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Recent measurements with Ru^{2+} oxygen sensors, using doped sapphire crystals, both as a calibration aid and an integral temperature sensor

(Invited Paper)

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ABSTRACT

Recent measurements are presented, using an improved, real-time, all-optical sensor for simultaneous measurement of dissolved oxygen and temperature. The sensor has a small Cr^{3+} - doped sapphire (ruby) thermal monitoring crystal mounted at the probe tip, to which is bonded a Ru^{2+}-based oxygen-indicator membrane. The probe may be interrogated in real-time, using, for both temperature and oxygen monitoring, the same combination of blue LED, light source, optical filter set, photomultiplier detector and digital processor. The ruby crystal also provides a fluorescent intensity reference, for possible on-line self-testing of the interrogation hardware. By examining the relative intensities from ruby and the membrane, mechanical damage, detachment or photo-bleaching of the sensing membrane may also be recognised. Recent developments of our novel, Ti^{3+} - doped sapphire fluorescence-lifetime calibration probe are reported. These confirm that the fluorescence lifetime of the probe can be thermally controlled in a reliable manner. This calibration probe then allows multi-point calibration of Ru^{2+}-chemical sensors.

INTRODUCTION

Many chemical sensors based on monitoring the fluorescence lifetime of indicators are now available, some commercially,. (Eg., the well-known Ru^{2+} dye chemical probe). These probes are recognised to have several advantages. The most attractive features are:- firstly, they do not consume oxygen, secondly, the probe tip has very low cost, and thirdly they are all optical. The latter feature ensures that they do not respond to electrical or electromagnetic interference, do not cause electrical safety hazards and cannot suffer from or catalyse galvanic corrosion.

One of the known problems with such sensors is that their response varies significantly with temperature. This dependence occurs with most fluorescent-lifetime-based chemical probes, as the occupancy and hence decay rates of electronic levels show considerable thermal variation. It is therefore desirable to mount a temperature sensor at the probe tip to monitor and correct for such thermal changes. Clearly, a simple thermocouple could be used, but this would require a separate interrogator and throws away one of the major attractions of the probe, its non-electrical nature. It is preferable to use an optical temperature probe that can be interrogated via the same optical lead as the chemical sensor, and use the same opto-electronic interrogation system as for the chemical measurand, giving an almost ideal solution.

An opto-electronics interrogation unit is required to determine the fluorescent lifetime of the sensor probe. This must excite the dye with a time-dependent waveform, and measure the temporal dependence of the returning signal. Clearly, when two different materials are present in the sensing probe, e.g. to provide thermal compensation, then some electronic processing scheme is necessary to separate out the individual temporal responses from these two materials.

Another problem with fluorescence-lifetime measuring chemical sensors is the need to avoid errors from undesirable changes in internal electronic or optoelectronic delays in the interrogation unit. In industrial quality assurance, there is usually a need to calibrate the interrogation system, using a standard plug-in probe. This should be capable of being checked independently of the external chemical sensing probe, as the latter could have its own variability due to changes in composition, support matrix, and of course oxygen level. Once this interrogator calibration has been performed, the chemical sensing probe can be either calibrated independently, or using the validated interrogator.

Basis of our novel in-probe thermal compensation method

As stated above, it is desired to include an optical temperature sensing element within the chemical sensing probe. The temperature sensitive component should be a material having predictable and thermally dependent fluorescent decay characteristics and with similar absorption and fluorescence spectra to the chemical sensing dye. This allows interrogation with the same optics as the chemical sensing membrane. As the source excites both materials, and the detector receives

fluorescence from both, it is necessary to have some means of distinguishing the fluorescent decay of this material from that of the dye. This can be achieved by frequency-domain processing. As an example of this, an earlier optical temperature compensation system[1] used an alexandrite crystal to monitor the temperature of a platinum tetraphenylporphyrin (PTPP) indicator, and the interrogation system measured the electronic frequency spectrum of the detected fluorescent signal. Although this worked in practice, it required a complicated high-frequency signal processing scheme, which generally tends to give an inferior signal/noise ratio.

