



## **Analysis of crude petroleum oils using fluorescence spectroscopy.**

Title	Analysis of crude petroleum oils using fluorescence spectroscopy.
Author(s)	Ryder, Alan G.
Publication Date	2005
Publisher	Springer
Repository DOI	<a href="https://doi.org/10.1007/0-387-23690-2_8">10.1007/0-387-23690-2_8</a>

Cite this chapter:

Ryder, A.G. (2005). Analysis of Crude Petroleum Oils Using Fluorescence Spectroscopy. In: Geddes, C.D., Lakowicz, J.R. (eds) Reviews in Fluorescence 2005. Reviews in Fluorescence, vol 2005. Springer, Boston, MA.  
[https://doi.org/10.1007/0-387-23690-2\\_8](https://doi.org/10.1007/0-387-23690-2_8)

## ANALYSIS OF CRUDE PETROLEUM OILS USING FLUORESCENCE SPECTROSCOPY

Alan G. Ryder\*

### 1. INTRODUCTION

Crude oil is defined as “a mixture of hydrocarbons that existed in the liquid phase in natural underground reservoirs and remains liquid at atmospheric pressure after passing through surface separating facilities” (joint American Petroleum Institute, American Association of Petroleum Geologists, and Society of Petroleum Engineers definition).<sup>1</sup> Crude petroleum oils are complex mixtures of different compounds (mainly organic), which are obtained from an extensive range of different geological sources.<sup>2, 3</sup> Their physical appearance can vary from solid black tars to almost transparent liquids. In their natural state within an oilfield reservoir or entrapped within Hydrocarbon bearing Fluid Inclusions (HCFI), crude oils will also contain varying amounts of gasses (carbon dioxide, methane, etc.).<sup>4</sup> This presents the analyst with considerable challenges when developing methods for the characterisation and analysis of crude oils.<sup>5</sup> The non-contact, non-destructive, quantitative analysis of crude petroleum oils is a highly desirable objective for both research (e.g. study of microscopic HCFI) and industry (e.g. real-time assessment of oil production). Satisfying the needs of both macroscopic and microscopic applications is not straightforward, however, optical methods offer a convenient route to achieving these goals. Fluorescence spectroscopy is the best available optical technique, because it offers high sensitivity, good diagnostic potential, relatively simple instrumentation, and is perfectly suited to both microscopy and portable instrumentation.

This review encompasses a survey of fluorescence techniques used for the analysis of crude petroleum oils. Specific sections focus on the analysis of bulk crude petroleum oils, HCFI, and oil spills. The review focuses mainly on advances and studies reported in the literature from 1990 onwards, and outlines some of the issues that need to be addressed to make fluorescence methods more reproducible and quantitative.

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## 2. PETROLEUM COMPOSITION

The chemical composition of crude petroleum oils varies enormously, and their characterization requires the application of considerable instrumentation and time resources. Both Hunt<sup>2</sup> and North<sup>3</sup> provide good foundations in the basics of petroleum composition, and the geological factors that influence changes in oil composition. Comprehensive, laboratory based chemical analysis of crude oils is done using a variety of gravimetric, solvent extraction, and chromatographic techniques.<sup>6, 7</sup> Details of these methods, their advantages, and disadvantages are outside the scope of this review. However, it should be noted that the different methodologies employed, could provide subtly different values for oil composition parameters. The primary compositional parameters reported in fluorescence studies (if at all, see table 1) tend to be obtained from column chromatography and in chemical terms are: an alkane (or paraffin, or aliphatic) fraction, an aromatic fraction, a polar (or resin) fraction, and an asphaltene fraction.<sup>8</sup> The asphaltene fraction is that portion of the oil, which is insoluble in pentane or heptane and comprises of high molecular weight species.<sup>9</sup> Often the methods used, and the results obtained for chemical composition will differ from laboratory to laboratory (depending on the chromatographic conditions used, and whether the light, volatile fraction has been removed prior to chromatographic analysis). Therefore much care should be taken in comparing fluorescence data and results from different crude oil studies. In general, for accurate characterisation of crude oil fluorescence one should have information on the source rock, its density (API gravity), and the gross chemical composition (alkane, aromatic, polar, and asphaltene concentration).<sup>10</sup>

**Table 1.** Survey of crude oil data provided for fluorescence studies from a selection of literature sources.

<i>Reference</i>	<i>Location</i>	<i>API gravity</i>	<i>Source rock</i>	<i>Chemical data</i>
Abu-Zeid, <i>et al.</i> <sup>11</sup>	Partial	No	No	No
Alpern <i>et al.</i> <sup>12</sup>	No	Yes <sup>a</sup>	No	Partial
Blanchet <i>et al.</i> <sup>13</sup>	Yes	Yes	No	No
Camagni <i>et al.</i> <sup>14</sup>	Partial	No	No	No
Hagemann & Hollerbach, <sup>15</sup>	Yes	Partial	Partial	Partial
Measures <i>et al.</i> <sup>16</sup>	Yes	Yes	No	No
Mullins, Mitra-Kirtley, & Zhu. <sup>17</sup>	No	No	No	No
Mullins and Downare. <sup>18</sup>	Partial	No	No	No
Quinn <i>et al.</i> <sup>19</sup>	No	Yes	No	No
Rayner & Szabo. <sup>20</sup>	Partial	Partial	No	No
Ryder, & Ryder <i>et al.</i> <sup>21, 22</sup>	Partial	Yes	No	Partial
Ryder <i>et al.</i> <sup>23</sup>	Yes	Yes	Yes	Yes
Ryder (2004), <sup>10</sup>	Yes	Yes	Yes	Yes
Stasiuk <i>et al.</i> <sup>24</sup>	Yes	No	No	Yes
Wang & Mullins. <sup>25</sup>	Partial	Yes	No	No
Zhu & Mullins. <sup>26</sup>	Partial	No	No	No

<sup>a</sup> Provided as oil density.

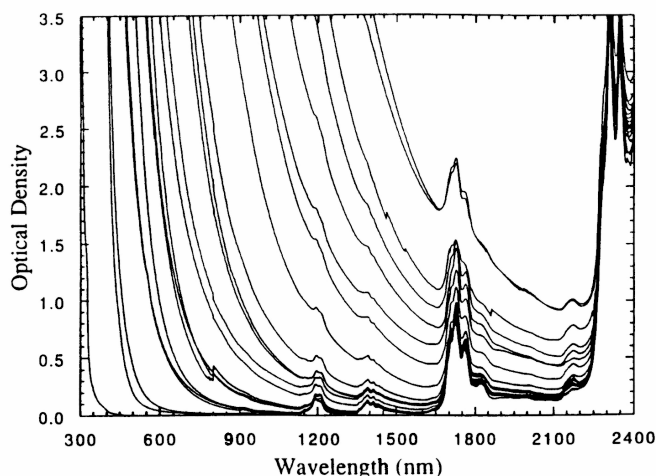
In many cases, the only information available for the crude oil being investigated is its location, source, or API gravity. API gravity is one of the simplest and most wide-ranging parameters used for describing crude oils. It is inversely related to the density by the formula:  $\text{API gravity} = ((141.5/\text{specific gravity at } 15.6^\circ\text{C}) - 131.5)$ . In general, the higher the API gravity value, the lighter the oil. Specific gravities of oils tend to be in the 0.73 to 1.0 range, with paraffin type oils being lighter than asphalt-base oils.<sup>3</sup> Despite the fact that it does not provide any significant chemical information, the parameter is easily measured and widely used. Table 1 gives a brief survey of some recent fluorescence studies of crude oils, clearly showing the dearth of chemical information usually provided. Therefore, one must be aware that when discussing petroleum fluorescence, qualitative descriptions dominate, and quantitative analysis may only apply to individual studies.

### 3. PETROLEUM OIL FLUORESCENCE

The use of fluorescence for the analysis of crude oils has been in use for the past 60 years particularly for mud logging where UV light is used to detect the presence of oil in drilling mud.<sup>27</sup> Fluorescence is also used in the analysis of core samples, again to identify the presence of oils.<sup>3</sup> The fluorescence of crude petroleum oils derives from the aromatic hydrocarbon fraction,<sup>28</sup> and this fluorescence emission is strongly influenced by the chemical composition (e.g. fluorophore and quencher concentrations) and physical characteristics (e.g. viscosity and optical density) of the oil. Unfortunately, crude petroleum oils encompass a very wide range of physical and chemical characteristics, making the fluorescence analysis of crude petroleum oils rather difficult.

Crude oils vary in appearance from black tars to clear liquids, indicating a complex absorption profile. This is shown experimentally in Figure 1, where the spectra of 22 oils of different compositions are displayed. It should also be noted that the shape of the absorption edge is very similar in each case. Examination of the electronic absorption spectra of crude oils reveals that the electronic absorption edge is similar to the "Urbach tail," in which the absorption coefficient depends exponentially on the photon energy.<sup>17</sup>

The absorption edge moves to the red, as crude oils get heavier (lower API gravity), and the distribution and number of chromophores increases. These absorption studies, in conjunction with fluorescence emission studies reveal that when the Urbach tail accurately describes the absorption, the dominant absorption process in the Urbach tail region, corresponds to excitation of the lowest-energy electronic transitions of the corresponding chromophores. In addition, Mullins and his co-authors suggest that the absorption tail gives a direct measure of the chromophores population distribution, with larger chromophores being present in exponentially decreasing quantities.<sup>17</sup>



**Figure 1.** Optical absorption spectra of 22 crude oils, with the heavier oils to the right of the plot. The vibrational absorption peaks are superimposed on a highly variable, continuous, monotonic electronic absorption profile. The spectra were collected in 2 mm pathlength cells and referenced against a 2mm cell filled with  $\text{CCl}_4$ . *Reproduced with permission from Ref [17] © 1995, Society for Applied Spectroscopy.*

In practical terms, this means that the excitation wavelength used for fluorescence spectroscopy of crude oils needs to be selected with care to enable efficient excitation of all crude oils types. From Figure 1 it is clear that visible excitation ( $>450$  nm) may not be suitable for the lightest crude oils and/or condensates. Furthermore, different excitation wavelengths results in the excitation of different fluorophore populations, which has an impact on the fluorescence emission produced. Another point, arising from the absorption studies, is the potential for inner filter and energy transfer effects to influence fluorescence emission. The chemical complexity of crude oils ensures that in most cases, there is a high probability the fluorescence emitted by one species will be absorbed by another fluorophore resulting in energy transfer. This is particularly true for UV or blue excitation. Therefore, for the study of bulk, undiluted oils, front-surface geometries are required,<sup>10, 21, 22, 23, 29</sup> and in all other cases, oils need to be highly diluted to minimise inner filter effects.

