Analytical Methods

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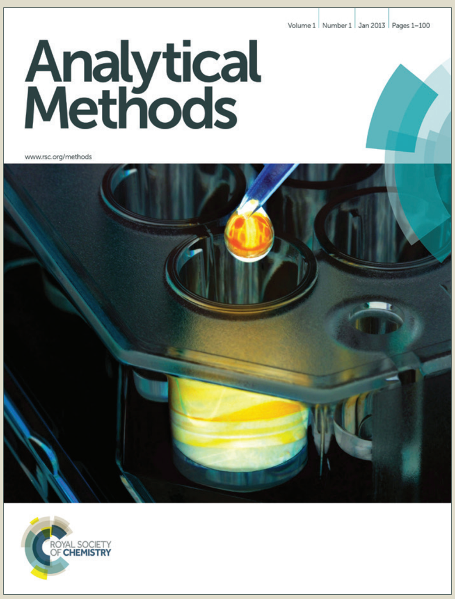
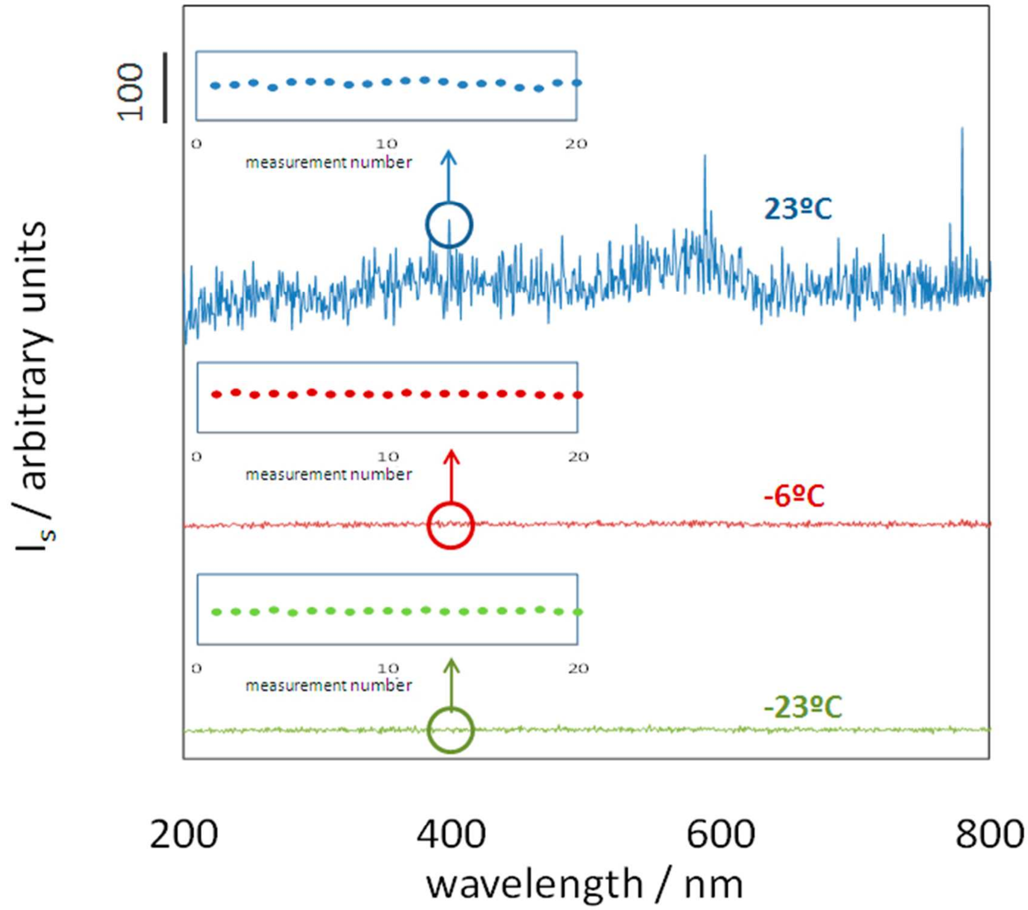


Graphical and Textual abstract

A study of uncertainty in CCDs, when used for fluorescence measurements, as a function of signal and temperature in detector. The latter has no practical influence on the signal-to-noise ratio and, consequently, on the limit of detection.



