HORIBA Scientific

JOBIN YVON Technology

Duetta™ with EzSpec™

Operation Manual

Manual PN: 5700004252

Revision 1.4.3

April 2023

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1 Introduction

1.1 About the Duetta™

The Duetta™ is a self-contained, fully automated spectrofluorometer system. Data output is viewed on a PC. All Duetta™ functions are under the control of EzSpec™ spectroscopy software. The main parts of the Duetta™ spectrofluorometer systems are:

- State-of-the-art optical components
- A personal computer
- EzSpec™ for Windows®, the driving software.

EzSpec™ is personalized according to the Windows® user ID at login. When a user opens EzSpec™, the dashboard remains how it was left upon last use by that user. All Duetta™ hardware are self-identifying to EzSpec™. The user interface will behave accordingly, including appropriate method types, options, electrical power, and signal out where appropriate. EzSpec™ is touch screen compatible.

This manual explains how to operate and maintain a Duetta™ spectrofluorometer. The manual also describes measurements and tests essential to obtain accurate data.

> ⓘ Note: This document is saved on the Duetta USB drive as well as the USB memory stick that is shipped with every Duetta instrument.

1.2 Disclaimer

By setting up or starting to use any HORIBA Canada Incorporated product, you are accepting the following terms:

You are responsible for understanding the information contained in this document. You should not rely on this information as absolute or all-encompassing; there may be local issues (in your environment) not addressed in this document that you may need to address, and there may be issues or procedures discussed that may not apply to your situation.

If you do not follow the instructions or procedures contained in this document, you are responsible for yourself and your actions and all resulting consequences. If you rely on the information contained in this document, you are responsible for:

- Adhering to safety procedures
- Following all precautions
- Referring to additional safety documentation, such as Material Safety Data Sheets (MSDS), when advised

As a condition of purchase, you agree to use safe operating procedures in the use of all products supplied by HORIBA Canada Incorporated, including those specified in the MSDS provided with any chemicals and all warning and cautionary notices, and to use all safety devices and guards when operating equipment. You agree to indemnify and hold HORIBA Canada Incorporated harmless from any liability or obligation arising from your use or misuse of any such products, including, without limitation, to persons injured directly or indirectly in connection with your use or operation of the products. The foregoing indemnification shall in no event be deemed to have expanded HORIBA Canada Incorporated's liability for the products.

HORIBA Canada Incorporated products are not intended for any general cosmetic, drug, food, or household application, but may be used for analytical measurements or research in these fields. A condition of HORIBA Canada Incorporated's acceptance of a purchase order is that only qualified individuals, trained and familiar with procedures suitable for the products ordered, will handle them. Training and maintenance procedures may be purchased from HORIBA Canada Incorporated at an additional cost. HORIBA Canada Incorporated cannot be held responsible for actions your employer or contractor may take without proper training.

Due to HORIBA Canada Incorporated's efforts to continuously improve our products, all specifications, dimensions, internal workings, and operating procedures are subject to change without notice. All specifications and measurements are approximate, based on a standard configuration; results may vary with the application and environment. Any software manufactured by HORIBA Canada Incorporated is also under constant development and subject to change without notice.

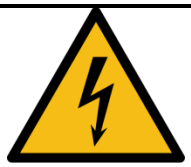
Any warranties and remedies with respect to our products are limited to those provided in writing as to a particular product. In no event shall HORIBA Canada Incorporated be held liable for any

special, incidental, indirect or consequential damages of any kind, or any damages whatsoever resulting from loss of use, loss of data, or loss of profits, arising out of or in connection with our products or the use or possession thereof. HORIBA Canada Incorporated is also in no event liable for damages on any theory of liability arising out of, or in connection with, the use or performance of our hardware or software, regardless of whether you have been advised of the possibility of damage.

1.3 Safety Summary

The following general safety precautions must be observed during all phases of operation of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture and intended use of instrument. HORIBA Canada Incorporated assumes no liability for the customer's failure to comply with these requirements. Certain symbols are used throughout the text for special conditions when operating the instruments:

Symbol	Definition
	⚠ **A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or similar that, if incorrectly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.** **HORIBA Canada Incorporated is not responsible for damage arising out of improper use of the equipment.**
	⚠ **A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or similar that, if incorrectly performed or adhered to, could result in damage to the product. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.** **HORIBA Canada Incorporated is not responsible for damage arising out of improper use of the equipment.**
	Ultraviolet light! Wear protective goggles, full-face shield, skin-protection clothing, and UV-blocking gloves. Do not stare into light.
	Intense ultraviolet, visible, or infrared light! Wear light-protective goggles, full-face shield, skin-protection clothing, and light-blocking gloves. Do not stare into light.
	Extreme cold! Cryogenic materials must always be handled with care. Wear protective goggles, full-face shield, skin-protection clothing, and insulated gloves.

Symbol	Definition
	Explosion hazard! Wear explosion-proof goggles, full-face shield, skin-protection clothing, and protective gloves.
	Risk of electric shock! This symbol warns the user that un-insulated voltage within the unit may have sufficient magnitude to cause electric shock.
	This symbol cautions the user that excessive humidity, if present, can damage certain equipment.
	Hot! This symbol warns the user that hot equipment may be present, and could create a risk of fire or burns.
	Read this manual before using or servicing the instrument.
	Wear protective gloves.
	Wear appropriate safety goggles to protect the eyes.
	Wear an appropriate face-shield to protect the face.
ⓘ Note:	ⓘ General information is given concerning operation of the equipment.

1.4 Risks of UV (ultraviolet) Exposure

⚠ **Caution: This instrument is used in conjunction with ultraviolet light. Exposure to these radiations, even reflected or diffused, can result in serious, and sometimes irreversible, eye and skin injuries.**

Overexposure to ultraviolet rays threatens human health by causing:

- Immediate painful sunburn
- Skin cancer
- Eye damage
- Immune-system suppression
- Premature aging

Do not aim the UV light at anyone.

Do not look directly into the light.

Always wear protective goggles, full-face shield and skin protection clothing and gloves when using the light source.

Light is subdivided into visible light, ranging from 400 nm (violet) to 700 nm (red); longer infrared, "above red" or > 700nm, also called heat; and shorter ultraviolet radiation (UVR), "below violet" or < 400nm. UVR is further subdivided into UV-A or near-UV (320–400 nm), also called black (invisible) light; UV-B or mid-UV (290–320 nm), which is more skin penetrating; and UV-C or far-UV (< 290 nm).

Health effects of exposure to UV light are familiar to anyone who has had sunburn. However, the UV light level around some UV equipment greatly exceeds the level found in nature. Acute (short-term) effects include redness or ulceration of the skin. At high levels of exposure, these burns can be serious. For chronic exposures, there is also a cumulative risk of harm. This risk depends upon the amount of exposure during your lifetime. The long-term risks for large cumulative exposure include premature aging of the skin, wrinkles and, most seriously, skin cancer and cataract.

Damage to vision is likely following exposure to high-intensity UV radiation. In adults, more than 99% of UV radiation is absorbed by the anterior structures of the eye. UVR can contribute to the development of age-related cataract, pterygium, photodermatitis, and cancer of the skin around the eye. It may also contribute to age-related macular degeneration. Like the skin, the covering of the eye or the cornea, is epithelial tissue. The danger to the eye is enhanced by the fact that light can enter from all angles around the eye and not only in the direction of vision. This is especially true while working in a dark environment, as the pupil is wide open. The lens can also be

damaged, but because the cornea acts as a filter, the chances are reduced. This should not lessen the concern over lens damage however, because cataracts are the direct result of lens damage.

Burns to the eyes are usually more painful and serious than a burn to the skin. Make sure your eye protection is appropriate for this work. NORMAL EYEGLASSES OR CONTACTS OFFER VERY LIMITED PROTECTION!

> ⚠ **Caution: UV exposures are not immediately felt. The user may not realize the hazard until it is too late and the damage is done.**

1.4.1 Training

For the use of UV sources, new users must be trained by another member of the laboratory who, in the opinion of the member of staff in charge of the department, is sufficiently competent to give instruction on the correct procedure. Newly trained users should be overseen for some time by a competent person.

1.5 Additional Risks of Xenon Lamps

⚠ Warning: Xenon lamps are dangerous. Please read the following precautions.

Among the dangers associated with xenon lamps are:

- Burns caused by contact with a hot xenon lamp.
- Fire ignited by hot xenon lamp.
- Interaction of other nearby chemicals with intense ultraviolet, visible, or infrared radiation.
- Damage caused to apparatus placed close to the xenon lamp.
- Explosion or mechanical failure of the xenon lamp.

1.5.1 Visible Radiation

Any very bright visible light source will cause a human aversion response: we either blink or turn our head away. Although we may see a retinal afterimage (which can last for several minutes), the aversion response time (about 0.25 seconds) normally protects our vision. This aversion response should be trusted and obeyed. NEVER STARE AT ANY BRIGHT LIGHT-SOURCE FOR AN EXTENDED PERIOD. Overriding the aversion response by forcing yourself to look at a bright light-source may result in permanent injury to the retina. This type of injury can occur during a single prolonged exposure. Excessive exposure to visible light can result in skin and eye damage.

Visible light sources that are not bright enough to cause retinal burns are not necessarily safe to view for an extended period. In fact, any sufficiently bright visible light source viewed for an extended period will eventually cause degradation of both night and color vision. Appropriate protective filters are needed for any light source that causes viewing discomfort when viewed for an extended period of time. For these reasons, prolonged viewing of bright light sources should be limited by the use of appropriate filters.

The blue-light wavelengths (400–500 nm) present a unique hazard to the retina by causing photochemical effects similar to those found in UV-radiation exposure.

1.5.2 Infrared Radiation

Infrared (or heat) radiation is defined as having a wavelength between 780 nm and 1 mm. Specific biological effectiveness “bands” have been defined by the CIE (Commission Internationale de l’Éclairage or International Commission on Illumination) as follows:

- IR-A (near IR) (780–1400 nm)
- IR-B (mid IR) (1400–3000 nm)
- IR-C (far IR) (3000 nm–1 mm)

The skin and eyes absorb infrared radiation (IR) as heat. Workers normally notice excessive exposure through heat sensation and pain. Infrared radiation in the IR-A that enters the human eye will reach (and can be focused upon) the sensitive cells of the retina. For high irradiance sources in the IR-A, the retina is the part of the eye that is at risk. For sources in the IR-B and IR-C, both the skin and the cornea may be at risk from “flash burns.” In addition, the heat deposited in the cornea may be conducted to the lens of the eye. This heating of the lens is believed to be the cause of so called “glassblowers’” cataracts because the heat transfer may cause clouding of the lens.

- Retinal IR Hazards (780 to 1400 nm): possible retinal lesions from acute high irradiance exposures to small dimension sources.
- Lens IR Hazards (1400 to 1900 nm): possible cataract induction from chronic lower irradiance exposures.
- Corneal IR Hazards (1900 nm to 1 mm): possible flash burns from acute high irradiance exposures.

Who is likely to be injured? The user and anyone exposed to the radiation or xenon lamp shards as a result of faulty procedures. Injuries may be slight to severe.

1.6 CE Compliance Statement

The Duetta™ spectrofluorometer is tested for compliance with both the EMC Directive 2104/30/EU and the Low Voltage Directive for Safety 2014/35/EU, and bears the international CE mark as indication of this compliance. HORIBA Canada Incorporated guarantees the product line's CE compliance only when original HORIBA Canada Incorporated supplied parts are used. Section 17 herein provides a table of all CE Compliance tests and standards used to qualify this product.

1.7 RoHS Declaration of Compliance

Manufacturer: HORIBA Canada Incorporated

Address: 347 Consortium Court, London, ON N6E 2S8, Canada

Products: Duetta and Duetta-Bio spectrometers

Restriction of Hazardous Substances Declaration of Compliance

Presence of Toxic and Hazardous substances or elements:
HORIBA Instruments Inc. is working toward compliance with EU Directive 2011/65/EU and China Directive SJ/Z11388-2009 for monitoring and control of the Restriction of Hazardous Substances (RoHS).

The following components have been evaluated and are in compliance with the aforementioned directives. HORIBA Instruments Inc. hereby declares this product is RoHS and RoHS2 compliant.

Part Number	Description	Lead (Pb)	Mercury (Hg)	Cadmium (Cd)	Hexavalent (Cr6+)	Polybrominated biphenyls (PBB)	Polybrominated diphenylethers (PBDE)	Hexabromo cyclododecane (HBCDD)	Bis (2-ethylhexyl) phthalate (DEHP)	Butyl benzyl phthalate (BPP)	Dibutyl phthalate (DBP)
5700007000	Duetta	O	O	O	O	O	O	O	O	O	O
5700004300	Duetta-Bio	O	O	O	O	O	O	O	O	O	O
5700012885	SampleSnap-1Pelt	O	O	O	O	O	O	O	O	O	O
5700012859	SampleSnap-4Pelt	O	O	O	O	O	O	O	O	O	O
5700012880	SampleSnap-PLQY	O	O	O	O	O	O	O	O	O	O
5700012886	SampleSnap-LN	O	O	O	O	O	O	O	O	O	O
5700012882	SampleSnap - FF	O	O	O	O	O	O	O	O	O	O
5700012883	SampleSnap-SS	O	O	O	O	O	O	O	O	O	O
5700012884	SampleSnap-PWD	O	O	O	O	O	O	O	O	O	O
5700012881	SampleSnap-WJ	O	O	O	O	O	O	O	O	O	O
5700006701	SampleSnap	O	O	O	O	O	O	O	O	O	O
5500357868	LampSnap	O	O	O	O	O	O	O	O	O	O
Maximum concentration value tolerated by weight of homogeneous materials		0.1%	0.1%	0.01%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%

Note:
O: Indicates that this toxic or hazardous substance contained in all of the homogeneous materials for this part is below the limit requirement.
X: Indicates that this toxic or hazardous substance contained in at least one of the homogeneous materials for this part is above the limit requirement.

Notice of environment-friendly use period (SJ/T11364-2014)

This logo for the restricted use of hazardous substances in electronic and electrical products includes three components: the content of hazardous substances in the electronic and electrical products, the environmental protection use period of the electronic and electrical products, and the recyclability of the electronic and electrical products as required by The People's Republic of China. This symbol represents that this product DOES NOT contain any hazardous substances exceeding the legal threshold. This product can be recycled after being discarded and should not be casually discarded. Additionally, when discarding this product, please comply with applicable law and/or regulations of the local government.
This logo appears on the instrument.

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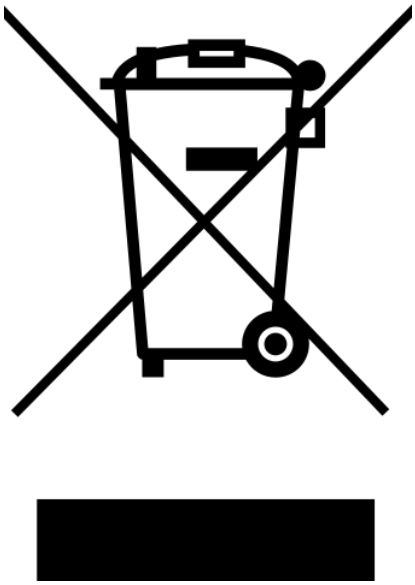
1.8 Waste Electrical and Electronic Equipment (WEEE)



WEEE Directive Symbol

This symbol on the product or its packaging indicates that this product must not be disposed of with regular waste. Instead, it is the user's responsibility to dispose of waste equipment according to local laws. The separate collection and recycling of the waste equipment at the time of disposal will help to conserve natural resources and ensure that it is recycled in a manner that protects human health and the environment. For information about where the user can drop off the waste equipment for recycling, please contact your local HORIBA Scientific representative.

2 Spectroscopy and the Duetta™

2.1 Introduction

A spectrofluorometer is an analytical instrument used to measure and record the fluorescence of a sample. While recording the fluorescence, the excitation, emission, or both wavelengths may be scanned. With additional accessories, variation of signal with time, temperature, concentration, polarization, or other variables may be monitored.

The Duetta™ spectrometer combines both fluorescence and absorbance measurements in one instrument with matching optical band pass resolution.

The main advantages the Duetta™ provides for simultaneous fluorescence and absorbance analysis include:

- Absorbance spectral information can be used to immediately correct the fluorescence spectrum for the inner-filter-effects (IFEs) involving both the excitation light-absorption and fluorescence reabsorption in the sample cuvette;
- 2-in-1 fluorescence and absorbance acquisition under the same band pass resolution eases true matching of spectral features required for accurate inner-filter-effect correction;
- 2-in-1 fluorescence and absorbance measurement allows monitoring of photobleaching of sample materials, which may be very sensitive to UV wavelengths of the exciting light used to measure the absorbance and excite fluorescence;
- Excitation and absorbance wavelengths are scanned from low-energy to high-energy (near infrared to UV) to reduce the exposure of the sample to UV and hence photobleaching;
- Absorbance data provide additional—and often vital—concentration-dependent information on non-fluorescent compounds in the sample;
- IFE correction often greatly reduces analysis and sample-preparation time, and increases accuracy by eliminating error-prone dilution procedures and record-keeping.

2.2 Introduction to Fluorescence Spectroscopy

2.2.1 What is an Absorbance or %Transmittance Spectrum?

The absorption of light is fundamental to understanding both absorbance, transmittance and photoluminescence spectroscopy. The Beer-Lambert law states that the optical density, or Absorbance (A) of a solution with a single absorbing species is proportional to the molar extinction coefficient, ε ($M^{-1}cm^{-1}$), the sample concentration, c (mol/L), and the path length of the cuvette or cell, l (cm). The absorbance is also calculated by knowing the transmittance, T of the solution.

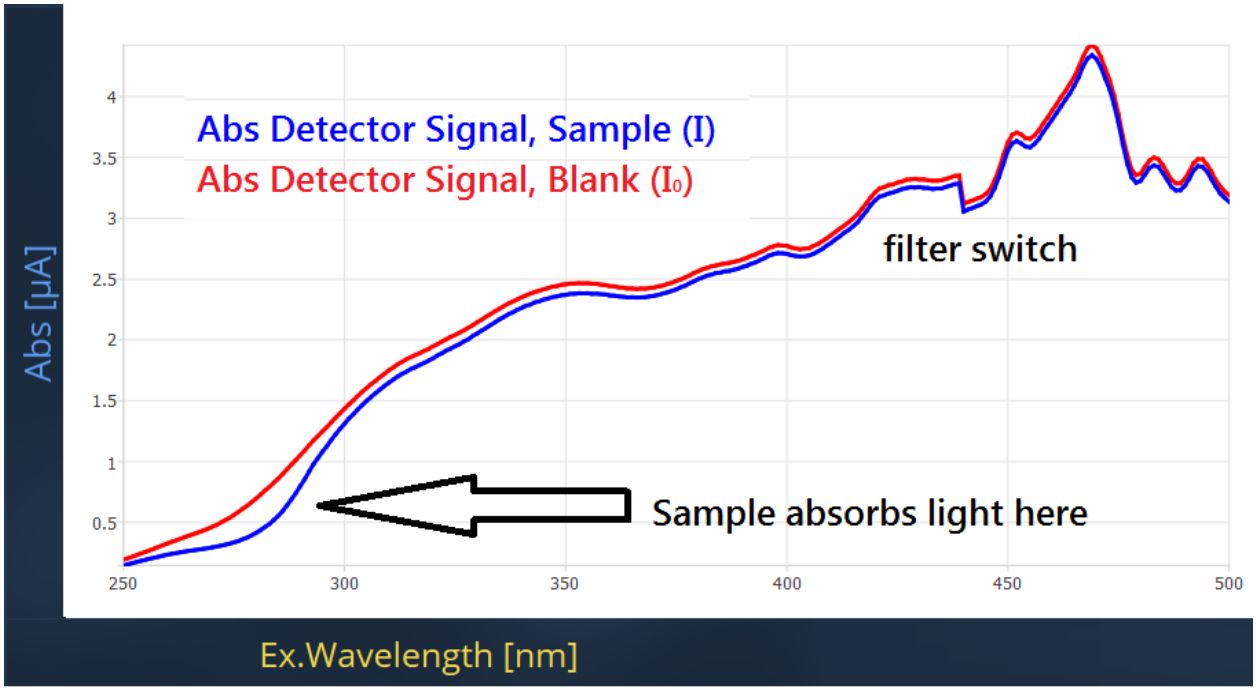
$$A = -logT = \varepsilon cl$$

To measure the transmittance of a solution, we use the incident light from the xenon lamp through the cuvette of the solution of interest. The measured incident light signal as measured through the sample is I. We compare the incident light, I, through the sample to the incident light through a cuvette of a blank solvent. This measured blank signal is called I_0. To calculate the transmission of the sample, T, we can use the other part of the derived Beer-Lambert Law below:

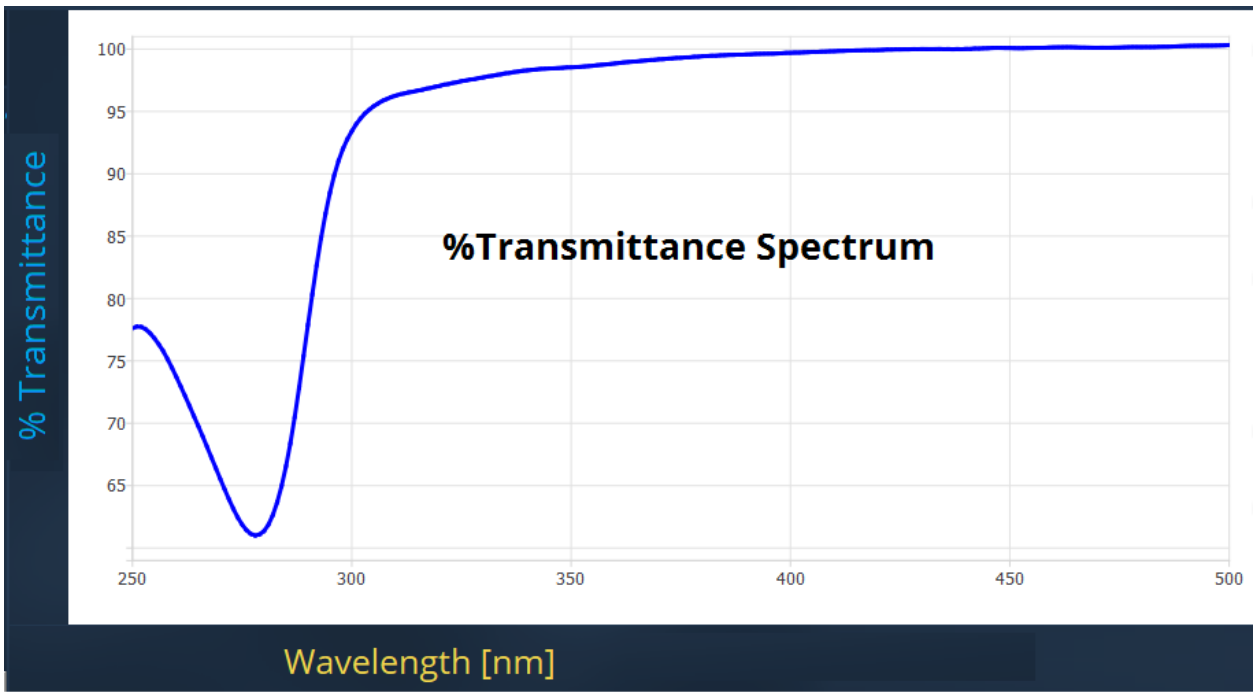
$$T = \frac{I}{I_0} \text{ and } \%T = T*100\%$$

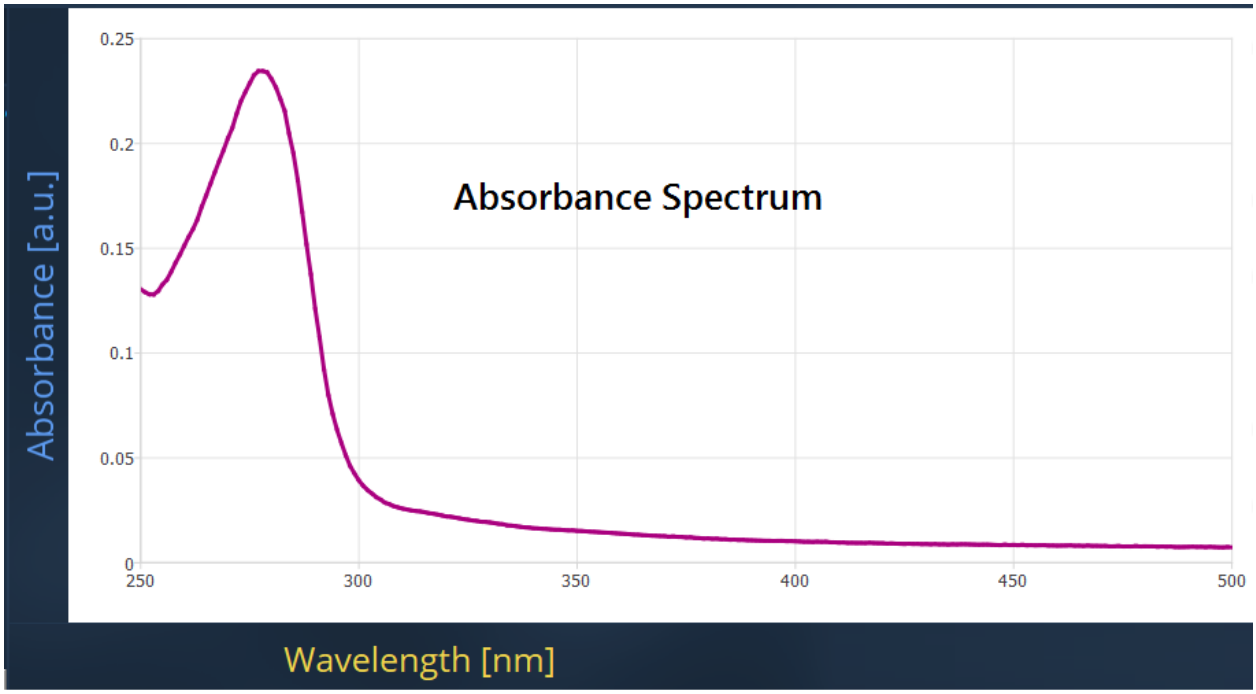
2.2.2 How is Absorbance or Transmittance Measured with Duetta?

To measure the absorbance or %Transmittance spectra, the Duetta will scan the excitation monochromator of Duetta across the wavelength range of the sample spectrum (from high wavelength to low wavelength). In doing this, the light from the xenon lamp is measured by the photodiode on the other side of the sample compartment, which is called the Absorbance detector on the Duetta (Abs). When an absorbance or %T spectrum is acquired, you will see the xenon lamp spectrum measured through the sample and again through the blank.