### 3.1. Steady-State Emission

In general, light oils (high API gravity) tend to have relatively narrow, strong fluorescence emission bands with a smaller Stoke shift than that found for of heavier oils (lower API gravity) where the emission tends to be weaker, broader, and red-shifted. Figure 2 shows the emission spectra obtained from a series of five crude oils, using 337 nm excitation. Similar results were later reported by Rayner and Szabo,<sup>20</sup> also using 337 nm excitation. These gross changes in fluorescence emission are due to the higher concentration of fluorophores and quenchers present in the heavier oils, which leads to a higher rate of energy transfer and fluorescence quenching, producing the broader, weaker, red shifted emission.<sup>18, 30</sup>

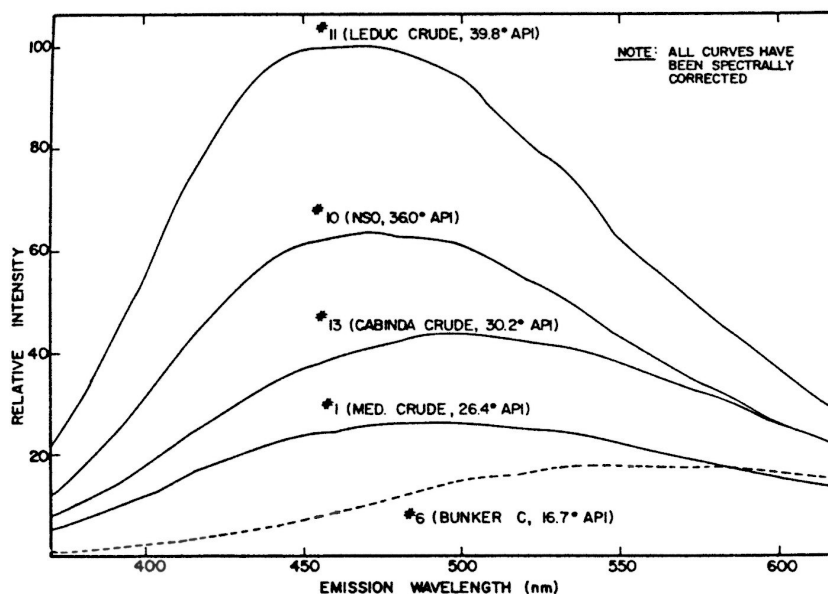
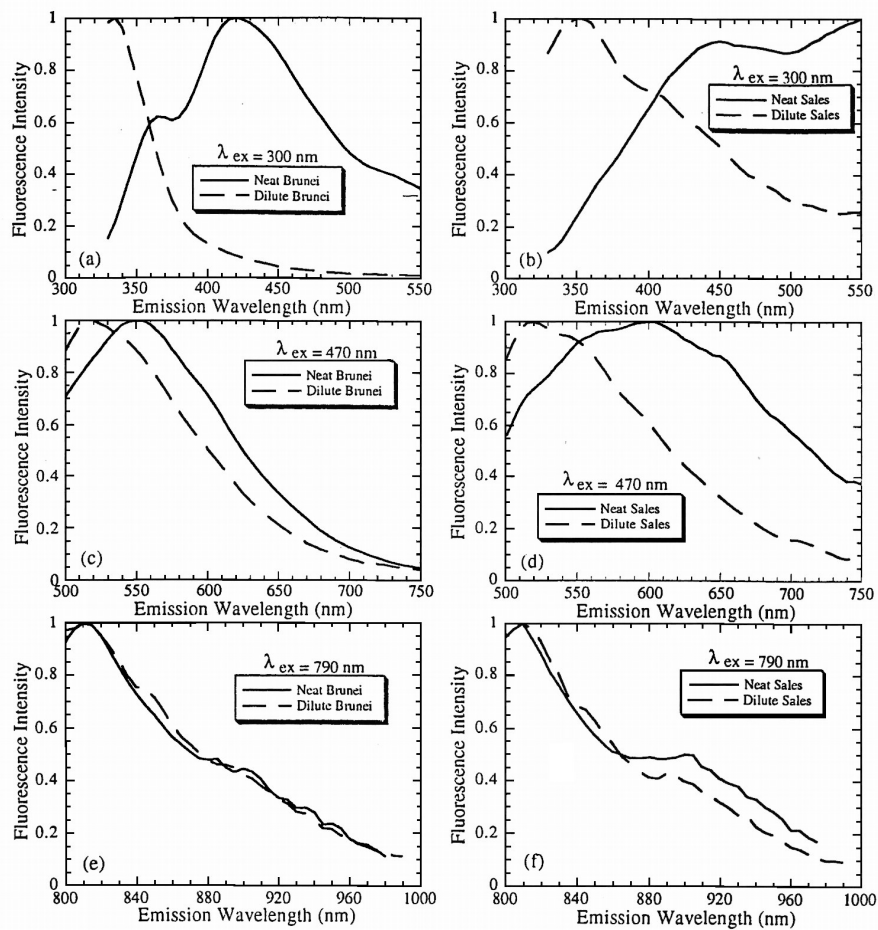
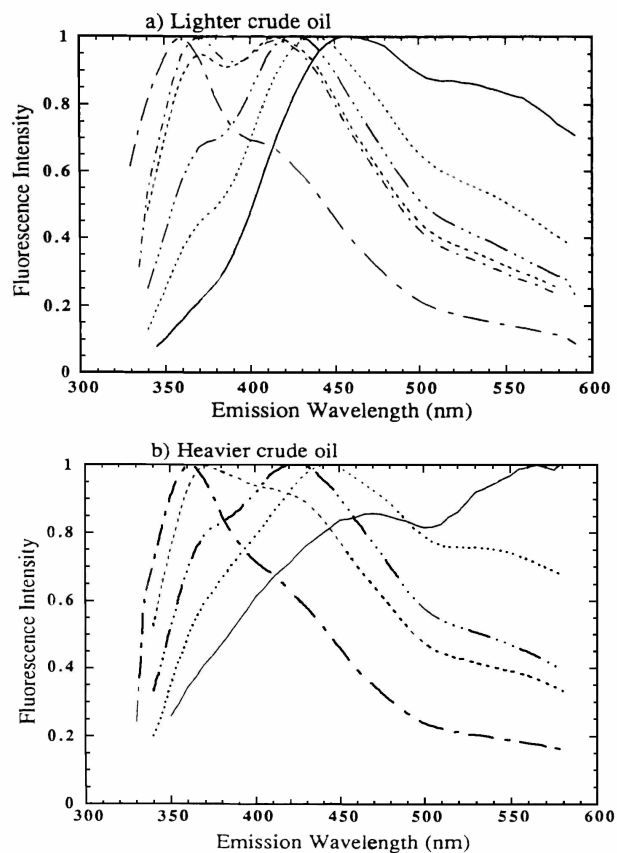


Figure 2. Fluorescence spectra of crude oils obtained using 337 nm excitation. *Reproduced with permission from Ref [16], © 1974, Optical Engineering.*

Energy transfer processes result in red shifted, and spectrally broadened emission while the quenching processes reduce emission intensity. The effect however diminishes with increasing excitation wavelength (Figure 3) until with near-IR excitation, the profile of the emission spectra of light, heavy, and diluted samples of each are very nearly identical. At these NIR excitation wavelengths, there is nearly no energy transfer. The effect can be quantified by diluting crude oils (Figure 4) with solvents such as benzene,<sup>18, 25</sup> n-heptane,<sup>25</sup> or cyclohexane.<sup>29</sup> In each case, dilution results in an increase in fluorescence intensity and a blue shift. Stern-Volmer plots constructed from highly diluted crude oils imply that the quenching and energy transfer processes depend linearly with crude oil concentration.<sup>18, 25</sup> Analysis of the wavelength dependence of the Stern-Volmer plots indicate that the collisional decay constants are greater at shorter emission wavelengths<sup>25</sup> and excitation wavelengths.<sup>18</sup>

