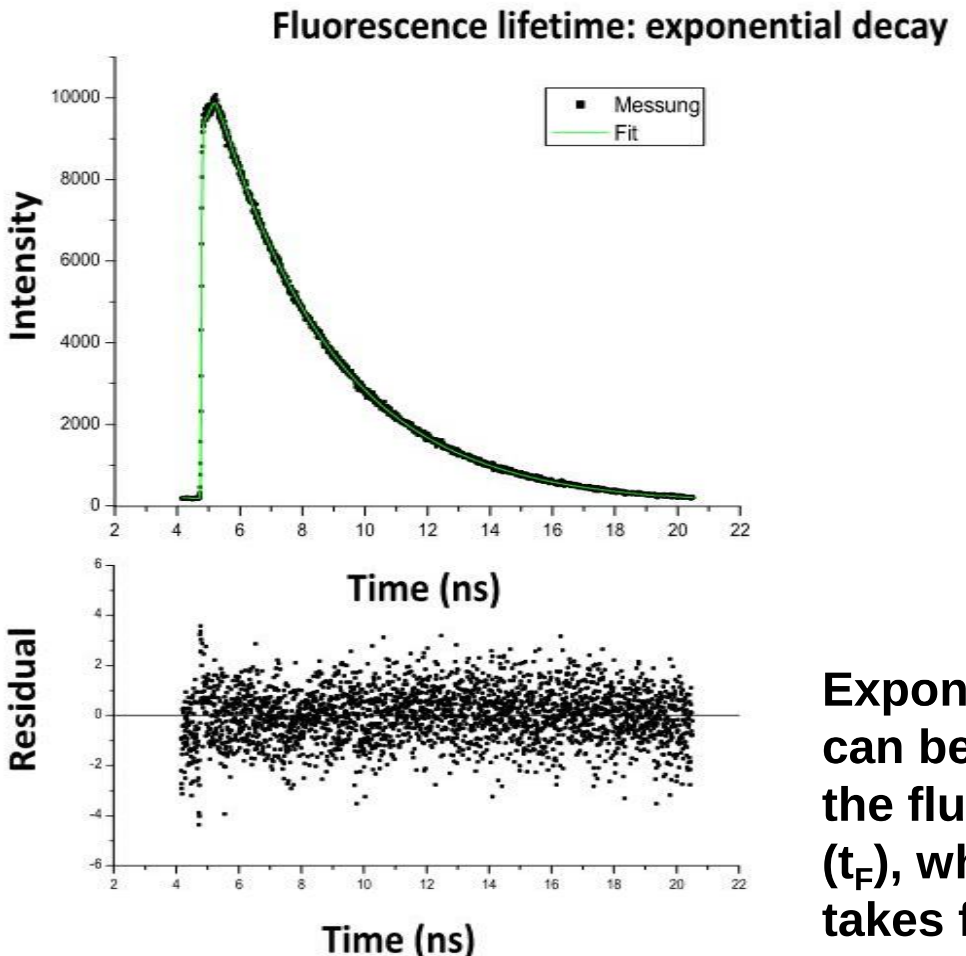
TCSPC(Fluotime 300): Capabilities

- 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 s
Sn
Tn
Vibrational Relaxation
10-14 - 10-11 s
S1
Inter System Crossing
10-8 - 10-3 s
Absorbance
10-15 s
T1
Fluorescence
10-9 - 10-7 s
Phosphorescence
10-4 - 10-1 s
S0

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?