In the above figure, the sample is clearly absorbing in the UV region between 250 and 300 nm. See the resulting %Transmittance and Absorbance spectra below, where there is a peak in the absorbance and a dip in the transmittance at around 280 nm.





Absorbance spectra can change linearly with the concentration of the absorbing species. If the extinction coefficient is known, the concentration can be easily calculated from the absorbance spectrum. The molar extinction coefficients for many compounds are published and are dependent on the solvent in which the chromophore is dissolved.

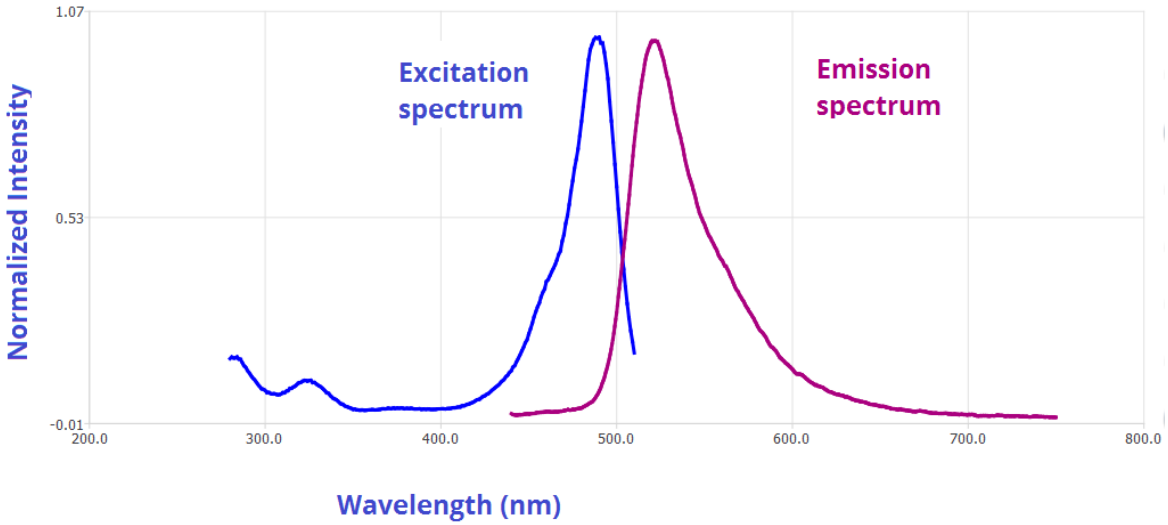
Knowing the wavelength region for which a sample absorbs is useful for characterizing the optical density, but it is also useful for choosing the appropriate excitation wavelength to use when measuring a fluorescence emission spectrum. Absorbance spectra can also be used to correct for the inner-filter effect, which is described in more detail further on in the manual.

2.2.3 What is a fluorescence spectrum?

Steady state fluorescence spectra are measured when molecules, excited by a constant source of light, emit fluorescence, and the emitted photons, or intensity, are detected as a function of wavelength. A fluorescence emission spectrum is when the excitation wavelength is fixed and the emission wavelength is scanned (or collected in the case of the CCD detector on Duetta) to get a plot of intensity vs. emission wavelength. Fluorescence is a type of photoluminescence, which broadly defined, is emission of photons by a molecule or molecules. There are other types of photoluminescence such as phosphorescence, chemiluminescence and electroluminescence.

A fluorescence excitation spectrum is 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. It is analogous to absorbance spectrum, but is a much more sensitive technique in terms of limits of detection and molecular specificity. 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 emission and excitation spectra for a given

fluorophore are mirror images of each other when viewed as fluorescence intensity vs. frequency. When viewed vs. wavelength the fluorescence emission spectrum is elongated at higher wavelengths (FWHMs are not the same). Typically, the emission spectrum occurs at higher wavelengths (lower energy) than the excitation or absorbance spectrum. The bumps in the excitation spectrum at 280 – 340 nm indicate molecular excitation to various levels of the second excited singlet state (S_2). There are no corresponding bumps in the (mirror image) fluorescence emission spectrum since molecules in higher excited singlet states usually rapidly decay to the first excited single state (S_1) via internal conversion, instead of fluorescence emission.



These two spectral types (emission and excitation) are used to see how a 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 other molecules that quench fluorescence 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.

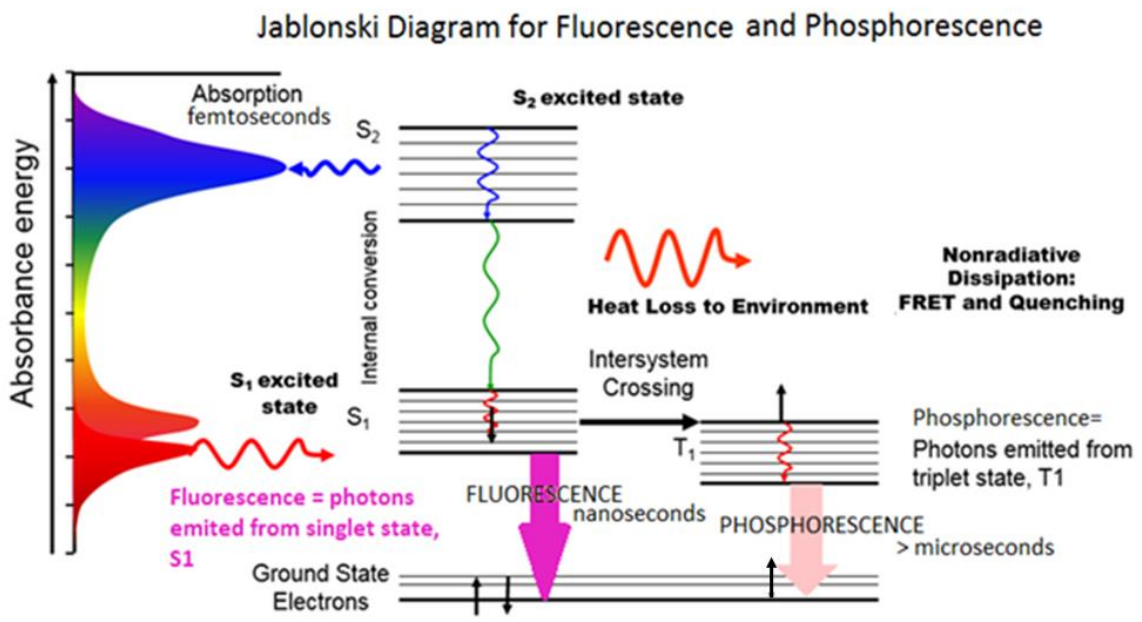
2.2.4 What is the Jablonski Diagram?

The figure below shows a Jablonski diagram, a schematic of the transition of electronic state of a molecule during the fluorescence phenomenon. The left axis shows increasing energy, where a typical fluorescent molecule has an absorbance spectrum. This spectrum shows the energy or wavelengths, where the molecule will absorb light.

If the incident light is at a wavelength where the molecule will absorb the photon, the molecule is then excited from the electronic ground state to a higher excited state, denoted S_1 or S_2 here.

The electrons then go through internal conversion, affected by vibrational relaxation and heat loss to the environment. A photon is then emitted from the lowest lying singlet excited state (S_1) in the form of fluorescence.

In conventional fluorescence, photons are emitted at higher wavelengths (lower energy) than the photons which are absorbed.

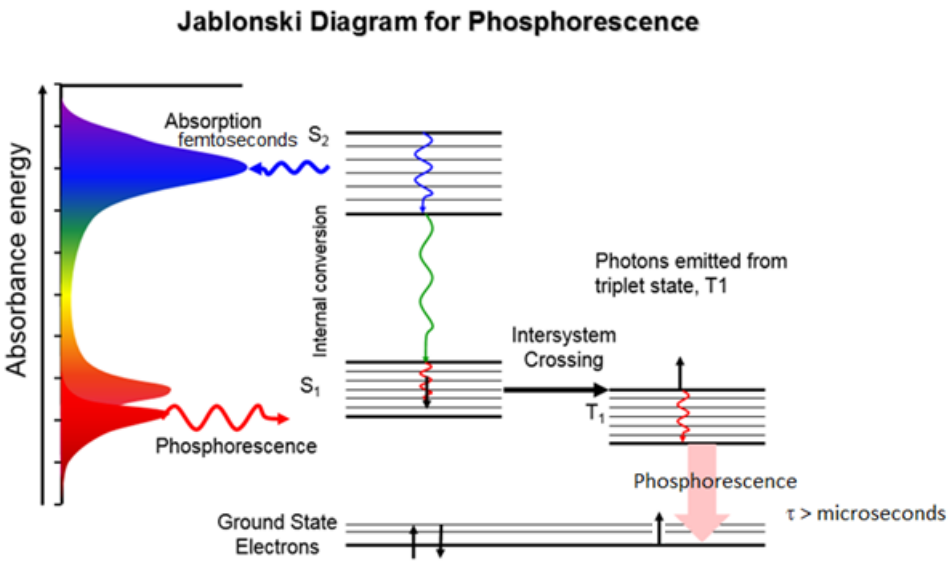


This diagram is extremely important to understand for any fluorescence spectroscopist. When measuring a fluorescence spectrum, one is typically looking at the intensity at which a molecule emits the wavelength or energy at which it emits, and also the time which the molecule spends in the excited state. This is the fluorescence lifetime, explained further in detail in coming sections.

Any number of things can affect these observables, including energy transfer to and from other molecules, quenching by other molecules, temperature, pH, local polarity, aggregation or binding. Understanding the mechanisms of these interactions can give one insight into what is being observed with a change in fluorescence spectra or lifetime. There are two non-radiative deactivation processes that compete with fluorescence: internal conversion from the lowest singlet excited to the ground state and intersystem crossing from the excited singlet state to the triplet state. This last process leads to the phenomenon called phosphorescence, explained in further detail later on.

2.2.5 What is phosphorescence?

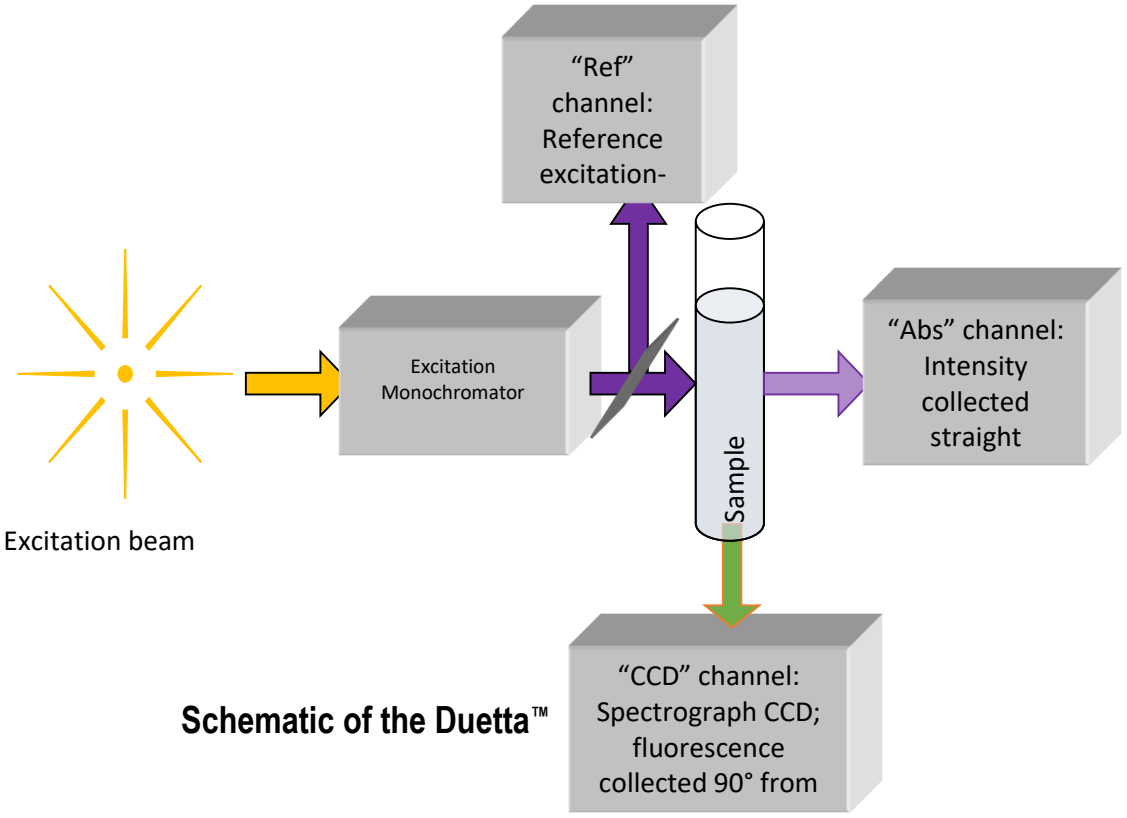
Phosphorescence is a process where the photon is emitted, not from a singlet excited state, but from a forbidden triplet state. The time scale of fluorescence emission is generally in the picosecond to nanosecond range, while phosphorescence typically lasts for microseconds, milliseconds, or even longer…minutes or hours. A pulsed source is typically used, such as a flash lamp or LED to measure phosphorescence spectra and decays on these longer time scales.



Phosphorescence measurements use a longer-lived pulsed source, such as a xenon flash lamp. The timing of the flashing lamp is used to measure spectra at different phosphorescence lifetimes. Duetta will measure steady-state phosphorescence emission, but not in a time-resolved mode since the xenon lamp is a CW lamp and not pulsed. HORIBA has other fluorometers that are compatible with various pulsed sources for time-resolved fluorescence and phosphorescence measurements.

More information is available at www.horiba.com/fluorescence

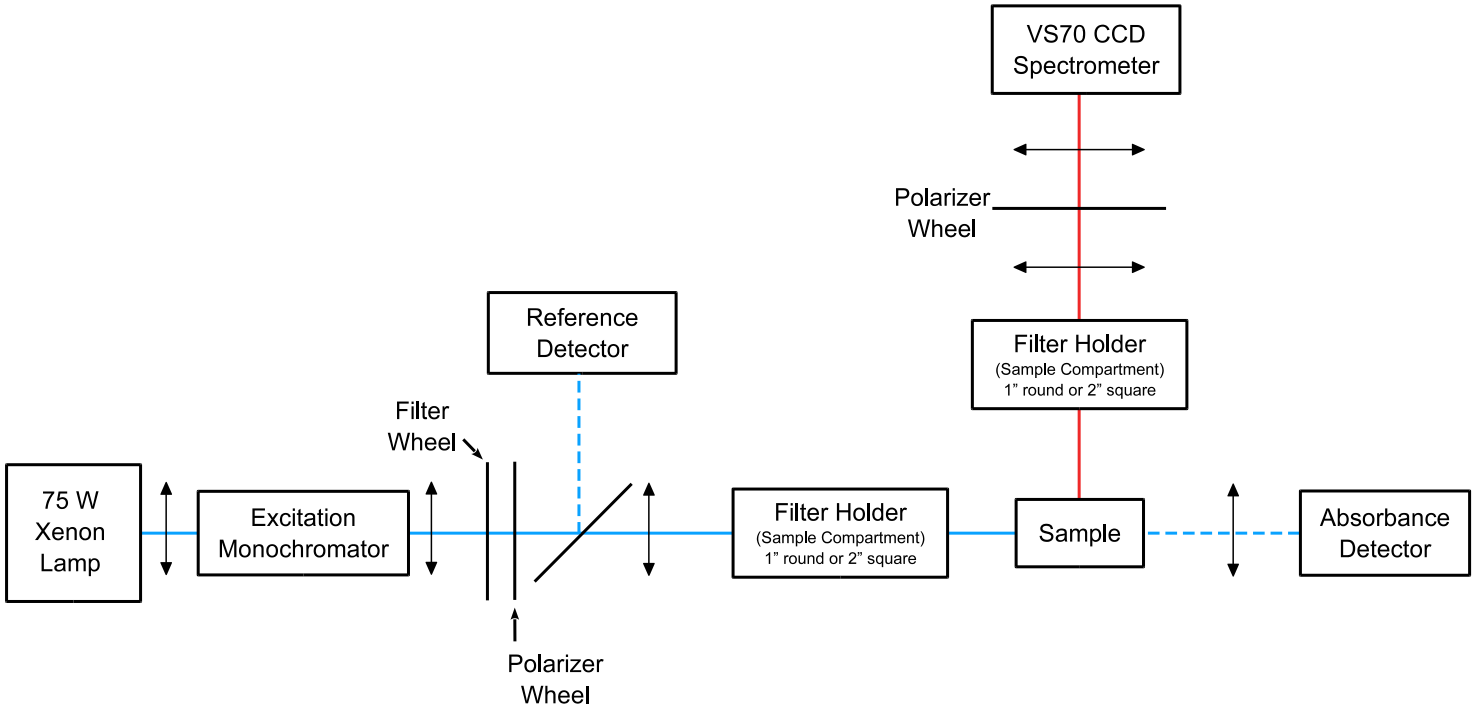
2.3 Basic Theory of Operation



Schematic of the Duetta™

A continuous source of light shines onto an excitation monochromator which selects a narrow band of wavelengths. The produced excitation light is then sent in two directions: 1) a small portion is reflected off a beam splitter to the reference detector ("Ref" channel) to be used as a normalization for excitation-lamp variations; and 2) the rest of the light is directed onto the sample, which emits luminescence in all directions. At right-angles to the excitation beam, some of the sample's luminescence is directed into a multichannel CCD detector ("CCD" channel), which reports a fluorescence spectrum. Co-linear with the excitation beam, a single-channel Absorbance detector ("Abs" channel) receives excitation light not absorbed by the sample plus luminescence from the sample and is used to measure an absorbance spectrum. The signals from the detectors are reported to a system controller and host computer, where the data can be manipulated and presented, using the EzSpec™ software.

2.4 Duetta™ Optical Block Diagram



2.5 Overview of Analysis of Samples

2.5.1 Spectral correction: Wavelength-dependent detector response

Because most studies rely on comparison to traceable spectral and concentration standard samples, the spectral correction of the EEM is of prime concern. A typical EEM scans the sample across the excitation wavelengths from about 250-1000 nm, and across the emission wavelengths from 250–1100 nm. Band pass and resolution are selectable from a set of fixed slit widths. To account for variations in the excitation beam's intensity, a reference detector, *Ref*, collects a small fraction of the excitation beam, and the emission CCD detector's output, *CCD*, is ratioed to the reference detector signal (*CCD*/*Ref*).

However, the instrument's optical responsivity is not ideal throughout the wavelength-range of the experiment, so a series of instrumental spectral correction factors must be used to obtain reproducible ideal spectra that are traceable to established, calibrated spectral standard samples, detectors, and light sources.

- Dark current signals must be subtracted from the *CCD* detector signal, and background signals must be subtracted from the *Ref* and *Abs* detector signals.
- The *CCD* and *Ref* detectors' signals must also be respectively multiplied by the excitation ($X_{correct}$) and emission ($M_{correct}$) spectral correction factors.

It follows that the final signal plotted as a function of wavelength in an EEM involves both the corrected reference signal, $Ref_{Correct}$,

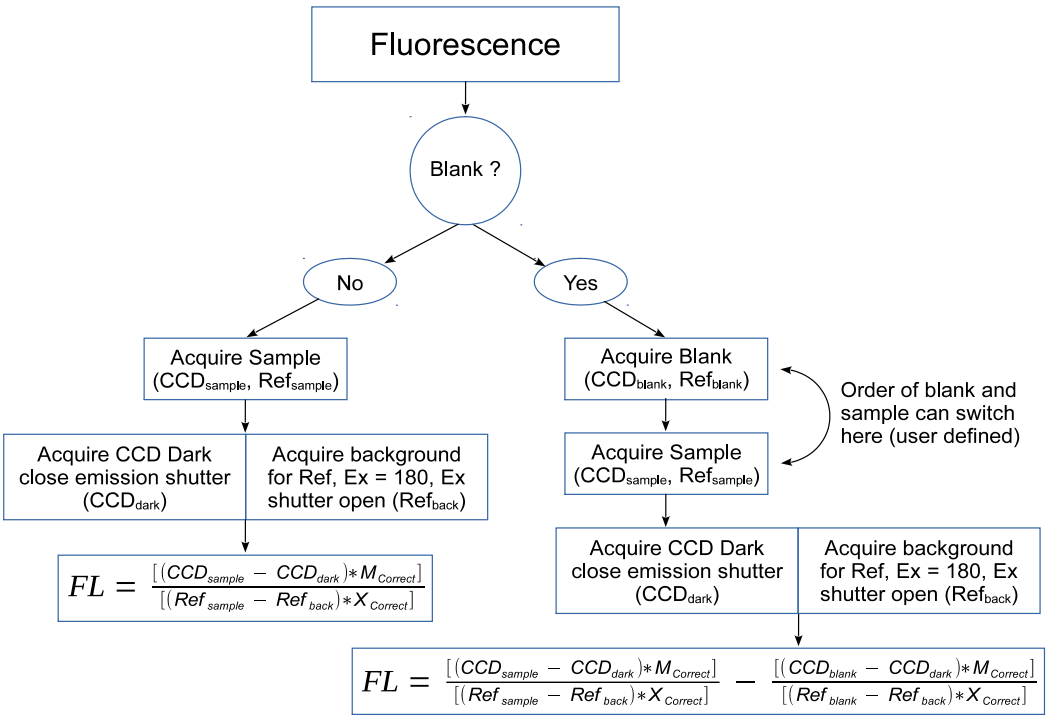
$$Ref_{correct} = \left(Ref_{sample} - Ref_{back}\right) * X_{correct}$$

and the corrected emission-detector signal, $CCD_{Correct}$,

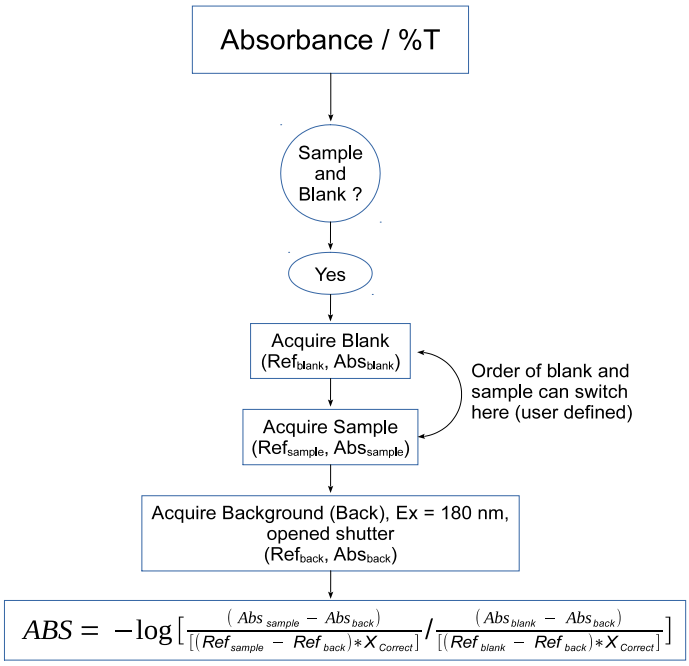
$$CCD_{correct} = \left(CCD_{sample} - CCD_{dark}\right) * M_{Correct}$$

The final fluorescence signals recorded are thus $CCD_{correct,\ sample}/Ref_{correct,\ sample}$ for the sample to be evaluated and $CCD_{correct,blank}/R_{correct,blank}$ for a blank sample as shown in the flowchart below.

> ⓘ Note: The emission correction factors are validated only in the range of 320-800 nm where suitable standard reference materials are available. No validation is provided for corrections outside this range.



Simultaneous to the EEM, the sample's spectral transmittance and absorbance properties can be recorded with the Duetta™. Within the Duetta™, the absorbance detector signal, $Abs_c = Abs$ – background signal, is used to calculate the absorbance (ABS) and transmittance (T) values. The absorbance detector's signal, Abs_c, is also corrected for the excitation-source intensity measured using the corrected reference detector signal ($Ref_{Correct}$) formulated below as $Abs_c / Ref_{Correct} = I_0$ from a blank and $I = Abs_c / Ref_{Correct}$ from the sample to be evaluated as per below:

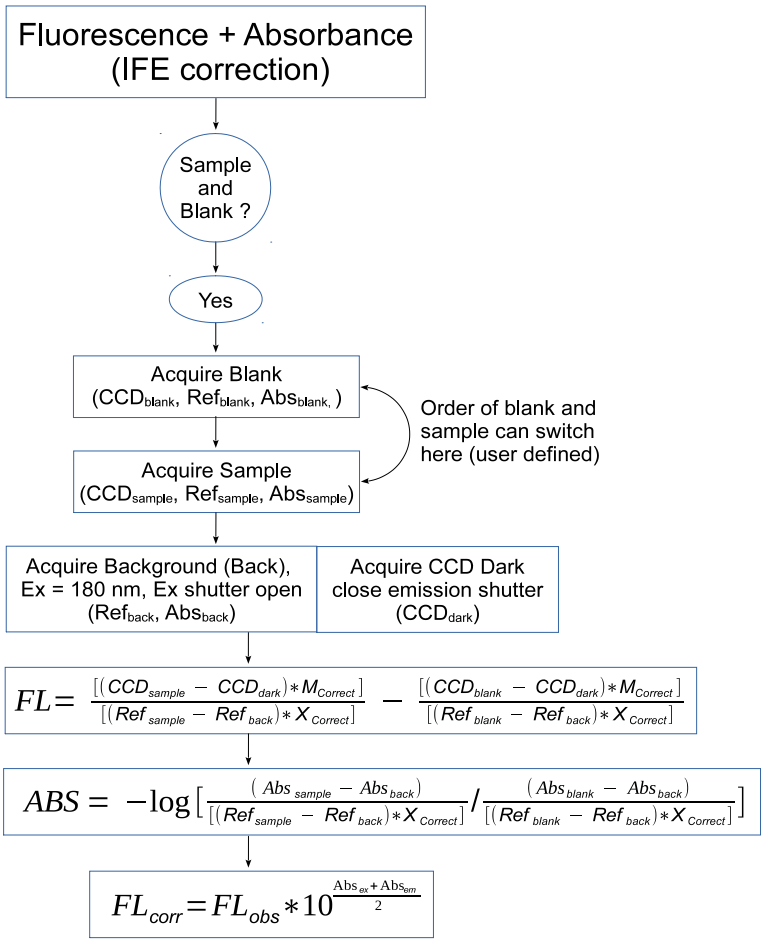


2.5.2 EEM spectral correction: primary and secondary inner-filter effects

Common, recommended practice is to correct the EEM data for inner-filter effects (IFE) using the parallel absorbance measurements from the sample and blank as mentioned above. One obvious criterion for accurate IFE is the requirement for the concentration of the sample to fall within the linear Beer-Lambert region for the absorbance spectral region associated with the EEM. The IFE algorithms used in EzSpec™ involve measuring the absorbance spectrum of the sample for the overlapping range of both the excitation and emission spectra to correct for both the primary and secondary IFEs. The basic IFE algorithm employed in the EzSpec™ software requires use of conventional 1 × 1 cm path-length cuvettes. The equation below is applied to each excitation-emission wavelength coordinate of the EEM:

$$F_{ideal} = F_{obs} \times 10^{\frac{Abs_{Ex} + Abs_{Em}}{2}}$$

where F_{ideal} is the ideal fluorescence-signal spectrum expected in the absence of IFE, F_{obs} is the observed fluorescence signal, and Abs_{Ex} and Abs_{Em} are the measured absorbance values at the respective excitation and emission wavelength-coordinates.