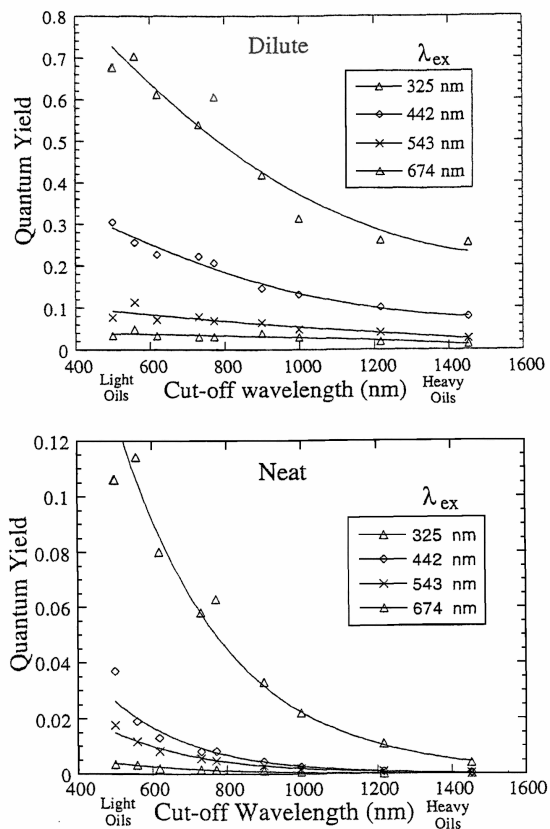


**Figure 3.** Fluorescence emission spectra for neat and dilute solutions of a light (Brunei) and a heavy (Sales) crude oil for 300-, 400-, and 790- nm excitation wavelengths. Collisional energy transfer produces large spectral differences between neat and dilute solutions for short excitation wavelengths. Collisional energy transfer decreases with increasing excitation wavelength. Determining the contribution of the nascent (dilute solution) emission to the neat spectrum allows the quantitative determination of collisional energy transfer. *Reproduced with permission from Ref [18] © 1995, Society for Applied Spectroscopy.*



**Figure 4.** The emission spectra with 316-nm excitation for (a) North Sea, the lighter crude oil, and (b) Sales, the heavier crude oil, at different concentrations. A substantial red shift with increasing concentration results from energy transfer. Dilution factors: (a) (---) 1:35,000; (- - -) 1:625; (- · - ·) 1:125; (— · — ·) 1:25; (····) 1:5; (—) neat. (b) (---) 1:35,000; (- - -) 1:625; (- · - ·) 1:125; (- · - ·) 1:25; (····) 1:5; (—) neat. *Reproduced with permission from Ref [25] © 1994, Society for Applied Spectroscopy.*

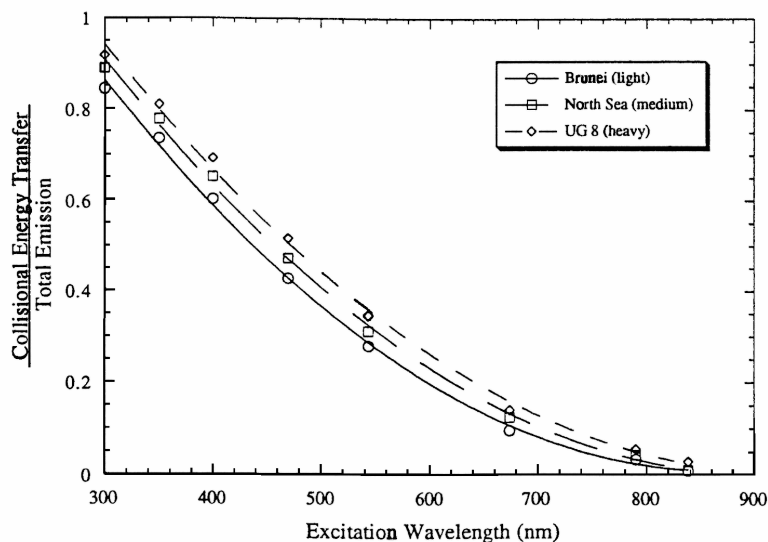
The quantum yields of crude oils are highly dependant on the excitation wavelength, with excitation in the visible or red being much less efficient than UV excitation (Figure 5). This is largely due to the reduction in optical absorption (Figure 1) and the increase in non-radiative decay pathways (internal conversion) with increasing excitation wavelength. The relative change in quantum yield was also demonstrated to be largely similar for a wide range of light to heavy oils, which can be accounted for by the energy dependence of internal conversion. Dilution of crude oils also increases the quantum yield by reducing the quenching rate.<sup>30</sup> Diluted heavy oils however, have lower quantum yields than diluted light oils due to their higher concentrations of larger (red absorbing) chromophores, which are more likely to undergo non-radiative internal conversion.



**Figure 5.** The absolute quantum yields for neat (bottom) and dilute (top) crude oils. The cutoff wavelength is an optical measure of the oil weight; heavy oils have a large cutoff wavelength. The cutoff wavelength is defined as being the point at which the optical density reaches a value of 3.0 for a 2mm optical pathlength. Quantum yields decrease greatly with increasing excitation wavelength due to internal conversion. Quenching from chromophores interactions causes neat crude oils to have much smaller quantum yields than dilute crude oils, especially for heavy oils. For the dilute case, the quantum yields of heavy oils are lower due to the greater fraction of large, aromatic chromophores. *Reproduced with permission from Ref [30] © 1995, Society for Applied Spectroscopy.*

The balance between collisional energy transfer and quenching is the dominant feature affecting crude oil fluorescence. Downare and Mullins<sup>18</sup> calculated (from emission spectra) and plotted the ratio of energy transfer to total emission against excitation wavelengths for three representative oils (Figure 6). This plot shows how influential the selection of excitation wavelength is on the photophysics of crude oil emission. This has obvious consequences for the consideration of excitation sources for petroleum fluorescence applications. For several studies in the 1970's and 80's, which employ 316 nm or 337 nm excitation sources, emission arises largely (>75%) from energy transfer, while more recent studies employing semiconductor light sources (380

nm LED or 405 nm violet laser diodes) produce emission that has a lower energy transfer contribution (75-60%). Therefore, direct comparison of experimental results between studies using different excitation sources needs to be approached with caution.



**Figure 6.** The ratio of energy transfer to total emission plotted against excitation wavelengths for three crude oils ranging from light to heavy, which exhibits several systematic trends. Collisional energy transfer varies from nearly 100% for the shortest wavelength excitation to nearly 0% for the longest wavelength excitation. All crude oils show nearly the same behaviour. Thus, for crude oils, the fraction of collisional energy transfer is not a function of chromophores concentration; all crude oils are in the high concentration limit. *Reproduced with permission from Ref [18], © 1995, Society for Applied Spectroscopy.*

The changes observed in the fluorescence emission of crude oils with increasing excitation wavelength are: a narrowing of the emission band,<sup>18, 21</sup> and reductions in the Stokes shift, quantum yield,<sup>30</sup> and fluorescence lifetime.<sup>21</sup> This decrease is caused by the complex interaction between energy transfer and quenching processes. At short excitation wavelengths, energy transfer processes dominate since most of the absorbing fluorophores have large bandgaps and can transfer energy to the large numbers of smaller bandgap molecules. At longer excitation wavelength, the excited fluorophores have small bandgaps and there are fewer molecules with smaller bandgaps for energy transfer, so most collisions result in quenching, with the subsequent reduction in fluorescence lifetime. Furthermore, as the bandgaps of the excited fluorophores decreases, there is an increased rate of internal conversion, which also contributes to the reduction in lifetime.<sup>21, 26, 30, 31</sup>

## 3.2. Time-Resolved Fluorescence

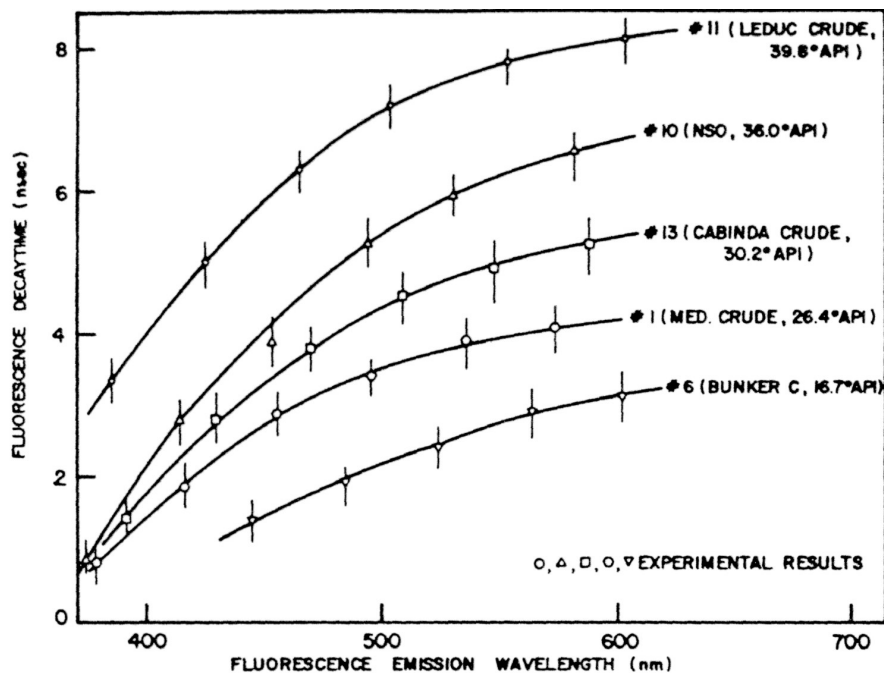


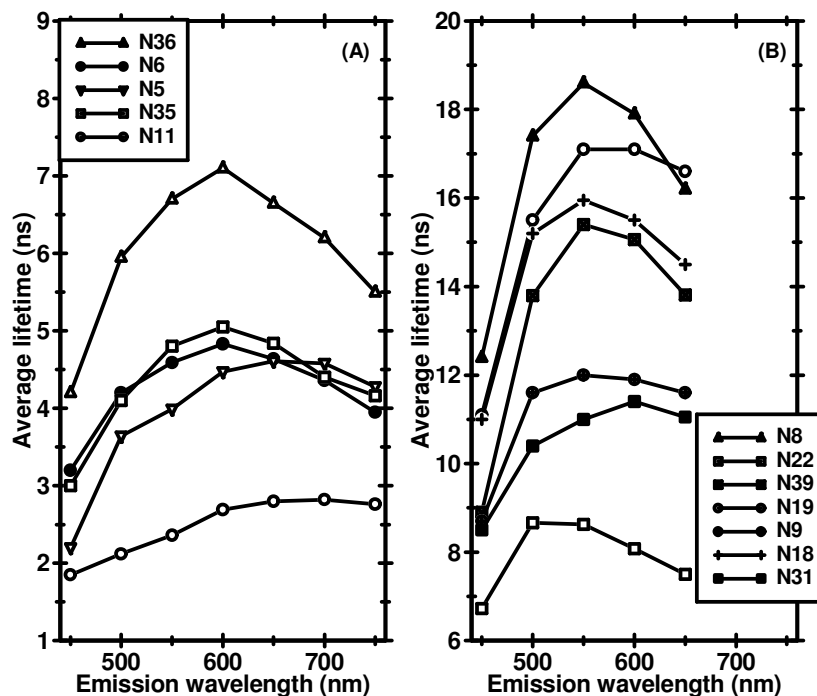
Figure 7. Variation of fluorescence decay time with emission wavelength for several crude oils. Data was collected with 337 nm excitation and using a single exponential fitting approach. Reproduced with permission from Ref [16], © 1974, Optical Engineering.