Uncertainty in CCD detectors with and without cooling devices when used for molecular fluorescence measurements

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The performance of CCD detectors for fluorescence measurements is evaluated through uncertainty studies, mainly as a function of both the signal intensity and the temperature of detector. Two CCD detectors have been used; one of them was furnished with a cooling device but the other one was not, and results are compared. The dependence of uncertainty on the instrumental signal was evaluated simultaneously with both detectors at temperatures ranging between -23 and 23 ºC. The tested detectors needed between 30 and 50 ºC increments in temperature to double dark noise (the random part of dark current). The temperature in detector affects uncertainty, but in fluorescence measurements this is basically related with dark noise; the consequence is that the temperature does not affect much the signal-to-noise ratio (*S/N*), so the presence of a cooling device does not provide a significant improvement in performance and the limit of detection (LOD) does not depend much on the temperature in detector. The quality of the two dimensional array does affect uncertainty, the value of *S/N* and the limit of detection (LOD). A good CCD detector can perform at a level similar to a spectrofluorometer furnished with a photomultiplier tube. Laboratory data are given to show how the three components of uncertainty (*dark noise*, *shot noise* and *flicker noise*) behave at different signal intensities and temperatures. Dark noise is the most important effect, shot noise has relevance only at high values of signal and flicker noise is practically irrelevant. The classical model of the dependence of *S/N* on the fluorescence signal is applied and the uncertainty constants that rule the performance of the apparatus used are given.

Introduction

Fluorescence measurements are relevant in analytical instrumentation (chromatography, automation, sensors, immunoassays, etc.) owing to high performance because of the very low background signal, which provides a high signal-to-noise ratio (*S/N*) and, consequently, low values of the limit of detection (LOD). The classical studies on uncertainty in fluorescence measurements[1] have been recently completed for the case of digital cameras,[2] that use charge-coupled devices (CCDs) as detection system, because of the number of laboratory applications of these instruments used for luminescence sensing and imaging.[3,4] UV-visible absorption and fluorescence have different working conditions: the former is usually taken under high light level conditions in detector, whereas low signals in detector are commonplace during fluorescence measurements; so the significant effects on the uncertainty of the signal may be different in both cases and uncertainty studies on absorption[5,6] may not apply in the same way to fluorescence. The same theoretical bases dictate uncertainty in both situations and they are well established since long ago,[1,7-11] but it is difficult to find laboratory data to back them up in the case of fluorescence; so there is no much experience in designing experiments to find how different factors affect uncertainty in fluorescence measurements. Some studies related to accuracy and precision have been approached in the case of digital cameras, but they mainly deal with the problem of luminescent background digital signals,[12] through the use of the hue (H) parameter of HSV colour-space[13] or upconversion for ratiometric methods.[14] The dependence of uncertainty (noise, N) on the analytical signal (I_S) can be expressed as:[1,7]

$$N = \sqrt{k_1^2 + k_2^2 I_S + k_3^2 I_S^2} \qquad (1)$$

where N can be obtained as the standard deviation, s, of the experimental signal, I_s; the term in k_1 corresponds to *background noise*, the term in k_2 corresponds to *shot noise* and the term in k_3 corresponds to *flicker noise*. Figure 1a shows a plot of eqn (1) as well as theoretical plots of the three limiting effects. According to it, for I_S values low enough N will be background limited; as I_S gets higher, shot and flicker noise will take relevance, and for I_S values high enough, flicker noise may become dominant.

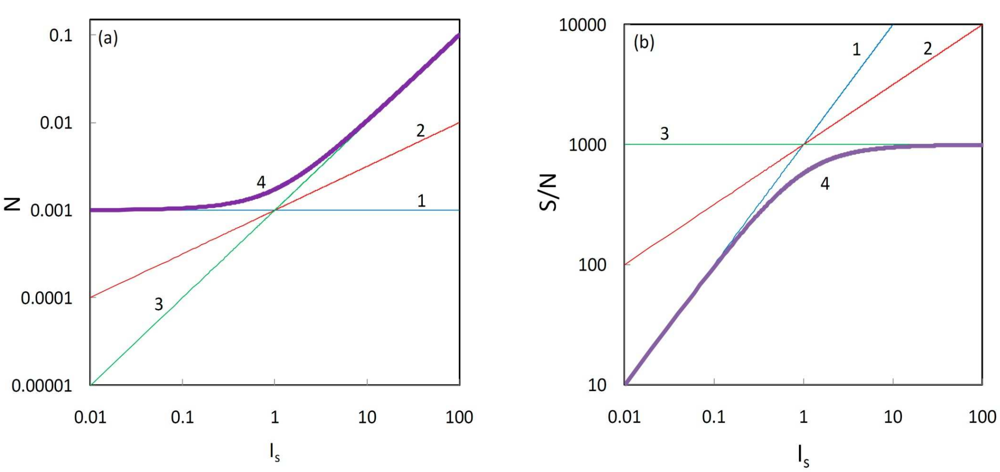

Figure 1. (a) Theoretical components of uncertainty expressed as N, the standard deviation of the photocathodic current (I_S) (eqn (1)). (1), background, including dark noise, reset noise, read-out noise and

non-uniformity; (2), shot noise; (3), flicker noise; (4), combined effect. (b) The same effects as in (a) but expressed as *S/N* (eqn (2)). Log scales are used. $k_1 = k_2 = k_3 = 0.001$.

An alternative to the use of *N*, with evaluating purposes, is the use of *S/N*:

$$S/N = \frac{I_S}{\sqrt{k_1^2 + k_2^2 I_S + k_3^2 I_S^2}} \tag{2}$$

Figure 1b shows the dependence of *S/N* on I_S. The use of *S/N* (a figure of merit) has the advantage that it may be compared for experimental data with quite different intensity units; so it is useful for comparing spectrometer performances.

