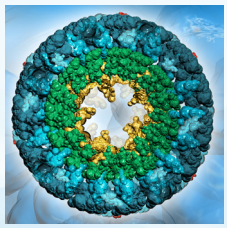
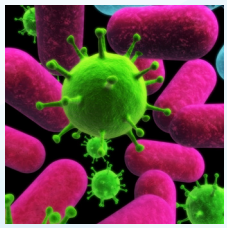
HORIBA Scientific

Fluorescence

How Inner-Filter Effects (IFE) can affect your fluorescence measurements: A case study - Colloidal InP Quantum Dots





Application Note
Life Sciences
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Introduction

During the last two decades, a great deal of attention has been focused on the optoelectronic properties of nanostructured semiconductors or Quantum Dots (QDs). QDs absorb over a broad range and have photoluminescence emission over a narrow range which can be tuned depending on the material from which the QD is made and the size of the QD. The applications of QDs continue to expand, i.e. in vivo imaging, light-emitting devices, photodetection or solar energy conversion [1].

Among the many ways to analyze QDs, fluorescence is one of the most common [2]. Fluorescence is a technology that is now routinely used in a variety of scientific domains: life science, material science, agri-food, environment. Fluorescence techniques have many advantages, including their relative ease of use; nonintrusive nature; very high sensitivity; potential combination with ghost, temporal, and spatial imaging for measurements; and suitability for multiplexing and remote sensing. However, one of the difficulties encountered involves the nonlinear relationship between the fluorescence intensity and the concentration of a fluorophore, called the Inner-Filter Effect (IFE) [3].

This well-known IFE depends on sample absorption and on instrument geometry and is usually significant even in samples with rather low absorption (the error is about 8% at an absorbance of 0.06 in a 1 cm square cell) [4].

We have recently shown that IFE correction is very useful for extending the dynamic range of an instrument to higher concentrations of solution, so that fluorescence data is collected easily and accurately [5].

Figure 1: Duetta 2-in-1 absorbance and fluorescence spectrometer

To do that, we use the new two-in-one absorbance and fluorescence spectrometer, Duetta™ (Figure. 1).

It can be used as a fluorometer, as a UV-Vis-NIR spectrometer to measure absorbance, or as an instrument that measures true molecular fingerprints via the simultaneous acquisition of fluorescence and absorbance, thus correcting for IFE in real time.

In this work, we analyze a new generation of QDs. By avoiding the use of the toxic heavy metals Pb and Cd, Indium Phosphide (InP) based QDs are a promising alternative that retains the spectral range of size tunable emission while expanding the scope of applicability of soluble semiconductor emitters. They show great potential to replace the widely applied cadmium-containing QDs in next-generation commercial display and lighting applications [6-7].

Methods and Results

InP/ZnS QDs were provided by Dr. Damien Boyer and Rodolphe Valleix, from the Institute of Chemistry of Clermont-Ferrand – UMR 6296 (France).

InP cores of QDs were synthesized by a conventional hot injection method [7]. Briefly, Indium (III) chloride (99 mg, 0.45 mmol), zinc (II) chloride (307 mg, 2.2 mmol) and 98% purity degassed oleylamine (5 ml) were mixed in a 100 ml three-necked flask with a magnetic stirrer. Then, the reactive medium was heated to 180°C under argon, upon reaching 180° C, the phosphorus source, tris-(triethylamino)-phosphine (0.45 ml, 1.6 mmol), was quickly injected in the above mixture. After this injection, the InP nanocrystals (NCs) growth occurred for 20 minutes. NCs were precipitated in absolute ethanol and suspended in chloroform.

For the ZnS shell, instead of cooling down the temperature, at t=20 min: 1ml of stoichiometric TOP-S ([TOP-S]=2.2 M) is injected in the reactive medium. At t=60 min: the temperature is increased to 200°C. At t=120 min: slow injection of 1 g of zinc stearate dispersed in octadecene (ODE) and the temperature is increased from 200°C to 220°C. At t=150 min: injection of 0.7 ml of stoichiometric TOP-S and the temperature is increased from 220°C t 240°C. At t=180 min: slow injection of 0.5g of zinc stearate suspended in ODE and the temperature is increased from 240°C t 260°C. At t=210 min: end of the reaction and the temperature is quickly cooled down. The synthesized QDs are precipitated with ethanol and suspended in chloroform.

All diluted samples were generated directly from the undiluted sample (A). The concentration of the main solution was about 65 g/L. The main solution was diluted approximately 10X (B) and approximately 100X (C) in chloroform. Fluorescence/Absorbance spectra were recorded from samples in 1-cm quartz cuvette.

The fluorescence Excitation Emission Matrix (EEM) was first collected on the undiluted sample in order to find the optimal excitation and emission spectral ranges. EEM conditions included an excitation from 300-700 nm with 5 nm step increments and an emission range of 300-750 nm with 0.05 s integration time on the CCD (Fig.2). Once the acquisition parameters were optimized, we measured the emission spectrum by exciting at 450 nm and by fixing the emission between 500 and 750 nm, with 0.05 s integration time. Simultaneous absorbance measurement was performed in order to apply the IFE correction. To do that, a solution of chloroform was used as a blank sample.