2.5.3 EEM spectral correction: blank-subtraction, Rayleigh-masking and Raman scattering

The current practice for EEMs involves measuring the excitation and emission scan-ranges, which includes their overlap regions. These overlap regions manifest in intense signals from the scattered photons from the monochromatic excitation source in the emission detector's response. These lines are caused by the first-order Rayleigh-scattering features consistent with the well-known grating equation. Additionally, another spectral feature is the Raman scattering line. The Raman scattering line is related to the Rayleigh scattering line by a constant energy shift. Most component libraries contain spectra for which the Rayleigh and Raman spectral features have been removed, and hence EEM data is usually processed to remove both the Rayleigh and Raman scattering features systematically. The EzSpec™ software package can remove both types of scattering peaks. Subtraction of the blank EEM from the sample EEM effectively removes the Raman scatter line. Applying a Rayleigh-masking algorithm based on the excitation and emission spectral bandwidth nullifies the signal intensities for the first-order Rayleigh lines.

A number of advanced algorithms described in the literature can also account for variations of the optical geometrical parameters of the cuvette path-length, beam- or slit-width, and positioning/shifting of the cuvette relative to the excitation and emission beam paths. However, the fixed optical geometry of the Duetta™ lends itself to the simple solution above because neither the slit-widths that determine the beam geometry, nor the path-lengths or overlap volume of the absorbance and emission paths are user-adjustable. Moreover, IFE corrections are generally only important when the absorbance values exceed 0.05 in a 1 cm path-length, so there is generally little information to be gained in the EEM from either an extended or shortened path-length cell. The fixed geometry of the Duetta™ further lends the use of the instrument to support valid intra- and inter-laboratory comparisons by eliminating variances in the chief parameters of absorbance and emission path-length. The fixed optical geometry also makes accurate and reproducible spectral correction easy as well as easy validation of such with standard traceable samples.

2.5.4 EEM spectral correction: nonlinear least-squares and multivariate spectral analyses

As required by the research community, the concerted application of the instrumental spectral corrections, Rayleigh-line masking, IFE correction, and normalization are readily enabled by the EEM-processing tools in our EzSpec™ software. As mentioned above, the purpose of the spectral corrections and EEM-processing is to make the identification and quantification easier of the components that are usually based on a reference-component library or model.

3 Requirements & Installation

3.1 Safety-training requirements

Every user of the Duetta™ must know general and specific safety procedures before operating the instrument. For example, proper training includes (but is not limited to):

- Understanding the risks of exposure to ultraviolet, visible, and infrared light, and how to avoid unsafe exposures to these types of radiation
- Handling xenon lamp bulbs, and their dangers
- Safe handling for all chemicals and other samples used in the instrument

3.2 Surface requirements

- A sturdy table- or bench-top
- Surface must hold 36 kg (80 lbs.).
- Surface should be about 66 cm × 91 cm (26 in × 36 in) to hold the Duetta™, computer, and accessories comfortably.
- Overhead clearance should be at least 76 cm (30 in).

3.3 Environmental requirements

⚠ **Caution: Excessive humidity can damage the optics.**

⚠ **Caution: For adequate cooling, do not cover, block, or obstruct the vents on the back side and underside of the instrument.**

The environmental requirements are:

- Temperature 15–30 °C (59–86 °F)
- Maximum temperature fluctuation ± 2°C
- Maximum relative humidity 75% non-condensing
- Low dust levels
- No special ventilation

3.4 Electrical requirements

⚠ Caution: HORIBA Instruments Incorporated is not liable for damage from line surges and voltage fluctuations. A surge protector is strongly recommended for minor power fluctuations. For more severe voltage variations, use a generator or uninterruptible power supply. Improper line voltages can damage the equipment severely.

⚠ Warning: The Duetta™ is equipped with a three-conductor power cord that is connected to the system frame (earth) ground. This ground provides a return path for fault current from equipment malfunction or external faults. For all instruments, ground continuity is required for safe operation. Any discontinuity in the ground line can make the instrument unsafe for use. Do not operate this system from an ungrounded source.

The Duetta™ operates from universal AC single-phase input power over the range of 100 - 240 V AC with a line frequency of 50 to 60 Hz. This AC input power is applied to a two-pole fusing power entry module located on the side of the instrument. This module incorporates two IEC approved, 2.0 A, 250 V, Time Delay fuses to protect against line disturbances or anomalies outside the system's normal operating range.

Have enough outlets available for:

- Host computer (PC)
- Monitor
- Duetta™

ⓘ Note**:** HORIBA Scientific recommends connecting the host computer, monitor, and printer to a single surge-protector, to make start-up more convenient, and to conserve AC outlets. Connect the Duetta™ to a separate line, if possible, to isolate the xenon-lamp power supply inside the Duetta™.

3.5 Unpacking and Hardware Installation

3.5.1 Introduction

The Duetta™ spectrofluorometer system is delivered in a single packing carton. If a host computer (PC) is ordered as a part of the system, the PC is delivered in a few clearly labeled boxes. All accessories, cables, software, and manuals ordered with the system are included with the delivery.

Examine the shipping boxes carefully. Any evidence of damage should be noted on the delivery receipt and signed by representatives of the receiving and carrier companies. Once a location has been chosen, unpack and assemble the equipment as described below. To avoid excessive moving and handling, the equipment should be unpacked as close as possible to the selected location.

ⓘ Note: Many public carriers will not recognize a claim for concealed damage if it is reported later than 15 days after delivery. In case of a claim, inspection by an agent of the carrier is required. For this reason, the original packing material should be retained as evidence of alleged mishandling or abuse. While HORIBA Canada Incorporated assumes no responsibility for damage occurring during transit, the company will make every effort to aid and advise.

⚠ **Caution: The spectrofluorometer system is a delicate instrument. Mishandling may seriously damage its components.**

Duetta™ carton contents

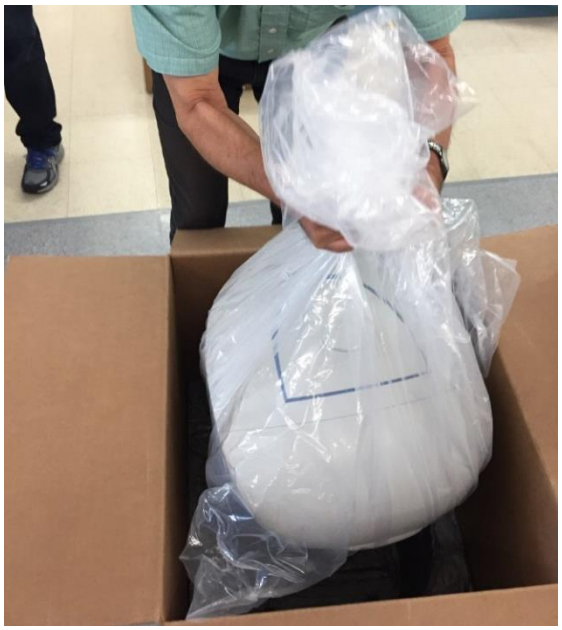
Quantity	Item	Part number
1 of:	Duetta™	5700007000
	Duetta-Bio ™	5700004300
1	USB cable	5500980087
1	USB stick with Duetta™ Operation Manual and EzSpec™ software package	5700973169
1	LampSnap	5500357868
1	Injection Port	5700006790
1	Air intake filter	5700006697
2	Custom filter holder insert 2 inch square	5700006644
2	Adapters for 1 inch round filters	5700004301
1	SampleSnap	5700006701
1 of	Mains Power cord (110 V) (220 V)	5500098015 5500098020

3.5.2 Directions for Installation

⚠ **Caution: Heavy Lifting Hazard!**

⚠ **Caution: Hand Crushing Hazard!**

1. Unpack the Duetta™.
    a. Move the shipping carton holding the Duetta™ to a location near its final location.
    b. When opening the Duetta™ shipping carton, examine each component for possible shipping damage.
    c. With a utility knife cut through the taping holding the top flaps of the shipping carton.
    d. Open the top flaps of the shipping carton.
    e. Remove the top foam injected cover from the carton.
    f. Remove the component boxes from the top level of the carton and place them on a table.
    g. Remove the next foam-injected layer from the carton.
    h. The Duetta™ is wrapped in a plastic bag sitting on a foam-injected bottom layer. Gather the top of the plastic bag, lift the Duetta™ out of the shipping carton and place it on a level surface near to its final location.



i. The Duetta™ is heavy.

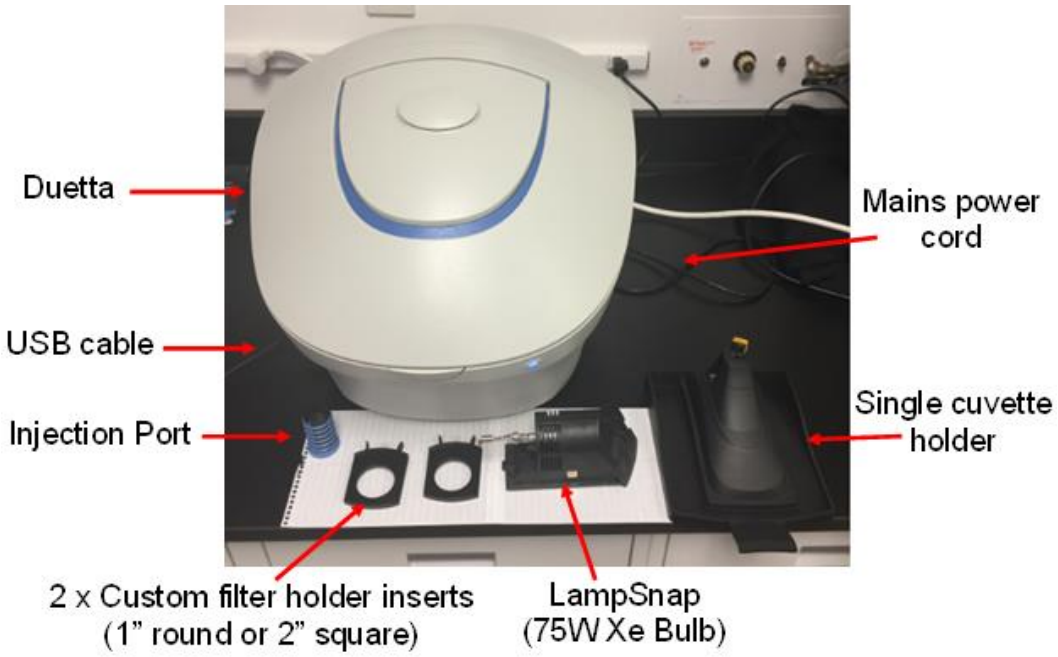
Do **NOT** lift the Duetta™ by the sides or with hands under the air intake or exhaust vents.

When moving or lifting the Duetta™, hold the bottom of the Duetta™ by the center front and center back.

j. Push the plastic bag down to the table, lift the Duetta™ up enough to remove the plastic bag from underneath, and then position the Duetta™ where the system will stay.

k. Inspect for previously hidden damage. Notify the carrier and HORIBA Scientific if any is found.

I. Check the packing list to verify that all components and accessories are present. The figure below depicts the individual system components.



Also included: EzSpec software and user manual on a USB stick

2. Setup the Duetta™.
   Do not install the power (mains) cord yet.

a. Remove the Lamp Assembly box from the shipping carton.




⚠ **Caution: The LampSnap is delicate. Mishandling may seriously damage it. Exercise extreme caution while installing or removing the lamp to avoid bumping the lamp against anything (otherwise it could cause lamp breakage or damage to the equipment).**

⚠ **Caution: Compact arc lamps contain highly pressurized gas, and present an explosion hazard even when cold. Wear face protection, such as a protective face shield, and gloves whenever handling lamps.**

⚠ **Caution: Do not operate the Duetta™ where volatiles are present (otherwise it could cause fire or explosion).**

⚠ **Caution: Never touch the quartz envelope with bare hands. Such handling may lead to deterioration and premature lamp failure. If the quartz envelope is dirty or has been accidentally handled with bare hands, clean the quartz envelope with an alcohol swab to remove any residue.**

⚠ **Caution: Do not operate the Duetta™ with any other lamp. Only the LampSnap is approved for installation in the Duetta™.**

⚠ **Caution: Use the LampSnap only in the Duetta™. Do not make any other use of the LampSnap (otherwise it could cause lamp breakage or damage to the equipment).**

b. On the lower right side of the Duetta™ open the lamp housing door by pushing down on the latch at the top of the door.

c. Pull the lamp access panel up and away from the Duetta™.

d. Rotate the blue lamp latch 90° left or right to unlock it.

e. Open the box and lift out the top foam layer to show the LampSnap module in its plastic bag.

⚠ **Caution: Never touch the quartz envelope with bare hands. Such handling may lead to deterioration and premature lamp failure. If the quartz envelope is dirty or has been accidentally handled with bare hands, clean the quartz envelope with an alcohol swab to remove any residue.**

f. Remove the plastic bag with the LampSnap from the box, open the bag, remove the LampSnap and place it on the table. Do NOT lay the LampSnap on the foam as this will cause static to attract dust and other particles to the lamp surface which will burn on the surface and shorten the life of the lamp.

g. Orient the front bottom edges of the LampSnap module with the top and bottom grooves in the lamp housing.

h. Slide the LampSnap module into the lamp housing.

i. Push on the tab on the end of the LampSnap module to make sure it is fully seated.

j. Lock the LampSnap module in place by rotating the blue latch 90° clockwise to an upright position. The latch should sit in a vertical groove below the center of the tab.

k. Re-install the lamp access panel.

3. Install accessories
   Install any accessories that arrived with the system, using the instructions that accompany the accessories.
   See section 12 for a detailed list of components and accessories.
4. Set up the computer.
   The information gathered by the Duetta™ is displayed and controlled through the host PC via EzSpec™ software. The host PC may be purchased from HORIBA Scientific or another supplier.
   a. Set up the host PC reasonably close to the Duetta™ system. The limitation is the length of the USB cable. The recommended location for the PC is just to the right of the spectrofluorometer, but other positions are possible.
   b. Follow the instructions for the host PC to set up the computer system, including the CPU, monitor, keyboard, and mouse.

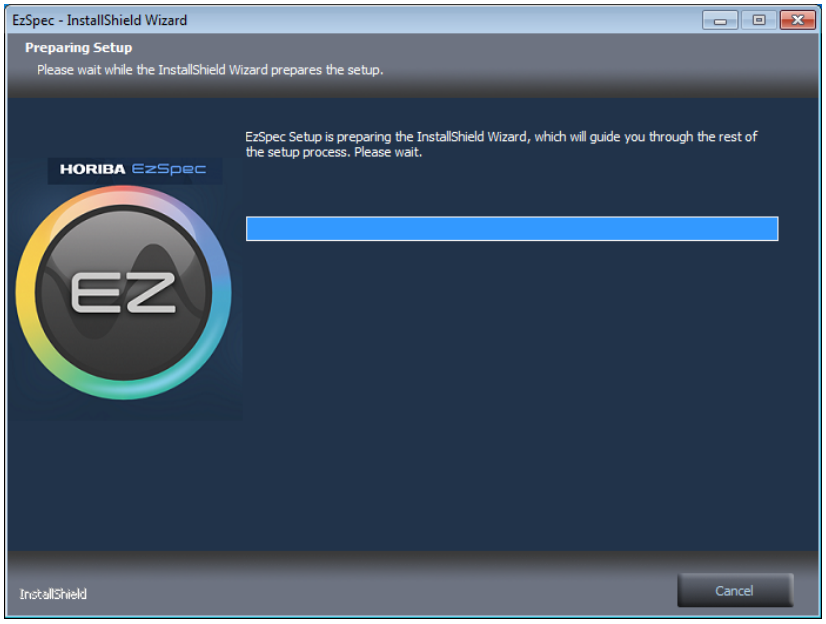
3.6 EzSpec™ Software Installation.

Before the EzSpec™ software can be installed, Windows® must be installed already and operating properly. Refer to the Windows® manual that came with the computer for installation instructions.

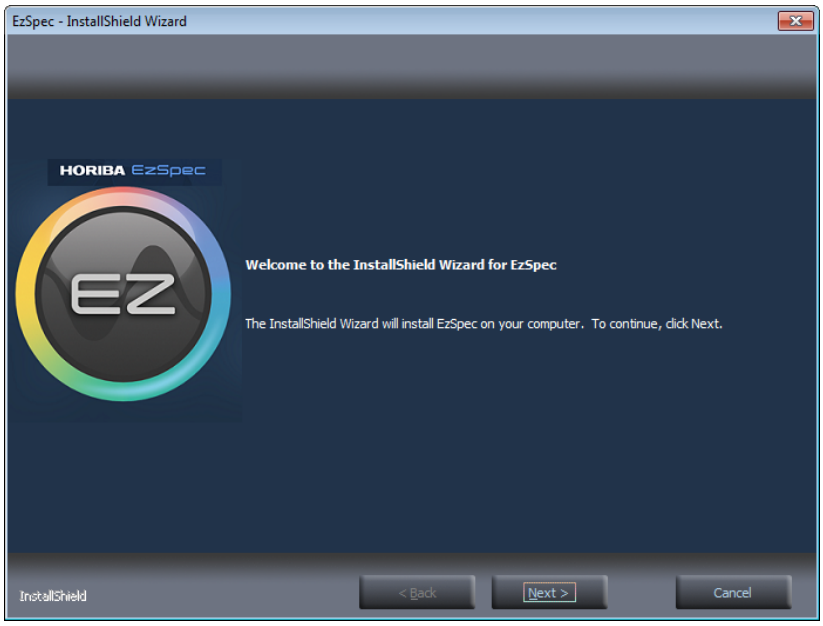
The Duetta™ is controlled by EzSpec™ spectroscopy software operating within the Windows® environment. If the computer and software were purchased from HORIBA Scientific, the EzSpec™ software installation is complete. If the computer is not from HORIBA Scientific, perform the installation. Contact a HORIBA Scientific Sales Representative for recommended specifications for a suitable host computer.

The EzSpec™ software is supplied on a USB stick.

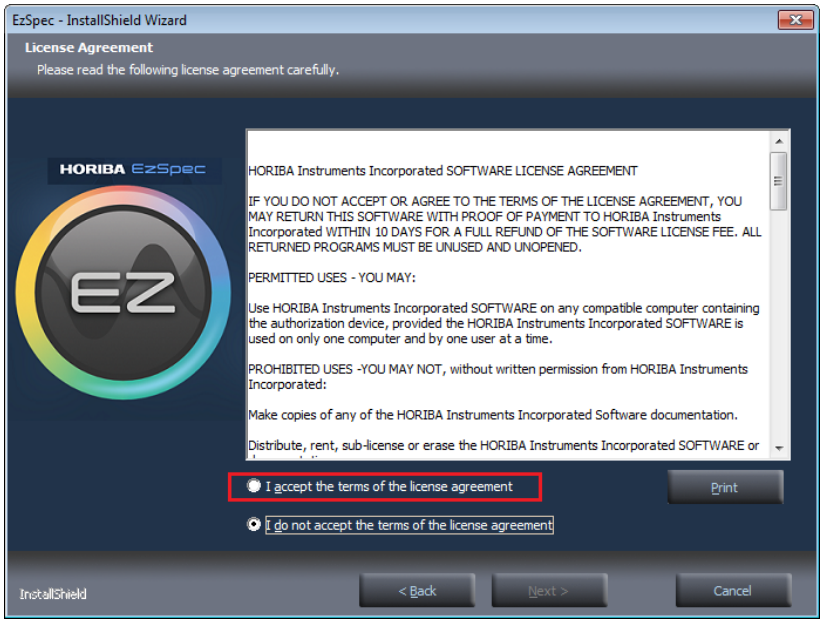
1. Insert the USB stick in a USB port on the computer.
2. In the EzSpec Software folder, double-click on **setup.exe**. The Preparing Setup window appears.



3. When the Preparing Setup window is finished, the Welcome to the InstallShield Wizard for EzSpec appears. Click **Next >**.

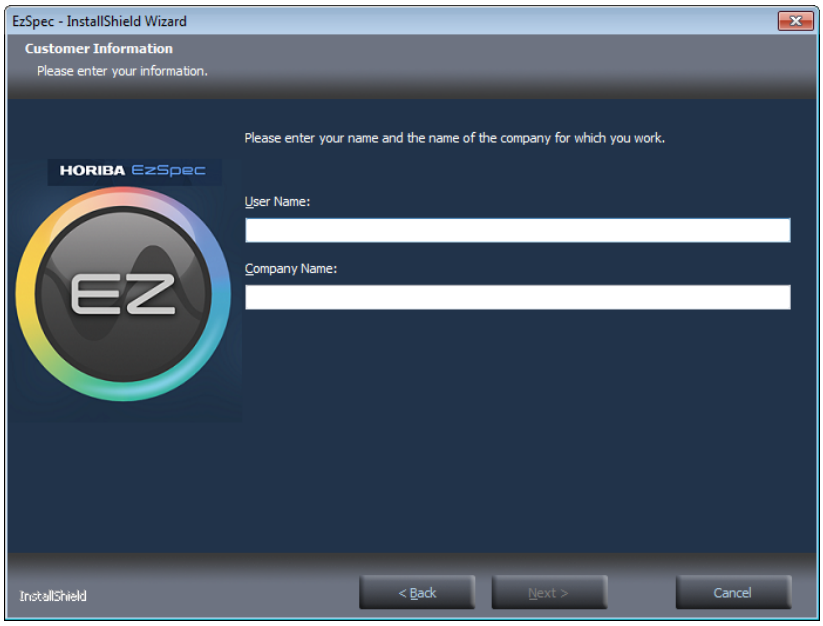


4. The License Agreement window appears.

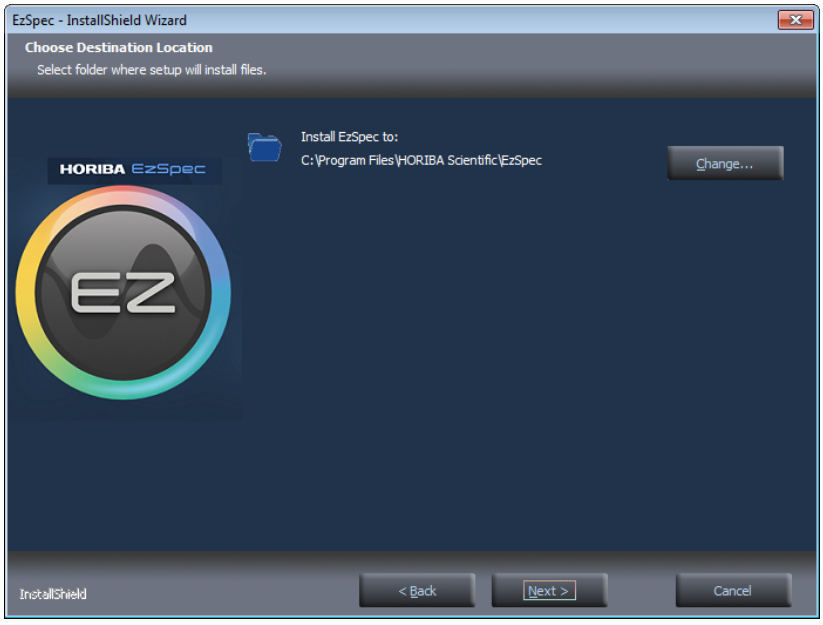


5. Click on I accept the terms of the license agreement, and then click on **Next >**.

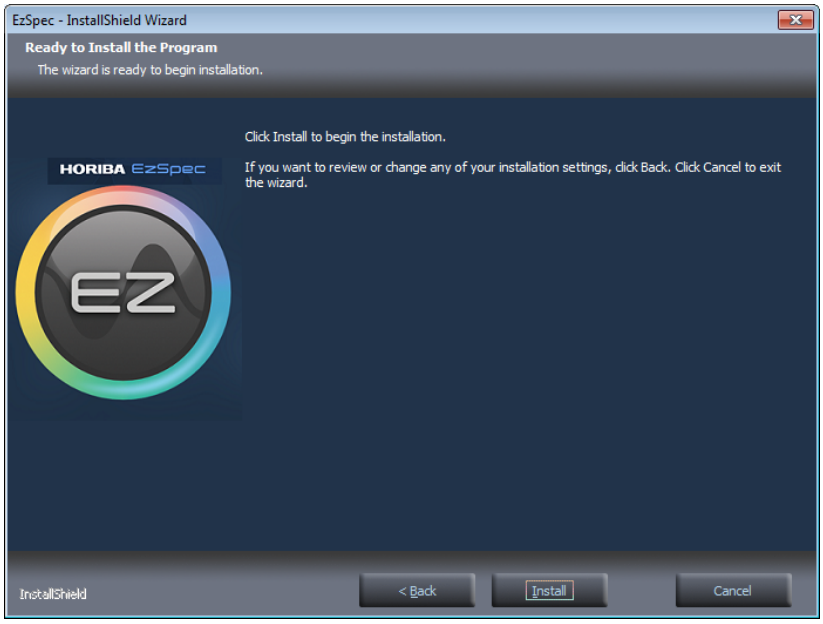
6. The Customer Information window appears. Enter your User Name and Company Name, and then click on **Next >**.



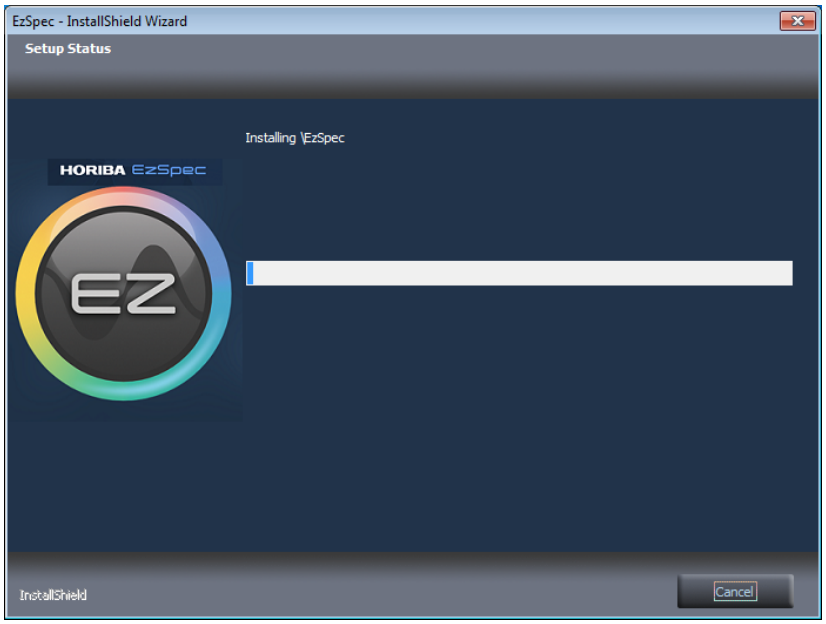
7. The Choose Destination Location window appears. Click **Next >**.



