Detection of Singlet Oxygen

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Introduction

Detection of singlet state of Oxygen ($^1O_2$) is of particular interest in a multitude of applications such as photodynamic treatment of cancer,[1] fine chemical synthesis,[2] and treatment of wastewater.[3]

$^1O_2$ shows a weak emission band at ~1270 nm with a natural bandwidth of approximately 18 nm. As the ground state of molecular oxygen is a triplet state, direct excitation, by the absorption of light, into the first excited singlet state is not possible. Therefore $^1O_2$ is often generated by chemical reactions or by photosensitisation, i.e. light is absorbed by other photoactive dyes that will be able to transfer the absorbed energy via their triplet states to the molecular oxygen.

As the signal at 1270 nm is so weak, detection of the $^1O_2$ emission has always been challenging. Germanium and InGaAs detectors were used in the past, but predominantly for steady state applications. Lifetime applications were very limited indeed, due to the further reduction of signal amplitude when the RC constant of the detector had to be kept low to achieve the necessary temporal resolution. Nowadays, photomultipliers (PMT) are available with sensitivity to 1400 nm and beyond. This greatly facilitates both steady state and lifetime measurements of $^1O_2$.

Even with modern detectors, it is essential to have a powerful excitation source and an efficient optical system. The FLS980 is the ideal spectrometer for this application.

Methods and Materials

Emission spectra were measured in a FLS980 Fluorescence Spectrometer equipped with a 450 W Xe lamp, a 60 W microsecond flashlamp, and single monochromators. A 400 nm blazed grating was used at the excitation and a 1200 nm blazed grating at the emission arm.

An NIR-PMT (Hamamatsu, R5509-72) with 0.5 s, 4 repeats and a two-stage TE-cooled InGaAs detector (Hamamatsu, G8605-23) with 0.1 s dwell and 20 repeats were used, both resulting in 2 s integration time. Higher diffraction orders were filtered by the built-in long wave-pass filters in the FLS980.

Samples of Erythrosine B (Sigma-Aldrich, 19,826-9) dissolved in anhydrous ethanol (Sigma-Aldrich, 459836) and tris (2,2'-bipyridyl)dichlororuthenium(II) hexahydrate Ru(bpy)$_3$ (Sigma-Aldrich, 544981) dissolved in D$_2$O (Sigma-Aldrich, 151882) were prepared air saturated with optical density (OD) 0.5 and 0.35, respectively. The Ru(bpy)$_3$ sample was additionally saturated with oxygen for approximately 10 minutes.

All samples were measured in quartz cuvettes of 10 mm path-length in right angle geometry. The Ru(bpy)[3] samples were excited at the absorption peak of 450 nm, while the Erythrosine B at 530 nm with excitation and emission

bandwidths of 15 nm.

To allow for direct comparison of the spectra between different detectors, the data was noise normalised by applying a scaling equal to the noise ratio at 1360 nm - 1400 nm, that is $f=I_{NIR\text{-}PMT}(\lambda)/I_{InGaAs}(\lambda)$, and consequently offset vertically.

Steady State Emission

The $^1O_2$ emission spectra displayed in Figure 1 were measured with a) an NIRPMT and b) an InGaAs detector. The $^1O_2$ emission around 1270 nm generated by erythrosin shows a single emission peak, however in Ru(bpy)$_3$ the $^1O_2$ emission is overlapped by the emission tail of the sensitizer molecule. It can also be seen in Figure 1b that, for the O$_2$ saturated sample, the background emission is reduced due to the quenching of the triplet state,[4] as has been observed in Ru(bpy)$_3$ in aqueous solutions.[5]