In the last few years, charge-couple devices (CCDs) are increasingly used for light measurements, either as portable detectors or for flow methods, chromatography, etc.[15-17] Most of the times they are used for absorbance measurements,[18,19] but also they are suitable for luminescence or Raman measurements, owing to their high quantum yield; however, uncertainty studies are scarce in the latter case.[20] Owing to the low signal in detector during fluorescence measurements it can be expected that uncertainty will depend much on background noise (eqn (1)); that is, on dark noise, reset noise, read-out noise and non-uniformity;[21] no one of them depend on the signal, but they strongly depend on the temperature of detector (T_{det}).[9,10,22] It is important to distinguish between dark noise and dark current, the former being the random part of the latter; so, non-uniformity (systematic) is included in dark current but not in dark noise. On the other hand, shot and flicker noise do not depend on T_{det} but they do depend on the signal (eqn (1)). According to this, the temperature of CCD detectors should specially be taken into account when low light level conditions are implied, and this correspond to the LOD area of fluorescence methods; this is the reason why internally cooled detectors can be expected to perform better in these conditions.[2] The effect of temperature on CCD detectors has been discussed in the literature,[23] but which is the actual importance of T_{det} in the performance of detectors used for fluorescence measurements?, in which extent the *S/N* value is affected by T_{det}?, which improvements in LOD of analytical methods can be expected when cooling devices are used? The answer to these questions is not easily found in literature, and no laboratory data seem to be available. This paper presents experimental data of uncertainty, values for k_1, k_2 and k_3 (eqn (1) and (2)) are given and *S/N* is shown as a function of the experimental signal and T_{det} for two CCDs of different quality (with and without cooling device). Conclusions on CCDs performance are drawn and some comparison with spectrofluorometer data is made. The paper is also intended to help those interested in exploring the possibilities of CCDs when they are used for fluorescence measurements.

Experimental

Reagents

All the chemicals were of analytical-reagent grade and used as received. Doubly distilled water was used throughout.

Stock solutions of 100 and 500 mg l^{-1} quinine in 5 x 10^{-2} mol l^{-1} sulphuric acid were used. From these, diluted solutions of quinine as fluorescent species between 8 x 10^{-4} and 125 mg l^{-1} were also prepared, also in 5 x 10^{-2} mol l^{-1} sulphuric acid.

Instrumentation, procedures and data processing

The charge-coupled device detectors from Ocean Optics (Dunedin, FL) USB 4000 (with a 25 μm slit and no cooling device) and QE 65000 (with a 100 μm slit and Peltier cooling device) together with the spectrofluorometer RF-540 from Shimadzu (Kyoto, Japan) were used; all of them were furnished with fused-silica cells for fluorescence measurements. The cuvette was always 1.0 cm side. Fiber optic assemblies were 2 m length and solarized; the inner diameter was 600 μm between light source and cell holder and 400 μm between cell holder and detector. The cell-holder CUV-UV, the light source DT-MINI-2-GS and the high power xenon light source HPX-2000 (all of them from Ocean Optics) were also used. A 150 W xenon lamp was used as light source in the RF-540. Light sources were turned on at least 30 min (CCDs) or 120 min (RF-540) before any measurement was taken. A Julabo (Seelbach, Germany) F26 water bath circulator was used for the cells' temperature control. A wine cooler between 9 and 18ºC, a refrigerator down to -6ºC and a freezer down to -25ºC were sometimes used for detectors temperature control.

Measurements with both USB 4000 and QE 65000 detectors were always taken simultaneously and from the same solution, making the most of the cell holder design with two opposite way out light paths at 90º to the incident light. Integration times of 1 *s* (light source DT-MINI-2-GS) and 0.5 *s* (light source HPX-2000) were used. Unless otherwise stated, measurements were acquired using the high power xenon light source. To change the solution in the cell (washing included) micropipettes and a peristaltic bomb were always used in order to avoid uncertainty due to cell positioning. The CCD cell holder was covered during measurements to avoid light fluctuations from the ambient. Most of the times the temperature in QE 65000 was fixed using the Peltier cooling device included, but sometimes the temperature in CCDs was fixed by introducing both of them simultaneously in the wine cooler, the refrigerator or the freezer; they provided good conditions to compare the experimental results obtained with both detectors. In these conditions, about 20 cm of fiber optics are also introduced in the cooling external device. It was checked that the response of QE 65000 was the same regardless the temperature was fixed by the Peltier system or by some external device. To study the effect of temperature upon the transmission of the fiber optic assemblies, about 20 cm of them (but not the detector) between cell and detector was introduced in the freezer (-23ºC), whereas the temperature in detector was controlled by the Peltier system. The most of the experimental data corresponding to CCDs were obtained in two series with two different light sources (low and high power) by two different people and with a six month interval of time; all this giving more robustness to the study.