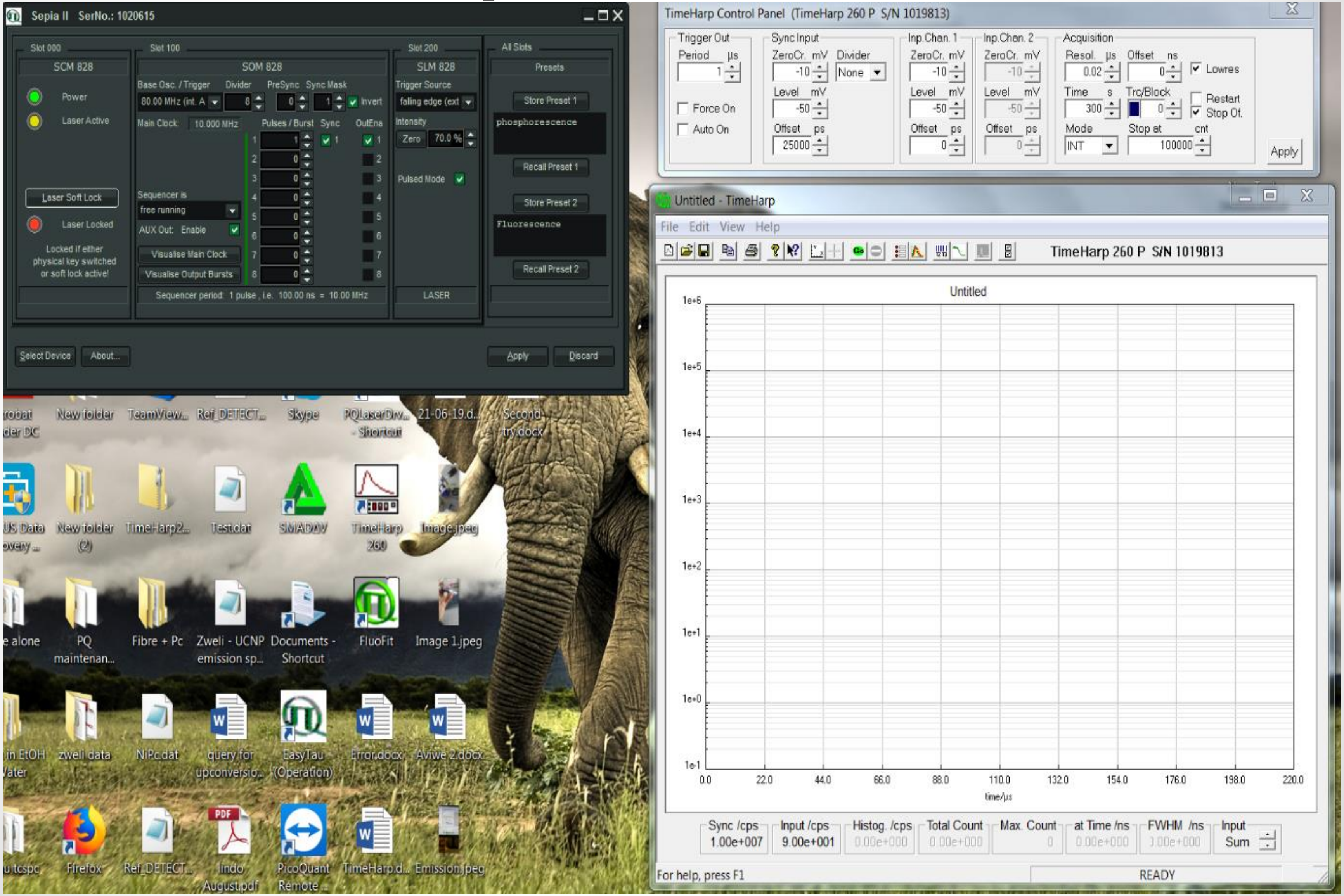
Lifetime measurements can give us information about:

