Duetta: A New Fluorescence and Absorbance Spectrometer Providing Unique Two-in-One Benefits, Plus High Speed and NIR Fluorescence Detection

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In 2018, the HORIBA Instruments Fluorescence Division introduced a mid-market analytical spectrometer, called Duetta™, the first such "value, mid-market" instrument from the division. For decades now HORIBA has had a global leading market share of high-end research instruments, and the introduction of this new instrument opens up new market opportunities for HORIBA. The design of this new spectrometer concept, the sales success in the market in just its first year, and the key markets and applications of Duetta are all presented.

Introduction and Market

In March of 2018, HORIBA introduced a new fluorescence and absorbance spectrometer called Duetta (Figure 1). It was introduced to penetrate a new fluorescence market for HORIBA, the so-called analytical bench-top market. This is a mature market with well-established companies offering rather simple, and affordable, bench-top scanning photomultiplier tube (PMT) fluorometers.

Prior to the introduction of Duetta, the HORIBA Fluorescence Division had been selling instruments only to the high-end research community and that is where HORIBA is very well known and has a very strong market leadership position. HORIBA's research steady state and time-resolved fluorometers, including the Fluorolog3, Fluoromax Plus, QuantaMaster 8000, DeltaFlex TCSPC systems and the Aqualog all serve the high-end research community. In particular, HORIBA has a significant market share at high-end fluorescence research.

The analytical bench-top market consists of lower priced scanning PMT fluorometers. These analytical fluorometers have served this mature market for decades now. Duetta was introduced to penetrate this new large market for HORIBA with an innovative and disruptive new instrument concept.

Principles of Fluorescence

A fluorescence excitation spectrum is acquired when the emission wavelength is fixed and the excitation monochromator wavelength is scanned. In this way, the spectrum gives information about the wavelengths at which a sample will absorb so as to emit at the single emission wavelength chosen for observation. The fluorescence excitation spectrum is analogous to absorbance spectrum, but is a much more sensitive technique in terms of limits of detection and molecular specificity. Fluorescence excitation spectra are specific to a single emitting wavelength/species as opposed to an absorbance spectrum, which measures all absorbing species in a solution or sample. The fluorescence emission and excitation spectra for a given fluorophore are mirror images of each other (Figure 2). Typically, the emission spectrum occurs at higher wavelengths (lower energy) than the excitation or

Figure 1 Duetta two-in-one fluorescence and absorbance spectrometer

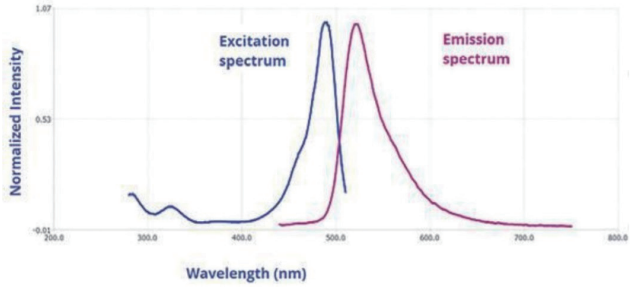

Figure 2 A fluorescence excitation spectrum (blue) and an emission spectrum (purple).

absorbance spectrum.

These two spectral types (emission and excitation) are used to see how a fluorescing sample is changing. The spectral intensity and/or peak wavelength may change with variants such as temperature, concentration, or interactions with other molecules around it. This includes quencher molecules and molecules or materials that involve energy transfer. Some fluorophores are also sensitive to solvent environment properties such as pH, polarity, and certain ion concentrations.

Features of Duetta

Fluorescence and absorbance are complimentary techniques. In fact, most research labs that use a fluorometer, also have a UV-Vis spectrophotometer for absorbance measurements. Absorbance is more widely used because it can work with a wider range of samples that do not fluoresce, but fluorescence is much more sensitive, and so it is preferable for fluorescing molecules. The HORIBA Fluorescence Division was able to deliver a Duetta two-in-one fluorescence (Figure 3) and absorbance spectrometer that offers much greater value, at the same price as a competitive bench-top instrument that can only measure fluorescence, but not absorbance.