For each sample a number of 20-30 consecutive background spectra were first acquired in the absence of light from the source (CCDs sources include a shutter, and a manual procedure was used for RF-540), then the shutter was open and 20-30 consecutive sample spectra (*raw* spectra) were taken; the respective differences raw–background provided a number of 20-30 *corrected* spectra for each sample, from which a mean (the signal, I_S or just S) and a standard deviation (the uncertainty, noise, N) were obtained. Excitation and emission wavelengths of 350 nm and 450 nm were respectively used.

Curve fittings were obtained with the Table curve 2D version 5.0.1 program.

Results and discussion

Signal-to-noise and temperature

Obviously, the extent of the fluorescence signal does not depend on temperature in detector, but the instrumental noise does depend on temperature in detector through the random component of dark current. Nevertheless, according to our experimental results this dependence is not high. Fig. 2 shows the process for the acquisition of a fluorescence signal in QE 65000 CCD at three different temperatures (-23, -6 and 23ºC).

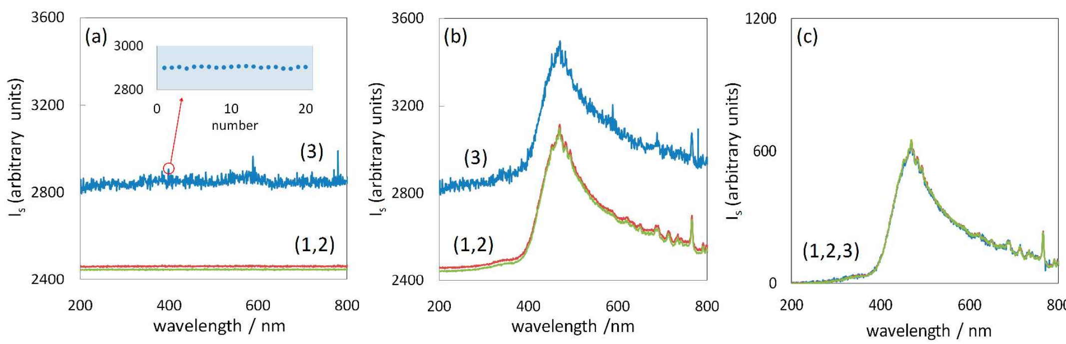

Figure 2. Effect of temperature on the experimental signal in a CCD detector (QE 65000) used for fluorescence masurements. Spectra: (a) dark current; (b) raw spectra; (c) spectra corrected to background. Quinine / mg l^{-1}: (a), 0; (b) and (c), 0.2. Temperature/ºC: 1, -23; 2, -6; 3, 23. Inset: 20 data points consecutively acquired with the shown pixel.

The temperature in detector strongly affects background (Fig. 2a) and raw signals (Fig. 2b), but the corrected signals (Fig.2c) are affected only in a much lower extent.

At first sight this is unexpected, because according to the propagation of random errors theory, the uncertainty of the difference

$$I_{S(corrected)} = I_{S(raw)} - I_{S(background)} \quad (3)$$

is given by:

$$s^2_{I_{S(corrected)}} = s^2_{I_{S(raw)}} + s^2_{I_{S(background)}} \quad (4)$$

where the symbol s represents the standard deviation of I_S. If the photonic current (I_S) is not very high, as it usually happens in fluorescence, it can be approximately written that

$$s^2_{I_{S(corrected)}} \cong 2s^2_{I_{S(raw)}} \cong 2s^2_{I_{S(background)}} \quad (5)$$

and consequently,

$$s_{I_S(corrected)} \cong \sqrt{2} \times s_{I_S(raw)} \cong \sqrt{2} \times s_{I_S(background)} \quad (6)$$

but Fig. 2 shows that (23ºC, spectrum (3))

$$s_{I_S\,(corrected)} < s_{I_S\,(raw)} \left(\cong s_{I_S\,(background)}\right) \tag{7}$$

as it can be seen comparing Fig. 2a, 2b and 2c. The disagreement shown by eqn (6) and (7) can be explained by the fact that one of the main sources of uncertainty in CCDs is non-uniformity,[7] which has not only a random component (Fig. 2a, inset) but also a very important systematic component. The systematic component (spatial uncertainty) is compensated for, in fluorescence measurements, by operating experimentally according to eqn (3), whereas the random component (temporal uncertainty) follows eqn (6). So only a part (but not the most important one) of the apparent uncertainty in Fig. 2a and 2b contributes to the final uncertainty. Because of that, differences in T_{det} have not the consequence it could have originally been expected (compare differences in uncertainty between spectra in Fig. 2b and 2c). That is, cooling of detector is not so important as expected if only Fig. 2a and 2b are taken into account, because cooling affects only the random component of uncertainty.

Fig. 3 shows *S/N* data obtained with QE 65000 CCD at four representative concentrations of quinine when temperature is fixed either with Peltier system or with external devices. Similar data can be found for the rest of concentrations and for data obtained with USB 4000 CCD.