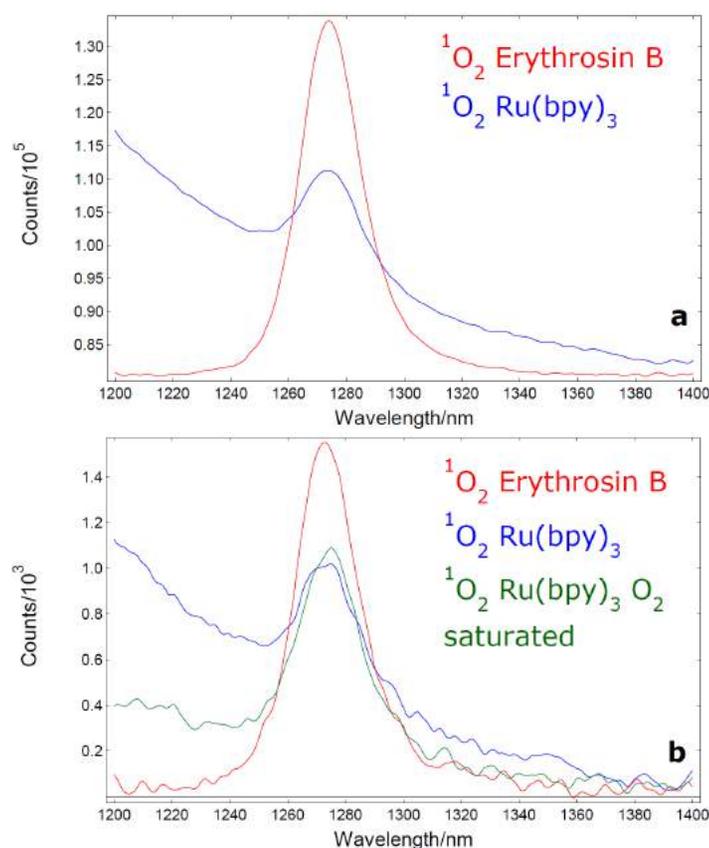Figure 1: Emission spectra of $^1O_2$ in Erythrosin B and Ru(bpy)[3] under λ_{exc}=530 nm and λ_{exc}=450 nm, respectively, obtained with a) NIR-PMT and b) InGaAs detectors. The experimental conditions were $\Delta\lambda_{exc}=\Delta\lambda_{em}$=15 nm, integration time 2 s/step and step of 1 nm for all samples.

The estimated signal to noise ratio (SNR) of the $^1O_2$ emission generated from erythrosin B is 195 for the NIR-PMT and 45 for the InGaAs.

The difference in the SNR is a result of integration, quantum efficiency, active area, sensitivity and different sources of noise in the detectors:

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1. Noise. The NIR-PMT is a single photon counting detector. Working in this mode means that the only source of noise is photon noise (Poisson noise) which is the square root of the measured signal (or more specifically square root of signal plus detector dark counts). The InGaAs detector has a lock-in amplifier to minimise noise fluctuations and so will be noise limited by the noise equivalent power (NEP).

2. Sensitivity: The difference in sensitivity between single photon counting and analogue detection, in particular the fact that single photon counting is intrinsically digital and only accepts signal counts above a noise floor.

3. Active area. The NIR-PMT has an active area of 24 mm compared to 3 mm for a standard InGaAs chip. For small monochromator slits this will not have an impact, but for weak samples, when the monochromator slits are opened so that the focus on the detector is larger than the active area, the increase in sensitivity will be limited.

4. Quantum efficiency. The InGaAs detector actually has almost 2 orders of magnitude higher quantum efficiency than the NIR-PMT at 1270 nm which additionally depends on the operating temperature of each detector.

5. Integration. The analogue detection works with an integration time that is set by the electrical circuit, whereas with single photon counting the integration time is directly set by the length of time the individual photons are counted.

The higher source of noise inherent in an analogue detector means that the NIR-PMT will have a higher SNR and therefore be more sensitive at 1270 nm, even though the InGaAs may have higher quantum efficiency.

In order to obtain a direct comparison of the $^1O_2$ signal obtained by the two detectors, the emission spectra of $^1O_2$ from erythrosin B in Figure 1 have been noise normalised and are displayed in Figure 2.

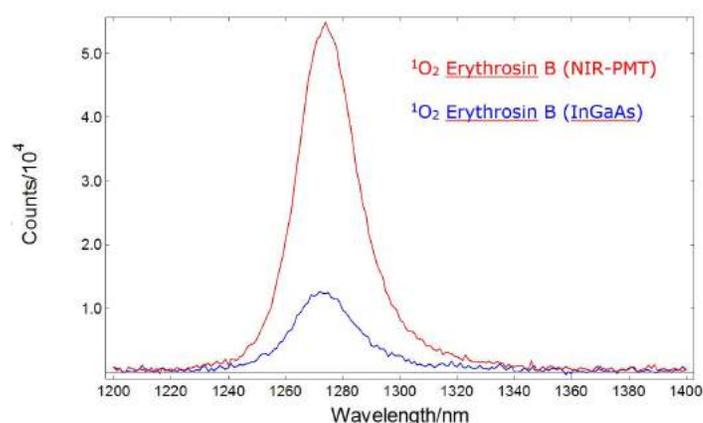Figure 2: Emission spectra of $^1O_2$ in Erythrosin B obtained by NIR-PMT and InGaAs detectors. The spectra have been scaled to equal noise and offset, to allow for direct comparison between detectors.