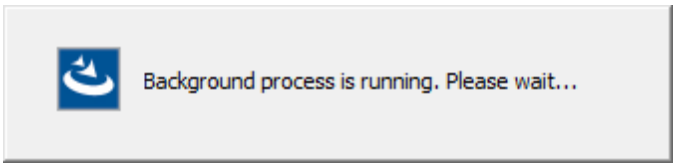
8. The Ready to Install the Program window appears. Click **Install**.



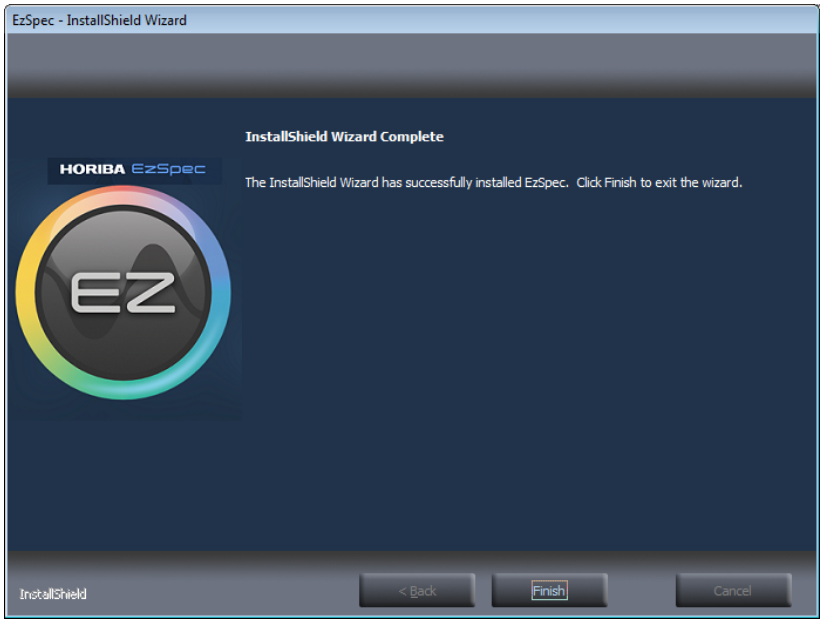
9. The Installing EzSpec window appears.
   This may take a few minutes.



10. You may see “Background process is running. Please wait…”



11. The InstallShield Wizard Complete window appears. Click **Finish**.



12. Plug one end of the power cord into the power receptacle on the back side of the Duetta™.

13. Plug one end of the USB cable into the USB receptacle on the left side of the Duetta™.

14. Attach the free end of the USB cable to a USB receptacle on the host computer.

4 Hardware Description

Warning: Do not open the instrument without proper training, appropriate protection, and having read this operation manual. The instrument contains dangerous voltages, ultraviolet, visible, and infrared radiation, and fragile light-sources. In addition, tampering with the optical components can irreversibly damage them.

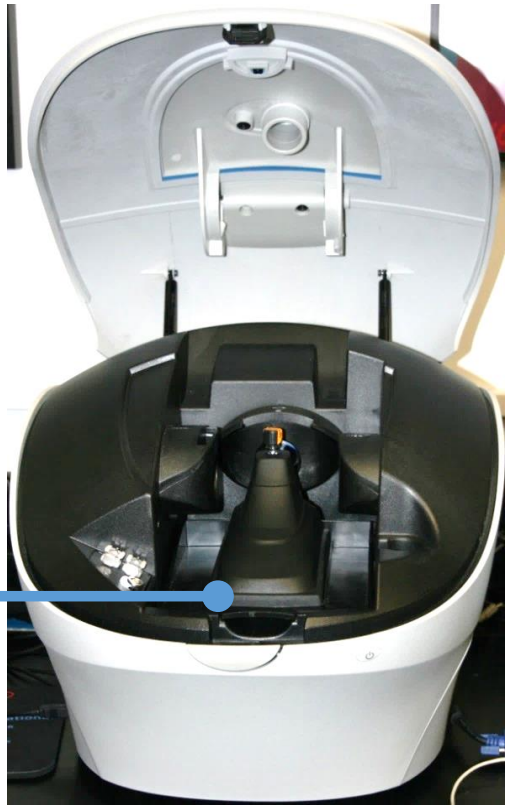
4.1 Hardware description

4.1.1 Sample Compartment - Changing a Sample Holder

1. Push on the button under the top cover to unlatch the cover.

The top cover swings up to show the sample compartment.

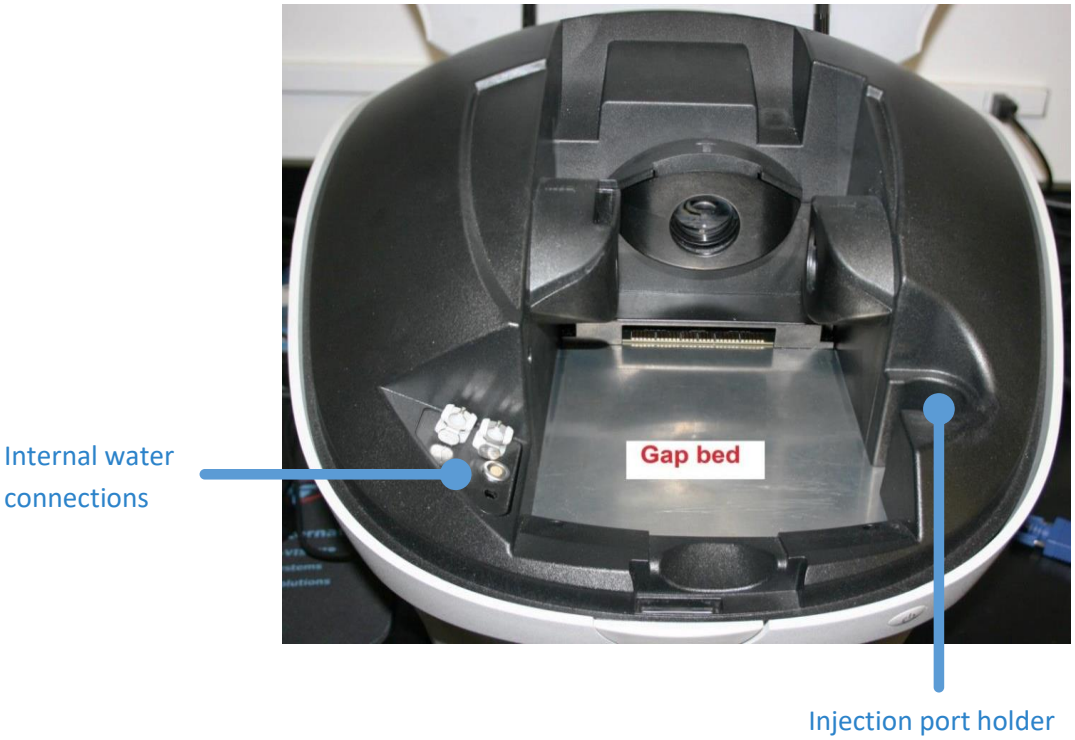
2. To remove the gap bed drawer containing a sample holder, pull the handle on the gap bed drawer back about 2 cm (1 in), and then lift the gap bed drawer up and out of the sample compartment.



Top view of a gap bed drawer with a single cuvette sample holder. All gap bed drawers have a raised edge on all sides to capture spills if necessary.

Bottom view of a gap bed drawer. On the bottom of each gap bed drawer there is a row of electronic contacts.

The electronic contacts on the bottom of the gap bed drawer fit into a socket with complementary contacts in the back of the sample compartment gap-bed. The self-identification of different gap bed drawers and sample holders takes place via these contacts.



On the left are internal water connections to help regulate optional temperature controlled sample holders.

On the right is a place to hold an injection port to be inserted into the injection access port.

3. Place the gap bed drawer with a sample holder onto the gap bed and slide it forward so the electrical contact row fits into the socket.
4. Pull the front edge of the top cover down until it latches.

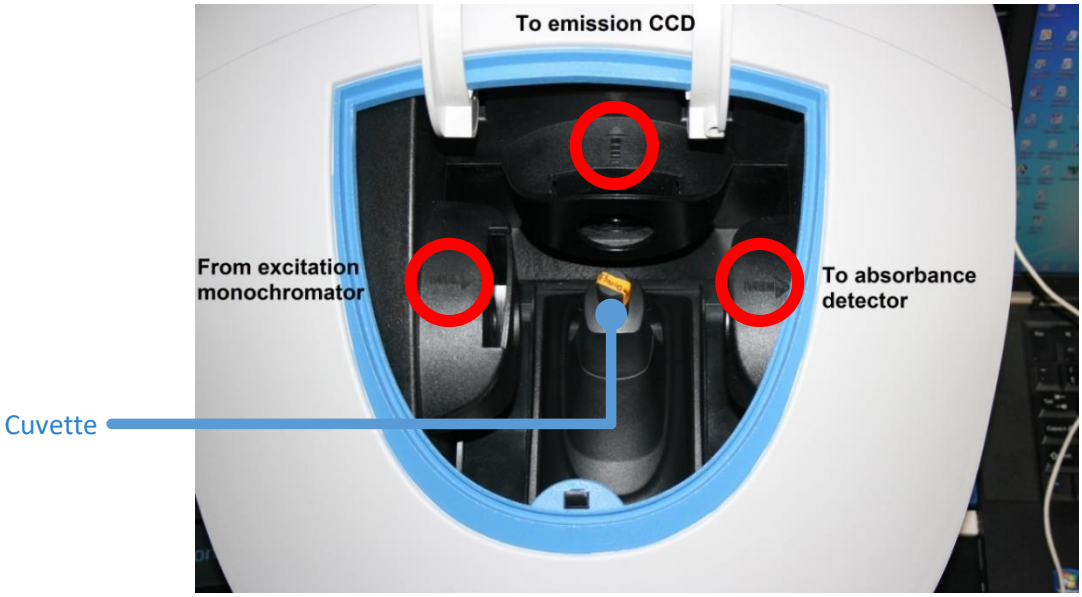
4.1.2 Sample Cover – Changing a Cuvette

1. Push down on the front edge of the sample cover to release it.

The sample cover springs up.

The sample cover provides access only for changing samples and user-accessible optical filters.

Embossed arrows show the directions of the light path.



2. Remove the cuvette and insert a new one.
3. Lower the sample cover until it latches.

4.1.3 User accessible optical filters

Other than controlling which filter position is in the optical path, the optical filters installed in the excitation or emission filter wheels are not accessible by the user.

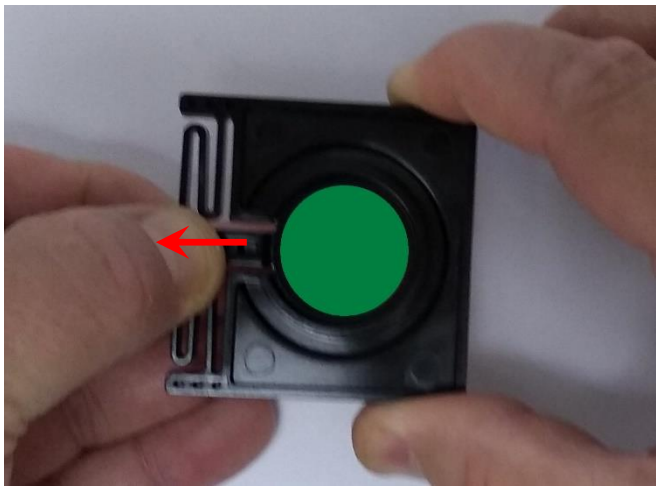
For other filter choices, filter holders with one-inch diameter filters (25 mm filters will also fit) can be placed in the excitation and emission optical paths in the sample chamber.

4.1.3.1 Assembling and inserting filter holders

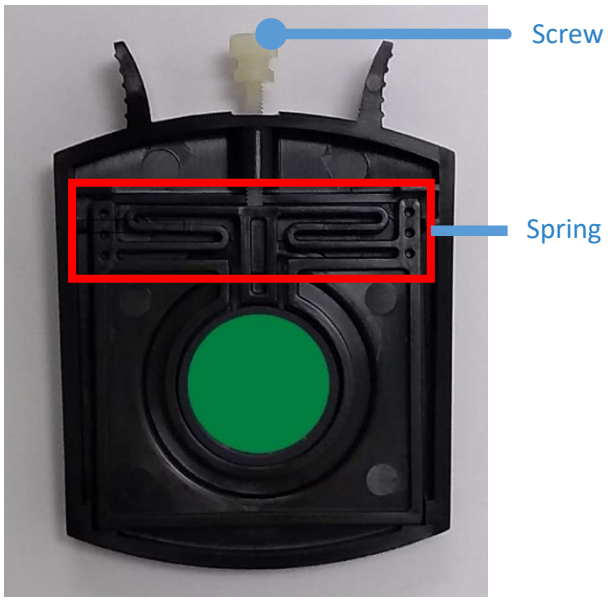
Each filter holder consists of a filter holder and a filter holder insert.

1. Place the filter holder insert on a table with the wide hole shoulder facing down.
2. Place a one-inch filter in the hole so that one edge of the filter is sitting on either the top or bottom inside edge of the hole.

3. Gently pull the top edge of the filter holder insert away from the filter so that the filter falls into and sits on the bottom of the hole.
4. With the curved side of the filter holder facing down, place the filter holder insert into the filter holder so that the wide shoulder of the filter holder insert fits into the filter holder hole.

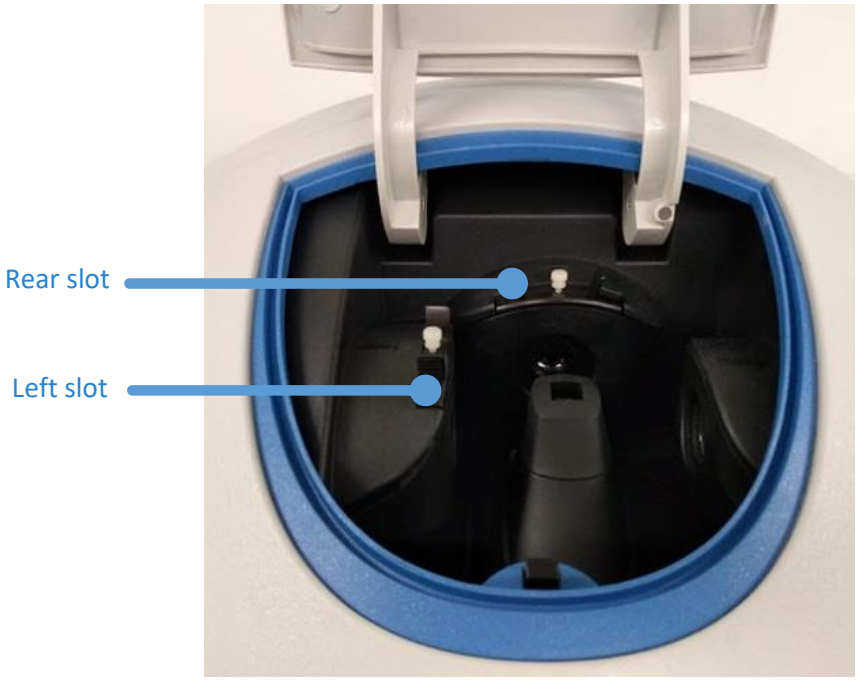


5. Insert and gently screw the screw in the hole in the filter holder to depress the spring above the filter enough to hold the filter securely in place.



⚠ **Caution: Do not hold the white plastic screw when carrying, inserting or removing a filter holder – this may loosen the screw enough that the filter could fall out and be damaged.**

6. With the sample lid open, the user accessible optical filter holders may be placed in the slots to the left and rear of the sample holder. The curved sides of the filter holders face the sample holder. To insert or remove the filter holder, hold the two black tabs on top of the filter holder.

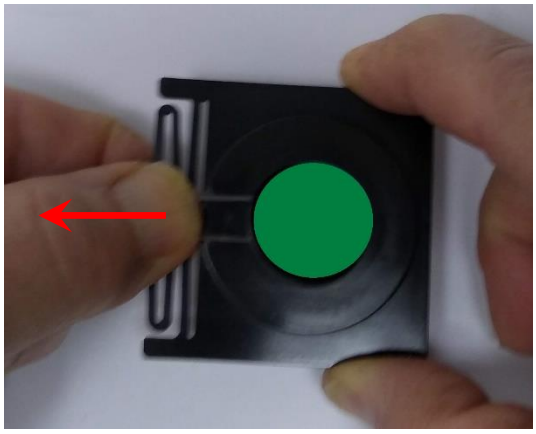


4.1.3.2 Removing filter holders

When a filter will not be used in the sample chamber, either:

Remove the filter holder with filter installed altogether from the sample chamber and store it in a safe place, or:

1. Remove the filter holder and place it flat side down on a clean paper on a flat surface.
2. Loosen the screw at the top of the filter holder so that the filter holder insert and filter fall on the paper.
3. You can either store the filter holder insert with filter installed in a safe place, or remove the filter from the filter holder insert.
    a. Lay the filter holder insert filter side down (wide shoulder facing up) above a clean sheet of paper on a flat surface.

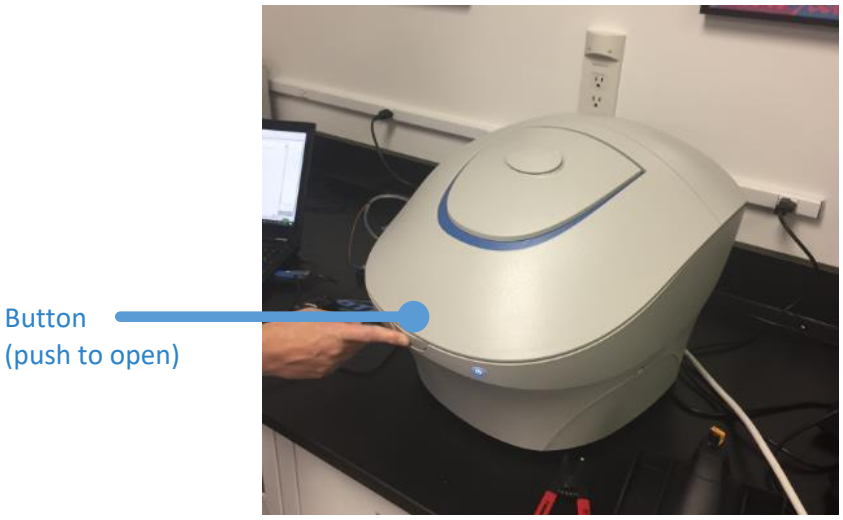


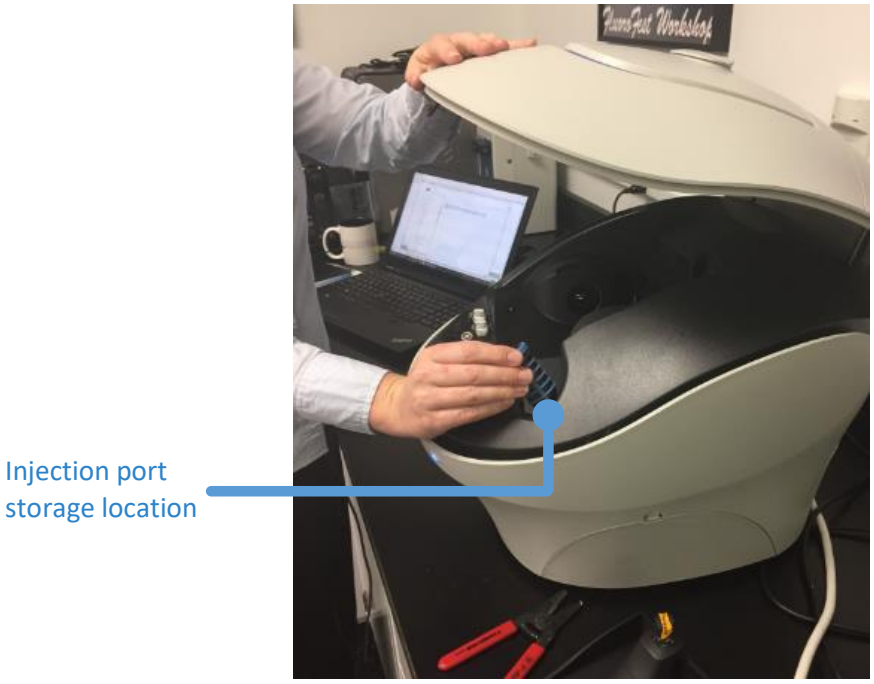
    b. Gently pull the top edge of the filter holder insert away from the filter so that the filter falls onto the paper.
4. If you want to insert an empty filter holder back in the slot the sample chamber, do not put the empty filter holder insert back into the filter holder.

4.1.4 Injection Port Installation and Use

Depending on the test, the technician may need to install the injection port to add a sample to the cuvette.

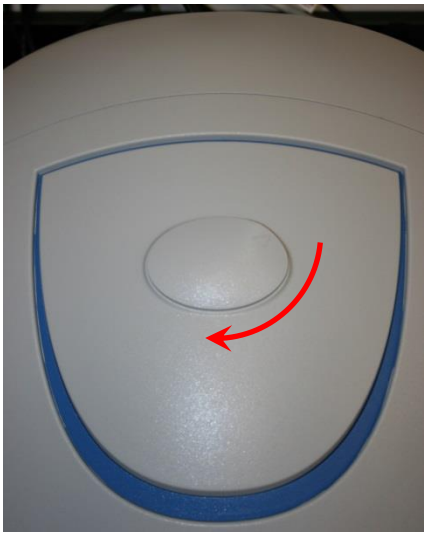
1. Open the top cover.



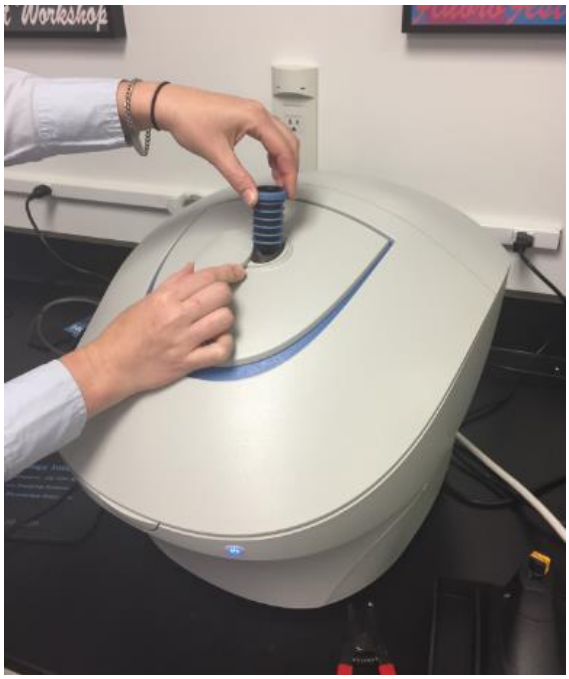


2. Remove the injection port from its storage location on the right hand side. Remember to check that the cuvette is uncapped. Close the top cover.

3. Rotate the right edge of the access port cover clockwise down to open it.



4. Install the injection port in the access port.
5. Inject a sample.



6. Rotate the access port cover back to close it.

5 System Operation

5.1 Introduction

This section explains how to turn on the Duetta™ instrument, check its calibration, and, if necessary, recalibrate the monochromators. While doing these procedures, how to define a scan, run a scan, and optimize system settings to obtain the best results is explained.

5.2 Safety Interlocks

5.2.1 75 W Xenon lamp power:

Lamp Access Panel Interlock – If the lamp is on and the lamp access panel is opened the lamp will be turned off. A wait time of at least 5 minutes is required for the lamp to cool before the Duetta™ can be restarted in order to turn the lamp back on or the lamp is turned back on via the software. The Duetta lamp will be locked out for at least a 5 minute wait time so the lamp will not be ignited while hot. Igniting the xenon lamp while hot can cause damage to the instrument.

5.2.2 Thermal:

Duetta main board:

- If the lamp fan is not operating, the lamp will be turned off.
- If the internal temperature of the Duetta™ rises above 60 °C, the lamp will be turned off.

CCD Detector:

- If the CCD detector's fan is not operating (or running slow), the camera cooling will be turned off.

5.2.3 Notification

EzSpec™ will warn the user if the Duetta™ top cover or the sample cover is open.

5.3 Duetta power switches

5.3.1 Rear power receptacle and switch

The power cord receptacle is located on the back panel beside the rear power switch.

Before inserting the power cord on the back of the Duetta™, make sure the rear power switch is in the OFF position (○ depressed).

Insert the power cord into the receptacle on the back of the Duetta™, and then into a power bar.

Switch the rear power switch to the ON position (— depressed), and then wait for 20 seconds or more before turning on the front power switch.

5.3.2 Front power switch

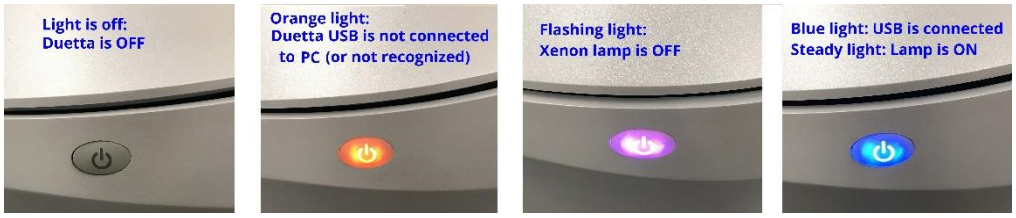
The front power switch is located on the front of the Duetta™ just below the top cover edge and to the right of the top cover latch button.

5.4 Turning on the system

1. The lamp must be installed and the side door closed.
2. Connect the USB cable from side of Duetta to a PC with EzSpec™ software installed.
3. Connect the power cable from an AC outlet to the back panel of the Duetta™, turn on the main power switch on the back panel, and wait 30 seconds.
4. Turn on the Duetta™.
    a. Push and hold the front power switch in for 1-3 seconds. If you hold the power button for longer than 5 seconds, the Duetta™ does not turn on. When switched on, the Duetta™ turns on, runs through self-diagnostics, starts the xenon lamp, and then initializes the Duetta™ hardware (you will hear this).