We have chosen to use time-domain processing, with ruby (Cr^{3+}-doped-sapphire) as our thermal indicator, to monitor the temperature of a Ru^{2+} dye indicator layer. Ruby has previously been used alone in sensing probes, solely for temperature monitoring[7,8] . Our combination has advantages over the previous system, as the ratio of crystal fluorescent lifetime to Ru^{2+} dye is far greater, and ruby, unlike alexandrite, is non-toxic. Using ruby, we have thus realised a much more convenient, high signal/noise, real time temperature compensation system that uses the same LED source, photo-multiplier detector, same optics, including the filter set, and even the same digital detection hardware as the Ru^{2+} dye chemical probe.

Further details of the probe will be given later.

Basis of our novel plug-in calibrator probe

The plug-in calibrator probe is simply a thermally-controlled Ti-sapphire crystal, with lifetime of similar order to that of the Ru^{2+} chemical indicator dye. The crystal is housed at, and coupled to, the end of a connectorised fibre lead, to form a plug-in replacement for the oxygen sensor probe. This can be inserted whenever calibration is required. Details of this probe, which has the capability to be thermally controlled to vary its lifetime, are given later.

Basis of our lifetime interrogation unit

There are two well known methods for interrogating fluorescent lifetime of chemical probes. Most Ru^{2+} chemical sensors have measured the fluorescent lifetime by monitoring the phase delay between the modulation of incident blue light from an LED and, by means of a photodiode, the detected fluorescent signal returning from the probe[5,6]. We have designed and constructed a similar system, but, for the work in this paper, we shall report results using our more sophisticated photon-counting system. The photon-counting lifetime sensor[4] offers several clear advantages over phase sensors:-

Firstly, by counting individual photons, it is possible to work at light levels below the detection limit of a photodiode-based system. This permits interrogation of dyes with low photo-bleaching thresholds and/or very low quantum efficiencies. As less returned fluorescent light is required, they may more easily be addressed via single fibres instead of thick fibre bundles, as often required for the insensitive phase-detection system.

Secondly, a photon counting system may more easily separate any excitation light from fluorescence. In conventional fluorescence measurement systems, some cross-talk light, albeit significantly attenuated in the filtering system, will appear at the detector and change the apparent phase of the returning fluorescence. This can occur due to imperfect filters or due to fluorescence in glues, etc, which generally have very much shorter decay time. Because it only measures signals after the pulsed light source has switched off, a photon counting interrogator is intrinsically immune to such cross-talk, as the elastic scattering signal and the short-decay fluorescence signals disappear as soon as the light source is switched off.

Thirdly, it is easier to design a photon-counting system to interrogate dyes having much faster decay times than it is with a phase-detection system. To maintain a large phase-angle variation when detecting fast decays, a high excitation frequency, and hence very fast detection system is required. It is more difficult to detect low-intensity, high frequency signals with a photodiode, making it difficult to design a simple phase-detection-based fluorescence lifetime monitor for weak signals, particularly when the fluorescence lifetime is much less than a micro-second. By using simple, yet fast, digital electronics and a photon counter, such high-speed decay times are easily measured, as the photon counter output dominates electronics noise of subsequent signal processors.

Fourthly, a photon counting system can more easily be configured to monitor the actual fluorescence decay curve of materials, by setting it to act as a boxcar detector system. A band-limited phase detector system cannot follow time decay curves and a high bandwidth one would be very noisy.

DETAILS OF EXPERIMENTAL APPARATUS

The combined temperature and oxygen sensing optrode

As stated above, to compensate for the temperature dependence of the Ru^{2+} chemical sensor, a small ruby crystal insert is used as a temperature sensor. The properties of the ruby crystal are ideal for this purpose, as the same optics can be used to excite and collect light from the Ru^{2+} sensor, because there is excellent overlap of their absorption and fluorescence spectra. The fluorescence lifetime of the crystal is reasonably strongly temperature dependent (Figure 1), yet ruby itself has not only stood the test of survival over geological time, it has proven stability under intense illumination (eg ruby laser systems).