The fluorescence lifetime of crude oils is very sensitive to composition, with heavy oils having shorter lifetimes than light oils (Figure 7).<sup>10, 16, 21, 22, 25</sup> The 1974 study also showed a positive correlation between API gravity for four crude oils, and the decay time being measured at  $\lambda_{\max}$ . The changes in fluorescence lifetime is a result of the interplay between energy transfer and quenching, and is also emission wavelength dependant, since each emission wavelength represents a different population of emitting fluorophores. The numerical value of the fluorescence lifetime is however, very dependant on the method by which it is calculated. In Figure 7, the lifetimes were calculated via a single exponential convolution process, which should not be used other than for the most qualitative of descriptions. Rayner and Szabo,<sup>20</sup> used Time Correlated Single Photon Counting (TCSPC) with a pulsed nitrogen lamp source to measure fluorescence lifetime data from a series of 13 oils, including five crude oils. They calculated lifetime values via a deconvolution method, the fitting of two decay terms, and the requirement that the residuals of the fit be randomly distributed about zero. This resulted in reproducible fits with three parameters being reported:  $\tau_a$ ,  $\tau_b$ , and  $F_a/F_b$ , with  $F_a/F_b$  being the ratio of the pre-exponential factors for the two lifetime components.

However, no attempt was made to correlate the lifetime data with chemical composition. In 1987, Abu-Zeid et al. revisited the use of fluorescence lifetimes as a potential tool for identifying oil slicks.<sup>11</sup> Unfortunately, their lifetime measurements did not include any pre-exponential factors so it is not possible to assess the true fluorescence lifetimes of the oils.

The complex composition of crude oils ensures that the measured fluorescence decay curve is the sum of a distribution of individual decay curves. As such, one needs to exercise caution in discussing fluorescence lifetimes. Wang and Mullins,<sup>25</sup> in their detailed study of the fluorescence lifetime of crude oils used a bi-exponential fit to describe the fluorescence decay curves. More recently, we have used the intensity-averaged lifetime ( $\bar{\tau}$ ) calculated using either bi- or tri- exponential fits as a reproducible and standardised lifetime measurement for crude oils.<sup>10, 21, 22, 23, 32</sup> Figure 8 shows the  $\bar{\tau}$  dependence with emission wavelength for a series of North Sea oils. In general, the wavelength at which the maximum average lifetime is measured occurs at longer wavelength for heavy crude oils than it does for light oils.<sup>16, 21, 22</sup> The variation in lifetime going from 450 nm to ~600 nm emission wavelength is more pronounced for the lighter oils as is seen from Figure 8. At a particular point, a maximum value for the average lifetime ( $\bar{\tau}_{\max}$ ) is recorded at a wavelength  $\lambda_{\tau_{\max}}$  (e.g. ~ 600 nm for N11 with 380 nm excitation), which is dependant on the chemical characteristics of each oil. Figure 8 also shows a noticeable decrease in the average lifetime at emission wavelengths greater than ~600 nm. This 'curved' wavelength dependence is due to the complex interplay between collisional energy transfer and quenching processes.<sup>21, 22</sup>

At short emission wavelengths, fluorophores have large bandgaps and can readily undergo collisional energy transfer with larger, aromatic, small bandgap molecules. The rate of energy transfer is dependant on the bandgap between the two populations and so the value of  $\bar{\tau}$  relative to the maximum lifetime ( $\bar{\tau}_{\max}$ ) is shortened the most at short emission wavelengths where the bandgap is greatest. As the emission wavelength increases the energy gap between donor and acceptor decreases, and the concentration of large aromatic acceptor molecules decreases. The first factor results in an increase in the energy transfer rate but the second factor results in a decrease in the rate, as there are fewer acceptor molecules. Overall, the effect of both factors results in a net overall decrease in the rate of energy transfer leading to an increase in  $\bar{\tau}$  until  $\bar{\tau}_{\max}$  is reached at a wavelength  $\lambda_{\tau_{\max}}$ .  $\lambda_{\tau_{\max}}$  is red-shifted as the API gravity of the oils decreases which is evident from Figure 8.



**Figure 8.** Plots of intensity averaged fluorescence ( $\bar{\tau}$ ) lifetime vs. emission wavelength for a series of medium-heavy oils, API gravity < 40° (A), and light oils, API gravity > 35° (B). The lifetimes were measured using a 380 nm LED excitation source and TCSPC instrumentation. *Reproduced and adapted with permission from Ref [22], © 2002, Society for Applied Spectroscopy.*

At emission wavelengths longer than  $\lambda_{\tau_{\max}}$ , collisional quenching takes over as the dominant effect because as the emission wavelength increases, the bandgap between excited fluorophores and acceptor molecule gets smaller. Eventually the bandgap is so small that the acceptor molecules act as quenchers since they can de-excite themselves (internal conversion), and so collisional quenching dominates resulting in a decrease in  $\bar{\tau}$  relative to  $\bar{\tau}_{\max}$ . The excited fluorophores can also undergo internal conversion the rate of which is inversely proportional to the bandgap and this leads to a decrease in  $\bar{\tau}$ . The greater degree of lifetime change associated with lighter oils is due to the comparatively dilute concentration of fluorophores which so the average lifetime is more sensitive to collisional and internal conversion processes. With heavy oils, the concentration of fluorophores and quenchers is so high that small changes in the concentration of donor or quencher species will have very little effect on the average lifetime. The decrease in lifetime attributable to internal conversion is far outweighed by collisional quenching for the heavy oils.

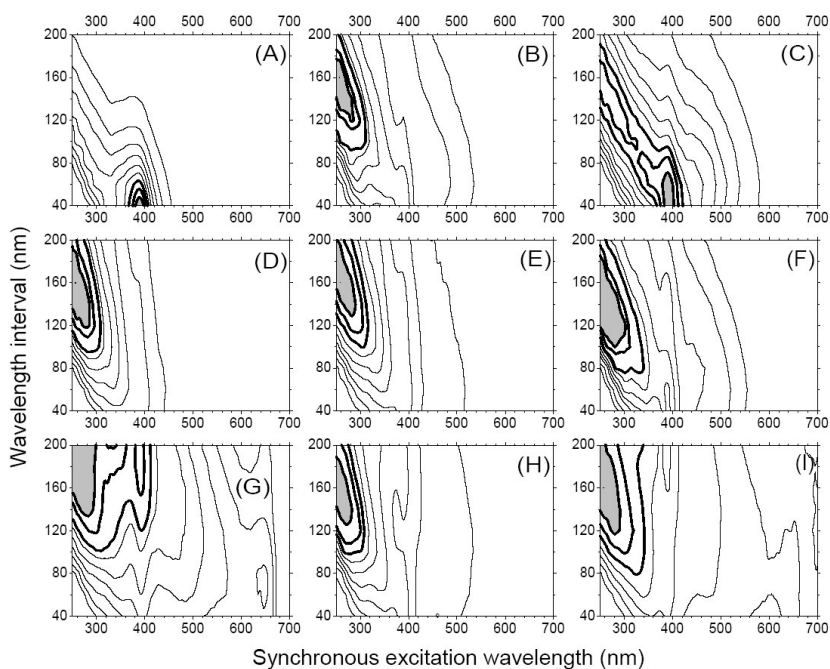
### 3.3. Multidimensional Techniques

Multidimensional techniques involve the collection of multiple fluorescence parameters and offer a more detailed method for studying petroleum photophysics or providing a spectroscopic fingerprint for identification purposes. Excitation-Emission Matrix (EEM) methods are a convenient way of mapping the fluorescence space that complex oils occupy.<sup>33, 34</sup> They can be used in the laboratory to determine the optimum excitation-emission parameters for simpler measurements/instrumentation, or as a method for the discrimination of oil types. The main drawbacks of EEM include time-consuming data collection and the requirement for complex instrumentation. Synchronous Fluorescence Spectroscopy (SFS) has been applied to the study of petroleum-based materials since the mid-1970's.<sup>35, 36</sup> Constant wavelength mode SFS has been used to study crude oils,<sup>37</sup> motor oils,<sup>38, 39</sup> and asphaltenes.<sup>40</sup> SFS has also been applied to the study of Shale oils,<sup>41</sup> fluid inclusions,<sup>42</sup> and reservoir compartmentalization.<sup>43</sup> Constant energy SFS was used for the analysis of both crude oil and gasoline.<sup>44</sup> 3-D fluorescence spectra have been used to discriminate oils into condensate, light oil, and heavy oils.<sup>45, 46</sup> Smith and Sinski<sup>47</sup> studied the red-shift cascade,\* which has also been used to investigate the degradation of aged petroleum by mycobacteria.<sup>48</sup> More recently, Total Synchronous Fluorescence Scan Spectroscopy (TSFS) has been used to discriminate different refined and crude petroleum liquids.<sup>49, 50</sup>