Front power button indicator

The front button will be blue if the PC USB connection is connected, or orange if the USB connection is not connected properly or if the Duetta™ is not recognized by the PC in the hardware manager. The button will be steady if the lamp is on, or flashing blue and pink if the lamp is off (this also applies if the lamp has been turned off by the EzSpec™ software). If the lamp is on and the USB is connected properly, the button will be steady blue.



When the lamp has been turned off from the **Top Menu**, **Lamp**, **Turn Lamp OFF**, the front button will be flashing blue and pink indicating that the **Lamp Status** is OFF.

ⓘ Note: After turning the lamp OFF, whether by the software, by opening the lamp cover interlock, or by turning the instrument front power button OFF, the Duetta will not reignite the lamp again for at least 5 minutes. Igniting a hot xenon lamp may cause damage to the instrument.

⚠ **Warning: You must wait a minimum of 2 minutes after turning the lamp off before installing new firmware to prevent EMI from a hot lamp on restart.**

ⓘ Note: Each time the xenon lamp is ignited adds one more hour to lamp use. HORIBA Scientific suggests leaving the Duetta™ and lamp on during brief periods of inactivity.

5. Wait 1 hour for the lamp and Duetta to warm up before acquiring data.

   The Status bar will show **Status: IDLE, Instrument warming up…** for 1 hour after the lamp is turned on.

   If you want to analyze data or print results, you can do that immediately.

6. Turn on all peripheral devices for the host PC.
7. Peripherals include any printers or plotters, or other devices such as temperature controllers, water or other circulating devices, stop flow.
8. Start the host computer.
    a. Switch on the host computer.
    b. Login to Windows® with your Windows® ID. This is used as the user ID for EzSpec™ including “Administrator”.
    c. Click on the EzSpec™ icon  on the Windows® desktop.
    d. The **EzSpec Launcher window** appears.

5.5 Turning off the system

On the front of the Duetta™ press and hold the power button for more than 3 seconds to turn the lamp off. The power button should then be flashing to indicate the lamp power is off.

The internal exhaust fans run for 5 minutes after turning the Duetta™ off to cool the lamp. When the fans have stopped running the system is off.

If the lamp needs to be replaced, follow the instructions starting on page 226 of this manual.

> ⓘ Note: For day-to-day use the power cord may be left connected, and the back panel mains power switch may be left on and only reset when needed.

5.6 EzSpec™ Launcher window

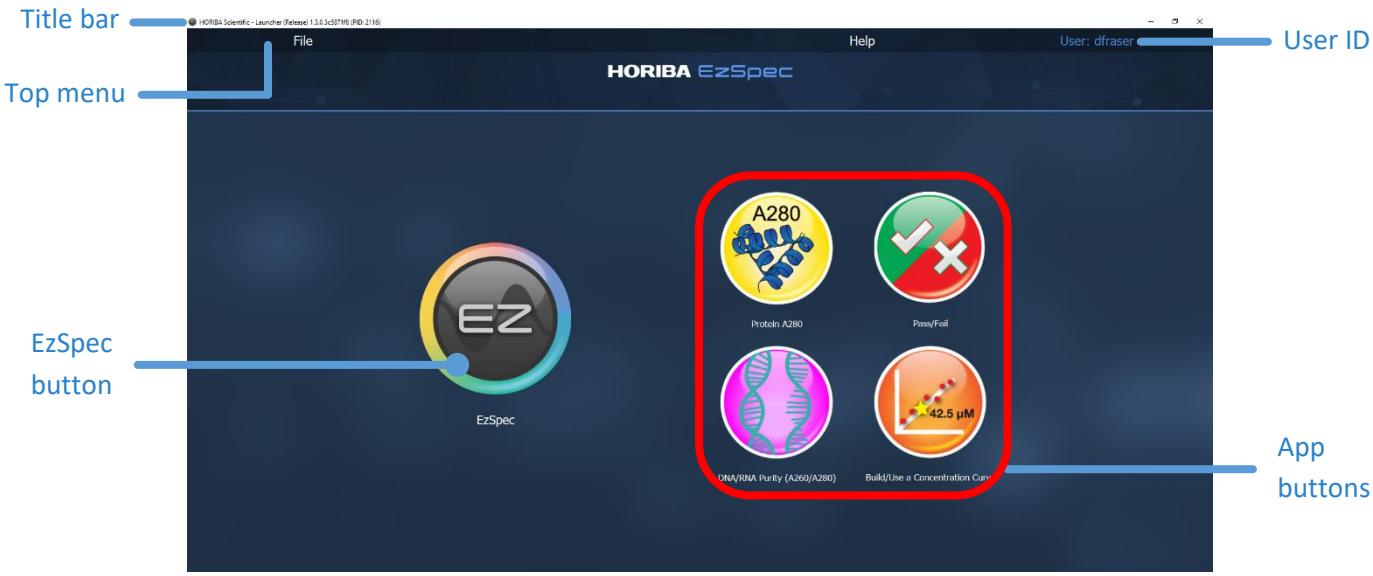
At the top of every window is the title bar. On the left end, this shows HORIBA Scientific, the name of the software component (this example: "Launcher (Release)"), the software version number, and a code for the software component. On the right end are the standard Windows® buttons: **Minimize**, **Restore Down/Maximize**, and **Close**.

When you open EzSpec™, it uses your Windows® user ID to recognize you and report your user ID on the right end of the top menu bar.

Across the top menu bar is the Top Menu, seen on all EzSpec™ windows.

> ⓘ Note: there are some differences in the Top Menus between the Launcher window and the EzSpec™ and App windows.

The Launcher window shows the EzSpec button on the left, as well as Application ("App") buttons on the right.

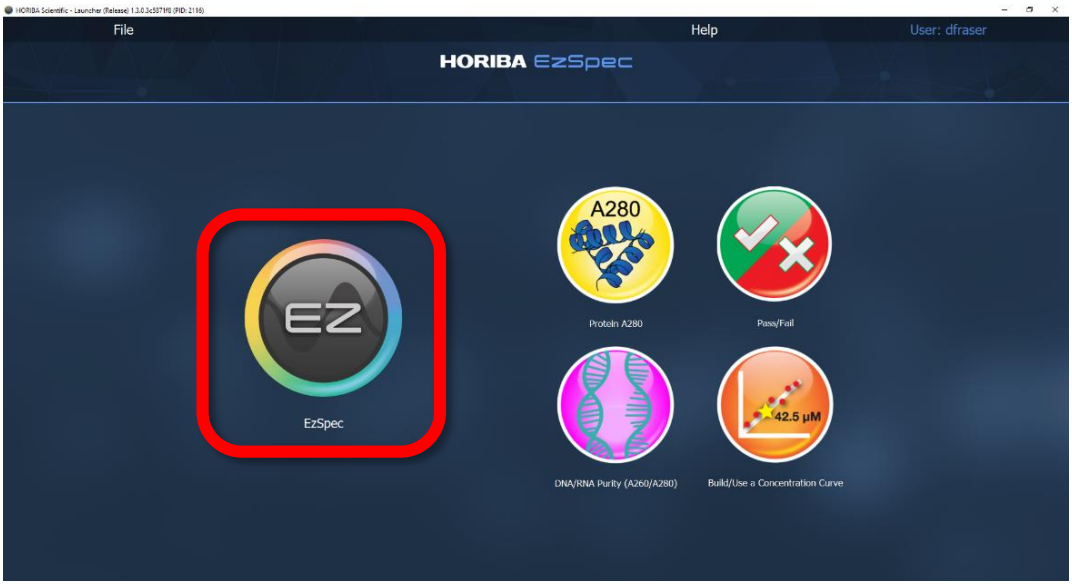


Clicking on the EzSpec button switches to the EzSpec Dashboard menu. The EzSpec application is where the user can create setup methods, acquire data, and process and report the data. EzSpec™ is described in section 5: EzSpec. The top menu bar is also described therein.

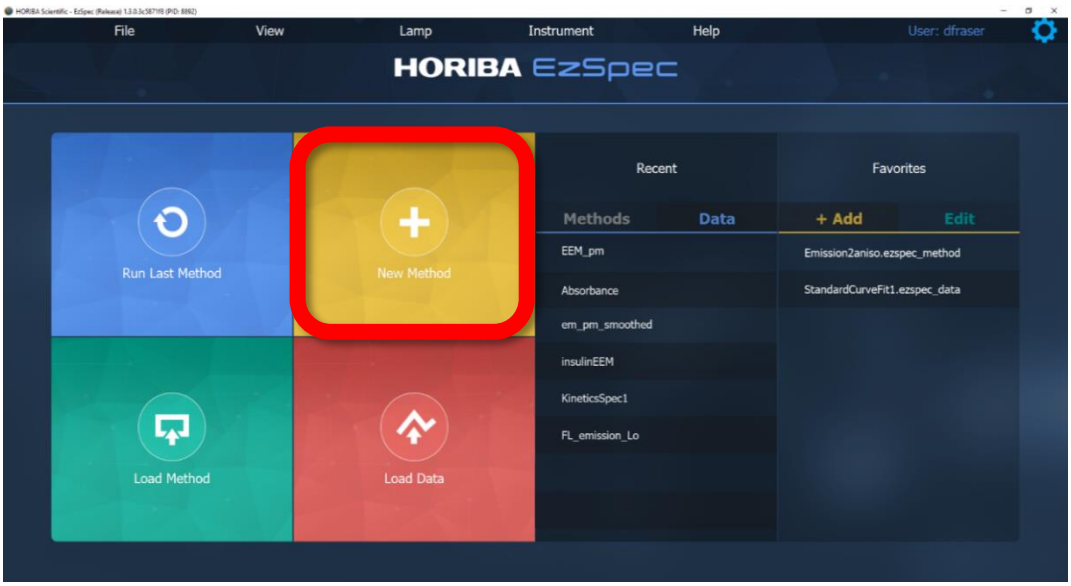
Clicking on other application buttons opens the named App. Apps are very specific methods created by HORIBA and focused on a single measurement or series of measurements to answer a specific question. Apps are described in section 8. New apps may be added in future updates of EzSpec™.

5.7 Measuring an emission spectrum in EzSpec™

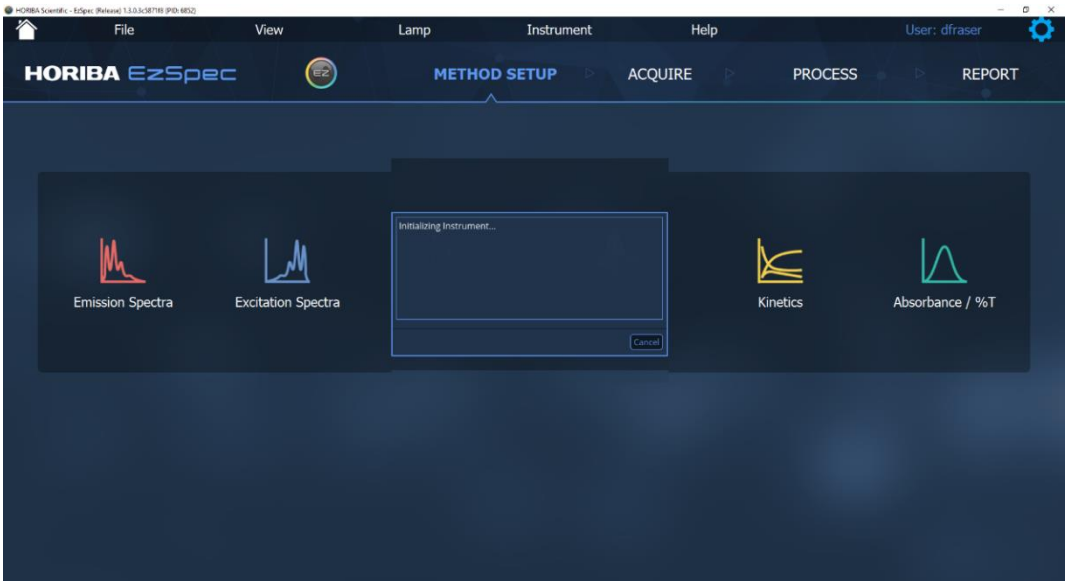
1. On the EzSpec Launcher window, click on the **EzSpec** application button.
   The EzSpec Dashboard appears. For a new installed EzSpec the Recent and Favorites menus will be blank.



2. Click on the **New Method** button.



The Method Setup menu appears, and the **Initializing Instrument** notice appears for several seconds and then disappears.



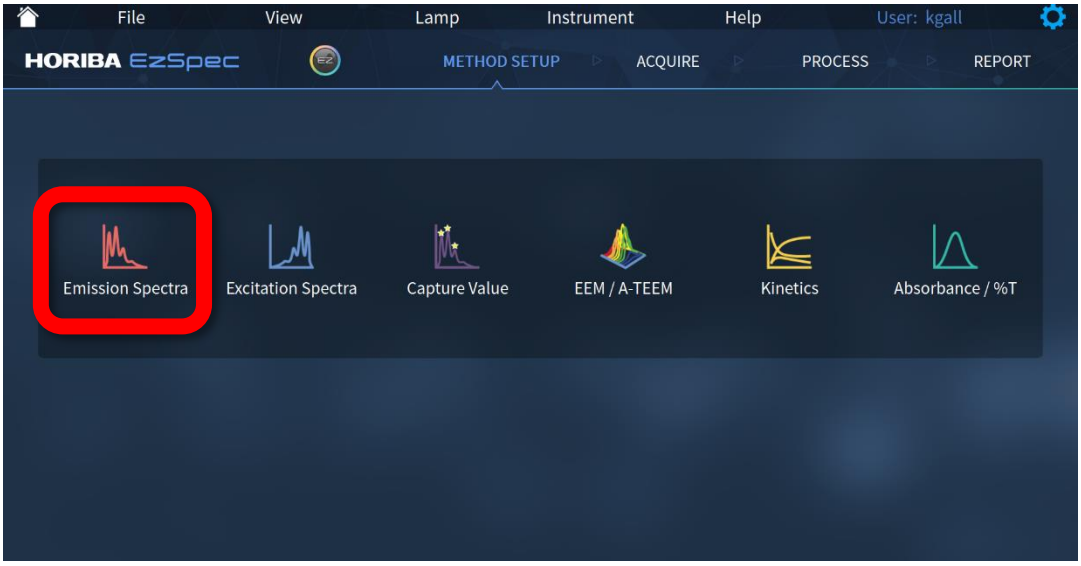
The Method Setup menu shows the main data types that can be acquired. Capture Value has two sub-types: Single Point and Standard Curve. Kinetics has two sub-types:

- Emission Spectra
- Fixed Wavelengths

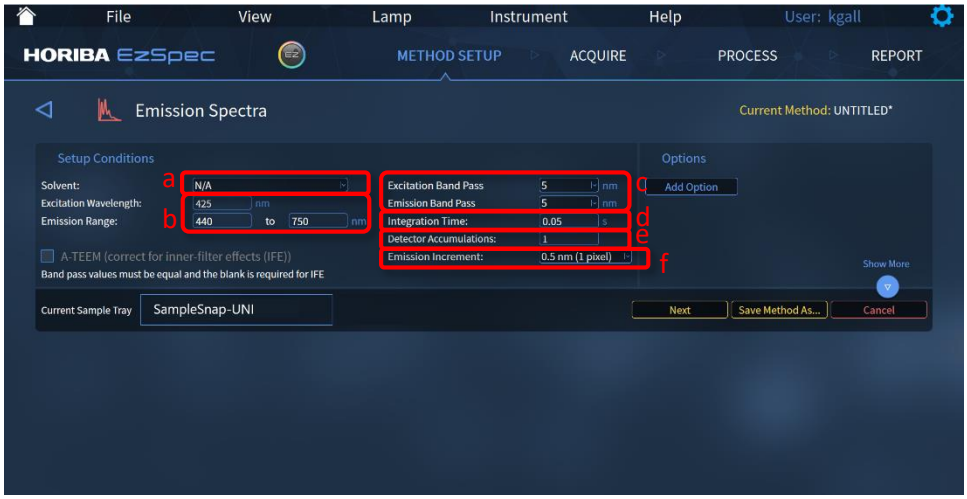
Absorbance / %T has two sub-types:

- Absorbance Spectra
- Absorbance Kinetics

3. In the Method Setup menu, click on **Emission Spectra** to show the Setup Conditions.



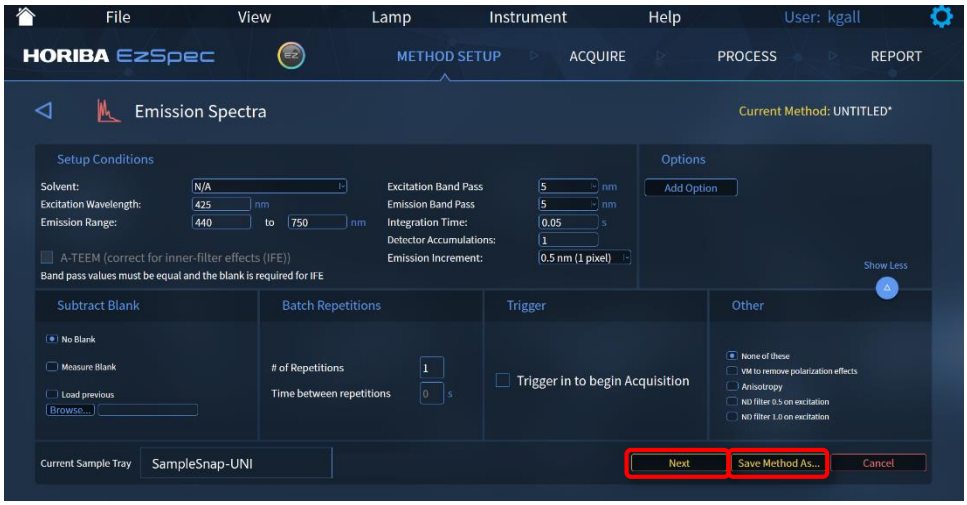
4. Select setup conditions.
    a. Select the **Solvent** in the pull down menu. If the solvent is not listed or the sample is not a solution, select N/A (not applicable).
    b. Select the **Excitation Wavelength** (range 250 to 1000 nm) and the **Emission Range** (250 to 1100 nm) values (Start and End).
    c. Select the **Excitation** and **Emission Band Pass** values from the pull down menus.
    d. Enter an **Integration Time** (range 0.05 to 600 seconds).
    e. Enter the number of **Detector Accumulations**. If this value is greater than 1, the spectrum will be repeated that number of times and those spectra will be summed together.
    f. Select an **Emission Increment** from the pull down menu.



5. For more advanced options, click the **Show More** button.

6. Add any advance options to the method such as the loading or measurement of a blank, repeats of the measurement, trigger options, anisotropy, or the use of neutral density filters.

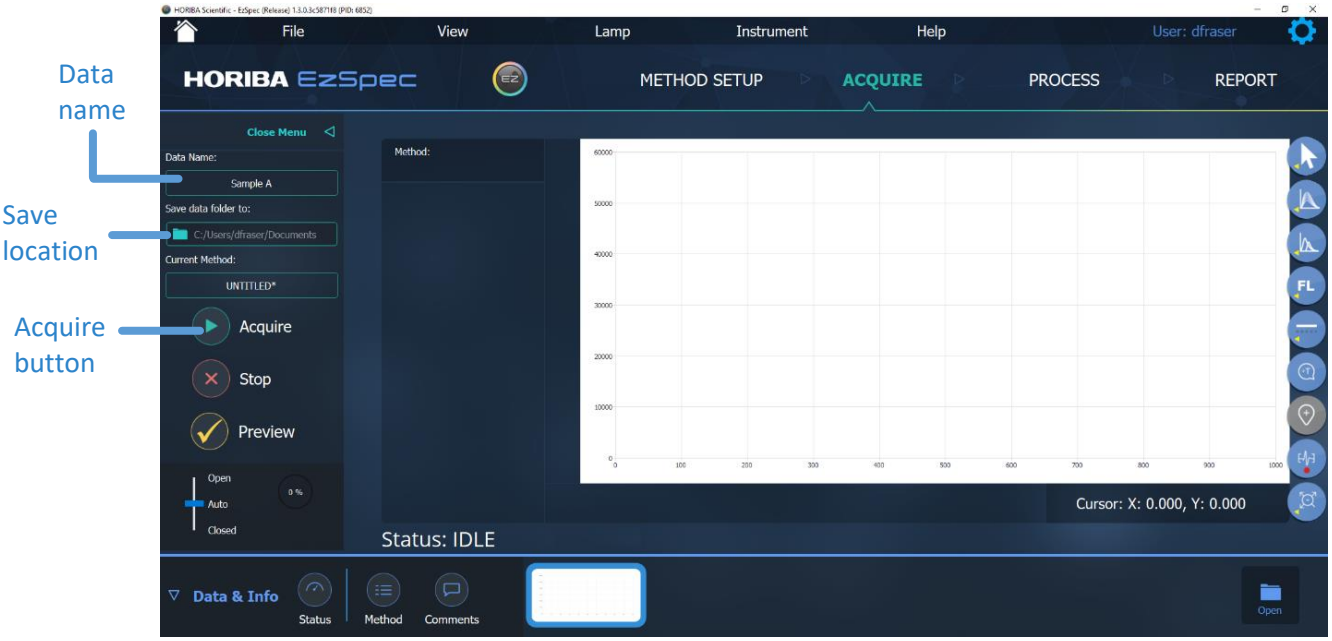
> **Note**: a blank measurement must be used to enable the **Acquire Absorbance and correct for inner-filter effects** checkbox.



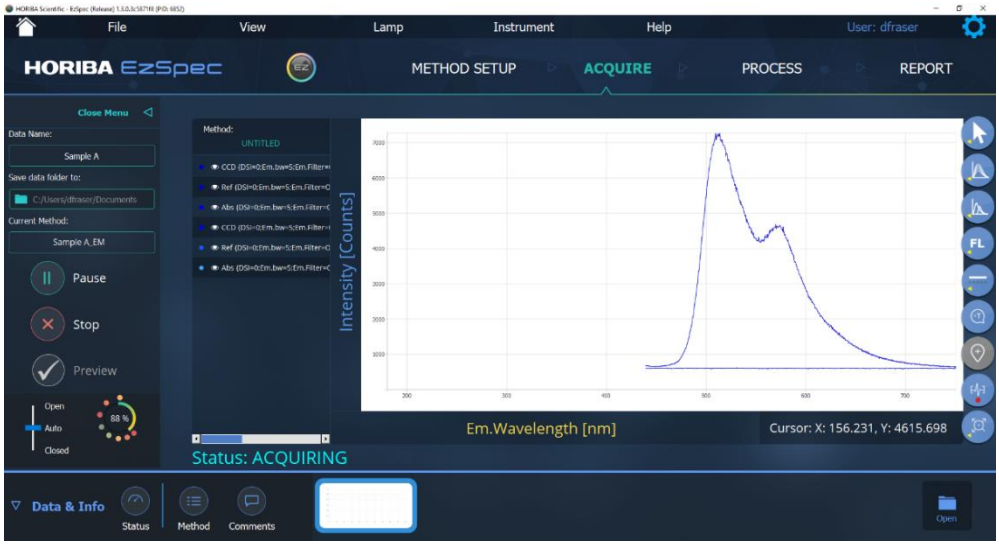
7. At this point, either click **Next**, or **Save Method As…** to be able to recall the method later.

> **Note**: Methods will always be saved automatically, even if not explicitly saved before acquisition. In the following example only **Next** was clicked.

8. Proceed to the Acquire menu. In the **Data Name** box enter the root name of the data file you wish to be saved for the measurement. The initial default Data Name is “Sample A”. Whatever is entered becomes the new default name.
9. Click on the folder icon and browse to select the location, or create a new folder, where the acquired data will be saved. A subfolder will be created with the data name, method type suffix, and timestamp for the acquisition.
10. Click the **Acquire** button to acquire the data.

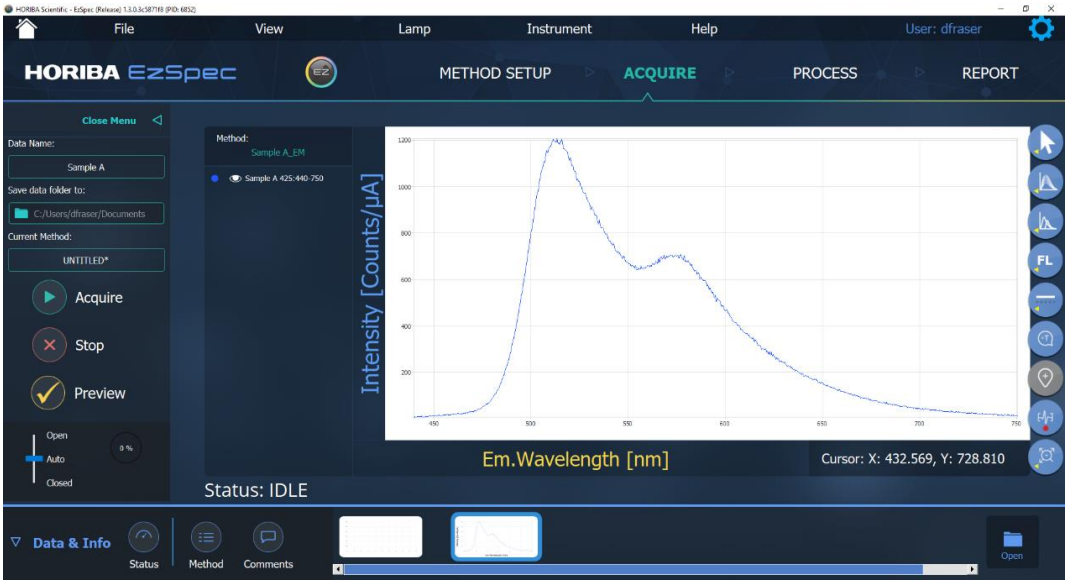


11. The **Acquire** button changes to **Pause**, the **Current Method** and the **Method** name at the top of the legend become Sample A_EM, the **Preview** button changes color to grey, and the **Status** below the graph changes first to “PREPPING” in yellow and then to “ACQUIRING” in light blue. As the raw data is acquired, the Progress wheel reflects the percentage of the measurement that is completed.



12. When the acquisition is completed, the **Pause** button changes back to **Acquire**, the **Preview** button changes back to yellow, the finished spectrum appears in the graph, and a new blank thumbnail is inserted at the left end of the Data & Info bar.

    The first thumbnail on the very left side is always the live acquisition thumbnail to which newly acquired data is added. The blue outline around a thumbnail means it is the graph or table being viewed in the Graph Window above. The curve names in the legend are labeled as: Data Name Excitation value or range: Emission value or range. In this example the curve name is “Sample A 425:440-750”.



6 EzSpec™

6.1 Introduction

This section presents an introduction to the EzSpec™ application where the user can set parameters to acquire, process, and report data, and save these steps of method setup, acquisition, data processing, and reporting.