Offering fluorescence and absorbance in one unit is already a big benefit for Duetta compared to its competitors. However, beyond just simply being capable of two different types of experiments, Duetta also uses simultaneously the absorbance information from the sample to correct the measured fluorescence signal for inner filter effects (IFEs), which are the absorption and the reabsorption of light occurring at higher concentrations. In this way, the absorbance data enhances and extends the quality of the fluorescence data by correcting for primary and secondary IFEs (Figure 4). Using the absorbance spectrum, the IFE correction is applied to the measured (observed) fluorescence intensity using the following equation:

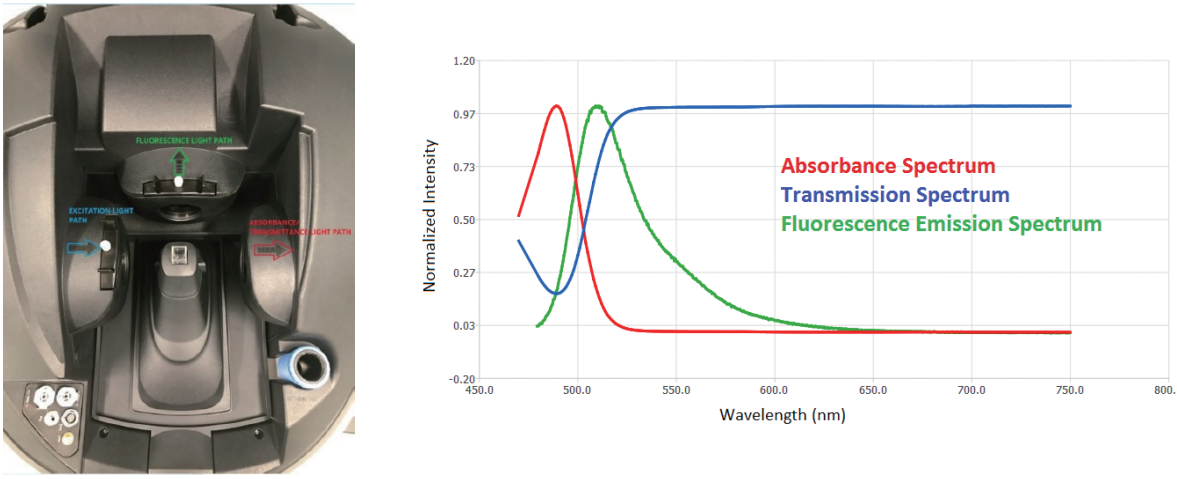

Figure 3 (Left) Duetta sample compartment shows the transmission/absorbance (red) detection optical path and the simultaneous fluorescence optical path (green) at 90 degrees from the excitation beam (blue). (Right) Duetta acquires multiple types of data in a single instrument.

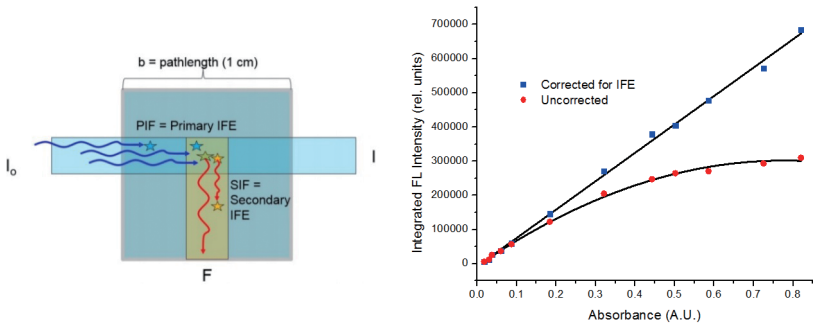

Figure 4 (Left) Top view diagram of a cuvette with sample solution inside, showing incident light (I_o), transmitted light (I) and the path of fluorescence detection at 90 degrees (F). Primary and secondary IFEs on the detected fluorescence intensity are shown with blue and yellow stars, respectively. (Right) The IFE is a well-known physical phenomenon. This experiment shows that the fluorescence of fluorescein at varying concentrations exhibits a non-linear response due to IFE reabsorption (red), and the corrected linear response of Duetta (blue) when IFE correction is implemented. Thus, extending the concentration range wherein the fluorescence signal is quantitative.

$$F_{corr} = F_{obs} \times 10^{\frac{Aex + Aem}{2}}$$

where F_{corr} is the corrected fluorescence intensity, F_{obs} is the measured or observed fluorescence intensity, A_{ex} and A_{em} are the absorbance values of the sample at the excitation and the emission wavelength, respectively.