The ruby crystal has a lifetime around one thousand times longer than that of the chemical sensor, so, with pulsed optical excitation, both lifetimes can be easily distinguished, in the time domain, from their components in the returning fluorescence signal.

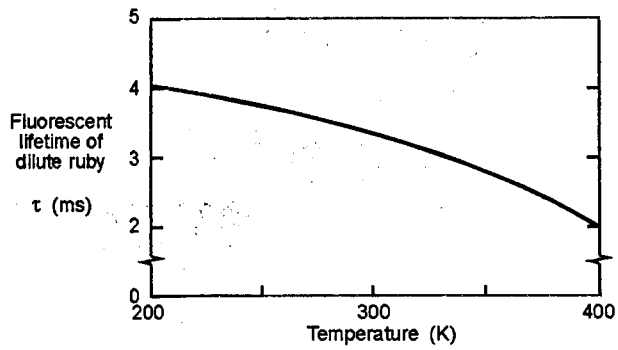


Figure 1 Fluorescent lifetime of ruby crystal plotted as a function of temperature. Note, around room temperature, the fluorescence lifetime is over one thousand times longer than the Ru^{2+} dye lifetime[2].

Several optical arrangements are possible for the combined ruby and membrane probe, based on a single 600μm core 630μm cladding diameter fibre for excitation and collection. Figure 2 shows a simple flat-plate design of such a probe. Here, a circular disc of ruby is bonded to the polished, ferruled, fibre end. The chemical sensing membrane is bonded to the ruby disc. Incident light emerging from the fibre passes through the disc, exciting the ruby crystal, and the remaining light falls on the membrane. Fluorescence from both the membrane and the ruby is partially coupled back into the fibre.

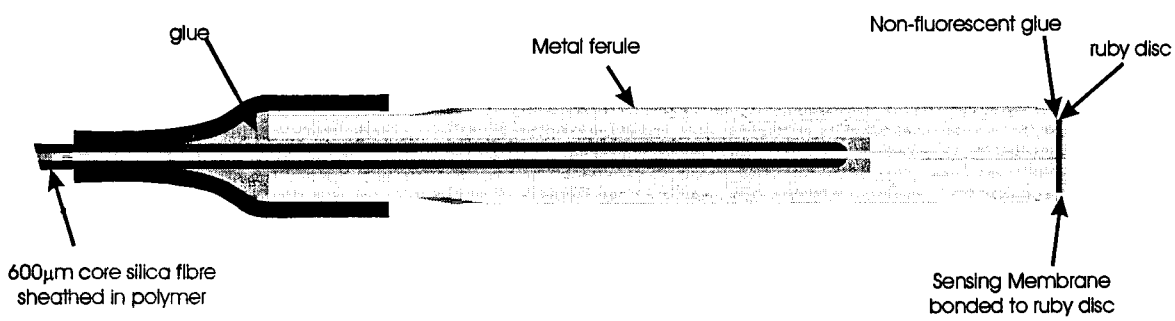


Figure 2 Temperature-compensated probe design, employing a simple disc of ruby bonded to the probe end, with the chemical sensing membrane bonded to the disc.

Figure 3 shows an alternative design for a temperature-compensated probe. Excitation light emerging from the fibre passes through a small ruby sphere, exciting the crystal. Light remaining at the far side of the sphere is focussed to a spot on the chemical sensing membrane. The resultant fluorescence is collected by the sphere (the wide collection angle improves signal/noise ratio), and re-launched back down the fibre. A small proportion of the fluorescent light resulting from excitation of the ruby sphere is also captured by the fibre.

The latter design has the disadvantage of increased photo-degradation due to smaller membrane excitation area, (unless the incident power is reduced), but the advantage of enhanced membrane fluorescence collection.