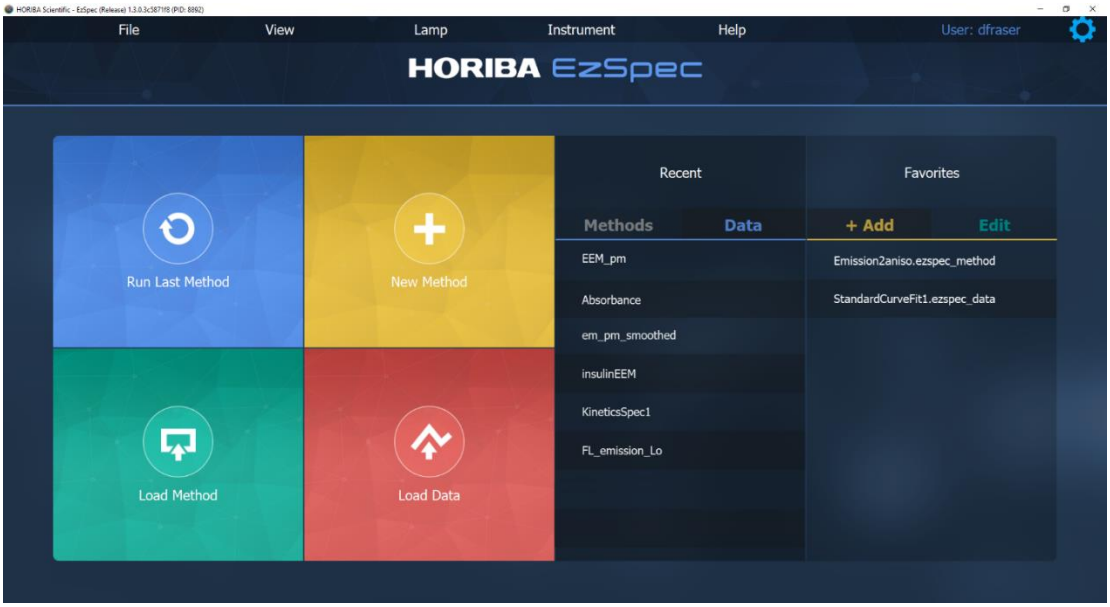
As an aid to using EzSpec™, letting the mouse cursor move over or rest on most buttons and enterable fields will show a tool tip (white text on a black background) giving information about the purpose of a button, or the complete text of an enterable field.

6.1.1 Terminology

The terms “curve” and “trace” mean the same thing and are used interchangeably in this manual.

6.2 Dashboard

On the launcher page, clicking the EzSpec Application button switches the launcher page to the Dashboard menu:



On the left are four buttons.

1. **Run Last Method**: Clicking this button automatically loads the last method from the current user and skips to the Acquire menu in EzSpec™. This is the same as clicking on the **File**, **Run Last Method** in the Top Menu.
2. **New Method**: Clicking this button opens the Setup menu so the user can select a method type and enter new parameters in an empty method setup. This is the same as clicking **File**, **New Method** in the Top Menu.
3. **Load Method**: Clicking this button opens the Load EzSpec Method File browser window so the user can select a previously saved method file. The method opens in the Method Setup menu for that acquisition type, such as Setup – Emission spectra and it becomes the **Current Method**.
4. **Load Data**: Clicking this button opens the Load EzSpec Data File browser window so the user can select a previously saved **.**ezspec_data file with the method used to create the data. The data opens in the graph window.
    a. Load multiple data files into same graph/thumbnail
    b. Load multiple data files into separate graphs/thumbnails

On the right are two menus: **Recent** and **Favorites**.

When opening an existing version of EzSpec, the Recent and Favorites lists may show names. A newly installed EzSpec will have blank lists.

1. **Recent menu**. This has two submenus: Methods and Data.
    a. **Methods**: Click or touch on Methods to show a list of the last 8 method files that have been open and saved. Holding the mouse cursor over or touching and holding a method name in the list shows the full path and file name of the method file. Click on the method file name to open the setup menu with the method loaded.
    b. **Data**: Click or touch on Data to show a list of the last 8 data files that have been open and saved. Holding the mouse cursor over or touching and holding a data file name in the list shows the full path and file name of the data file. Click on the data name to open the Acquire menu with the data loaded.
2. **Favorites** menu. This menu shows a list of the up to 8 data or method files that can be saved by the user. The Favorites list may be rearranged by the following:
    a. **+Add**: Click or touch on +Add to open a browser window to select a method or data file to add to the Favorites list.
    b. **Edit**: Click or touch the Edit button to be able to change the order of the favorites list by dragging a favorite up or down or deleting a favorite from the list by clicking the minus sign to the left of the item.

6.3 Top Menu

The **Top Menu** is the same for the EzSpec Dashboard and all Apps, but only shows **File** and **Help** on the Launcher. Some submenus differ between EzSpec and the Apps.

The **Top Menu** and submenus described here give access to all EzSpec™ operations.

Each heading in the **Top Menu** represents a group of related commands.

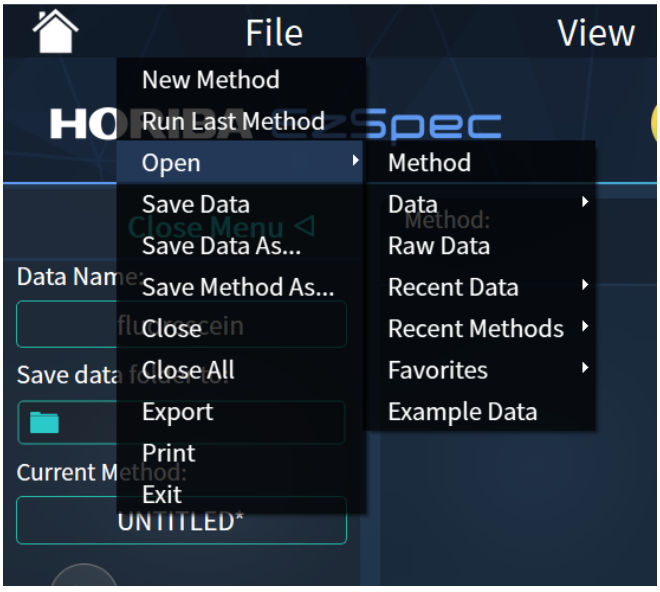
Click on a **Top Menu** heading to open a pull down menu. Click on an item in a pull down menu to open an experiment setup menu, a dialog, another pull-down menu, or perform some other operation.

- The Dashboard button is at the left end of the top menu bar for all EzSpec™ windows, except for the Dashboard menu itself. Clicking the Dashboard button returns to the EzSpec Dashboard.
- Click on the blue toothed wheel at the right end of the Top Menu, to see “Language”. Click on “Language” to see a listed of languages supported and the current language.

6.3.1 File Menu

The **File Menu** on the EzSpec **Top Menu** lets you open, save and close methods and data, export data and images, and print a report.

This picture of the **File Menu** was taken when **Acquire** is the active menu.



6.3.1.1 New Method

Clicking **New Method** opens the Method Setup menu so the user can select a method type and enter new parameters in a default or the last used method setup. This does the same action as clicking on the **New Method** button on the Dashboard.

The user then can change parameters in the method setup. Methods are files that contain all the experimental parameters required to control the Duetta™ to acquire data. They can also store comments about the data acquisition.

Only one method setup can be open at the same time.

6.3.1.2 Run Last Method

Clicking **Run Last Method** runs the last method from the current user. This does the same action as clicking on the **Run Last Method** button on the Dashboard.

6.3.1.3 Open

Click **Open** to open a sub menu as:

- **Method:** Click Method to open a browser window to select a Method file to open. This does the same as clicking on the **Load Method** button on the Dashboard.
- **Data:** Click Data to open a browser window to select a Data file (*.ezspec_data) to open. This does the same action as clicking on the **Load Data** button on the Dashboard, and clicking on the **Open** button on the Bottom bar. The data opens in the graph window (on either the Acquire, Process, or Report menu), and a thumbnail of the graph is inserted on the bottom bar to the right of the live acquisition thumbnail and to left of all the other open thumbnails so that other thumbnails are shifted to the right.
- **Raw Data:** Click Raw Data to open a browser window to select a Raw Data file (*.ezspec_raw) to open. The data opens in the current Graph window. Raw data contains a trace/table column for each detector in each state of acquisition. For example, a separate trace for each automated filter wheel position is created during an excitation or absorbance spectral acquisition.
- The raw data file cannot be overwritten or resaved as the raw data file format. It can only be resaved after opening in EzSpec to the .ezspec_data format (or exported), so the original raw data file is always saved and never changed.
- **Recent Data:** Click Recent Data to show a list of the last 8 data files that have been opened.
- **Recent Methods:** Click Recent Methods to show a list of the last 8 method files that have been opened.
- **Favorites**: Click Favorites to shows a list of the data or method files that have been saved by the user on the dashboard.
- **Example Data:** Click to browse example data provided with the software.

6.3.1.4 Save Data

Click **Save Data** to save the open data set (the data in the current Main graph), using the current file name.

Warning: If you make any changes (including Remove Selected – see page 121) to the data and then save the data using the original file name, the original file will be overwritten and some original data may be lost. If you make any changes to EzSpec data and want to save the changes you should save the data with a new name, using **Save Data As…**.

6.3.1.5 Save Data As…

Click **Save Data As…**to save the active data. A browser window opens to allow you to select a folder and enter a filename for the data. If you select the name of an existing data file, you will be prompted by the message

"*Filename* already exists. Do you want to replace it? Yes No"

6.3.1.6 Save Method As…

Click **Save Method As…** to save the active method, including any changes. A browser window opens to allow you to select a folder and enter a name for the method. This option is always active, even if there have been no changes to the active method.

If the method is new and has not yet been saved, then the default filename is "UNTITLED.ezspec_method".

If you select the name of an existing method file, you will be prompted by the message

"*Filename* already exists. Do you want to replace it? Yes No".

A Method file contains setup and optional comments. Method files can be saved at any point in the software (Method Setup, Acquire, Process, or Report).

6.3.1.7 Close

If the data has been saved including any changes to the data, then clicking **Close** closes the active data in the graph window (closes the graph page and the corresponding thumbnail) and opens the graph corresponding to the next thumbnail on the left.

If the data has not been saved (including changes), the user is prompted:
"Do you want to save the data?"

- Clicking **Yes** opens a browser window to allow the user to select a folder and enter a name for the data file.
- Clicking N**o** closes the data without saving changes.

6.3.1.8 Close All

Clicking **Close All** closes the active data in the graph window and all the thumbnails to the right of the blank thumbnail on the bottom bar, and opens the blank graph in the graph window.

6.3.1.9 Export

Opens the Export dialog from the Report menu. This command is described under Report.

6.3.1.10 Print

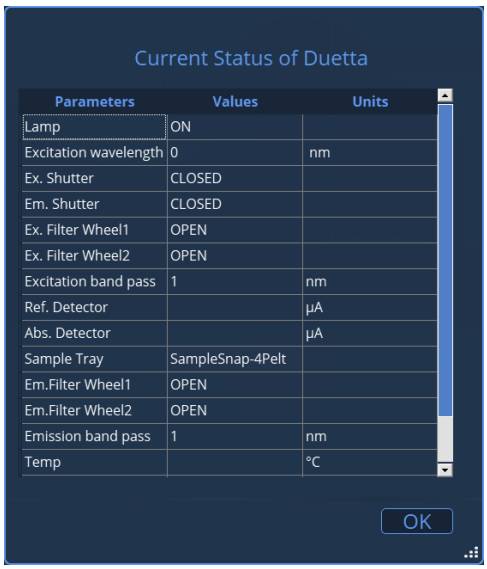
Click **Print** to open the **Report Generator** window as opened by the **Report**, **Report Generator**, **Quick Report** command.

6.3.1.11 Exit

Click **Exit** to close the entire **EzSpec** application, and return to the **EzSpec Launcher** page.

6.3.2 View menu

1. **Graph** – This command requires either of the Acquire, Process, or Report menus to be open. This does the same action as clicking on the **2D Graph View** button. Click **Graph** to show the current data as a 2D graph in the current display menu screen (either Acquire, Process, or Report menu). This always shows the current data as a 2D graph, no matter how it was acquired or saved. If no data has been loaded or acquired, the current window will not change.
2. **Table** – This command requires either of the Acquire, Process, or Report menus to be open. This does the same action as clicking on the **Table View** button. Click **Table** to shows a table with the Method Setup information in several rows, followed by rows of data. If no data has been loaded or acquired, the current menu will not change.
3. **Method** – This does the same action as clicking the **Method** button on the Bottom Bar. Click **Method** to show a 2 column table with all the Method Setup options and their values for the method corresponding to the currently displayed data.
   See an example at **Data & Info bar**, **Method**, page 121.
4. **Status** – This does the same action as clicking the **Status** button on the Bottom Bar. Click **Status** to show the current Duetta™ hardware information, including the current wavelength of the excitation monochromator, slit settings, filter wheels, any SampleSnap accessories, Reference detector and Absorbance detector readings if the instrument is acquiring data, or a message if the Duetta™ is not connected.

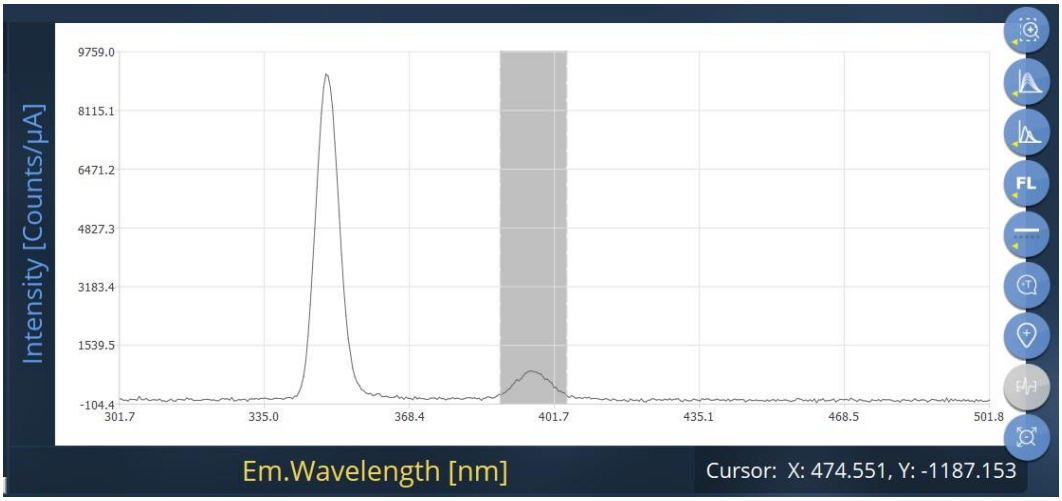


5. **Comments** – Click **Comments** to open a window to show the comments attached to the current method. To enter or edit comments, click the **Comments** button on the Data & Info bar.
6. **Raman Indicators** – When this is checked a vertical grey band is shown on the Graph Window where the expected Raman peak(s) from the solvent are located. This is true for any spectral fluorescence graph. The band width is dependent on the band pass used.

   The range can be calculated by:

$$Raman\ peak\ position\ \pm\ (excitation\ bandpass\ +\ emission\ bandpass)\ *\ 0.75$$

   For example, for an emission spectrum in water, at 350 nm excitation, 5 nm band pass on both slits, the range of the Raman marker would be 397 nm ± 7.5 nm = 389.5 to 404.5 nm.



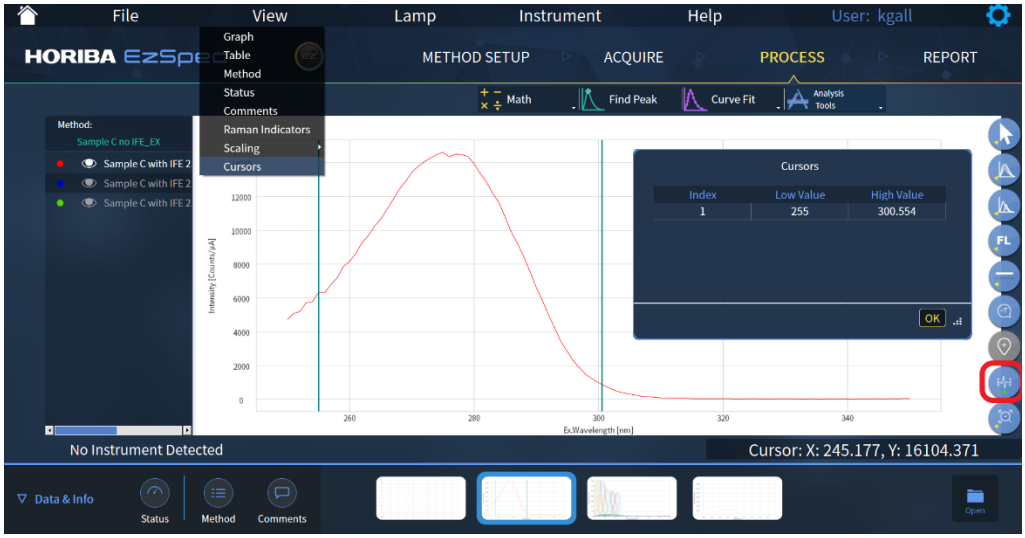
   Raman Indicators should be enabled for the following method types:

   - Emission spectra
   - Excitation spectra
   - Kinetics vs. Emission Spectrum

   If the experiment is Capture Value, Calibration Curve, or Kinetics, and the emission wavelength value is set at the expected Raman wavelength for a selected solvent (as defined in the Setup pull down menu), a popup will occur in the setup as soon as the solvent and emission wavelength are selected and this value can be compared. To view or hide the Raman indicator, go to the View menu and select/unselect Raman indicators as needed. The Raman indicators are only viewable when a solvent is selected in the Method used for the acquired data.

7. Scaling: Select to scale x or y axis to logarithmic or linear scaling in graph view. This is useful for viewing calibration curve data where concentrations or x-axis values are not linearly spaced.

8. Cursors: View the x-axis values or set the x-axis values of the range cursors



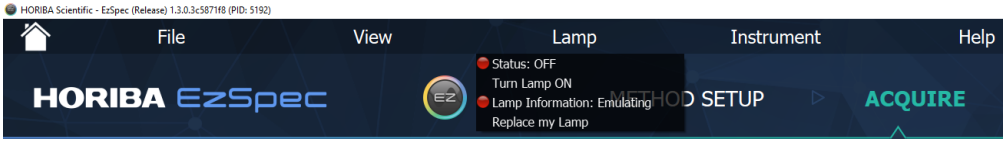
6.3.3 Lamp menu

On the Top Menu, click on **Lamp** to display the Lamp menu.

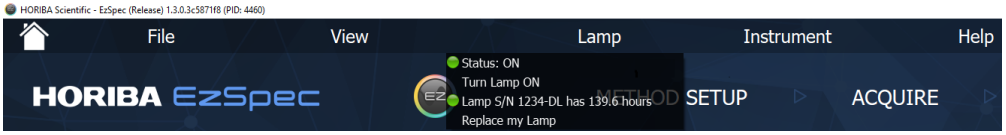
Status shows whether the lamp is on or off. **ON** is marked by a green dot. **OFF** is marked by a red dot. See the following examples.

Lamp S/N shows the serial number of the lamp and the total number of hours the lamp has been on. If the number of hours is 800 or less, the dot before **Lamp S/N** is green. If the number of hours is greater than 800, the dot before **Lamp S/N** is red and a notification symbol will appear on the top menu between View and Lamp.

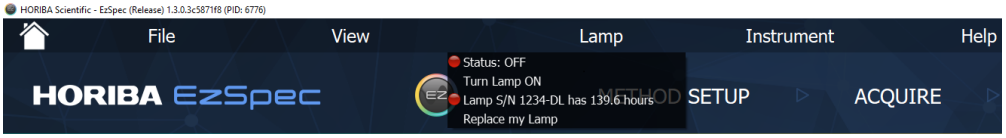
When the Duetta™ is turned off or not connected to the computer, the Lamp menu will display the following information:



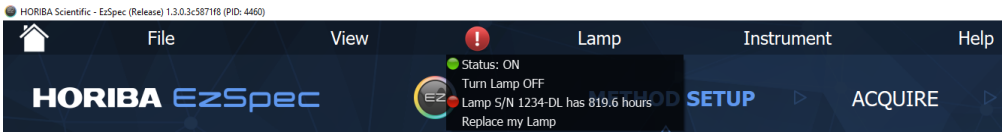
When the Duetta™ is turned on and connected to the computer, and the lamp is installed and turned on and has not exceeded 800 hours, the Lamp menu will display the following information:



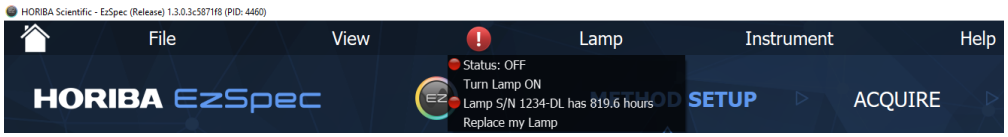
When the Duetta™ is turned on and connected to the computer, and the lamp is installed and turned off and has not exceeded 800 hours, the Lamp menu will display the following information:



When the Duetta™ is turned on and connected to the computer, and the lamp is installed and turned on and has exceeded 800 hours, the Lamp menu will display the following information:



When the Duetta™ is turned on and connected to the computer, and the lamp is installed and turned off and has exceeded 800 hours, the Lamp menu will display the following information:



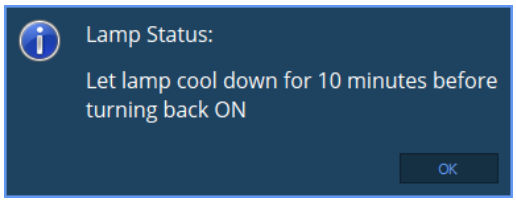
6.3.4 Turn Lamp OFF

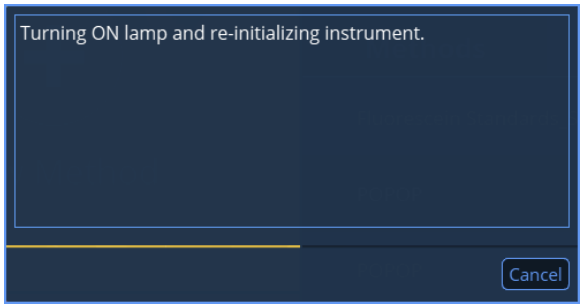
> ⓘ Note**:** Each time the xenon lamp is ignited adds one more hour to lamp use. HORIBA Scientific suggests leaving the Duetta™ and lamp on during brief periods of inactivity.

When the lamp is ON, click **Turn Lamp OFF** to turn the lamp off. The lamp will be turned off, the front Power button on the Duetta™ will start alternating between bright pink and bright blue, and the following message will appear:

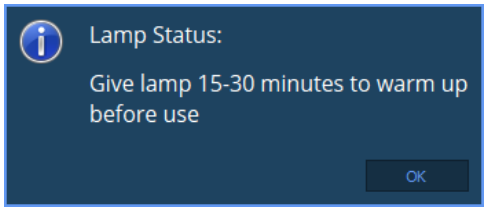
6.3.5 Turn Lamp ON

When the lamp is OFF, click **Turn Lamp ON** to turn the lamp on. The lamp will be turned on, the front Power light will change to bright blue, and the following message will appear for several seconds:



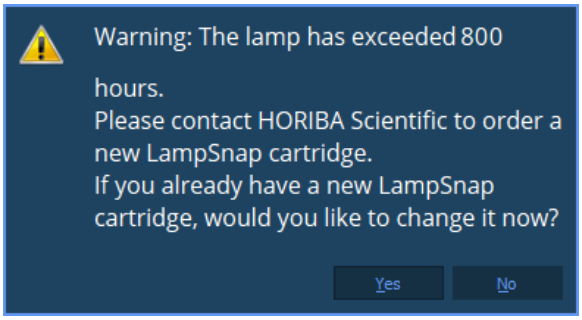


Then the Lamp Status prompt appears:



Click **OK**.

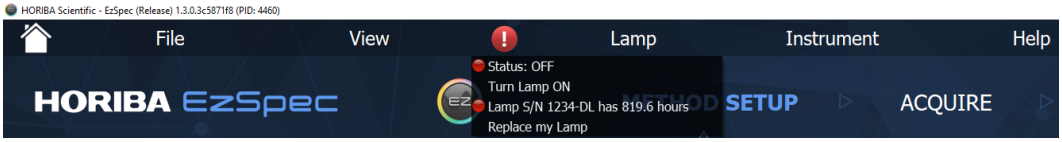
When the lamp hours exceed 800, each time the instrument is started, this prompt is displayed:



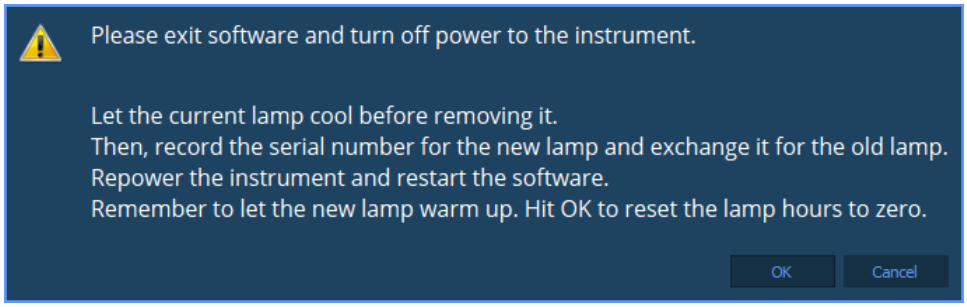
Clicking **No** will close the prompt.

If the user ignores the warning, the Top Menu shows a notification until the lamp hours are reset:

Clicking on **Lamp** will show the following Lamp menu:



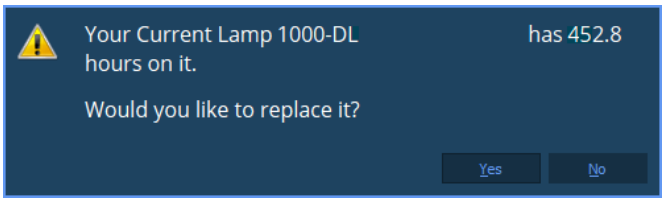
Clicking **Yes** will display the following prompt:



Click **OK** and follow the procedure for changing the lamp in section 9.2 of this manual.

Clicking **Cancel** will close the prompt.

6.3.6 Replace My Lamp



Click this button when you want to replace the lamp.

The user is prompted:

- Clicking Yes will display the Please exit software and turn off power to the instrument prompt above.
- Clicking **No** will close the prompt.