Another key aspect of the design of Duetta is it uses a CCD for detection of fluorescence emission spectra. All of the competitors' instruments in the mid-market have a single channel PMT at the exit port of a scanning emission monochromator. Duetta, with its core HORIBA CCD detection technology, is able to collect the entire emission spectrum in just 50 milliseconds, compared to other scanning fluorometers that may take many minutes. So Duetta can collect an entire emission spectrum in less than a blink of an eye. This is the very big difference between Duetta and traditional scanning spectrofluorometers. As an example, Duetta can measure time evolution of the interaction between BSA and ANS (Figure 5).

The CCD detector also gives Duetta an additional advantage of detecting further into the near infrared (NIR) than is possible with a traditional PMT. PMT detectors in bench-top fluorometers typically cannot detect light with wavelength above 850 to 900 nm, although some offer slight wavelength extensions with additional PMT's. The Duetta CCD, however, is able to measure all the way to 1,100 nm (Figure 6), much further in the NIR than all of its competitors. There are some very exciting applications of NIR fluorescence research in new probe development, nanomaterials, semiconductors and life sciences, so having a value priced instrument that works at the NIR gives Duetta a big advantage in many areas of research.

Excitation emission matrix (EEM) molecular fingerprinting is another exciting application for Duetta. Traditional scanning spectrofluorometers have been used for some time now to collect molecular fingerprints, in the form of a fluorescence EEM. Sometimes also referred to as 3D Fluorescence, an EEM is a three dimensional data set consisting of fluorescence excitation wavelength, fluorescence emission wavelength, and fluorescence intensity. With a scanning spectrofluorometer, this data set is acquired by sequentially scanning a series of emission spectra, at varying excitation wavelengths and then reconstructing the resultant data set three dimensionally. This three dimensional data set can be used with third party chemometrics software for sample component analysis, as is done with other analytical techniques such as FTIR, HPLC and MS. There are in fact many scientific papers published citing the use of competitive mid-market scanning spectrofluorometers for fluorescence EEM component analysis in many disciplines including food sciences, water research and pharmaceuticals.

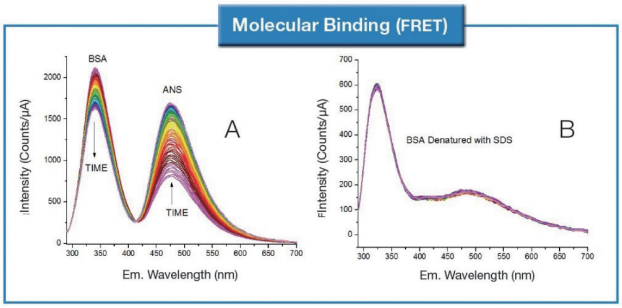

Figure 5 On the left, Image A, Kinetic spectral scans of native BSA protein with rapidly added ANS ($3x10^{-6}$ M). Spectra taken every 100 ms after ANS addition. As ANS binds to native BSA, the BSA Trp emission decreases and the ANS emission increases as a result of FRET, due to proximity of excited Trp and ANS. On the right, Image B, Kinetic spectral scans of SDS-denatured BSA after addition of ANS. Much higher ANS concentration ($4x10^{-5}$ M) is required to affect binding to denatured BSA; no FRET is observed due to increased distances between ANS and Trp.

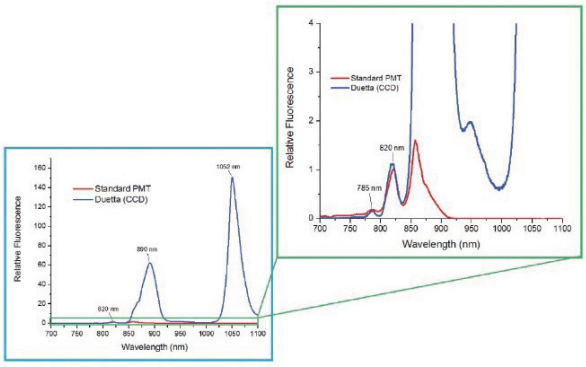

Figure 6 Neodymium laser glass emission spectrum. Ex: 575 nm, 5 nm band pass, Em: 700-1100 nm on fluorometer with PMT (red) and Duetta (blue). Standard PMT sensitivity falls off around 850 nm, while the Duetta CCD detector has sensitivity up to 1100 nm.

Applications of Duetta

Fluorescent molecules and materials come in all shapes and sizes. Some are intrinsically fluorescent, such as chlorophyll and the amino acid residue tryptophan (Trp), phenylalanine (Phe) and tyrosine (Tyr). Others are molecules synthesized specifically as stable organic dyes or tags to be added to otherwise non-fluorescent systems. There are entire catalogs of these fluorescence molecules available. Typically, organic fluorescent molecules have aromatic rings and pi-conjugated electrons in them. Depending on their size and structure, organic dyes can emit from UV to NIR.