Time-Resolved Emission

Although the emission spectrum of $^1O_2$ can be measured with InGaAs detectors, the measurement of its lifetime requires a photon counting detector. For this reason, the

decay of $^1O_2$ monitored at 1270 nm was measured with an NIR-PMT and is displayed in Figure 3. It can be seen that the exponential fit resulted in a lifetime of 15.3 µs for $^1O_2$ from Erythrosin B in ethanol and 59.47 µs for Ru(bpy)$_3$ in D$_2$O, in agreement with reported decay values.[6]

Fit results

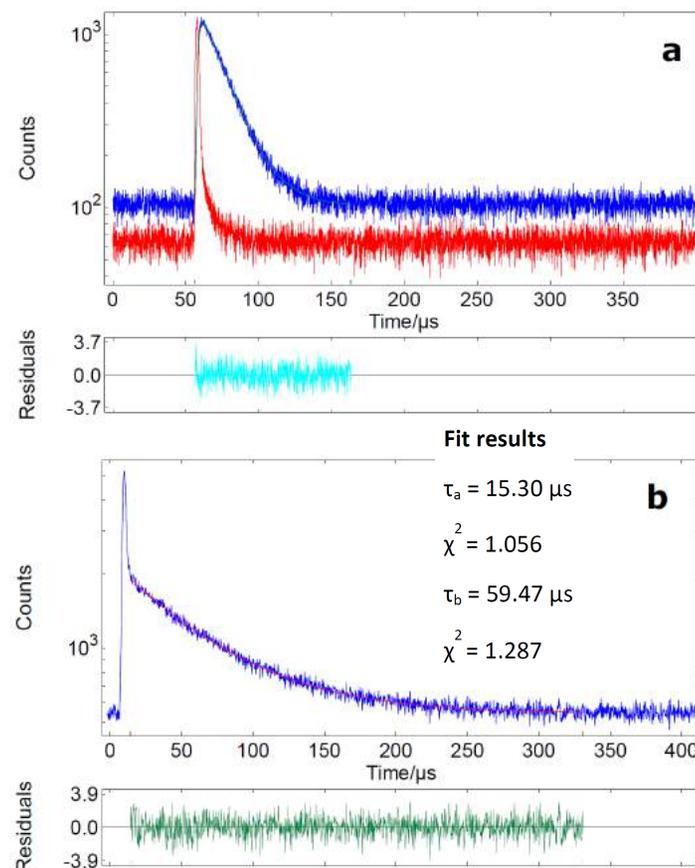τ_a = 15.30 µs

χ^2 = 1.056

τ_b = 59.47 µs

χ^2 = 1.287

Figure 3: Lifetime decay of a) Erythrosin B in Ethanol and b) Ru(bpy)$_3$ in D$_2$O.

The time-resolved emission spectra (TRES) can be obtained from time-resolved scans across the emission of $^1O_2$. In this way, the emission of $^1O_2$ can be distinguished from phosphorescence emitted by the sensitiser. The emission spectra generated by Ru(bpy)$_3$ are displayed in Figure 4 from 200 µs to 400 µs, with a step of 20 µs.

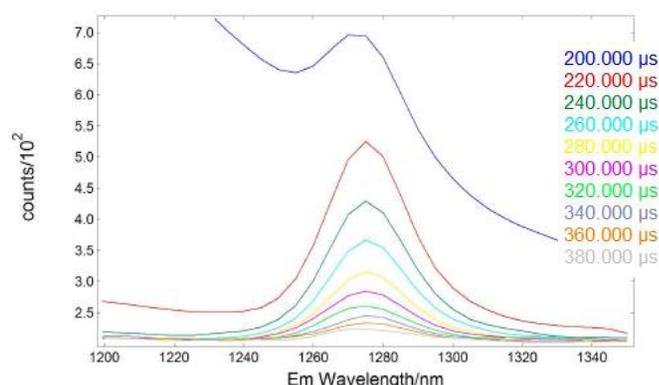Figure 4: Time-resolved emission spectra (TRES) of $^1O_2$ generated from Ru(bpy)$_3$.

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Conclusion

Steady-state and time-resolved emission spectra of $^1O_2$ generated by Erythrosin B and Ru(bpy)$_3$ are reported in this technical note. In addition, the lifetimes were fitted in single exponentials and are in good agreement with reported lifetime values. It can be concluded that both steady-state and timeresolved measurements of $^1O_2$ can be obtained with single photon counting detectors such as NIR-PMT, while InGaAs detectors can be used for steady-state measurements.

References

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