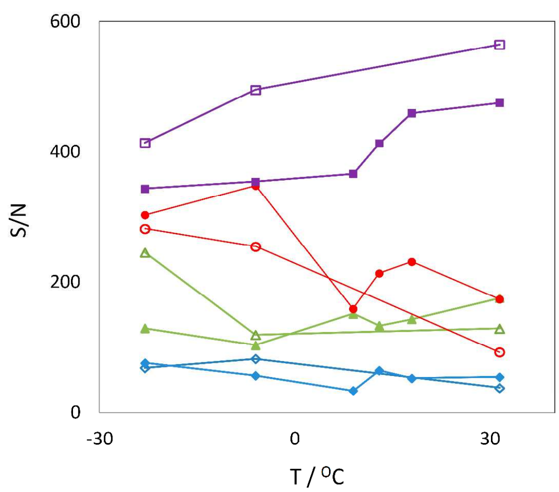

Figure 3. The value of S/N as a function of temperature in detector and fiber optics. Detector, QE 65000. Quinine/mg l^{-1}: rhombs, 0.2; triangles, 0.8; circles, 2; squares, 10. Open marks, detector temperature regulated by the Peltier system (fiber optics at ambient temperature). Filled marks, detector temperature regulated by some external device (about 20 cm of fiber optics stayed at the temperature of detector).

According to Fig 3 it is not relevant whether T_{det} is obtained with a peltier system or not. Moreover, the data do not show a clear dependence of *S/N* on T_{det}. In fact, it could be expected that *S/N* diminishes as T_{det} increases, whenever *N* increases with T_{det}; nevertheless *S/N* does not appreciably change with T_{det} and because the value of *S/N* determines the value of the limit of detection (LOD), the latter should not be essentially dependent on T_{det} when fluorescence measurements are taken. These results can be considered surprising and in order to take more experimental support about the temperature effect on fluorescence detectors, new measurements were performed to determine how T_{det} affects LOD. Different calibration lines were obtained at several T_{det} values; the obtained results are given in Figure 4 (IUPAC criterion, 3*s*).[24] As it can be seen, T_{det} has no practical influence in the value of LOD experimentally obtained. Consequently, it can be concluded that the presence of cooling devices in CCD detectors does not appreciably improve their performance when they are used for fluorescence measurements. This agrees with some precedent according to which only in some special applications cooling a CCD detector has a positive influence on results.[2] The different conditions of humidity in devices did not appreciably result in different detector behaviour.

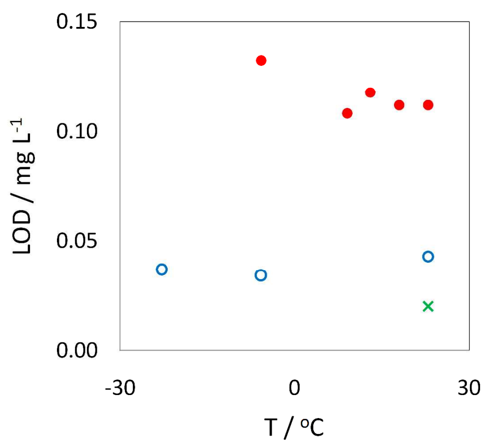

Figure 4. The limit of detection as a function of temperature in detector. Detector: filled circles, USB4000 (external cooling device); open circles, QE65000 (Peltier device); cross, RF-540.

According to Fig. 4, QE 65000 performs better than USB 4000; the reason is not the presence of a cooling device included, but it should probably be charged to a lower number of defects in the two dimensional array, as a consequence of a more careful making process.[21] On the other hand, the value of LOD obtained with QE 65000 is close to the one obtained with RF-540 spectrofluorophotometer, proving the high performance of these small detectors.

The temperature may affect the optical fiber transmission, depending on the material of the cladding.[25] In our case we have found a small dependence of the signal on the temperature of fibers, but does uncertainty depends on the temperature of fiber optics?, and, what is more important, does *S/N* depend on temperature of fiber optics?. This is important because some measurements in this paper have been acquired with a part of the fiber (about 20 cm) inside the external devices used to control the temperature in detector (wine cooler, refrigerator and freezer). Fig. 3 shows how temperature in fiber optics affects the value of *S/N* in QE 65000 CCD. The conclusion is that the influence of the temperature of fiber optics on noise is so low (at least at the temperatures tested) that the effect is included in the variability of measurements or, in other words, the temperature in both detector and fiber does not affect significantly the value of *S/N*.

The uncertainty of the dark noise signal was controlled along wavelengths 200-800 nm at -23ºC, -6ºC and 23ºC. No significant change or shift was observed in any case, so multi-wavelength analysis can be developed with similar results in the whole UV-visible range.

Signal-to-noise and analytical signal

Fig. 5 shows the typical behaviour of USB 4000 and QE 65000 CCDs at room temperature compared with the RF-540 specrofluorophotometer furnished with a photomultiplier tube. Similar profiles were found for temperatures ranging from -23ºC to ambient.