- 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

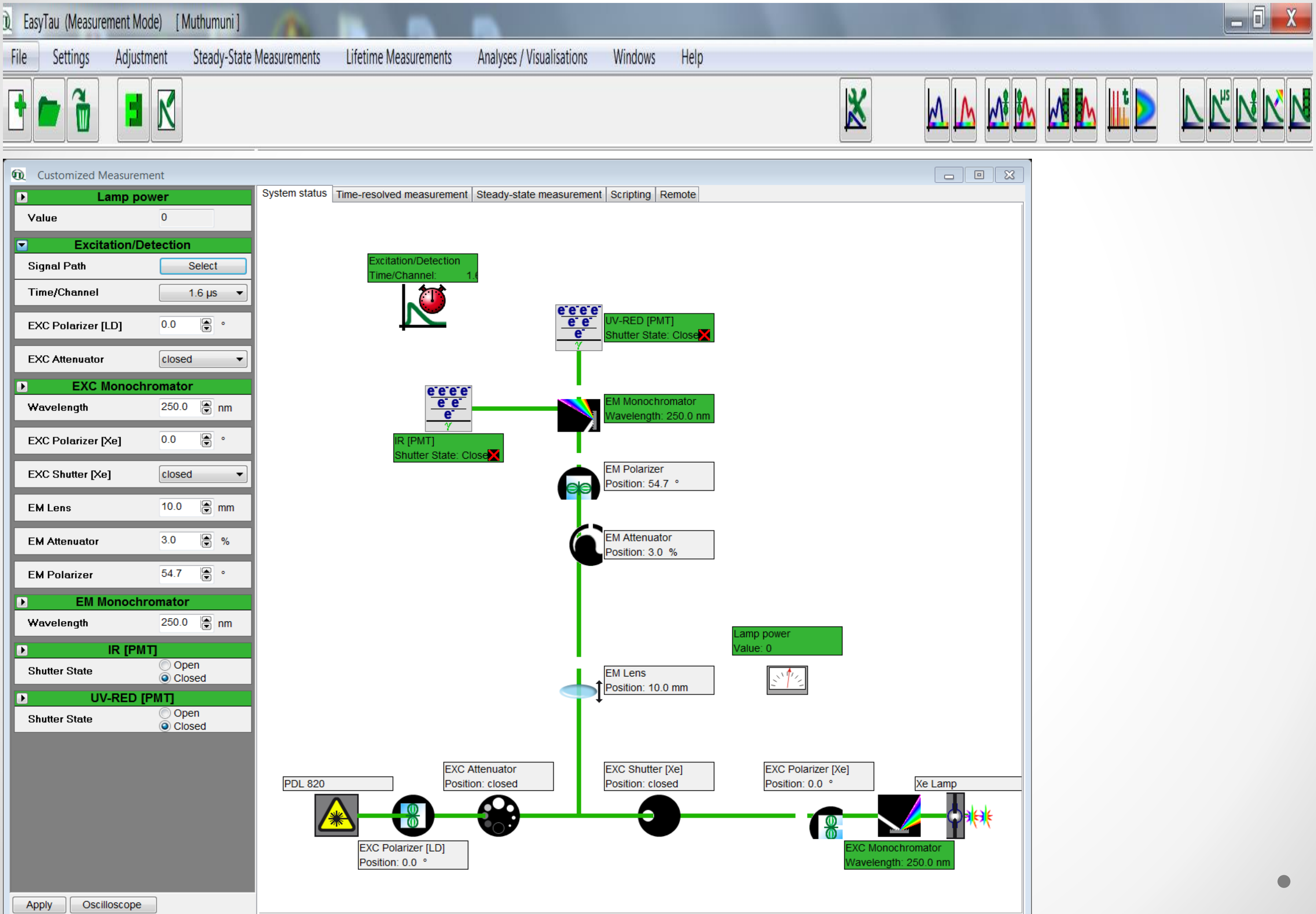
Fig. 1: Sepia PDL820 and Time Harp software



Sample prep..

