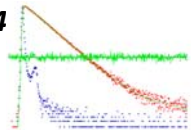


# The measurement of singlet oxygen lifetime sensitised using Rose Bengal

***The study of singlet oxygen ($^1O_2$) is of interest, principally, as it is a highly reactive species. It can be produced by photosensitisation, usually of a molecule such as a dye or porphyrin. Thus, by the appropriate selection of sensitiser, the presence of oxygen and light, $^1O_2$ can be selectively generated. From a biological aspect it has the ability to damage and destroy cells, which has lead to interest in its use as an anticancer agent in photodynamic therapy (PDT).***

## Sensitised production of $^1O_2$

There are several types of molecules that can be used as sensitisers to generate $^1O_2$. Common ones employed in PDT include porphyrins, although dyes such as Methylene Blue and Rose Bengal have been frequently used. The photosensitisation reaction that can occur when oxygen collides with a photosensitising agent (*PA*), sometimes referred to as a type II reaction, can be summarised below.

$$PA(S_0) \xrightarrow{h\upsilon} PA(S_1) \xrightarrow{k_{ISC}} PA(T_1)$$

$$PA(T_1) + {}^3O_2 \xrightarrow{k_{ET}} PA(S_0) + {}^1O_2$$

Where $S_0$ and $S_1$ are the singlet ground and excited states, $T_1$ the first excited triplet state, $k_{ISC}$ and $k_{ET}$ the rate constants for intersystem crossing and energy transfer. $^3O_2$ is the ground state of oxygen.

In practical terms this means that light is used to excite the photosensitiser (usually in the visible) and the emission from $^1O_2$ monitored (in the near infrared, NIR). The decay time of $^1O_2$ is usually in the microsecond time range and depends on the solvent used.

## Measurement of the $^1O_2$ lifetime

The emission of $^1O_2$ occurs close to 1275nm, in the near infrared part of the spectrum. This means that a NIR sensitive detector is required and instrumentation which can measure decay times on the microsecond time scale.

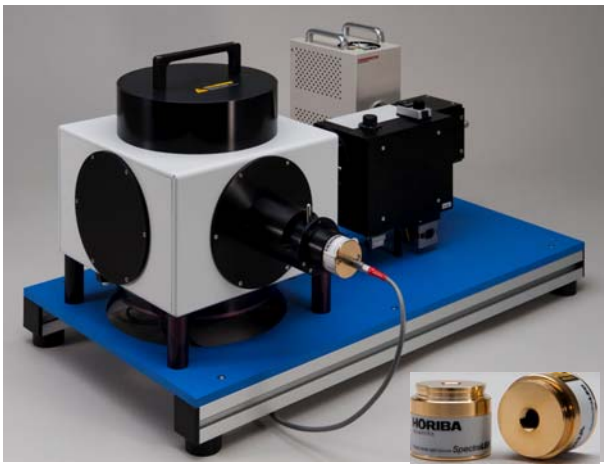
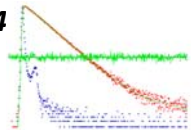



Fig. 1. TemPro-01 optical platform with NIR detector. Inset SpectraLED excitation sources.

The photosensitising agent, Rose Bengal, requires an excitation wavelength around 530nm. The *HORIBA Scientific TemPro-01*, equipped with a S-535 excitation source (*SpectraLED* emitting at 535 nm) operating on the phosphorescence timescale and a H10330-45 detector, is well suited for such a measurement. This equipment is shown in Fig. 1. The *SpectraLED* pulse rate is automatically adjusted to suit the time range and its duration can be altered which allows further control over the source intensity. These parameters are set by the *DataStation* control software. The apparatus phosphorescence time ranges, with a resolution from 83ns / channel is well suited for measuring lifetimes from a microsecond onward. It is also capable of coping with the high level of “dark counts” or noise associated with the use of NIR sensitive detectors for lifetime measurements



**Time-resolved decay of Rose Bengal sensitised $^1O_2$ emission in different solvents.**

To demonstrate the measurement of the sensitised decay of $^1O_2$ two solvents were chosen for this work; ethanol, where a time-resolution of 83ns/chnl was employed and acetone, where 167ns/chnl was selected. Making use of a lower resolution can be useful if a shorter data acquisition time is desired. In the following results it can clearly be seen that both time ranges yield a good quality of data, with *HORIBA Scientific DAS6* analysis software used to fit the data to a single exponential decay model.