Some categories of fluorescent molecules and materials are:

- Amino acids (Trp, Phe, Tyr)
- Base pair derivatives (2-AP, 3-MI, 6-MI, 6-MAP, pyrrolo-C, tC)
- Chlorophylls

- Fluorescent Proteins (FPs)
- Organic dyes (fluorescein, rhodamine, N-aminocoumarins and derivatives of these)
- Rare earth elements (lanthanides)
- Semiconductors
- Quantum dots
- Single Walled Carbon Nanotubes (SWCNTs)
- Solar cells
- Pigments, brighteners
- Phosphors

HORIBA Duetta technology has advanced the EEM technique by taking advantage of the fact that Duetta can simultaneously acquire the absorbance and the fluorescence of the sample and the absorbance data for IFE correction. HORIBA calls this advanced EEM technique Absorbance-Transmission Excitation Emission Matrix or A-TEEM™. By IFE correction, the A-TEEM molecular fingerprint is a much more absolute representation of the true molecular fingerprint than a traditional EEM. Therefore, when a researcher uses third party multivariate chemometrics analysis software, the A-TEEM data provides a much more robust component analysis than can be achieved with just a simple EEM from a scanning fluorometer.

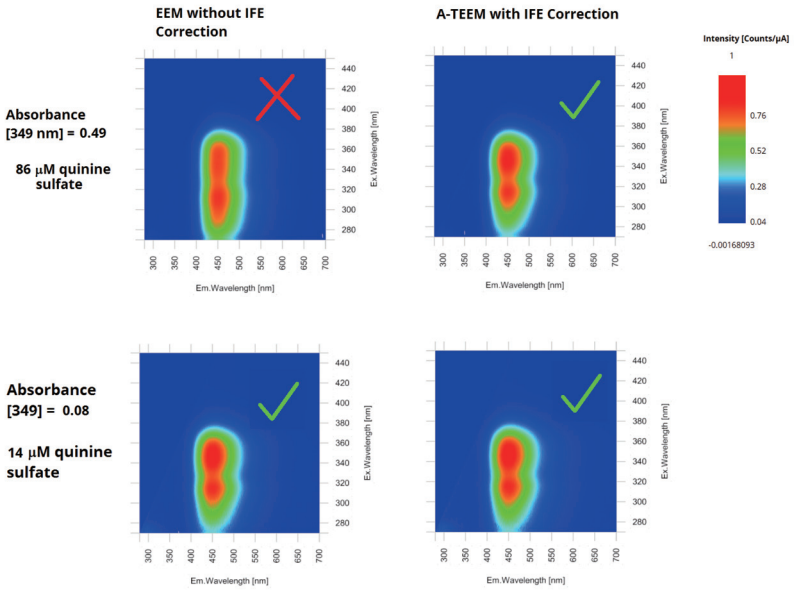

Figure 7 Fluorescence EEMs of two different concentrations of quinine sulfate in tonic water diluted in 0.1 M perchloric acid (aq.) with and without IFE corrections applied.

Figure 7 indicates how even a small difference in concentration of a fluorescence molecule can have a significant effect on the shape of an EEM fingerprint, but, with proper IFE correction and A-TEEM, the fingerprint remains the same.

Our customer demonstrates the power of EEM spectroscopy for nanomaterial development for biomedical imaging. Using the power of A-TEEM spectroscopy, this study demonstrates that the A-TEEM fingerprint is able to resolve unique fluorescence properties in different silver clusters (Figure 8[1]). The authors also used parallel factor analysis (PARAFAC) to support the presence of three distinct compounds from an A-TEEM fingerprint that consisted of a complex mixture of silver clusters. This work also benefited from the extended NIR detection of the CCD inside Duetta, since some components emitted above 850 nm.