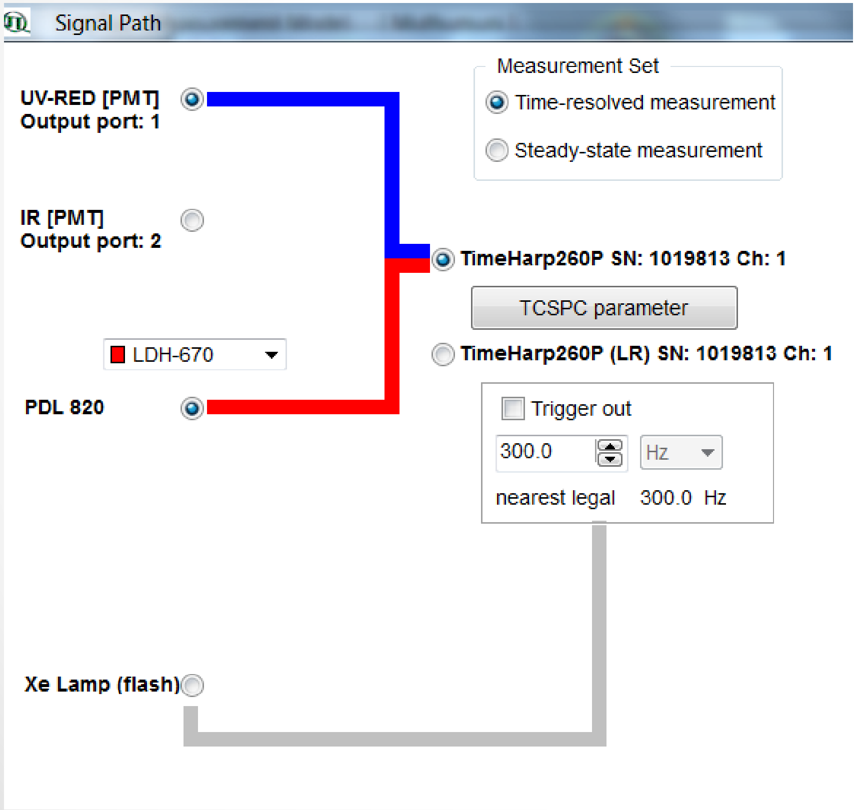
- Absorbance (0.2 to 0.3)
- Dimethylformamide (Organic solvent)
- Deuterated water

Steps to follow..