The compositional diversity of crude petroleum oils is evident in Figure 9 where the TSFS plots of nine different oils are displayed. For comparison purposes, and to account for instrument instability / sampling effects, each TSFS plot was normalised to the point of maximum fluorescence intensity. This allows for a general comparison between the different oils based on the identity of the emitting species. All the TSFS plots show a general diagonal contour trend from short  $\lambda_{\text{ex}}$  /large  $\Delta\lambda$ , to long  $\lambda_{\text{ex}}$  /short  $\Delta\lambda$  which represents a maximum fluorescence emission in the 350 – 500 nm range for these excitation wavelengths. This diagonal trend represents the extensive impact energy transfer processes have on crude petroleum oil fluorescence. The top row of Figure 9 shows the TSFS plots of 3 light oils (API > 40°) with low polar concentrations (<4%). There are considerable differences in the plot topology and this is due to changes in the aromatic concentration (as measured). The measured aromatic concentration increases across the top row from 1.8% to 6.6%, and then to 18.2% for (C). Oil (A) is classed as a late maturity oil and as such, most of the larger polyaromatic species will have been broken down to alkanes and small aromatic species. This results in a very tight TSFS contour plot centred at  $\lambda_{\text{ex}} = 390$  nm,  $\Delta\lambda = 40$  nm, indicating a somewhat homogenous, and restricted mixture of fluorophores, with an emission maximum around 430 nm. The more diverse and wider ranging contour plot of oil (B) cannot be explained just on the basis of a ~4% increase in aromatic concentration, but also by a change in the type of aromatic species present. Since most crude oil fluorophores are aromatic, it follows that the increase in aromatic concentration causes TSFS contours to spread out over a larger parameter space. The primary process driving this is the increased rates of collisional energy transfer from small to large aromatic species.

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\* The red shift cascade is the greater degree of energy transfer, which occurs at high concentrations of crude oils. The excitation energy will continue to cascade to larger fluorophores, producing greater red shifts in the emission spectra.<sup>25</sup>



**Figure 9:** Total Synchronous Fluorescence Scan plots for 9 different crude petroleum oils recorded from 250 nm to 700 nm over a wavelength interval of 40-200 nm in a front surface sampling geometry: (A) 7703: API = 50.6, polar = 1.6%; (B) 7197: API = 45.1, polar = 1.9%; (C) 7058: API = 40.1, polar = 3.5%; (D) 7062: API = 36, polar = 17.2%; (E) 7093: API = 30.9, polar = 11.5%; (F) 7633: API = 24.8, polar = 3.73%; (G) 7169: API = 21.6, polar = 20.3%; (H) 7130: API = 15.6, polar = 24.6%; (I) 7033, API = 12.8, Polar = 26.3%. *Reproduced with permission from Ref [50], © 2003, Journal of Fluorescence Spectroscopy.*

Generally as a crude oil matures, the aromatic fraction is gradually reduced and therefore it would seem possible to assess the maturity of the oils by measuring the changes in TSFS topography. Unfortunately, this is probably only applicable to oils from a single source, because the second row of Figure 9 shows that for oils with similar aromatic concentrations as C (D & E), the topography of the TSFS contour plots are significantly different. This is caused by a relatively higher polar concentration, which results in increased rates of collisional quenching, with the greatest effect being observed at  $\lambda_{\text{ex}} \sim 400$  nm and  $\Delta\lambda$  of <100 nm. In the TSFS plot for (F), the contours extend further out into the red because this oil has a relatively low polar concentration leading to a reduced quenching rate. The bottom row of Figure 9 shows the TSFS plots for some heavy oils, all of which have relatively large concentrations of polar constituents. This results in much weaker fluorescence intensity, but apart from (G), the TSFS topography does not appear to be very different from the TSFS in the preceding rows. Case (G) is a unique in that it is heavily degraded which has resulted in the formation of a much wider

range of fluorophores as evidenced by the spread of high intensity contours into more of the parameter space.

The large degree of similarity between some of the TSFS plots is largely due to the fact that normalisation was done at the point of maximum fluorescence intensity and this obscures the huge differences in fluorescence intensity observed for the various samples (~200 fold between light (A) and heavy oils (I)).<sup>50</sup>

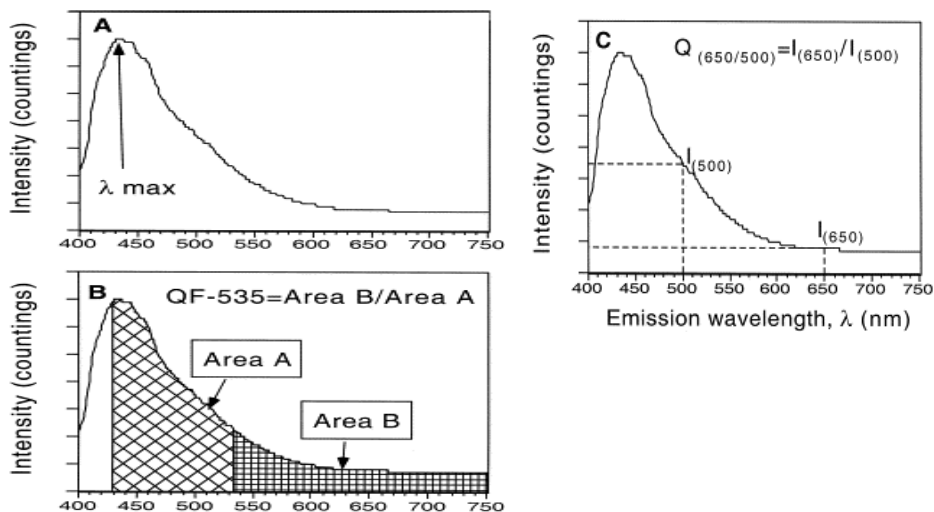
### 3.7. Instrumentation

The instrumentation for laboratory based fluorescence analysis of petroleum fluids is not that significantly different from that employed for routine biophysical or chemical physics measurements. In the author's laboratory at NUI-Galway, standard fluorescence steady-state instrumentation (Perkin-Elmer LS50B) and time-resolved instrumentation (TCSPC instrumentation assembled from commercial components) has been used. Apart from using front-surface excitation for all measurements, there are no significant novel requirements.<sup>10, 21, 22, 23, 32, 50</sup> Most of the novel instrumentation research involves the development of systems for specific applications. Examples include using fibre optics coupled with fluorescence and reflectivity measurements for quantifying the amounts of oil, water, and gas in a continuous flow,<sup>51, 52</sup> and fibre optic systems for the analysis of solid materials particularly from core samples,<sup>53</sup> and soil (or water).<sup>54, 55</sup> Several studies have discussed the utility of various excitation sources, such as tuneable dye lasers<sup>56</sup> and LED's<sup>57, 58</sup> for oil fluorescence applications.

## 4. QUALITATIVE AND QUANTITATIVE OIL ANALYSIS

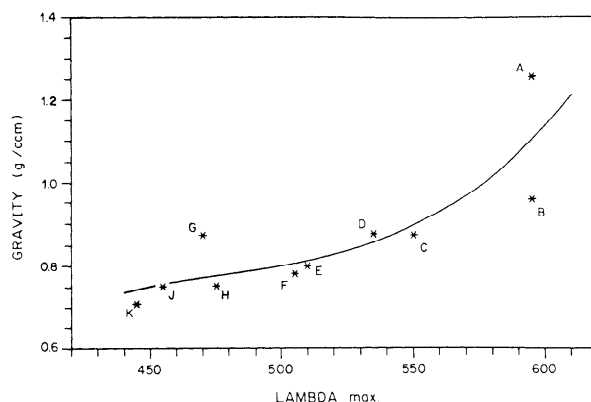
The use of fluorescence methods for the qualitative and quantitative analysis of crude oils is fairly common, covering diverse applications from oil spill identification to the study of microscopic petroleum fluid inclusions within rocks. The correlation of different fluorescence parameters with chemical (or physical) characteristics of bulk crude oils have been used to develop qualitative or quantitative models for industrial or research applications. The selection (or indeed discovery) of the best fluorescence parameters for quantitative crude oil analysis is a complex issue and has not yet been fully resolved. One of the key challenges however, is to develop a method that is suitable for any crude oil type from any source. In this section, we survey the use of both steady-state and time-resolved fluorescence parameters for the analysis of crude oils.