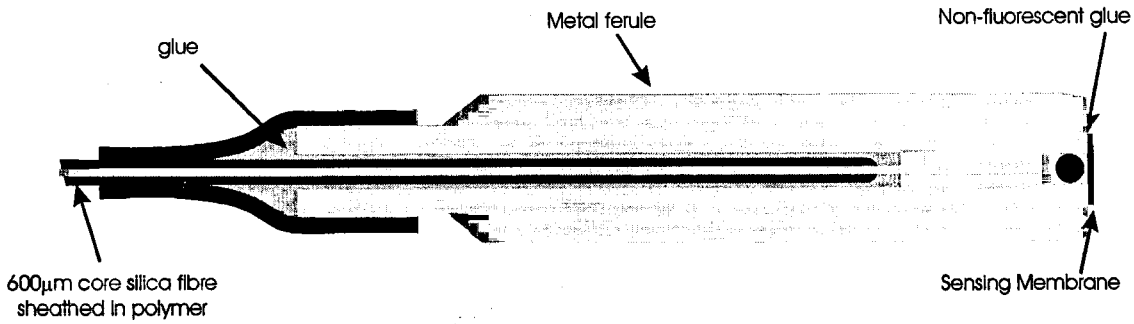


Figure 3 An alternative temperature-compensated probe design, based on a small ruby sphere insert. The sphere focusses light from the fibre down to a small spot on the membrane, and has a wide acceptance angle for light returning from the membrane, hence increasing signal/noise for the same input power.

The photon-counting fluorescence lifetime interrogation unit

Until recently, photon counting has been confined to large expensive instruments or the laboratory bench. By using new compact and low-cost, modular photon counting heads (e.g. Hamamatsu type H6180 and new red-sensitive modified versions), we have constructed a highly compact and versatile photon-counting monitor for interrogating the fluorescence lifetime of the new combined probe.

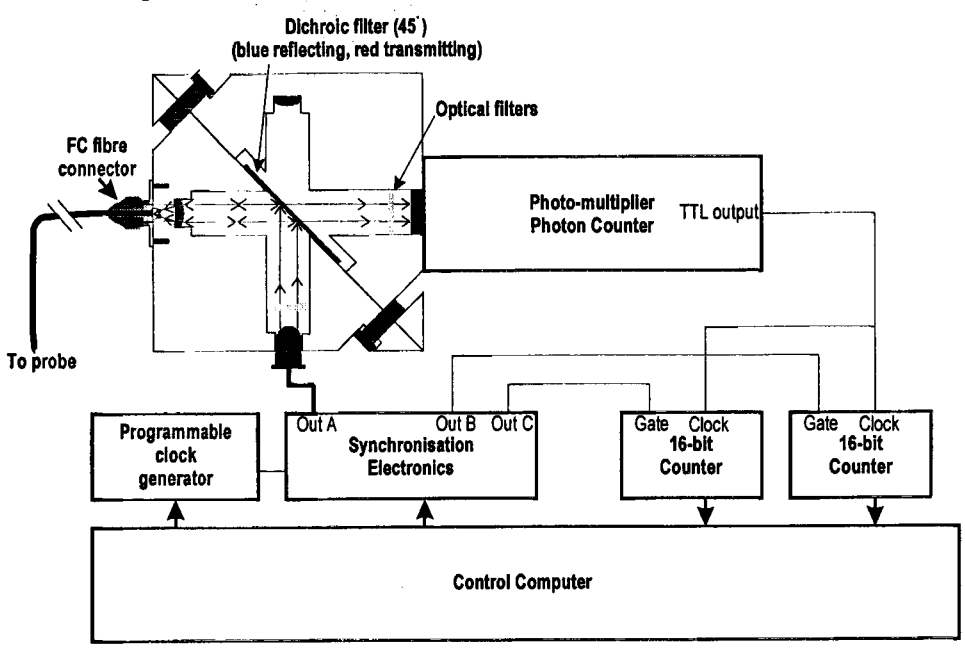


Figure 4 Schematic arrangement for the photon counting based interrogator

A high-intensity blue LED, with suitable optical filtering, illuminates the dye layer, which is bonded to the distal end of a 600 µm core 630µ m cladding, silica-based fibre probe. The returned fluorescence light is also optically filtered, in this case to reject excitation light, and falls on a modified Hamamatsu type H6180-01 photon counting module. The arrangement is shown in Figure 4.