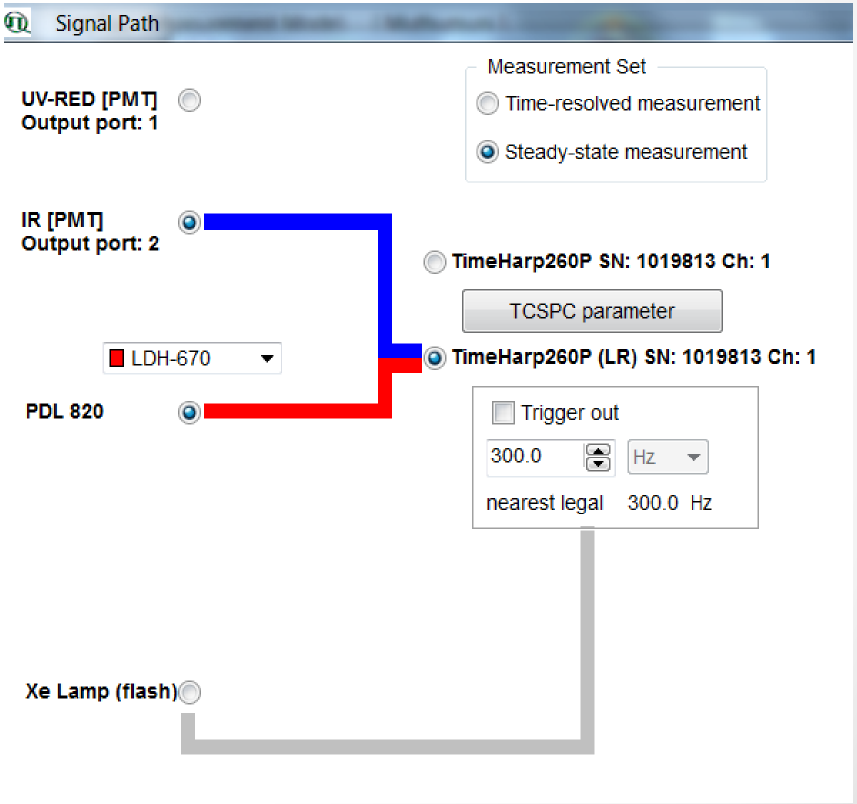


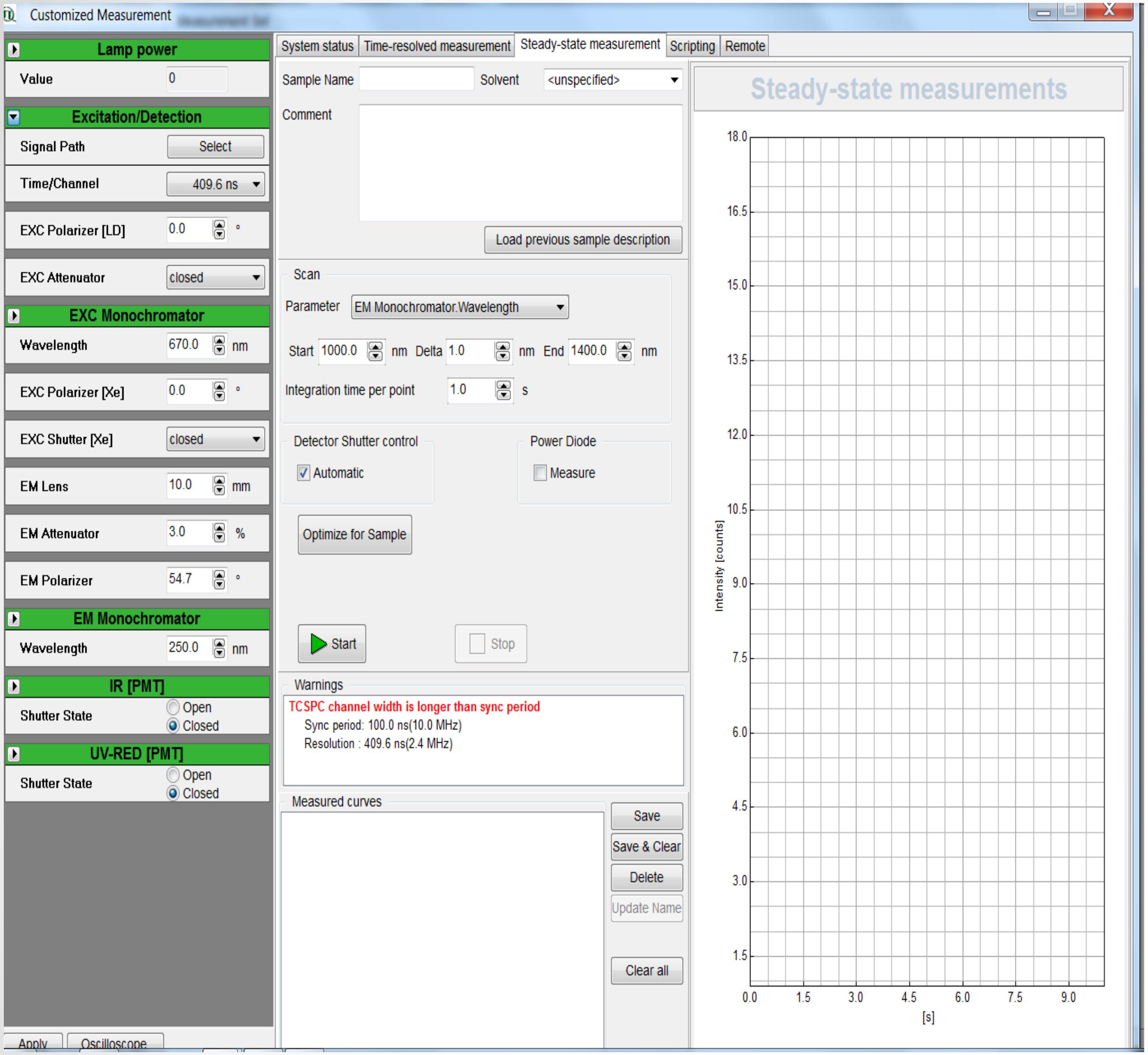
Conti…

τ_F

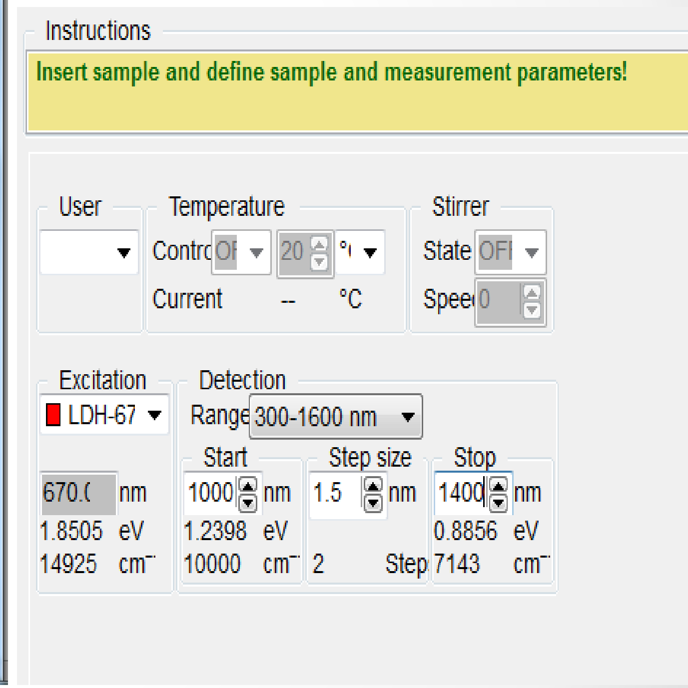


Φ_Δ



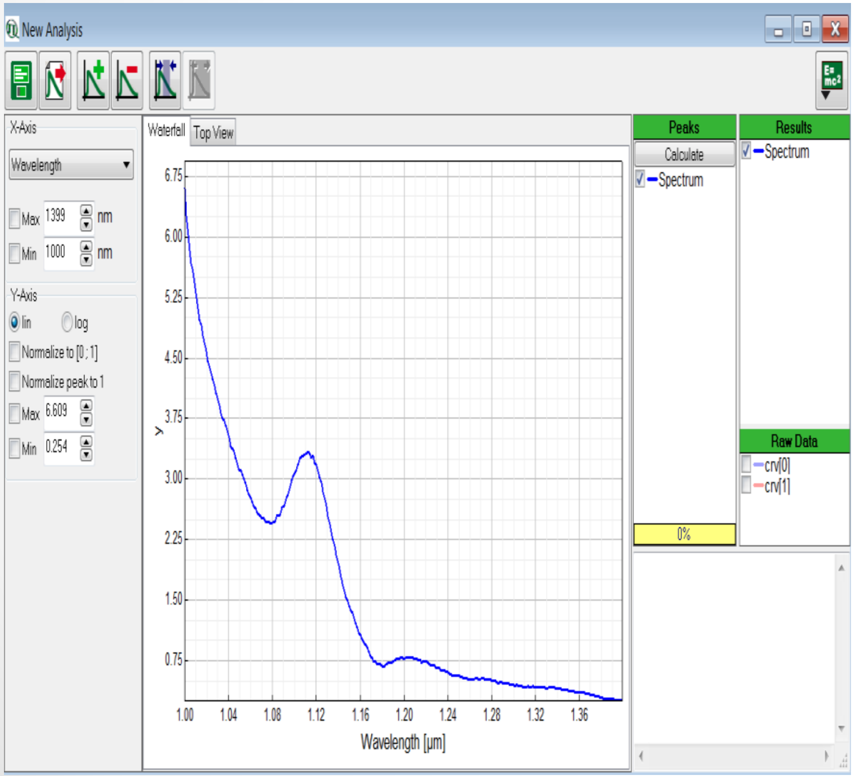


Emission icon