## 4.1. Steady-State

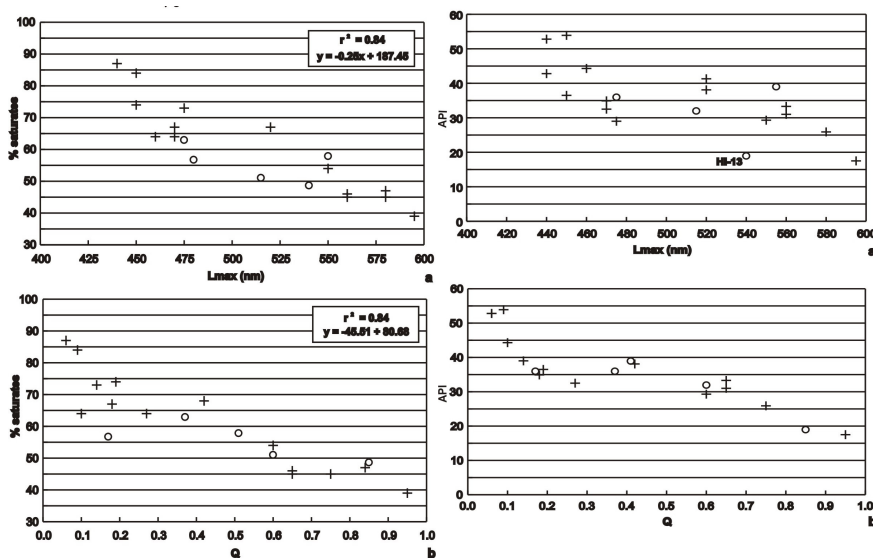


**Figure 10.** Definitions of fluorescence emission parameters. (A) The wavelength of maximum intensity,  $\lambda_{max}$ ; (B) The QF-535 factor; and (C) The  $Q_{650/500}$  (Red-Green-Quotient) factor. *Reproduced with permission from Ref. [68], © 2001, Elsevier.*

Three of the most common steady-state parameters used within the geological science community for oil analysis are illustrated in Figure 10. The first and most obvious fluorescence parameter to consider is the emission wavelength and in particular the point of maximum emission ( $\lambda_{max}$ ). Hagemann & Hollerbach,<sup>15</sup> correlated  $\lambda_{max}$  with the density (g/ccm) of a series of crude oils (Figure 11). The wavelength of maximum fluorescence emission ( $\lambda_{max}$ ), using a  $365 \pm 30$  nm excitation source, was later demonstrated to correlate with saturate and aromatic concentrations (Figure 12) for a series of mostly Canadian crude oils.<sup>59</sup> Using a 380 nm LED source and a small set of North Sea oils, it was found that the width of the emission band correlated better with API gravity than did  $\lambda_{max}$ .<sup>22</sup> However, the data set was too restricted and there was no detailed gross compositional data available.

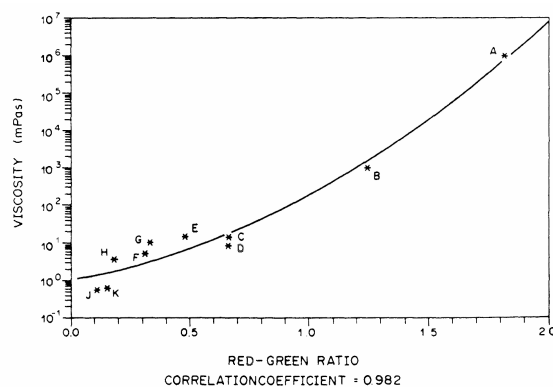


**Figure 11.** The variation in the fluorescence parameter  $\lambda_{max}$ , with decreasing gravity of the crude oils and condensates. (A) Papa Playa, biodegraded; (B) Georgsdorf, Germany, Heavy Oil; (C) Shell Monarch, USA, low mature; (D) Arco McCone, USA, low/high mature; (E) Arlesried, Germany, low/high mature; (F) Hassi-Messaoud, Algeria, low/high maturity; (G) Amerado USA Morton, low/high maturity; (H) east Merenie, Australia, high mature; (J) Amerdo F.E. Weedeman, USA, condensate; (K) Bilabari, Nigeria, USA, condensate. *Reproduced with permission from Ref [15], © 1986, Pergamon Journals Ltd.*



**Figure 12.** Plots of fluorescence parameters,  $\lambda_{max}$  (top) and Q(bottom) versus oil saturate concentration (left) and API gravity (right). The crosses represent data acquired from bulk crude oils while the circles are data obtained from Hydrocarbon bearing fluid inclusions. *Reproduced with permission from ref. 59, © 1997, Elsevier.*

A second parameter, the Red-Green ratio shows a better correlation with oil viscosity, which is linearly related to oil density (Figure 13). However, this was not pursued further and chromaticity diagrams based on the emission data were used to discriminate the various oil samples. More methods based on these chromaticity diagrams have been used for the analysis of hydrocarbon bearing fluid inclusions, and are discussed in the section on fluid inclusions. Unfortunately, these methods are dependant on the use of a set excitation wavelength, usually 365 nm from a Mercury arc lamp.<sup>15, 28</sup> This means that the results cannot be compared directly with those obtained using other sources such as a 337 nm nitrogen laser,<sup>16</sup> or 380 nm LED sources.<sup>22</sup> Another study using the Red-Green ratio demonstrated a correlation between this factor and an oil quality parameter based on the combined concentration of the aromatic, resin, and asphaltene fractions.<sup>12</sup> However, the method does not seem to have been used to any great extent by other researchers. This study also raises the potential for extracting hydrocarbons into epoxy resins for fluorescence analysis. However, this has to be approached with caution since no data is presented on the effects of immobilization on quenching and energy transfer rates. It would be expected that the fluorescence behavior of oils entrapped in epoxy resins would be significantly different from the free bulk oils.



**Figure 13.** Relationship between the Red-Green-Quotient and viscosity of the crude oils and condensates. (A) Papa Playa, biodegraded; (B) Georgsdorf, Germany, Heavy Oil; (C) Shell Monarch, USA, low mature; (D) Arco McCone, USA, low/high mature; (E) Arlesried, Germany, low/high mature; (F) Hassi-Messaoud, Algeria, low/high maturity; (G) Amerado USA Morton, low/high maturity; (H) east Mereenie, Australia, high mature; (J) Amerdo F.E. Weedeman, USA, condensate; (K) Bilabari, Nigeria, USA, condensate. *Reproduced with permission from Ref [15], © 1986, Pergamon Journals Ltd.*

Another emission ratio method is the QFT-II method developed by Texaco in the 1990's for oil well logging.<sup>60</sup> QFT-II uses 254 nm excitation and measures fluorescence intensity at emission wavelengths of 287 and 365 nm from solvent extracted material. The method was calibrated using a set of 70 crude oils (no data given on source or chemical composition) using two different extraction solvents (hexane and *iso*-propyl-alcohol). The method is designed for on-site analysis and only yields an estimate for API gravity, and is unsuited for heavy oils (API gravity <15).

The ratio of the 535-750 nm flux to the 430-535 nm flux of fluorescence emission spectra using 365 nm excitation was also used as a parameter to characterise crude oils but no assessment of its quantitative accuracy was provided.<sup>28, 70</sup> In general, there are several practical disadvantages in using fluorescence intensity based measurements for oil characterisation particularly for remote sensing applications. The main problem lies in the difficulty in the accurate, reproducible measurement of absolute fluorescence emission intensity.<sup>†</sup> These measurements can be affected by instabilities in the excitation source, detector electronics, sample turbidity, and photobleaching.<sup>61, 62</sup>

#### 4.2. Time-Resolved

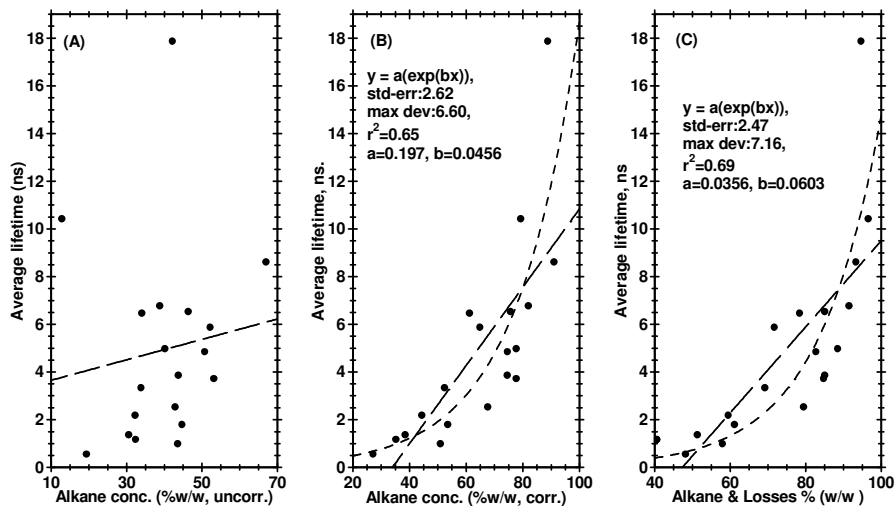
Time-resolved fluorescence measurements are largely insensitive to the negative factors that affect steady-state measurements and are more easily referenced, making sensing applications more robust.<sup>63</sup> Time resolved fluorescence techniques,<sup>64</sup> which have also been employed for characterization of petroleum products, are not only largely free of these artefacts,<sup>22, 25</sup> but in addition contain information that is lost in the time-averaging process inherent in steady-state methods. In the case of crude oils, which are complex mixtures of fluorophores, time-resolved fluorescence measurements offer the best approach for fully revealing the influence of quenching and energy transfer processes on fluorescence behaviour.

In 1987, the fluorescence lifetimes measured at a range of different emission wavelengths for a selection of Kuwaiti and Australian oils were measured using a 337.1 nm excitation source.<sup>11</sup> Regrettably, no chemical compositional data was supplied for the oils, and the reported lifetime data is incomplete (no fractional intensities supplied). A photophysical model for the fluorescence lifetime behaviour of crude petroleum oils detailed the complex balance between energy transfer and quenching that governs the fluorescence lifetimes of crude oils was derived from dilution studies on several oils.<sup>25</sup> This study used a variety of different excitation sources but did not try to correlate chemical composition with fluorescence lifetime data.