To provide the necessary wavelength-selective coupling between excitation LED, fluorescent dye, and detector, an optical filter block based on a dichroic filter has been employed. The indicator layer is excited with modulated radiation of wavelength around 470nm, derived from an ultra-bright blue LED (HP type HLMP-CB15) passing through a blue

transmitting, red-blocking filter. This light is coupled to the fibre via a 45° dichroic filter, configured as a beam splitter, to reflect the blue excitation light wavelength. The fluorescence is collected by the same (i.e. the exciting) fibre. The red fluorescence signal, returning via the fibre, is now transmitted by the dichroic beam-splitter to impinge, via a final long-pass (blue-blocking) filter, onto a photo-multiplier detector.

The dichroic beam-splitter cross-over wavelength is 510nm, chosen to optimize the fluorescence-signal/crosstalk ratio. The final long-pass filter has a cutoff at 590nm.

Figure 5 plots the photon arrival rate (intensity) as a function of time. Simple digital electronics is used to count the number of photons arriving in two pre-defined time intervals (time "buckets") designed to fall in the "exponential" (dyes often have more complex decays, e.g. bi-exponential) decay region after the excitation pulse (see Figure 5). Simple statistical averaging may be applied by illuminating the dye with several thousand pulses, and accumulating all the photons arriving in each of the intervals over this time. These two accumulated values, C_{ab} from the photons arriving in bucket A, and C_{cd} from the photons arriving in time interval B , are passed to the control computer. They are processed to calculate the dye's effective fluorescence decay constant or give, in more complex decays, a measure of the lifetime that can be calibrated against the measurand (e.g. pO_2).

The photon-counting system can be easily re-configured, under software control, to act as a "boxcar" counting system, counting in many small sequential buckets so that a time-resolved plot of fluorescence decay can be easily recovered. This spin-off feature is extremely useful for probe and optics diagnostics.

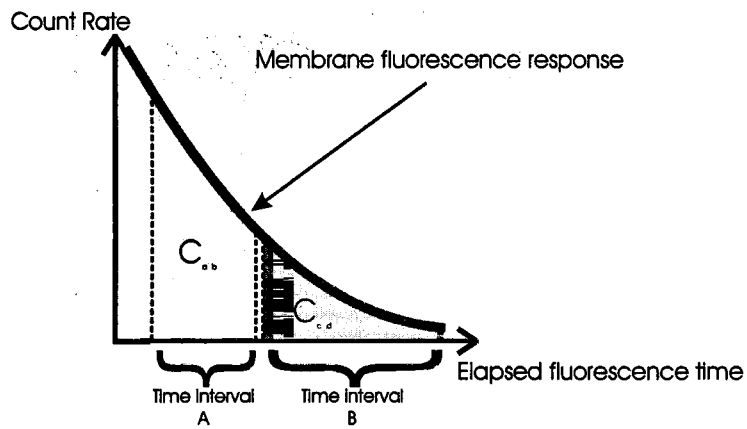


Figure 5 Measurement of a single fluorescence decay lifetime by photon counting detection. The number of photons arriving during time interval A and time interval B are accumulated (C_{ab} and C_{cd}) and processed to derive the fluorescence decay constant.

Simultaneous temperature and oxygenation measurement

After calibration, the single-decay measuring interrogator described above may be easily adapted to give a measure of both temperature and dissolved oxygen, using the detected fluorescence signal from the combined probe, and simply altering the control algorithm to operate the electronics at two clock frequencies sequentially.

At the slow clock frequency, the photon-counting buckets monitor light arriving a significant time after the end of the excitation pulse. When the time interval A opens, the fluorescence from the dye will have already decayed to a negligible degree, leaving only the fluorescence from the ruby (Figure 6). Photons accumulated during these wide time intervals are therefore related only to the ruby decay time constant.

Conversely, at high clocking frequency, the sensor measures only light occurring soon after the end of the excitation pulse, (Figure 7). As the ruby decays slowly, its fluorescence contribution is almost constant over this short time scale (and its value is known from the slow-clock rate measurements). By subtracting the ruby fluorescence intensity, the returned values C_{ab} and C_{cd} may be processed to derive the dye's fluorescence lifetime.