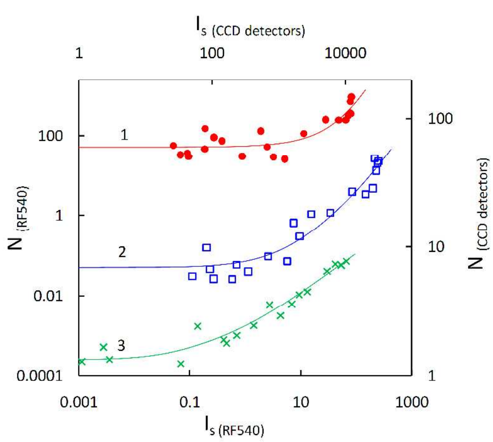

Figure 5. Dependence of uncertainty (N) on the signal (I_S) at room temperature. The axes for CCDs and for RF-540 are different and both of them are in arbitrary fluorescence units. Detector: (1), USB4000; (2), QE65000; (3) RF-540. Experimental data points together with regression lines are represented.

The regression lines can be found in each case according to eqn (1), and they provide the value of the uncertainty constants. Table 1 collects the values of k_1, k_2 and k_3 at every temperature tested. The spectrofluorometer RF-540 follows the expected behaviour; blank noise is dominant for low signals, then uncertainty is proportional to the square root of the signal and finally uncertainty is flicker noise limited. In the case of CCDs a similar tendency is observed but k_1 is clearly higher than k_2 and k_3; in fact, k_3 has no practical relevance, so the estimation of error associated to its value makes no sense (Table 1). The value of the uncertainty constants is always lower for QE 65000 than for USB 4000 showing the superior quality of the former. On the other hand, k_1 in CCDs is temperature dependent (Table 1) but k_2 and k_3 are not. According to literature, the increase of dark current with T_{det} approximately doubles for each 5 ºC increase in temperature;[26] this means an exponential dependence experimentally observed, [12,21,27] but this corresponds to the systematic component, whereas k_1 is related to the random component of that dependence. To make an estimation of the dependence of k_1 on T_{det} it can also be taken into account, for instance, that the *thermal dark current* from a photomultiplier tube can be given by;[28]

$$i_{th} = CAT^2 e^{-\frac{E_C}{kT}} \quad (8)$$

where C is a constant, A is the surface area of the cathode at a temperature T and E_C is the energy (work function) required to release a single electron from the surface of the cathode. In an equation such as (8) the exponential energy term is usually so dominant that a plot of $\ln i_{th}$ versus $1/T$ will be linear, within the usual experimental error, regardless the exponent of T in the pre-exponential term is 0, 0.5, 1 or 2. Because of that, in the case of CCDs the dependence of k_1 upon T_{det} can tentatively be approached by an Arrhenius-type equation:

$$k_1 = Ae^{-\frac{B}{T_{det}}} \quad (9)$$

where A and B are constants and T_{det} is in Kelvins. Making eqn (9) a linear relation:

$$\ln k_1 = \ln A - \frac{B}{T_{det}} \quad (10)$$

Eqn (10) is plotted in Fig. 6 using k_1 data from CCD detectors in Table 1. From the regression lines it can be deduced that the value of k_1 (the dark noise) in USB 4000 approximately doubles for each 50 ºC increase in temperature, whereas in QE 65000 it doubles for each 30 ºC approximately. The value of k_1 in QE65000 is more dependent on T_{det} than in the case of USB4000. This can be due, at least in part, to the way in which both detectors are constructed, but one should be cautious respect to other possible effects. In fact, the experimental value obtained for k_1 is apparent and can collect different effects, including the random part of stray light, which is not T_{det} dependent but a kind of flicker noise that obviously will distort the value of k_1. In any case, the dependence of k_1 on T_{det} is quite low for both detectors and accounts for the low dependence of N on T_{det}.

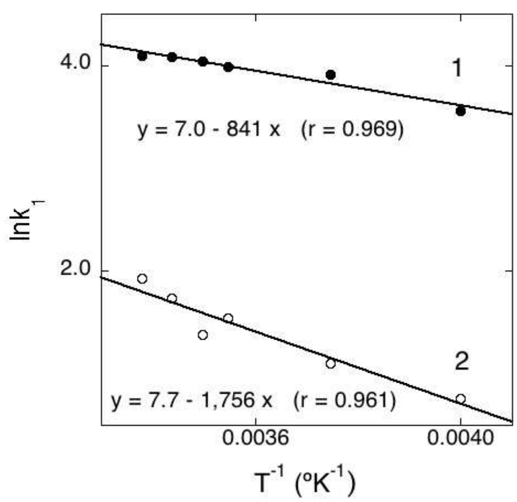

Figure 6. Arrhenius-type plot for the dependence of k_1 (dark noise) on the absolute temperature in detector. Detector: (1), USB4000; (2), QE 65000.