From this work, the authors concluded that "Based on these findings, EEM spectroscopy can be implemented as a powerful technique for determining the purity of complex mixtures, especially when other techniques, including mass spectrometry, fail to provide adequate

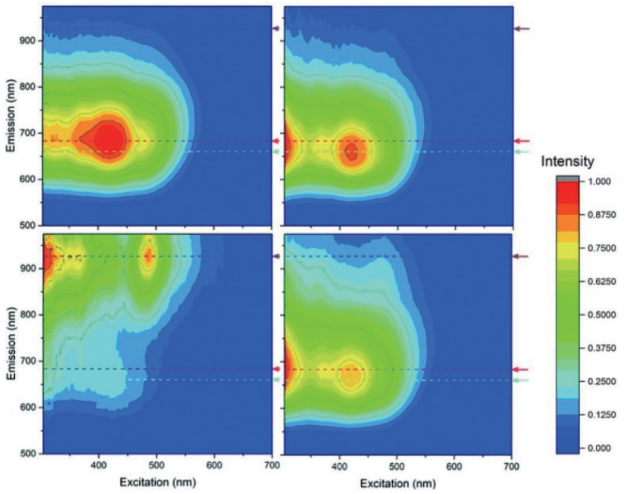

Figure 8 Fluorescence EEM spectra of Ag_A (top left), Ag_B (top right), Ag_C (bottom left) and the crude sample (bottom right). Dotted lines/arrows indicate the relative position of observed emission maxima. All plots are normalized to an intensity of 1 and were corrected for IFEs. The quantum yields of emission for Ag A, B and C are 0.323%, 0.232% and 0.083%, respectively. Reprinted with permission from [1] H. Ramsay, D. Simon, E. Steele, A. Hebert, R. D. Oleschuk and K. G. Stamplecoskie, RSC Adv., 8, 2018, 42080, Copyright 1999 the Royal Society of Chemistry.

characterization of a given material." This publication is a compelling testimonial to the power of A-TEEM spectroscopy as an analytical technique, and points to a valuable and significant benefit that Duetta provides with its unique A-TEEM molecular fingerprinting technology.

Solvent dependent sensitization of two rare earth elements, Ytterbium (Yb) and Neodymium (Nd), via an intramolecular excimer was studied (Figure 9[2]). Yb has spectral emission bands around 1,000 nm and Nd has two emission bands around 890 and 1050 nm, all well within

the range of Duetta. This publication also relied on UV-Vis absorbance data collected from Duetta. So this particular publication touched on a number of key aspects of Duetta, namely, Fluorescence and Absorbance, NIR Detection and novel Probe Development.

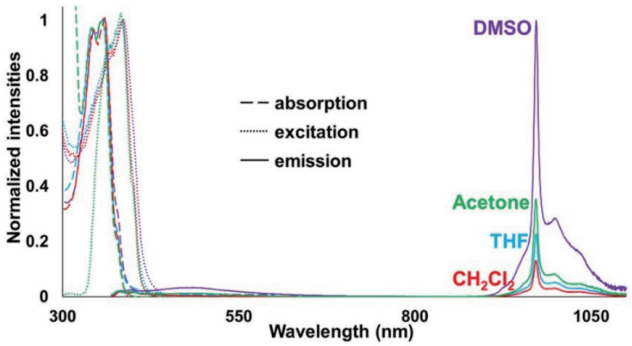

Figure 9 Absorption (dashed), excitation (dotted) and emission (solid) spectra of $(Pyr_2POAc)_3Yb(thf)$ in various solvents (2.6×10^{-6} mol L^{-1}). Excitation collected for emission centered at 970 nm. Emission collected with excitation at 355 nm. Slit widths: 5 nm (excitation/emission). Reprinted with permission from [2] Min Deng, Nathan D. Schley, and Gael Ung, Inorg. Chem., 57, 15399 (2018), Copyright 2018 American Chemical Society.

Conclusions

Duetta has been a tremendous success for HORIBA, far exceeding our commercial forecast. HORIBA has also been honoured to receive a number of awards for Duetta, including the 2018 Pittcon gold award for innovation. However, beyond commercial success, the customer feedback has been tremendous. One person told me “I love it. I want to hug it”, and that is just something no one has ever said to me when describing a scientific instrument. Duetta is inspiring a whole new host of scientists, who love to work with this unique instrument, and apply it to new scientific challenges. To receive such recognition from our peers, and such accolades from our customers, is very gratifying for the entire applications and R&D teams who worked tirelessly to develop this exciting new instrument.The future of fluorescence looks very bright indeed with the introduction of Duetta.

* This content is based on our investigation at this publish unless otherwise stated.

References

[1] H. Ramsay, D. Simon, E. Steele, A. Hebert, R. D. Oleschuk and K. G. Stamplecoskie, RCS Adv., 8, 42080 (2018)

[2] Min Deng, Nathan D. Schley, and Gaël Ung, Inorg. Chem., 57, 15399 (2018)

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