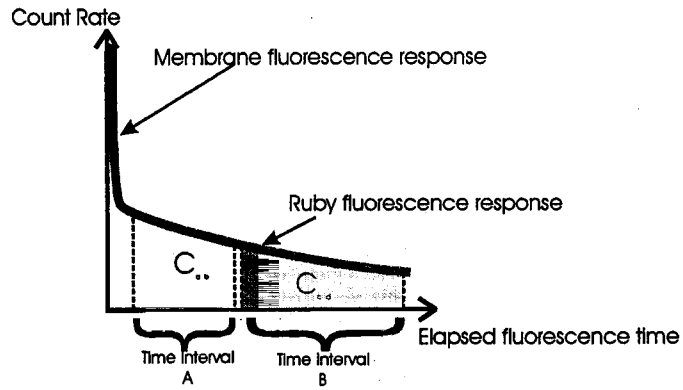


Figure 6 Illustration of long-time-scale (slow clock-rate) count rate against time after LED pulse

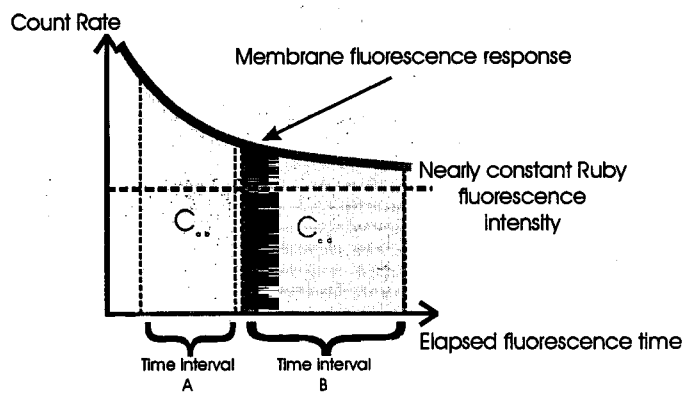


Figure 7 Illustration of short-time-scale (fast clock) count rate against time after LED pulse

The Ti^{3+}- doped-sapphire plug-in calibration standard

Ti^{3+}-doped-sapphire makes an ideal calibrator for interrogators designed to monitor ruthenium sensors. Firstly, its absorption (Figure 8) and spectral emission (Figure 9) curves overlap with those of the Ru-based membrane, so the calibration probe may be interrogated with the same optics as the oxygen sensor. (The fact that the fluorescence occurs at longer wavelengths is not a significant disadvantage when long-pass receiving filters are used). Secondly, its fluorescence lifetime (Figure 10) is of the same order as the effective lifetime of the chemical sensor, and is conveniently tunable with temperature in a well defined manner.

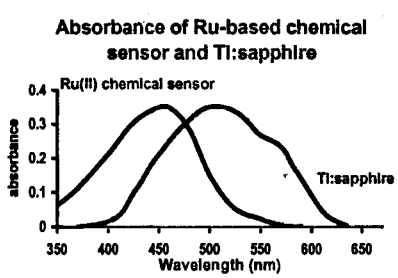


Figure 8 Absorption spectra of Ru chemical sensor and Ti-doped-sapphire

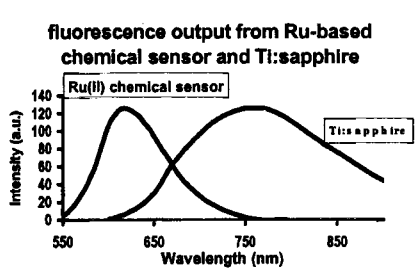


Figure 9 Fluorescence spectra from Ru^{2+} chemical sensor and Ti-doped sapphire

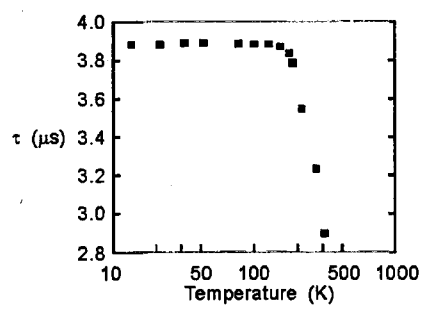


Figure 10 Variation of fluorescent lifetime of Ti-doped sapphire with temperature

As mentioned above, the calibration probe is simply a small cuboid of Ti^{3+}- sapphire, bonded on the distal end of a 600 μm core 630μm cladding, silica-based fibre. The Ti^{3+}- sapphire crystal (2 x 2 x 23mm) was bonded onto the fibre tip, with its long axis aligned with the fibre axis. This arrangement is shown in Figure 11.