The time-resolved decay of Rose Bengal sensitised $^1O_2$ emission in ethanol is shown in Fig. 2, along with the fit and weighted residuals.

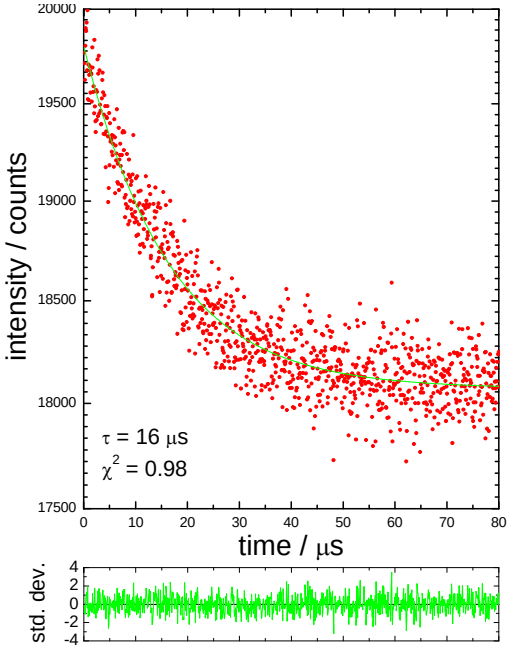



Fig. 2. Decay (red) of $^1O_2$ in ethanol, monitored at 1275nm with excitation of Rose Bengal made using a SpectraLED-535. The fit and weighted residuals (green) are shown and the lifetime obtained was 16µs.

Fig. 2 clearly shows a good fit to the single exponential decay model. The decay shown in Fig. 3 was measured in the same manner, but using acetone as a solvent. In this case it was expected that a longer-lived decay would be obtained and this proved to be correct. A lifetime of 50µs was recovered from the *DAS6* analysis. Again a good fit was obtained using a single exponential decay model.

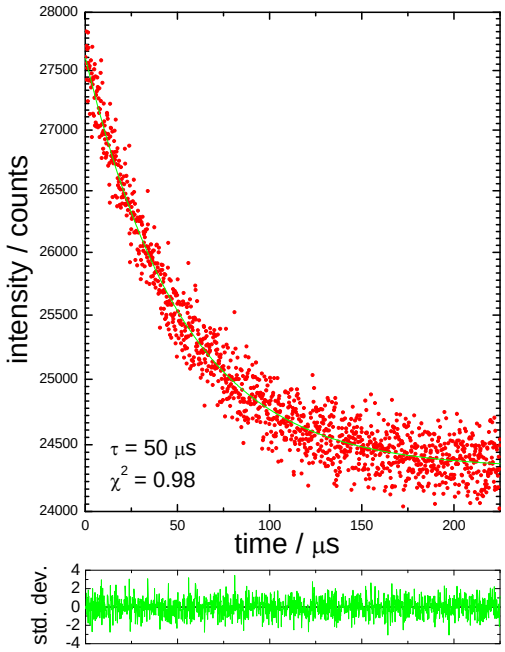



Fig. 3. Decay (red) of $^1O_2$ in acetone, monitored at 1275nm with excitation of Rose Bengal made using a SpectraLED-535. The fit and weighted residuals (green) are shown and the lifetime obtained was 50µs.

## Summary

The time-resolved emission of $^1O_2$ monitored at 1275nm after excitation of a photosensitiser, Rose Bengal, using a *SpectraLED* source could be measured using the *TemPro* system equipped with a NIR detector. Good fits were made to a single exponential decay model and the influence of solvent on the time-resolved kinetics was clearly evident.