Summing up for fluorescence measurements in CCDs, the uncertainty is mainly due to background noise (k_1 term), and shot noise (k_2 term) is significant only when high enough values of I_S are implied; flicker noise (k_3 term) is practically irrelevant (flicker noise in CCDs can usually be neglected because of the low signals involved).

To check the confidence of the uncertainty constants obtained (Table 1), the behaviour of the global uncertainty can be simulated and compared with experimental data in a different way to Fig. 5. This has been done by simulating the dependence of

S/N on I_S (eqn (2)), using the values of k_1, k_2 and k_3 from Table 1. Fig. 7 shows the results obtained for data at room temperature.

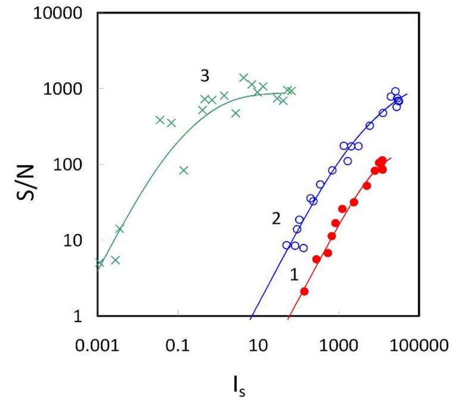

Figure 7. Dependence of S/N on the experimental signal (I_S) at room temperature. Detector: (1), USB4000; (2), QE65000; (3), RF-540. Experimental data points together with the simulated behaviour of *S/N* in each case (lines, eqn (2)) are represented.

Good agreement between simulated uncertainty and experimental data was found, including data from RF-540, which obeys the same pattern than CCDs, but in this case the effect of flicker noise is reached owing to the large I_S interval covered. On the other hand, the comparison of both CCDs shows that *S/N* for USB 4000 depends mainly on dark noise (k_1) showing practically a linear dependence on I_S, whereas *S/N* for QE 65000 extends for a higher I_S interval and the effect of shot noise (k_2) can clearly be appreciated at higher I_S values. Similar results were found for the rest of temperatures in the case of CCDs.

The whole of experimental data obtained at every temperature is shown in Fig. 8 as a plot of *S/N* versus I_S for the two CCDs tested. The dependence of *S/N* upon I_S can

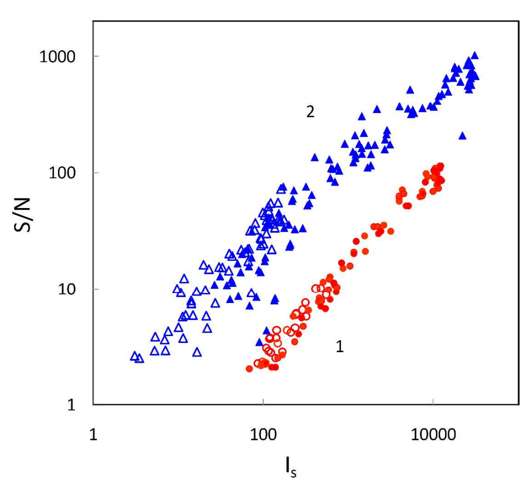

Figure 8. The whole of *S/N* experimental data obtained at several temperatures as a function of the experimental signal. Data corresponding to the same temperature have not been hyphenated because they do not show different patterns and they interweave one with another. Detector: (1), USB4000; (2), QE65000. Open data points were obtained by operator (1) using a low power light source; filled data points correspond to operator (2) and they were obtained using a high power light source. The passage of time between operator (1) and (2) was six months approximately.

be considered according to expected, taking into account the additional variability introduced by the different temperatures used. Data are coherent regardless light source, operator and passage of time; obviously, higher values of *S/N* were obtained when a high power light source was used (Fig. 8).

Conclusions

When CCDs are used as detectors of fluorescence uncertainty is mainly due to dark noise, shot noise is significant only when high values of the signal are involved and flicker noise has no practical relevance. The temperature of detector does not significantly affect the value of *S/N*. This is mainly due to the low effect of T_{det} on dark noise (the random component of dark current). Dark noise doubled for each 30 ºC or 50 ºC in temperature for QE 65000 or USB 4000 respectively. As a consequence, the dependence of the limit of detection (LOD) on T_{det} is very limited and the presence of cooling devices, some times included with equipment, do not appreciably improve the performance for this kind of measurements. The values of *S/N* and LOD do depend on some other characteristics such as the quality of the two dimensional array, the quantum yield, etc. CCDs can be used as fluorescence detectors with a performance similar to spectrofluorometers, provided enough quality of the two-dimensional array and high power sources are used. All this can probably be applied to any kind of cameras, whenever background can be compensated for by a well-defined reference frame or spectrum.

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References

1 J. D. Ingle, Jr. and S. R. Crouch, *Spectrochemical Analysis*, Prentice Hall, Englewood Clifs, New Jersey, 1988. Ch. 5.