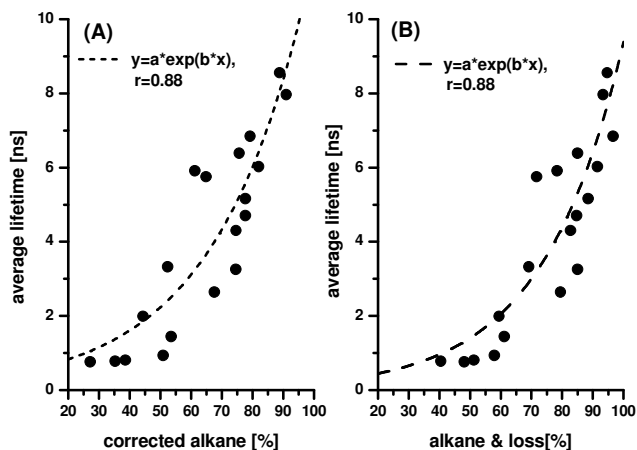
More recently, we have revisited the use of intensity average fluorescence lifetime for the characterisation of crude petroleum oils and have sought to correlate fluorescence lifetime data with physical characteristics (API gravity) and compositional factors such as alkane, aromatic, polar, and sulphur concentration.<sup>21, 22</sup> Figure 14 shows the plot of lifetime versus alkane concentration (calculated 3 different ways) for a series of 23 oils. The oils studied are topped oils, where the light hydrocarbon fraction was removed, and they encompassed a wide range of sources and compositions.<sup>10, 32</sup> Using a longer excitation wavelength (405 nm) resulted in a slightly better correlation for both corrected alkane (Figure 15) and polar concentration (Figure 16).<sup>32</sup> In each case, however, there is still considerable scatter about the best-fit line, preventing the development of accurate, quantitative models.

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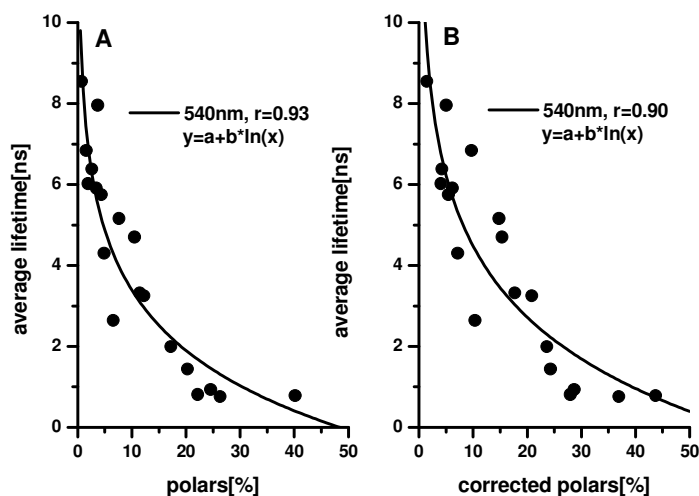
<sup>†</sup> Fluorescence ratio methods like the red-green quotient and the QFT-II avoid the problem of absolute emission intensity measurements.



**Figure 14.** Plot of average fluorescence lifetime at an emission wavelength of 500 nm (380 nm LED excitation) versus (A) uncorrected alkane concentration, (B) corrected alkane concentration and (C) sum of uncorrected alkane concentration and column losses. *Reproduced with permission from Ref [22], © 2004, Society for applied Spectroscopy.*



**Figure 15.** Plot of average fluorescence lifetime ( $\bar{\tau}$ ) versus: A) corrected alkane concentration; B) sum of alkane concentration and column losses at the emission wavelength of 540 nm. The best exponential growth fit and the value of correlation coefficient  $r$  for each concentration are also plotted. *Reproduced with permission from Ref [32], © 2004, Society for applied Spectroscopy.*



**Figure 16.** Plot of average fluorescence lifetime ( $\bar{\tau}$ ) versus: A) measured polar concentration; B) corrected polar concentration; at the emission wavelength of 540 nm. The best logarithmic fit and the value of correlation coefficient  $r$  for each concentration are also plotted. *Reproduced with permission from Ref [32], © 2004, Society for applied Spectroscopy.*

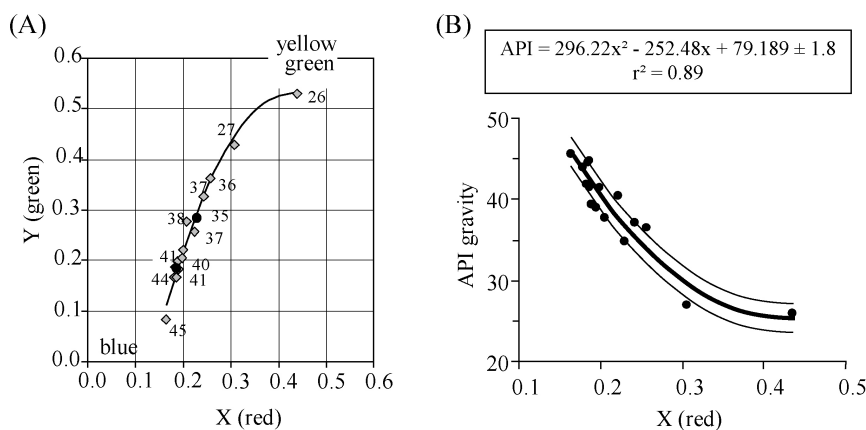
The data plotted in Figures 15 and 16 highlight the difficulty in developing all inclusive fluorescence methods suitable for any oil type. The wide diversity of sources from which the test oils were derived almost certainly contributes to the large scatter in the plots. The use of simple lifetime models is another potential source of error, since they cannot fully account for the complex decay kinetics of crude oils. Further investigations are underway to try to resolve this issue and will be reported in the near future. The use of fluorescence lifetime ratios for quantitative analysis was also investigated,<sup>21,22</sup> and the initial results were promising, but, these studies used a restricted set of oils from the North Sea. The application of lifetime ratios to the more diverse oil sample sets does not result in accurate correlations suitable for analytical use.<sup>65</sup>

## 5. APPLICATIONS: FLUID INCLUSION STUDIES

Hydrocarbon-containing fluid inclusions (HCFI) are small, generally  $< 10 \mu\text{m}$  in diameter, micro-cavities filled with fluid trapped during crystallization or healing of the fractures in minerals such as quartz, fluorite, or calcite.<sup>66, 67, 68</sup> The fluids entrapped within HCFI are fossilised samples of oils, gases, aqueous solutions, and solids, whose composition may not have changed since the trapping event. The accurate determination of the chemical composition of entrapped fluids can provide (together with micro-thermometric studies) essential information about the crystal growth, temperature, and

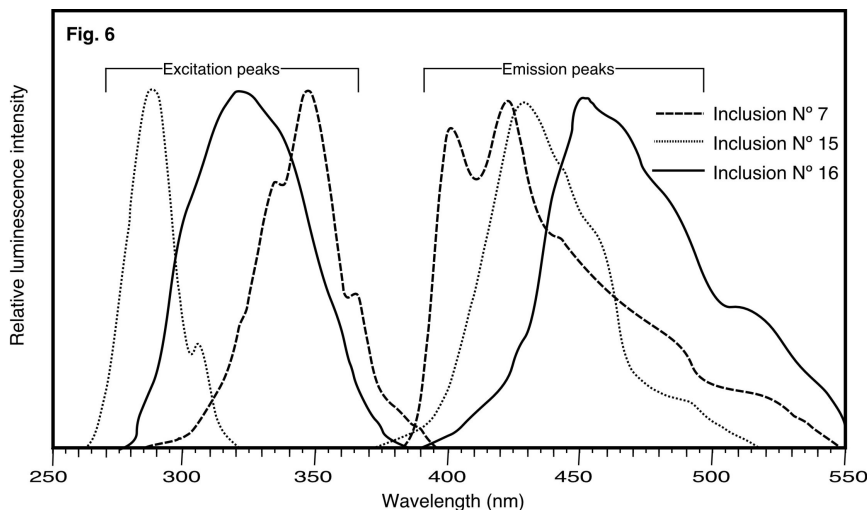
timing of fluid migration. This information is of significant importance to the petroleum exploration industry, particularly in regard to the study of petroleum reservoirs. The analysis of HCFI is done either by crushing bulk rock samples and extracting the entrapped fluid for analysis, or by the analysis of single fluid inclusions. The oil composition data obtained from bulk fluid inclusion analysis (by crushing) suffers from a variety of problems including: sample destruction, mixing of fluids from heterogeneous fluid inclusions, and contamination from materials within the rock sample itself.<sup>69</sup>

Fluorescence based methods are widely used for studying HCFI and the most common identification method is by observing their fluorescence colour under UV illumination.<sup>70,71</sup> The use of visually determined fluorescence colour, is widely used as a qualitative guide for assessing the maturity of oil in HCFI.<sup>67</sup> Unfortunately, the use of fluorescence colour is intrinsically prone to error, and does not yield quantitative results. In addition, there are issues with instrumental variation (excitation wavelength, emission filters, etc.) and reproducibility.<sup>72, 73, 74, 75</sup> However, when calibrated with oils from the same basin/reservoir, it has been used to show variation in HCFI composition (changes in API gravity) in a single fluid inclusion assemblage (Figure 17).<sup>13</sup>



**Figure 17.** Reference oil fluorescence results: (A) – calibration oil chromaticity coordinates. Black solids indicate 3/9A-6 well oils. Solid circles are indexed by their API gravity. Note the colour evolution towards green-yellow with decreasing API gravity; (B) Oil API gravity versus red chromaticity parameters. (Reproduced with permission from ref. [13]).