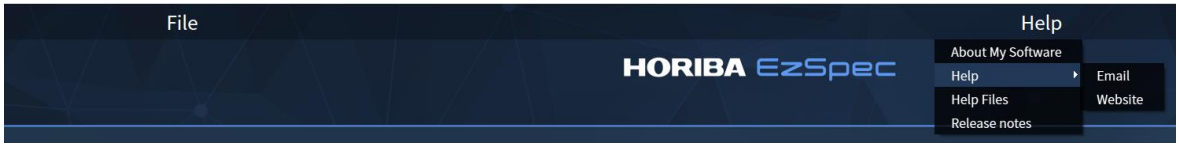
6.3.7 Instrument menu

The image below is from the EzSpec Top Menu.

By clicking on **Initialize…** the user can initialize the Duetta™ to reconnect the hardware with the software without having to restart the software.

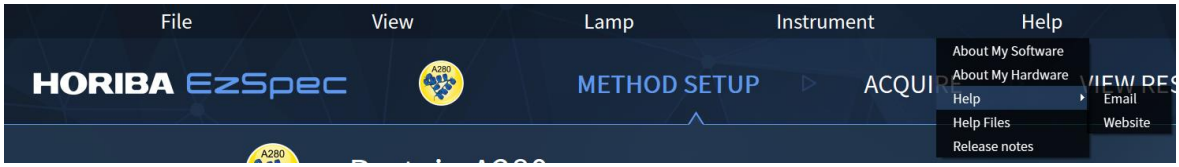
6.3.8 Help menu

The image below is from the Launch window.



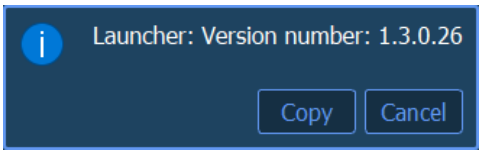
The image below is from the **EzSpec Method Setup** window. Only the EzSpec Method Setup window shows both **Run Test Report** and **Collect Instrument Information** as active options.

The image below is from the **A280** app. Other Apps show the same Help menu:

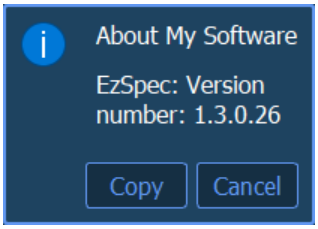


6.3.8.1 About my Software

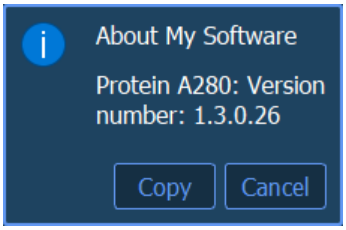
The **Launcher** window, **About my Software** displays the software version.



The **EzSpec** window, **About my Software** displays the software version.



The **Protein A280** window, **About my Software** displays the software version.



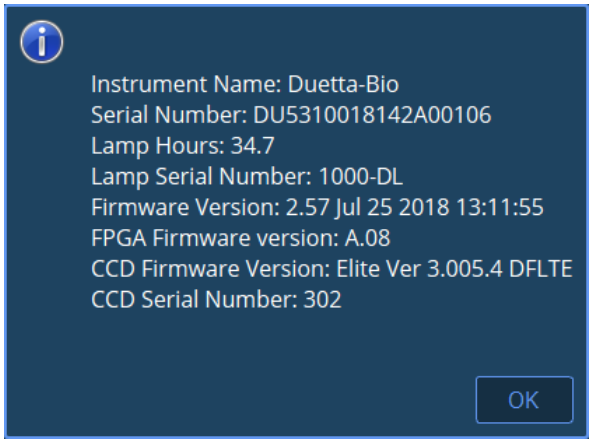
Click **Copy** to copy the text, that you can then paste into a document.

Click **Cancel** to close the displayed information.

6.3.8.2 About My Hardware

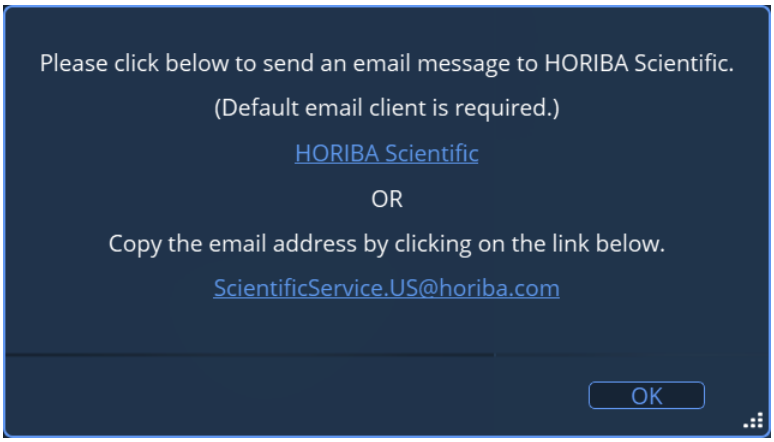
About my Hardware displays the:

1. Instrument Name: Duetta or Duetta-Bio
2. Instrument **Serial Number**
3. Number of Lamp Hours
4. Lamp Serial Number
5. Duetta Firmware Version
6. FPGA Firmware version
7. CCD Firmware Version
8. CCD Serial Number



6.3.8.3 Help (Email and Website)

Email shows the following instruction:



If your email is active, then clicking on the **HORIBA Scientific** link will start a new email to Horiba Scientific.

Or, clicking on **ScientificService.US@horiba.com** will copy this string which you can then paste into a document.

Website

Clicking on **Website** opens your web browser to the HORIBA Scientific website, Duetta page.

6.3.8.4 Help Files

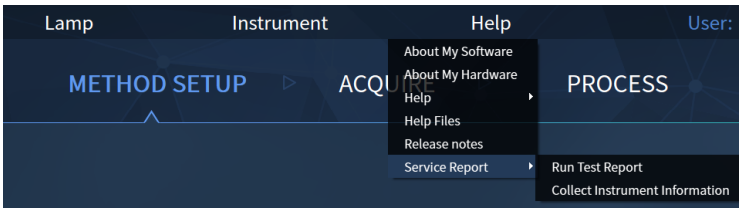
Clicking on **Help Files** opens the Duetta™ with EzSpec™ Operation Manual (this manual).

6.3.8.5 Release Notes

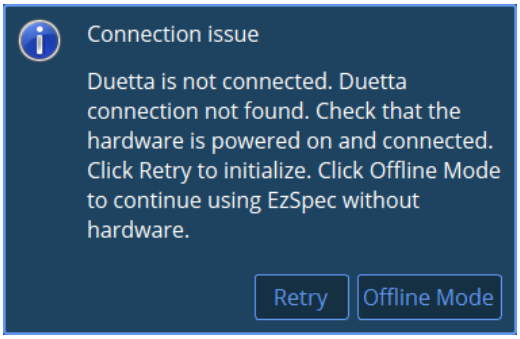
Clicking on **Release Notes** will open up a PDF file containing the product release notes for the current version of EzSpec.

6.3.8.6 Service Report

Click on Help, Service Report, Run Test Report.



When the Duetta is turned OFF or is not connected to the computer, the **Connection issue** prompt appears.

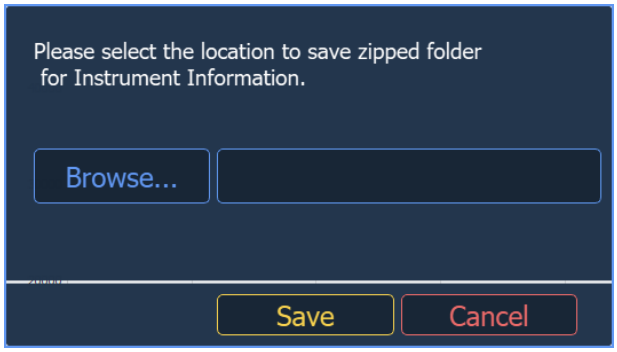


Click the **Retry** button to initialize the Duetta.

Click the **Offline** button to continue using EzSpec without the Duetta.

If the Duetta is **ON** and connected, but the lamp is not turned **ON**, then the Help menu closes.

When the Duetta is turned ON, is connected, and the lamp is **ON**, then EzSpec first asks for a folder location in which to save the **Instrument Information.**

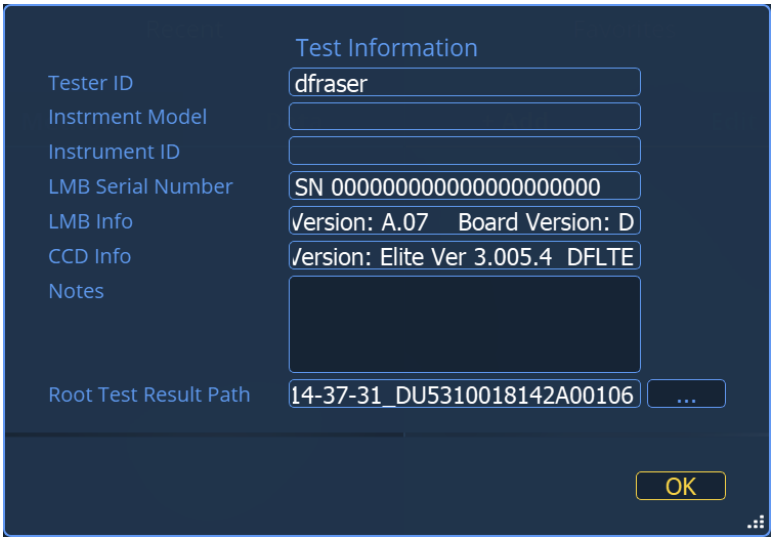


Click the **Browse…** button and select or create a folder for the Test Information.

Then click **Save**.

Click the **Cancel** button to exit the **Run Service Report** procedure.

The **Test Information** window appears.



Click **OK.**

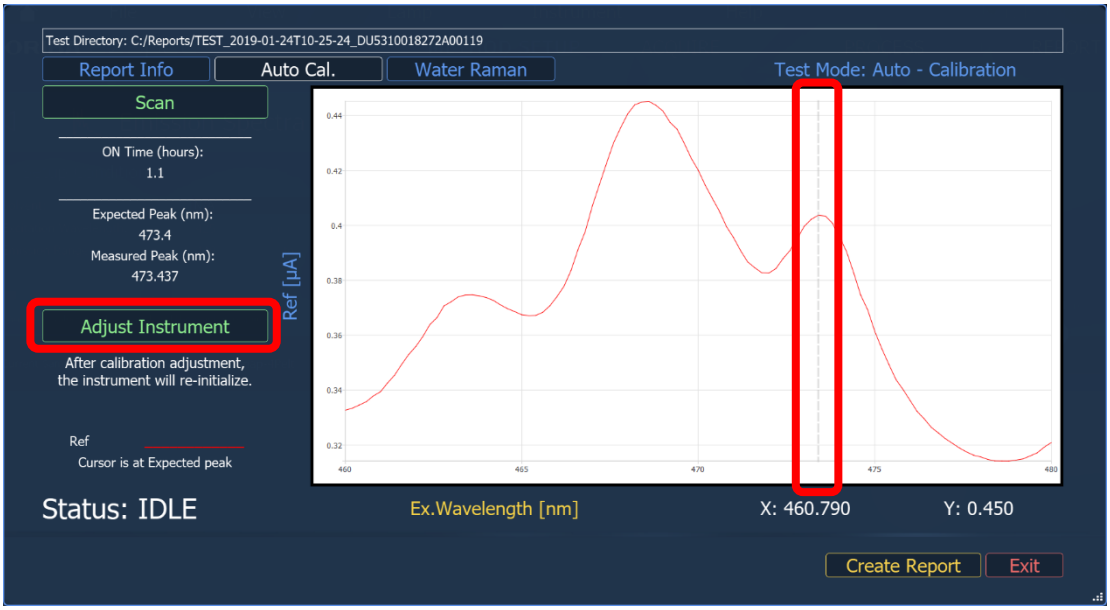
The **Test Mode: Auto - Calibration** window with three buttons at the top appears. The first tab **Report Info** will return to the test information window in the previous step, which can be edited.

The second button is **Auto Cal**. **Auto Cal** will not run until the xenon lamp has been on for more than one hour to let the system warm up and equilibrate properly.

Click the **Auto Cal** tab and then the **Scan** button. No sample is required.

Wait until the scan finishes.

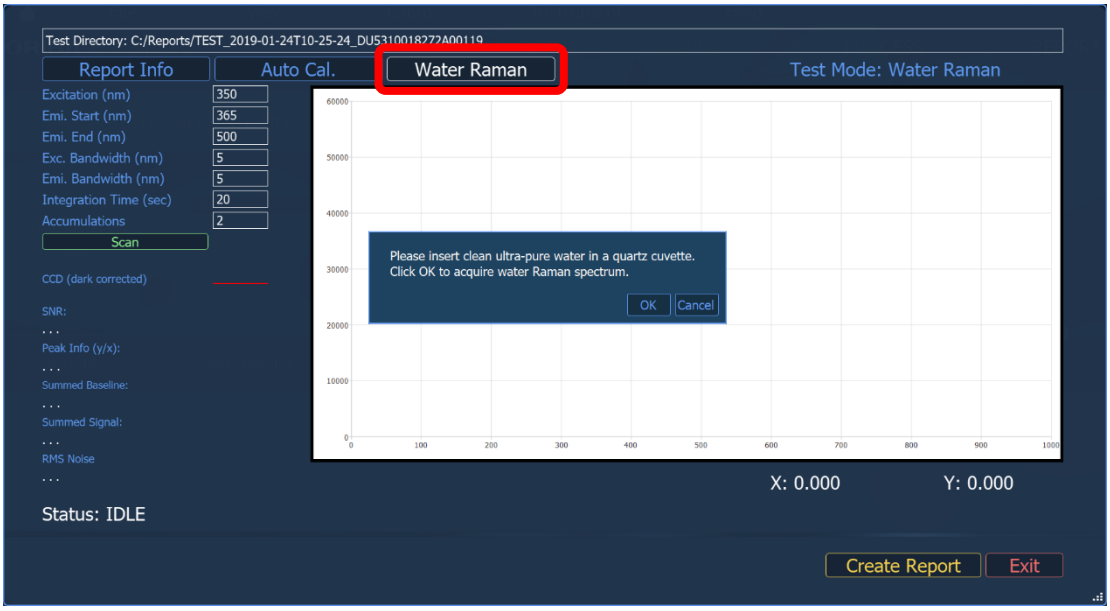
Autocal will scan the excitation monochromator and measure the output of the xenon lamp from 460 to 480 nm using the Reference detector, and find the position of the peak which is supposed to be at 473.4 nm indicated by the dashed line.



If the 473.4 nm peak is found more than 1 nm away, click **Adjust Instrument** and the software will recalibrate the wavelength position, reinitialize the instrument, and rescan the xenon lamp peak.

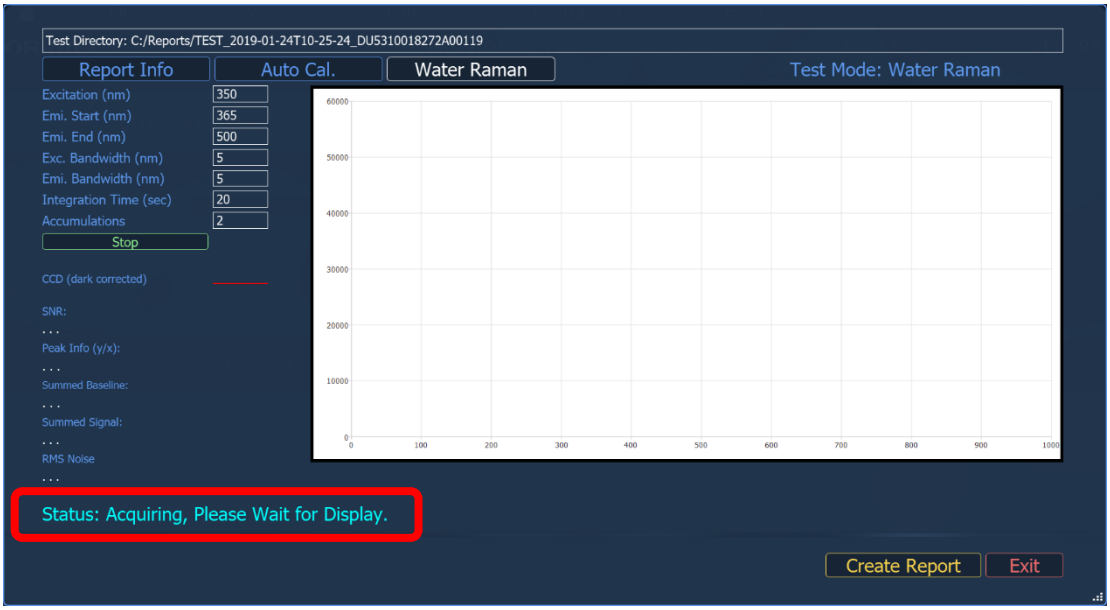
Next, the **Water Raman** button can be selected to measure the water Raman spectrum.

A blank Water Raman window appears with a prompt to insert clean ultra-pure water in a quartz cuvette.



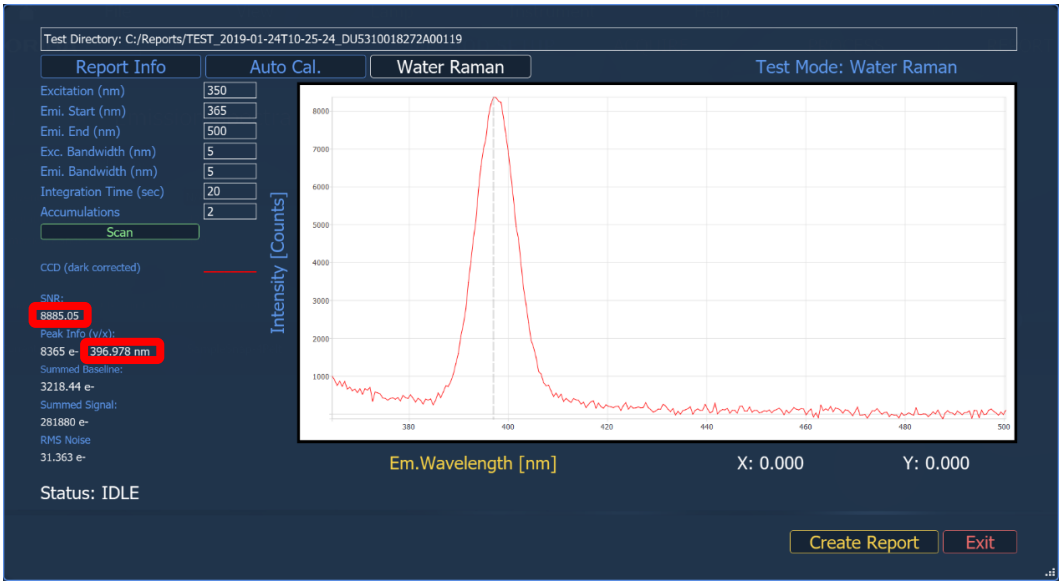
Insert the cuvette in the cuvette holder in the Duetta™.

Click **OK**. Acquiring the Water Raman spectrum will take 80 seconds.



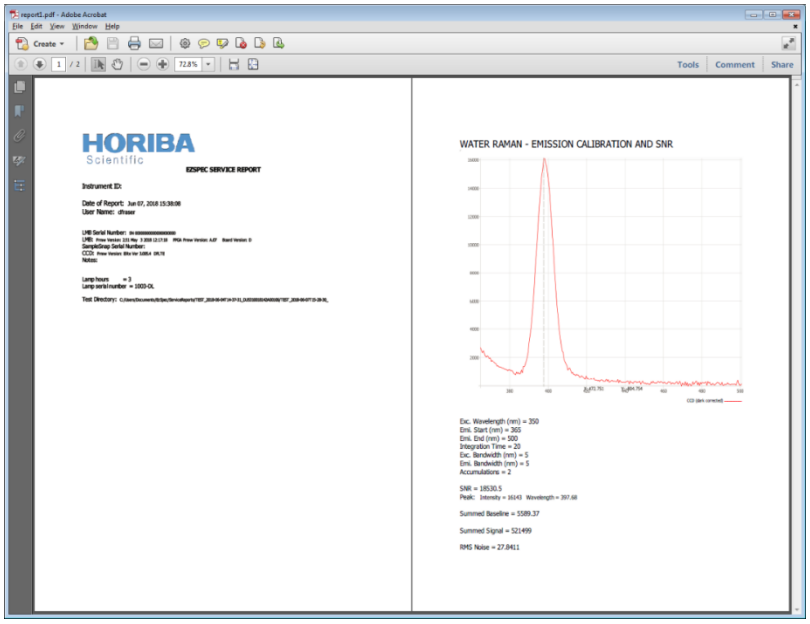
The result appears in the **Water Raman** window.

The water Raman signal-to-noise ratio (SNR) should be above 6000:1 and the peak should be at 397 nm +/- 1 nm. If the SNR is below 6000:1 as reported by the Service Report, or if the peak is not near 397 nm, please refer to the Troubleshooting section of the manual (Section 11). Also, create the PDF report (Create Report) so that there is a record to send to the HORIBA Service department if required.



Click **Create Report**.

The report appears.



Click **File**, **Save As**….

A **Save As** window appears for you to select a file name and location for the report.

Enter a file name and select a folder. Click **Save**.

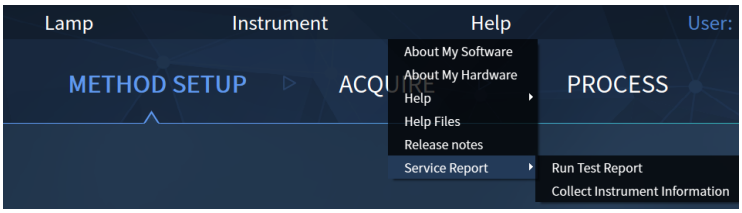
On the **Test Mode**: **Water Raman** window, click **Exit**.

The calculation of the above Water Raman Signal to Noise Ratio is performed as follows:

> ⓘ Note: The method setup has no Blank, no Inner Filter Effects, and no correction for the Reference channel.

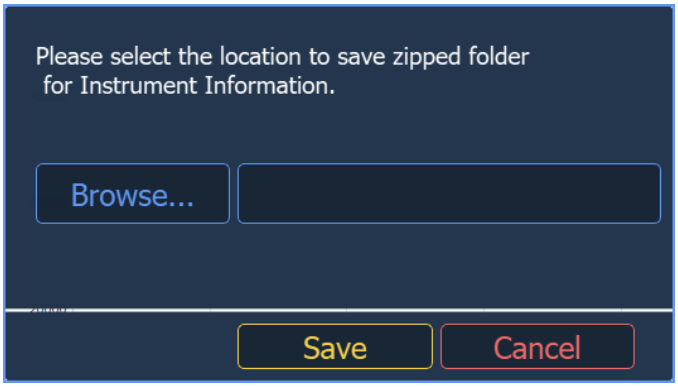
1. Convert the signal to electrons. For the Duetta™, for Best Dynamic Range mode, the conversion factor is 3.3 e-/count. The Summed Baseline and Summed Signal values are sums of the data after conversion to electrons.
2. Interpolate the emission region from 365 nm to 452.5 nm to 0.5 nm increments
3. Sum the interpolated signal from 394.5 to 399.5 nm (5 nm around 397 nm which is the “Peak Signal”) to get the Summed Peak value.
4. Sum the interpolated signal from 447.5 to 452.5 nm (5 nm around 450 nm) to get the Summed Baseline value.
5. Take the region from 447.5 to 452.5 and calculate the point-to-point noise (take the difference between each pair of adjacent points).
6. Take the average of those values.
7. Take the above average and divide by 5 to calculate the “Noise”.
8. SNR = (Summed Peak - Summed Baseline)/ (Noise).

6.3.8.6.1 Collect Instrument Information



Click this command to save the last log files created for the instrument and software. A prompt will ask the user to save them as a zip file.

This zip file can be provided to the HORIBA Service department in the event of any issue with the instrument. Use the Contact HORIBA menu for website and email address information in the event of any issue.

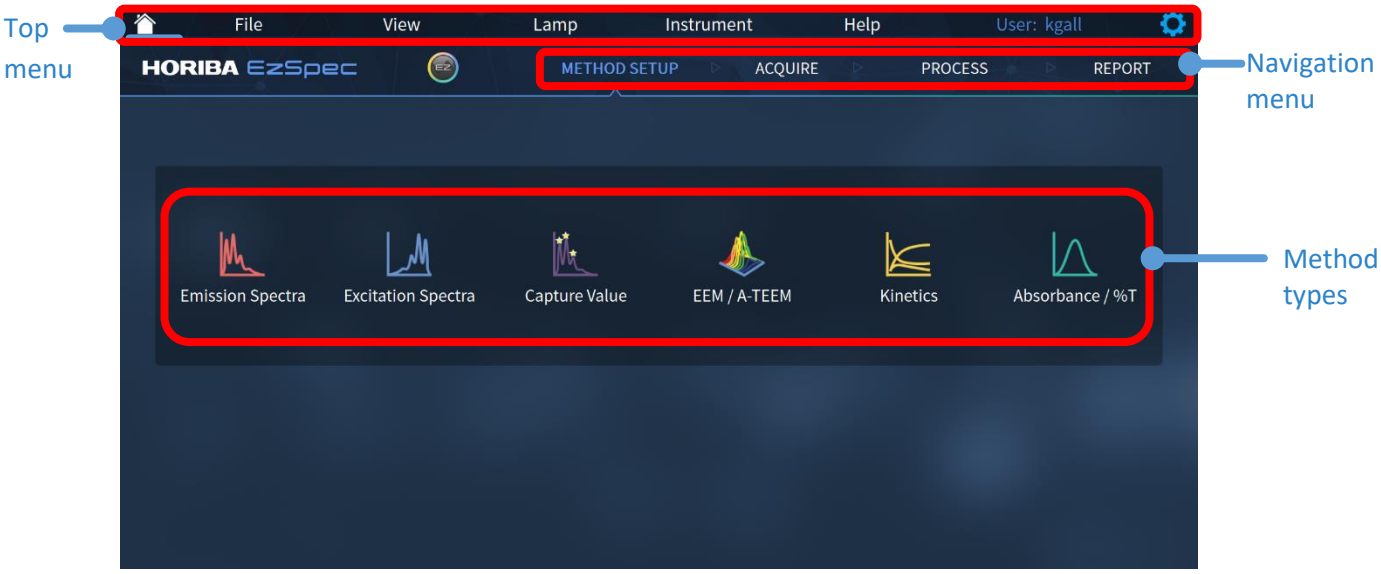


For any error detected by the software, the last log files will automatically be saved in a time-stamped zip file along with the last data and method collected when the error occurred.

Use the Contact HORIBA (Help) menu to report any issues. Please supply the log files, the TEST data folder, and the last Service Report file (.pdf file).

6.4 Method Setup

On the Dashboard, clicking on the **New Method** button, or on the EzSpec application top menu clicking on **File, New Method** displays the **Method Setup menu**.