2 U. Krüger and F. Schmidt, *Metrologia*, 2009, **46**, S252-S259.

3 A. Hakonen and N. Strömberg, *Chem. Commun*., 2011, **47**, 3433-3435.

4 A. Hakonen and N. Strömberg, *Analyst*, 2012, **137**, 315-321-

5 L. D. Rothman, S. R. Crouch and J. D. Ingle Jr., *Anal. Chem*., 1975, **47**, 1226-1233.

6 J. Galbán, S. de Marcos, I. Sanz, C. Ubide, and J. Zuriarrain, *Anal. Chem*., 2007, **79**, 4763-4767.

7 R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, Wiley, New York, 2000, ch. 4.

8 J. M. Harnly and R. E. Fields, *Appl. Spectrosc*, 1997, **51**, 334A-351A.

9 *Kodak Application note, CCD Image Sensor Noise Sources*, **2005**. Revision 2.1. (Available from world wide web).

10 T.J. Fellers and M.W. Davidson, *Hamamatsu Articles, Concepts in Digital Imaging Technology, CCD Noise Sources and Signal-to-Noise Ratio,* http://hamamatsu.magnet.fsu.edu/articles/ccdsnr.html (last seen 2014).

11 H. Mark and J. Workman, *Chemometrics in spectroscopy*, Academic Press, London, 2007, pp 223-338.

12 E. W. Bogaart, W. Hoekstra, I. M. Peters, A. C. M. Kleimann and J. T. Bosiers, *IEEE T. Electron dev*., 2009, **56**, 2462-2467.

13 A. Hakonen, J. E. Beves and N. Strömberg, *Analyst*, 2014, **139**, 3524-3527.

14 R. J. Meier, J. M. B. Simbürger, T. Soukka and M. Schäferling, *Anal. Chem*., 2014, **86**, 5535-5540.

15 R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, Wiley, New York, 2000, pp 183-202.

16 G. H. Rieke, *Detection of light: from ultraviolet to the submillimeter, Cambridge* University Press, Cambridge, 1994, ch. 6.

17 K. L. Ratzlaff and S. L. Paul, *Appl. Spectrosc*., 1979, **33**, 240-245.

18 J. Galbán, S. de Marcos, I. Sanz, C. Ubide, and J. Zuriarrain, *Analyst*, 2010, **135**, 564-569.

19 E.P. Tsaousoglou, S.D. Bolis and C.E. Efstathiou, *J. Automated Meth. Manag. Chem.,* 2003, **25**, 35-42.

20 G. Zonios, *Appl. Opt*., 2010, **49**, 163-169.

21 D. Teyssieux, L. Thiery and B. Cretin, **Rev. Sci. Instrum**., 2007, *78*, 034902.

22 R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, Wiley, New York, 2000, p. 55.

23 J. R. Janesick, *Scientific Charge-Coupled Devices*, SPIE Press, Bellingham, Washington, 2001.

24 L. A. Currie, *Pure Appl. Chem*., 1995, **67**, 1699-1723.

25 W F. Yeung and A. R. Johnston, *Appl. Optics*, 1978, **17**, 3703-3705.

26 R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, Wiley, New York, 2000, p. 188.

27 R. Widenhorn, L. Mündermann, A. Rest and E. Bodegom, *J. Appl. Phys*., 2001, **ss**, 8179-8182.

28 J. D. Ingle, Jr. and S. R. Crouch, *Spectrochemical Analysis*, Prentice Hall, Englewood Clifs, New Jersey, 1988, p. 112.

Table 1. Experimental values of uncertainty constants (eqn (1)). Because I_S and N in eqn (1) are in the same arbitrary units, the constants k_1, k_2 and k_3 have not defined units. Standard deviation is given in parenthesis. The parameter r^2 is the determination coefficient of the regression line.

Detector	T (ºC)	k_1	k_2	k_3	r^2
RF-540	23	0.0002(0.002)	0.001(0.002)	0.00113 (0.00006)	0.97
USB 4000	23	60 (4)	0.7 (0.4)	0.006 (0.004)	0.81
	18	59 (4)	1.0 (0.3)	0.00002 (1)	0.86
	13	57 (5)	1.0 (0.4)	0.000008 (4)	0.80
	9	54 (4)	0.7 (0.4)	0.007 (0.004)	0.87
	-6	50 (4)	1.0 (0.4)	0.000008 (6)	0.84
	-23	35 (5)	0.9 (0.4)	0.000008 (12)	0.88
QE65000	23	7 (2)	0.20 (0.04)	0.0008 (0.0004)	0.93
	18	6 (1)	0.20 (0.03)	0.0005 (0.0005)	0.95
	13	4 (1)	0.21 (0.03)	0.0011 (0.0002)	0.97
	9	5 (2)	0.23 (0.04)	0.0002 (0.002)	0.93
	-6	3 (1)	0.19 (0.03)	0.0007 (0.0004)	0.99
	-23	2 (1)	0.22 (0.03)	0.00009 (0.005)	0.95