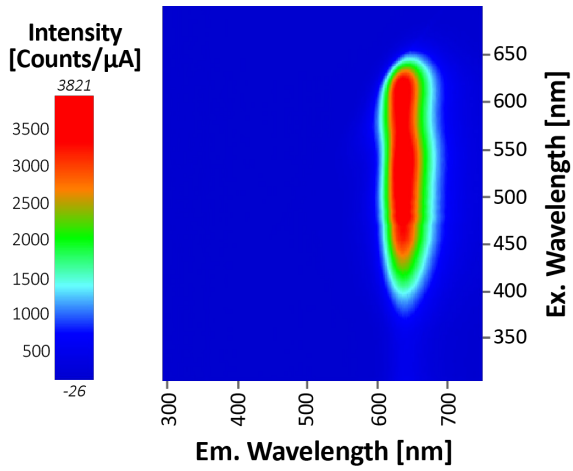

Figure 2: Excitation Emission Matrix (EEM) used to quickly determine optimal spectral ranges for further 2D spectral analysis.

Fig. 3 shows absorbance spectra for the three samples. Absorbance values ranges from 1.22 to 0.015 o.d. at 586 nm.

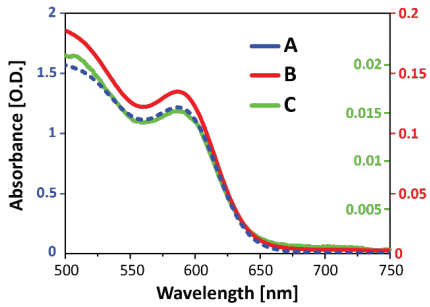

Figure 3: Absorbance spectra for InP_ZnS Quantum Dots (QDs) at different concentrations

In Fig. 4 the emission spectra with and without IFE correction are shown. All spectra are corrected for detector dark noise, spectral correction and lamp intensity. A and B fluorescence spectra are affected by significant and predictable distortions caused by concentration-dependent IFE. In this case, some of the excitation light does not properly reach the center of a cuvette due to strong light absorbance from the highly concentrated molecule(s), making the fluorescence intensity at the detector lower than expected.

Table 1 records the wavelength shift of the maximum peak as concentration increases, up to a value of 15 nm.

Sample	o.d. @586 nm	λMAX (nm) uncorrected	λMAX (nm) IFE corrected
A	1.22	640	625
B	0.13	627	625
C	0.015	625	625

Table 1: Recorded wavelengths of the maximum peak with and without IFE correction.

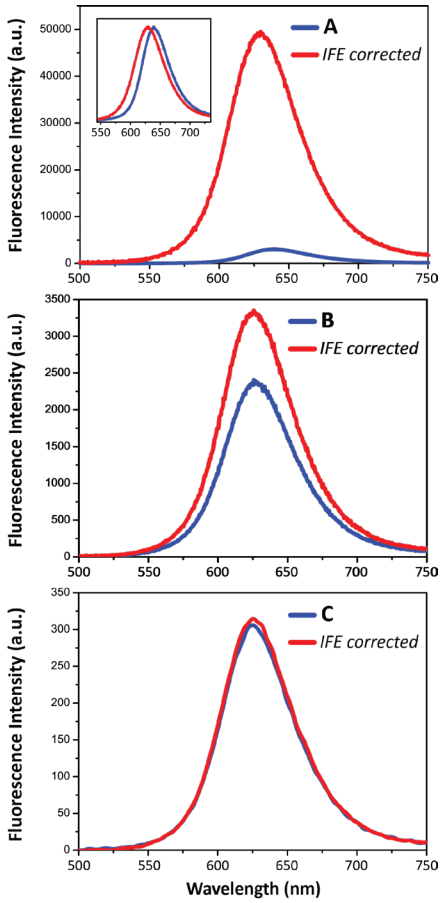

Figure 4: Emission spectra of A (the inset shows the normalized spectra), B and C Quantum Dots (QDs) with and without IFE correction applied.

Summary

Inner Filter Effects (IFEs) can significantly affect fluorescence emission spectral profiles, distorting their general shape, shifting the spectral position of the peak maximums and decreasing the emission intensities. Thus, when recorded with a standard spectrofluorometer, the detected fluorescence does not coincide with the true fluorescence emission of the sample, even for small fluorophore concentrations.

These fluorescence spectra distortions can be critical in display, lighting and bioimaging applications where reliability and a precise knowledge of the QDs emission properties are required.

IFE correction restores the correct emission profiles corresponding to the expected ones under so-called infinite dilution conditions. The Duetta™ 2-in-1 fluorescence and absorbance spectrometer is able to measure true molecular fingerprints, which requires the simultaneous acquisition of fluorescence and absorbance, correcting for IFE in real time.

References

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