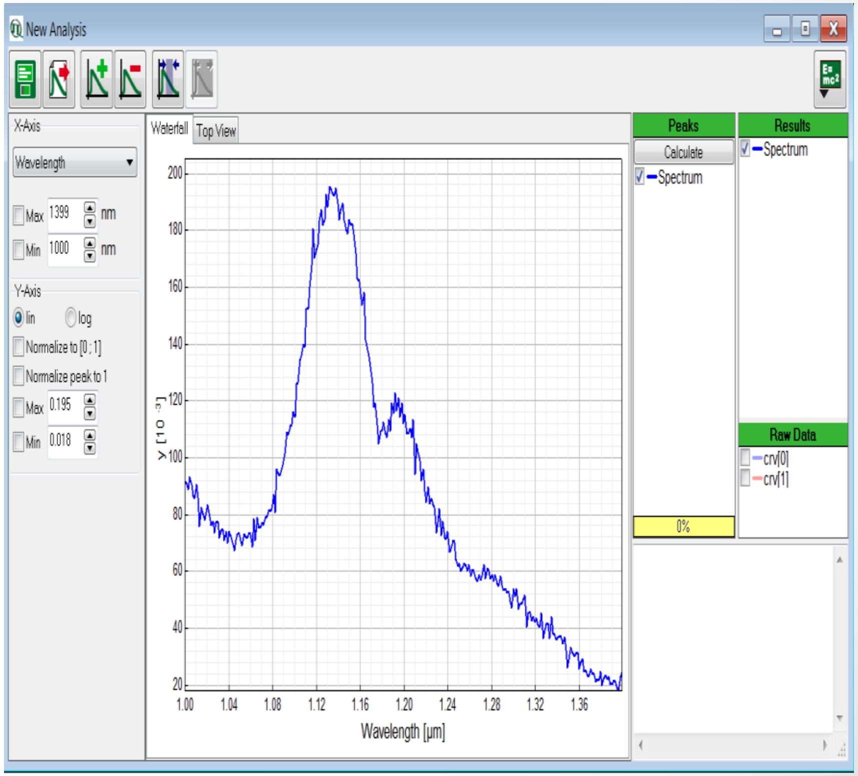


Near-Infrared phosphorescence spectrum

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 $\Phi_\Delta{}^R$ and $\Phi_\Delta{}^S$