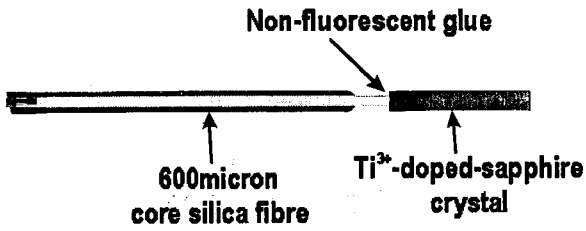


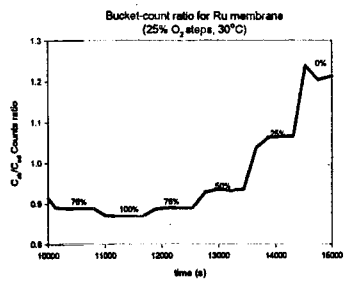
Figure 11 Mechanical design for simple Ti-doped-sapphire calibration standard probe

The fibre end and the crystal were potted into a metal housing which formed a compact oven for thermal control. This oven was governed by a simple PC-based temperature controller, to provide a portable, low-cost, multi-point fluorescence calibration aid. This can, whenever desired, be used to provide (or check) the calibration of the interrogator over a range of lifetime values, depending on the set temperature of the crystal oven.

RESULTS

Simultaneous, real-time temperature and dissolved oxygen results

In order to test the combined sensor, an oxygenation rig was constructed. This bubbled air/nitrogen mixtures, via a glass-frit filter, into water held in a temperature-controlled container. The input gas mixture was set using mass-flow control of N_2 and air supplies, under the management of a control computer. The sensor's response to numerous pre-set levels of dissolved oxygen (0,25,50,75,100% dissolved O_2, set by the gas mixture used) was obtained at several pre-set temperatures (30°,35°,40°C), Figure 12. In this first test, an averaging time constant of 100s for oxygenation and 15mins for temperature was applied, giving a good s/n ratio despite the rather low effective measurement duty cycle of 45% for O_2 measurement and 13% for temperature. This processing inefficiency was primarily due to initial software limitations in our program which controls the LED source repetition rate and data acquisition cycle. In particular, it appears advantageous to increase the duty cycle for the temperature measurement, which is only around 13% so far. We expect that, by the time of the conference, we will have improved our interrogation system to allow a shorter averaging time.



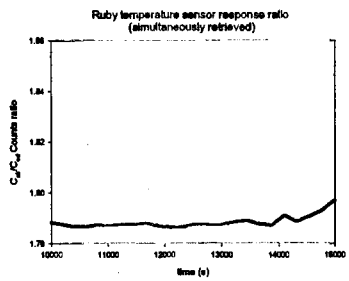
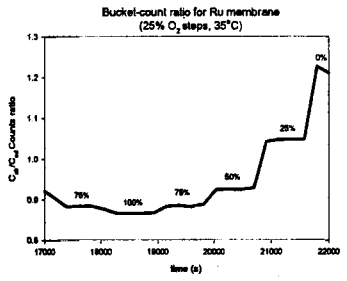


Figure 12a Measurements at 30°C



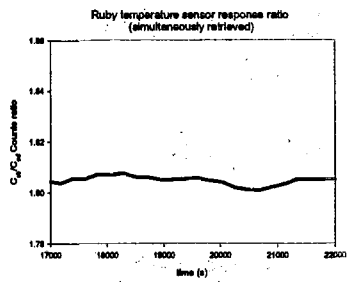
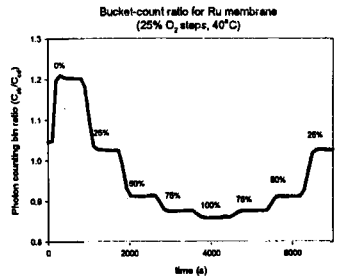