The red-green quotient  $Q$  ( $Q = \text{intensity } 650 \text{ nm} / \text{intensity } 500 \text{ nm}$ ), and the wavelength of maximum fluorescence emission intensity ( $\lambda_{\text{max}}$ ), were found to correlate with API gravity and gross chemical composition (%w saturates, aromatics, polars, and asphaltenes) for a set of HCFI synthesized using a sample set of Canadian crude oils.<sup>59</sup> Both  $Q$  and  $\lambda_{\text{max}}$  were also shown to correlate well with gross chemical composition of Athabaska bitumen sub-fractions.<sup>24</sup>



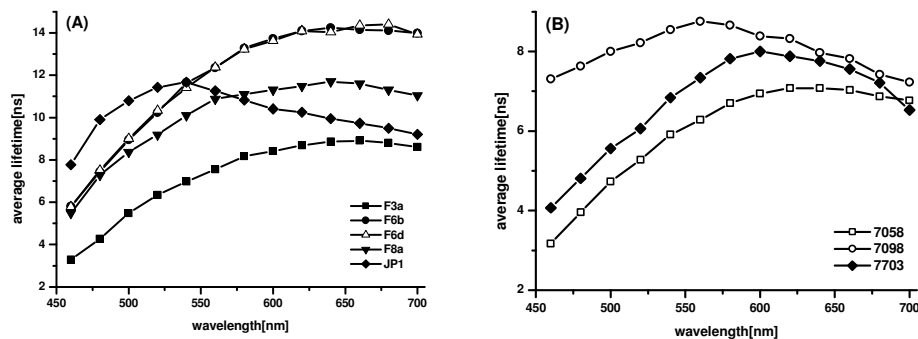
**Figure 18:** FLEEMS measurements of inclusion showing optimum excitation and optimum emission spectra for each inclusion. Excitation slit = 10 nm. Emission slit = 10 nm. Scanning speed = 60 nm per min. All spectra are normalized to same fluorescence intensity level for ease of comparison. Measuring microscope objective: Olympus D Apo UV 100 x , N.A. 1.3 oil immersion. *Reproduced with permission from Ref. [76] © 1996, Elsevier Science Ltd*

Coupling a standard steady-state fluorimeter to an epi-fluorescence microscope using fibre optics enabled the collection of excitation, emission, and synchronous excitation-emission from microscopic sample areas, such as single HCFI.<sup>76</sup> When the monochromators are set to zero order it enabled the collection of the emission spectrum at optimum excitation conditions, and the excitation spectrum at optimum emission conditions (Figure 18). This methodology was used to discriminate different HCFI on the basis of these spectra or via values extracted such as optimum excitation wavelength or the Stokes shift. Kihle also demonstrates the collection of synchronous fluorescence excitation-emission spectra from individual inclusions.<sup>76</sup>

However, steady-state based fluorescence measurements for inclusions suffer from several drawbacks (apart from those mentioned in section 4.1). The apparent fluorescence intensity and spectral distribution can adversely be influenced by the physical properties of the sample such as geometry, opacity, sample turbidity, and the scattering properties of both the actual inclusion and host mineral. The last factor is particularly important in the context of HCFI studies where there is a large variation in sample type. Photobleaching of synthetic and natural HCFI has been shown to cause changes in the fluorescence emission intensity.<sup>61</sup>

In 1987, McLimans outlined the potential for using fluorescence lifetime measurements for HCFI analysis.<sup>67</sup> He showed that fluorescence lifetime (355 nm excitation) increased on going from condensate, to very mature, to moderately mature, to immature oils. There was also a noticeable increase in lifetime for all oils with emission wavelength over the 400-600 nm range. However, no data was provided on the method for calculating fluorescence lifetime or about the chemical composition / source of the oils samples, nor was any HCFI data presented.

Recently, in NUI-Galway, fluorescence lifetime data has been acquired from a series of HCFI and correlated with data obtained from a test set of 23 crude oils.<sup>32</sup> The HCFI all emitted similar coloured fluorescence under 405 nm excitation, however, all were clearly discriminated on the basis of their lifetime measurements. Unfortunately, the study also showed that the fluorescence lifetimes of some HCFI oils were much longer than the lightest oils in the test set. This is probably because the oils in HCFI contain higher fractions of light hydrocarbon liquids and gases that are not present in the bulk oils sampled. This will necessitate the development of larger calibration datasets, which comprise of non-topped crude oils (or analogues) to account for the gases and volatiles entrapped in HCFI. It will be also necessary to fabricate artificial HCFI with entrapped oils of known compositions to further validate the methodology. Artificial HCFI can be synthesised in halite<sup>77</sup> at low temperature, and more recently from quartz at higher temperature.<sup>78</sup> Unfortunately, it is not clear that it will be possible to have a well defined (known chemical composition) oil in these HCFI as there is evidence that partitioning and/or thermal degradation can occur.



**Figure 19:** Plot of average fluorescence lifetime versus emission wavelength for: A) HCFI samples, B) several light crude oils, API gravity = 50.6° - 40.1°. Reproduced with permission from Ref. [32] © 2004, Society for applied Spectroscopy.

The use of lifetime based measurements for HCFI characterization, while still in its infancy does offer considerable benefits when compared to the more traditional colour, intensity, and spectral measurements. Finally, one novel application of fluorescence to inclusion studies involves using confocal microscopy to measure the volume of oil and gas within fluid inclusions by utilising the spatial resolution of modern confocal microscopes.<sup>79</sup>

## 6. APPLICATIONS: REMOTE SENSING/OIL SPILL IDENTIFICATION

Oil spill identification is another area where fluorescence based methods are being applied.<sup>80, 81</sup> The development of laser fluorosensors for oil spill identification was recently reviewed by Fingas, concentrates on ship-borne and airborne sensors using 308

or 355 nm ultraviolet excitation.<sup>82</sup> Studies by several groups have concentrated on the development of remote fluorosensors capable of measuring both emission spectra and fluorescence lifetimes,<sup>11, 83</sup> of crude oils on sea/water surfaces.<sup>14, 84, 85, 86, 87</sup> The photochemical weathering of crude oils on water can be monitored using fluorescence intensity, where there is a sharp decrease in intensity upon solar irradiation.<sup>8, 88, 89</sup> However, this decrease in intensity is probably due to light fraction loss by evaporation rather than the formation of any oxygenated species. The fluorescence intensity of crude oils is extremely sensitive to the alkane concentration, and any loss will result in large changes in fluorescence intensity.<sup>10, 32</sup> Chemometric methods such as Principal Component Analysis (PCA) have been applied with fluorescence emission spectroscopy to demonstrate the potential discrimination of oils types and weathering effects.<sup>90</sup> Computational models for discriminating or matching oil types vary from simple vector models<sup>91</sup> to more complex Artificial Neural Networks.<sup>92, 93</sup> ANN methods have also been used to help separate fluorescence signals from oil and humic substances in seawater.<sup>94</sup>

## 7. APPLICATIONS: SUNDRY TECHNIQUES

Asphaltene aggregation was studied by front surface fluorescence spectroscopy,<sup>95</sup> and the use of fluorescence depolarisation (FD) techniques have been shown to differences in asphaltene isolation methods,<sup>96</sup> measure asphaltene size,<sup>97, 98, 99</sup> and to track the effect of temperature.<sup>100</sup> Khorasani and Michelsen,<sup>101</sup> used PCA and fluorescence spectroscopy to derive a discrimination method for the assessment of maturity and oil generation from marine algal kerogens. The use of fluorescence to study the impact of crude oils on the environment is also becoming more important. Various fluorescence methods have been used to look at the Polycyclic Aromatic Hydrocarbon (PAH) content of crude oils and petroleum products,<sup>102</sup> and to characterise petroleum contaminated soils<sup>103</sup> and the formation of oil-mineral aggregates.<sup>104</sup> Fluorescence techniques have also been used to measure crude oil contamination in offshore sediments<sup>105, 106</sup> and the formation of oil in water emulsions is also studied by fluorescence.<sup>107</sup> There have been studies on estimating hydrocarbon levels in benthic organisms.<sup>108</sup> Fluorescence methods were also used to study the cracking kinetics of crude oils at high temperatures and pressures using a diamond anvil cell.<sup>109</sup> Time-resolved fluorescence spectra (plotted as contour maps) have also been proposed as a method for discriminating different oil types.<sup>110, 111</sup> The method, based on 250 nm laser excitation, shows potential, but as yet no quantitative reports have been shown.

## 8. CONCLUSIONS

The use of fluorescence spectroscopy in a wide variety of methodologies for the characterization and analysis of crude petroleum products is well established. Yet there are still considerable opportunities for the development of robust, accurate, and quantitative fluorescence methods for crude oil analysis. The rapid miniaturization and increasing capability of fluorescence instrumentation is further expanding the range of applications, particularly with regard to on-site and *in-situ* measurements. However, the wide range of compact, portable excitation sources now available, presents a problem to the crude oil fluorescence community. This diversity of excitation sources, each of

which excites different fluorophore populations within the crude oils, makes it increasingly difficult to compare quantitatively results from different studies. This combined with the fact that most research laboratories work on widely different crude oils further fragments this economically important research field. There is therefore, a need to develop a set of crude oil standards (either natural or synthesised in the laboratory), freely available to all, with which new methods and instrumentation can be calibrated, and the results from different laboratories more easily compared.

### 8.1. Acknowledgments

The author would like to acknowledge support from Science Foundation Ireland by way of the Grant Scheme for Investigators (Grant no. 02/IN.1/M231), and the National Centre for Biomedical Engineering Science (NUI-Galway) as part of the Higher Education Authority Programme for Research in Third Level Institutions.

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