- 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: $A_{abs}{}^R / A_{abs}{}^S$
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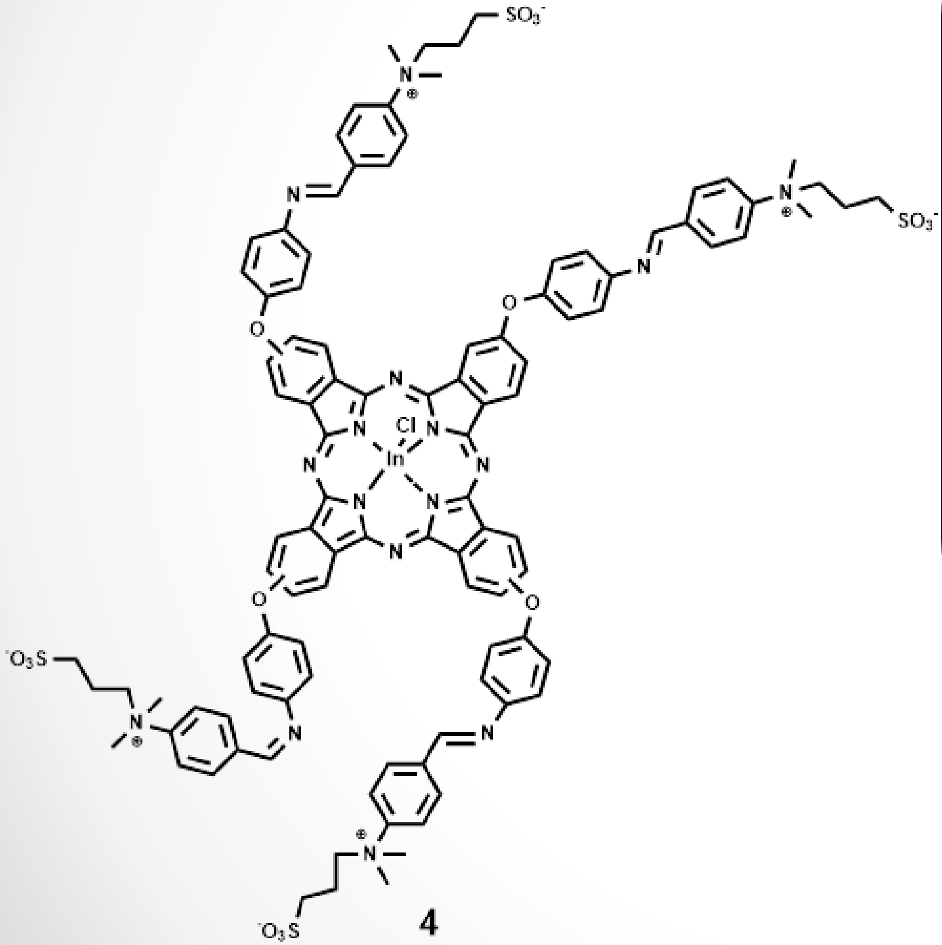
$$\Phi_\Delta^S = \Phi_\Delta^R \frac{r_S}{r_R}$$