Figure 12b Measurements at 35°C



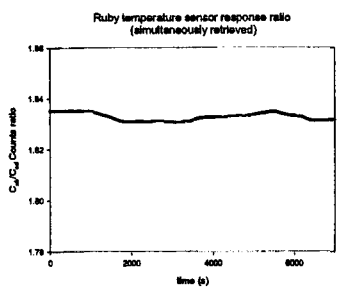


Figure 12c Measurements at 40°C

Figure 12 Responses of combined sensing probe at different temperatures (upper trace is $\%O_2$; lower trace is temperature)

By processing these sets of results, it is also possible to obtain graphical response curves of the Ru^{2+} membrane, in terms of bucket-count ratio, as a function of both oxygenation and temperature as shown in Figure 13.

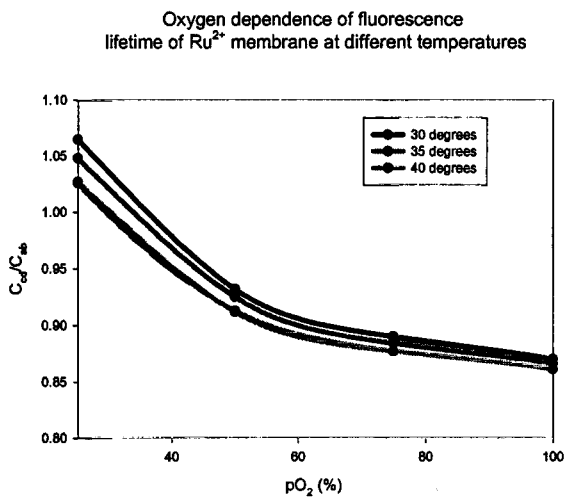


Figure 13 Response of Ru^{2+}-based membrane to oxygenation level at temperature of 30°, 35° and 40°

Results from Ti^{3+}-doped-sapphire calibration standard aid

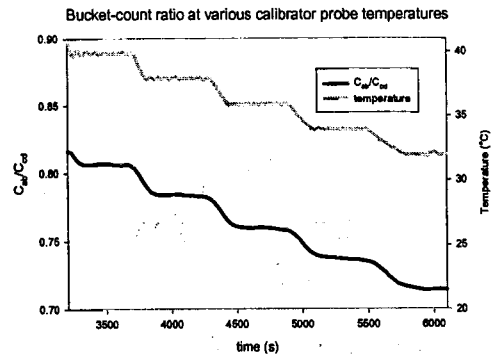


Figure 14 Real-time log of temperature and interrogated count ratio at several controlled temperatures

The PC-based temperature control system was programmed to sequentially set and hold the calibrator at a range of temperatures, (40,38,36,34, and 32°C), while a thermal monitor (LM35CZ having ±0.1°C accuracy) measured its temperature, and the previously described photon-counting interrogator measured its fluorescence lifetime. This is shown in Figure 14, showing stable fluorescence lifetime dependence with temperature. The calibrator's lifetime returned to these values over multiple thermal cycles.

CONCLUSIONS

We have described and presented the latest results from our real-time, simultaneous, temperature and oxygenation interrogation system.

The combined probe employs an oxygen-sensing Ru^{2+} sensor membrane, impregnated in silicone, and a Cr^{3+} - doped sapphire crystal (ruby) for temperature measurements. The presence of the ruby crystal not only provides a temperature sensing element, but also gives a reference signal to indicate mechanical damage or membrane detachment. It provides a reasonably constant fluorescence intensity, allowing detection of gradual but more significant changes in fluorescence light output from the membrane (e.g. changes due to photo-degradation).

We have combined a novel Ti^{3+}-doped-sapphire calibration standard with a temperature control system, to form a stable fluorescence lifetime reference capable of being set to different lifetime values simply by pre-setting its temperature.

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