Results



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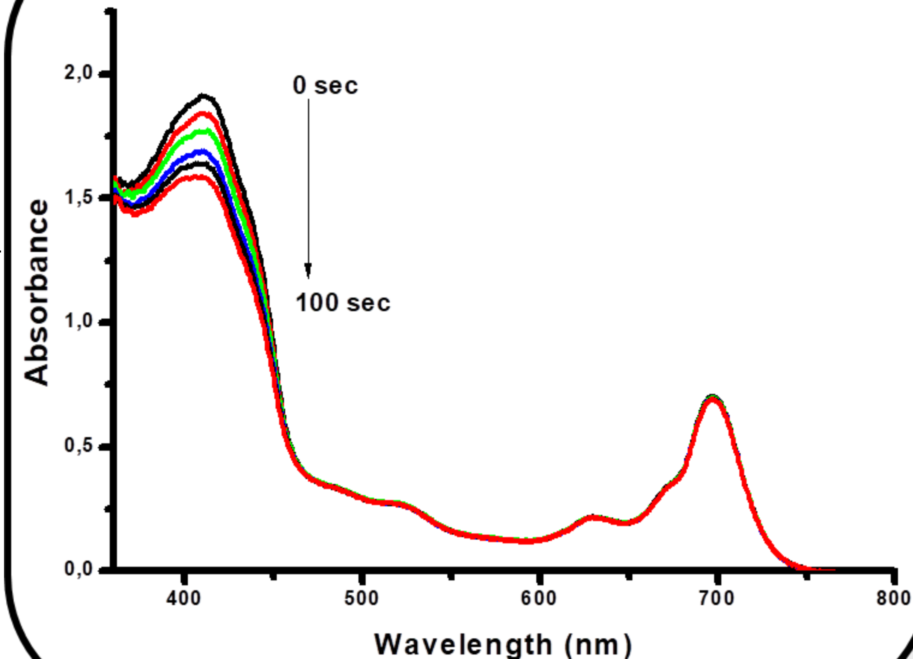

Previous calculated value is 0.61
The calculated value using TCSPC is 0.58