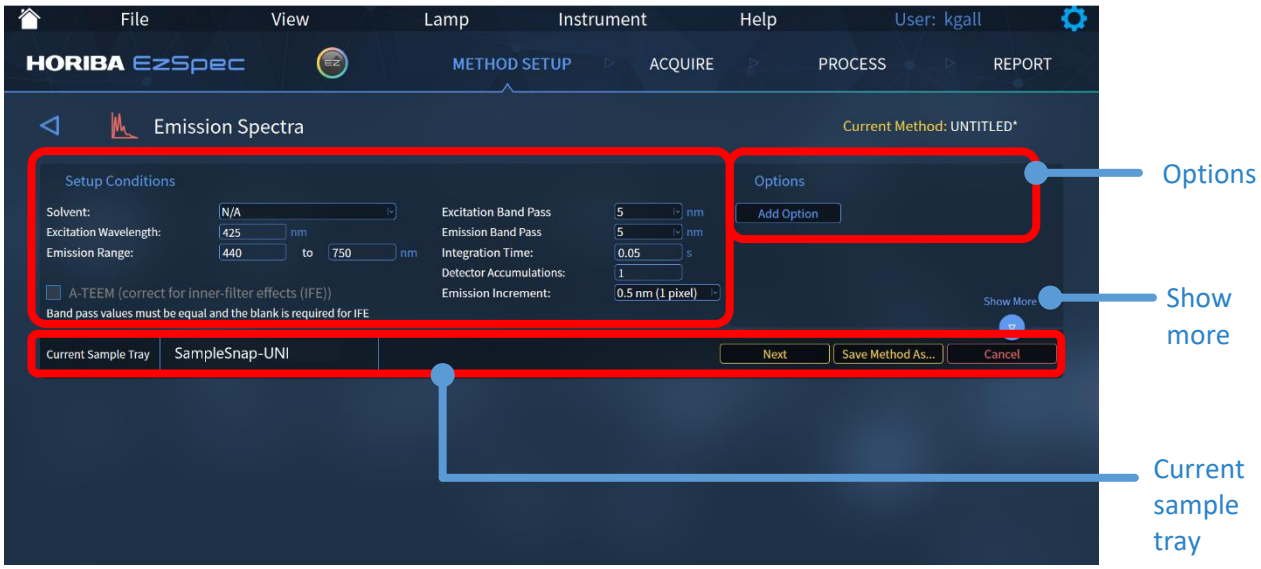
The top toolbar shows the Top Menu described earlier in this section, and below that, four navigation menu items: **Method Setup**, **Acquire**, **Process**, and **Report**. Clicking on one of these buttons opens the respective menu.

Click on a Method type button (and for some types on the sub-menu) on the Method Setup menu to open the last saved method setup for that method type. If the user has stayed in the Method Setup/Acquire/Process/Report (the user has not returned to the Dashboard or the Launch window), then returning to that method type will show the setup with the most recent settings, even if the setup had not been saved. The user then can change parameters as desired. If an unsaved setup is run to acquire data, then that method information will always be saved in the resulting data folder.

Methods are files that contain all the experimental parameters required to control the Duetta™ to acquire data. They can also store comments about the data acquisition.

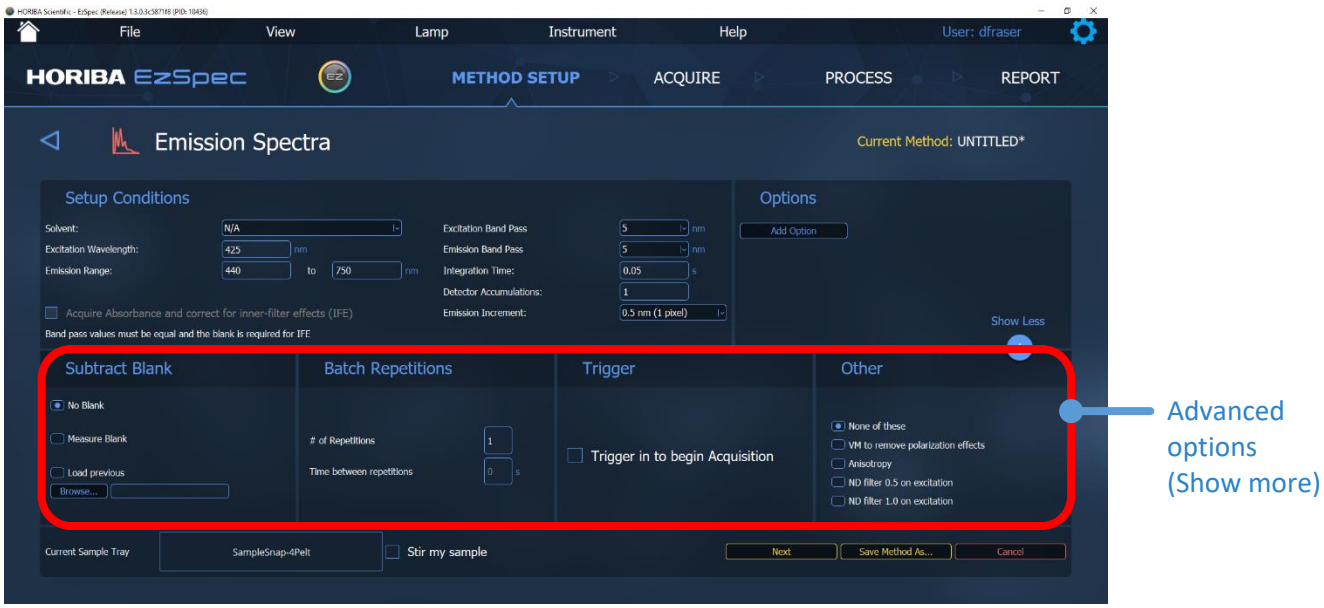
Only one method setup can be open at the same time.

For all Method Setup menus, the layout is similar. The top toolbar is the same as on the Main Setup menu. Below that on the left are the **Setup Conditions** specific to the current method type. To the right is the **Options bar** for hardware options. The image below shows the Temperature and Sample Changer options enabled.



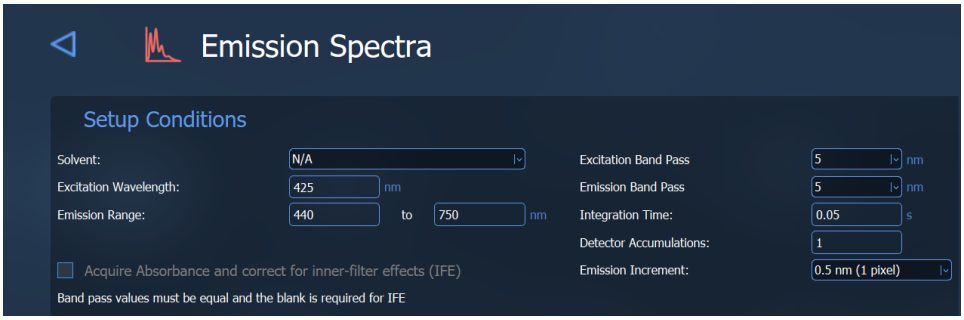
The Bottom bar shows the **Current Sample Tray** with the identified tray and three buttons: **Next**, **Save Method As…**, and **Cancel**.

Click on the **Show More** button to show advanced options above the bottom bar. The **Show More** button changes to **Show Less** when the advanced options are exposed.



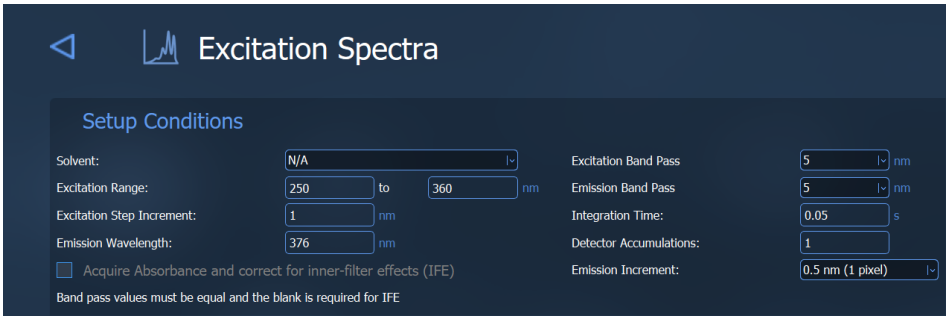
6.4.1 Method types

6.4.1.1 Emission Spectra



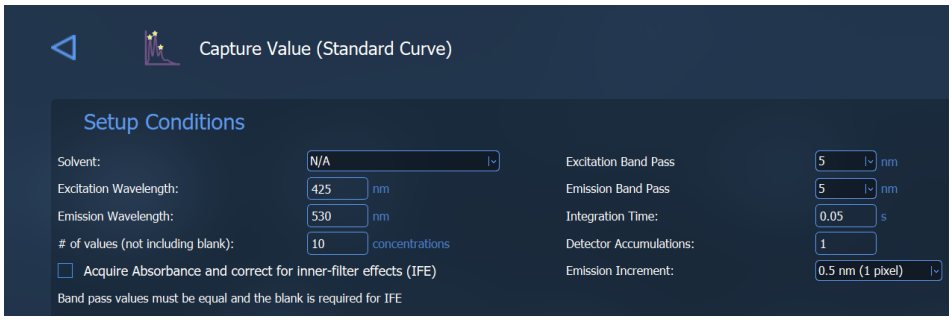
The data is displayed as signal intensity vs. emission wavelength for the emission range specified by the user. The default display is a 2D Graph.

6.4.1.2 Excitation Spectra



The data is displayed as signal intensity vs. excitation wavelength at the specified emission wavelength only. The default display is a 2D Graph.

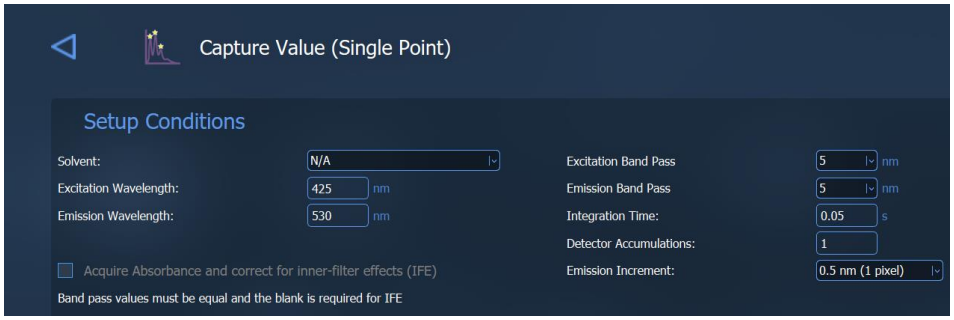
6.4.1.3 Capture Value (Standard Curve)



The data is displayed as single value intensity points at user specified concentrations. The default display is a 2D Graph. Before the acquisition of each sample, the user is prompted to insert a sample and enter a concentration value (numerical text entry) and units (alphanumeric text entry). Each value is added to a look up table with Sequence number, Intensity value, and Concentration value.

After the Concentration value and intensity value are recorded to the LUT, the user is prompted to change the sample, enter the concentration (keeping the same units as first input) and click **Next** to capture the next value. The intensities and concentration values are then processed by **Process**, **Analysis Tools**, **Create Standard Curve from Data**.

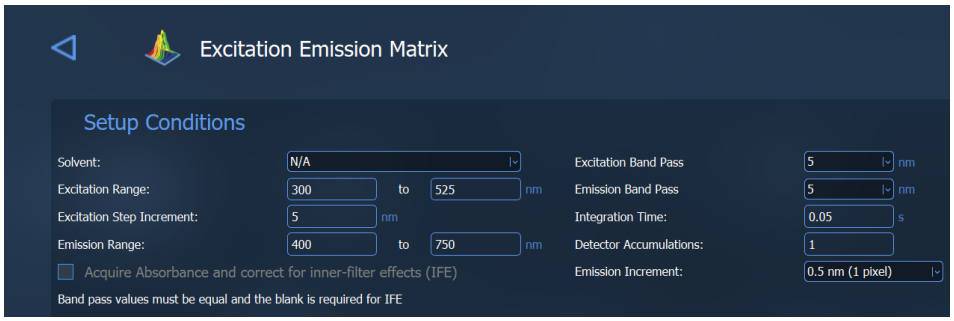
6.4.1.4 Capture Value (Single Point)



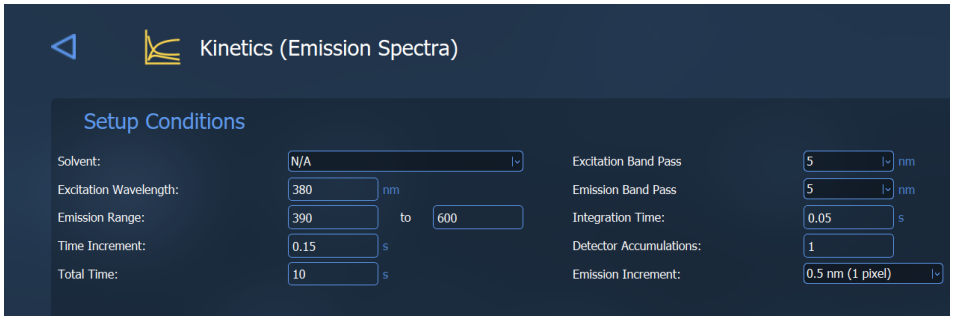
The data is displayed as single value intensity points at the user-specified wavelength.

6.4.1.5 A-TEEM/EEM

The data is displayed as signal intensity vs. excitation and emission wavelengths for the ranges specified by the user. An excitation emission matrix (EEM) corrected for inner-filter effects is called A-TEEM. The default display is a 3D Contour Plot.



6.4.1.6 Kinetics (Emission Spectra)

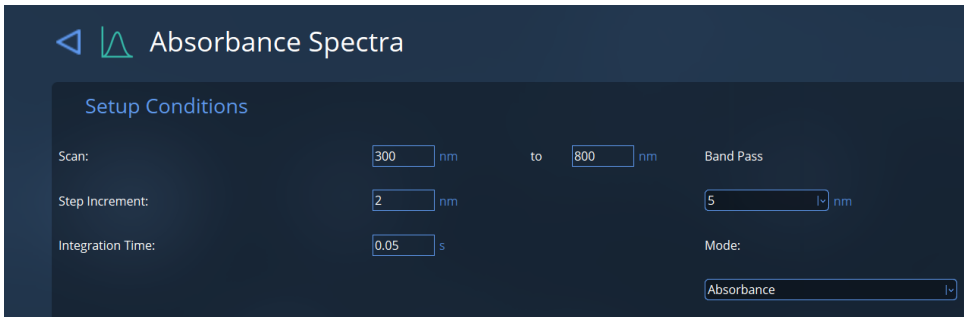


The data is displayed as signal intensity vs. time and emission wavelengths for the ranges specified by the user. The default display is a 3D Waterfall Plot.

6.4.1.7 Kinetics (Fixed Wavelengths)

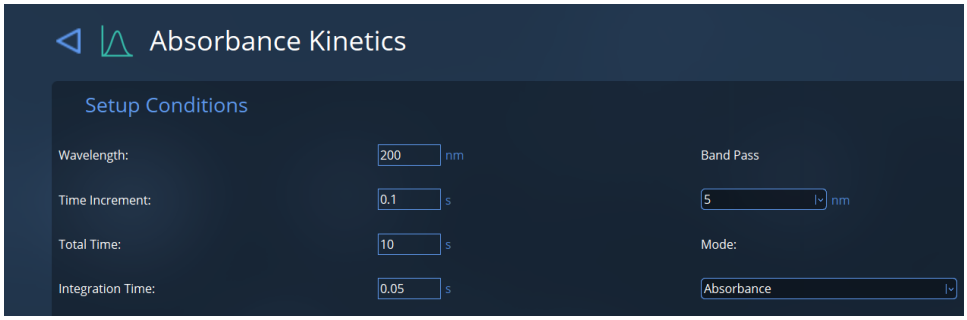
The data is displayed as signal intensity vs. time for the time range set by the user. Each excitation wavelength pair produces a separate trace. Click on the Add button + to add a new excitation wavelength pair. Click on the Delete button × to delete an excitation wavelength pair. The default display is a 2D Graph of overlaid intensity vs. time traces.

6.4.1.8 Absorbance / % Transmittance Spectra



The data is displayed as absorbance and/or % transmittance vs. wavelength for the ranges set by the user. The default display is a 2D Graph.

6.4.1.9 Absorbance / % Transmittance Kinetics



The data is displayed as absorbance and/or % transmittance vs. time for the time range and wavelength set by the user. The default display is a 2D Graph.

6.4.2 Setup Conditions

These setup conditions appear in various method setups.

> ⓘ **Note**: Emission data, including start and end wavelengths, will use the closest wavelengths that the CCD detector reports.

6.4.2.1 # of values

Applies to the Capture Value (Standard Curve) method type.

The user enters the number of values to be measured (one value per concentration).

6.4.2.2 A-TEEM (correct for inner-filter effects) checkbox

Applies to Emission Spectra, Excitation Spectra, Capture Value, and A-TEEM/EEM method types.

The user checks this checkbox to acquire absorbance data simultaneously with fluorescence data and correct the fluorescence data for inner-filter effects (IFE).

When this checkbox is checked, absorbance data and the spectral fluorescence data are acquired simultaneously.

Conditions:

- IFE can only be used and the checkbox is only active when the excitation and emission band pass settings are the same.
- A blank measurement is required. If the Sample Changer is used and the blank and sample positions are set, then this is done automatically. If a single cuvette holder is used, then during the acquisition the user is prompted to “Insert Blank” and then “Insert Sample”.

6.4.2.3 Band pass

Applies to Absorbance method types.

The user selects the slit width in band pass from the pull down menu. The allowed values are 1, 2, 3, 5, 10, 20 nm.

6.4.2.4 Detector Accumulations

Applies to Emission Spectra, Excitation Spectra, Capture Value, A-TEEM/EEM, and Kinetics method types.

The user enters the number of detector accumulations. If the number of Detector Accumulations > 1, the CCD accumulations are summed for the total number of accumulations.

6.4.2.5 Emission Band pass

Applies to Emission, Excitation, Capture Value, A-TEEM/EEM, and Kinetics method types.

The user selects the emission band pass from a pull down menu. The allowed values are 1, 2, 3, 5, 10, 20 nm.

6.4.2.6 Emission Increment

Applies to Emission, Excitation, Capture Value, A-TEEM/EEM, and Kinetics method types.

The user selects the emission increment value from the pull down menu. The allowed values are 0.5, 1, 2, 4 nm corresponding to binning width of 1, 2, 4, 8 pixels respectively, on the CCD. Using binning here will decrease the resolution, but increase spectral signal.

6.4.2.7 Emission Range

Applies to Emission Spectra, A-TEEM/EEM, and Kinetics (Emission Spectra) method types.

The Start wavelength is automatically filled in based on the selected excitation wavelength and slit settings, as: excitation wavelength + (sum of the excitation and emission band passes)*1.5.

The user can change the Start and End wavelengths in the enterable fields. The entered values can be between 250 and 1100 nm. If the **Acquire Absorbance and correct for inner-filter effects** checkbox is checked, then the end emission wavelength is 1000 nm to match the Absorbance range.

6.4.2.8 Emission Wavelength

Applies to Excitation Spectra, Capture Value, and Kinetics (Fixed Wavelength) method types.

Set the emission wavelength (nm) in the enterable field.

6.4.2.9 Excitation/Emission Wavelength Pair(s)

Applies to the Kinetics (Fixed Wavelengths) method type.

The user can click the Add button  to insert more blank pairs. To delete a wavelength pair, click the Delete button  to the right of the pair.

The user enters excitation and emission wavelength (nm) values in the enterable fields. If the list of wavelengths exceeds 4 pairs, a scrollbar appears on the right.

6.4.2.10 Excitation Band pass

Applies to Emission Spectra, Excitation Spectra, Capture Value, A-TEEM/EEM, and Kinetics method types.

The user selects the excitation band pass from a pull down menu. The allowed values are 1, 2, 3, 5, 10, 20 nm.

6.4.2.11 Excitation Range

Applies to: Excitation Spectra and A-TEEM/EEM method types.

Set the Start value in the enterable field. The entered value can be between 250 and 1000 nm, or zero (0).

Set the End value in the enterable field. The entered value can be between 250 and 1000 nm.

> ⓘ **Note**: 410 LP and 525 LP filters are used for automatic order sorting. The user may hear these filters switching during excitation scanning. The status/position of this filter wheel can always be viewed using the **Status** button in the Acquire menu.

6.4.2.12 Excitation Step Increment

Applies to: Excitation Spectra and A-TEEM/EEM method types.

This value is for user to fill in.

The entered value can range from a minimum 0.2 nm to a maximum of 20 nm.

EEM Setup: The default value is 5 nm or the last used setting for that user.

6.4.2.13 Excitation Wavelength

Applies to: Emission Spectra, Capture Value, EEM and Kinetics method types.

The user sets the excitation wavelength in the enterable field. The entered value can be from 250 to 1000 nm, or zero (0).

6.4.2.14 Integration Time

Applies to all method types.

The user sets the integration time (seconds) in the enterable field. The value can range from 50 ms (0.05 s) to 5 minutes (600 s) for all Fluorescence experiment types and 1 ms (0.001 s) to 5 minutes (600 s) for Absorbance/%Transmittance method types.

6.4.2.15 Mode

Applies to Absorbance method types.

The user selects what is to be displayed from either **Absorbance** or **% Transmittance** or **Both** in the pull down menu.

6.4.2.16 Scan

Applies to Absorbance Spectra method type.

The user enters the start and end values for the wavelength scan in the enterable fields. The entered values can be between 250 and 1000 nm. The end value must be larger than the start value.

6.4.2.17 Solvent

Applies to Emission Spectra, Excitation Spectra, Capture Value, EEM, and Kinetics method types.

Click on the solvent pull down menu and select a solvent from: aqueous/water/buffer, DMSO, methanol, ethanol, or toluene. Selecting “N/A” (for solid samples or solvents not in the list) means that no Raman peaks will be identified. The solvent choice will be used to detect where possible Raman peaks will appear. When viewing a graph, use **View**, **Raman Indicators** to view the positions of the major solvent Raman peaks.

6.4.2.18 Step Increment

Applies to the Absorbance Spectra method type.

The user enters the step increment between data points in the enterable field.

6.4.2.19 Time Increment

Applies to the Absorbance Kinetics method type.

The user enters the time increment between data points in the enterable field. The time increment must be longer than the integration time. If Duetta cannot attain the requested time increment under the method setup conditions, it will acquire the data as fast as possible, recording the actual time increment used.

6.4.2.20 Total Time

Applies to the Absorbance Kinetics, Kinetics Emission Spectra and Kinetics Fixed Wavelength(s) method types.

The user enters the total time for the experiment in the enterable field.

6.4.2.21 Wavelength

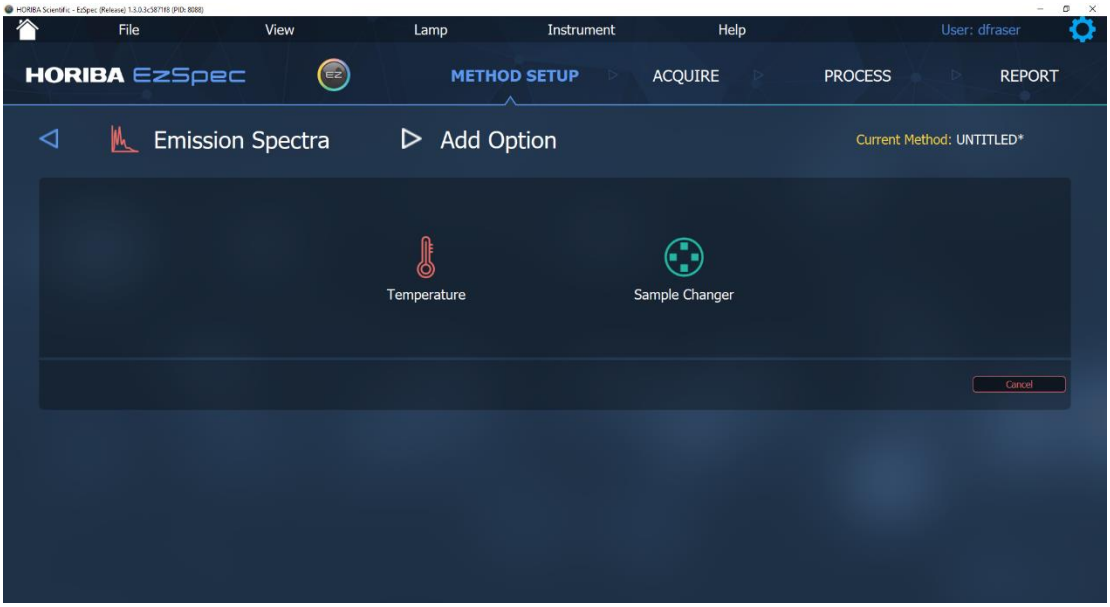
Applies to the Absorbance Kinetics method type.

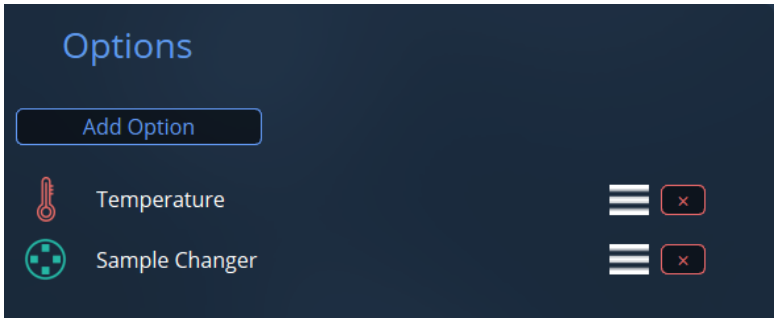
The user enters the value in the enterable field for the absorbance wavelength requested for this single wavelength kinetics acquisition. The entered value can be between 250 and 1000 nm.

6.4.3 Options

Click the **Add Option** button in the **Options** bar to open a menu of available options. These buttons are shown in color if the Duetta detects them, but greyed out if not detected.

When options have been added to the Option Bar, each option has Edit and Delete buttons. Click the **Edit** button to open the option menu for that option. The **Delete** button deletes the option from the Options Bar.





In EzSpec™, options are looped with the setup. The first added option is listed first in the list and does the outside loop. An option will loop (temperature or sample changer position) with the option below it (if one is selected) and the main experiment (for example Emission Spectrum). For the options example above, if the method type is an emission spectrum and the temperature option is set to Step Temperature, the sample will be set to Temperature #1, then the emission spectra acquired for the selected sample positions, then Temperature #2, acquire the spectra at the selected sample positions, and so on.

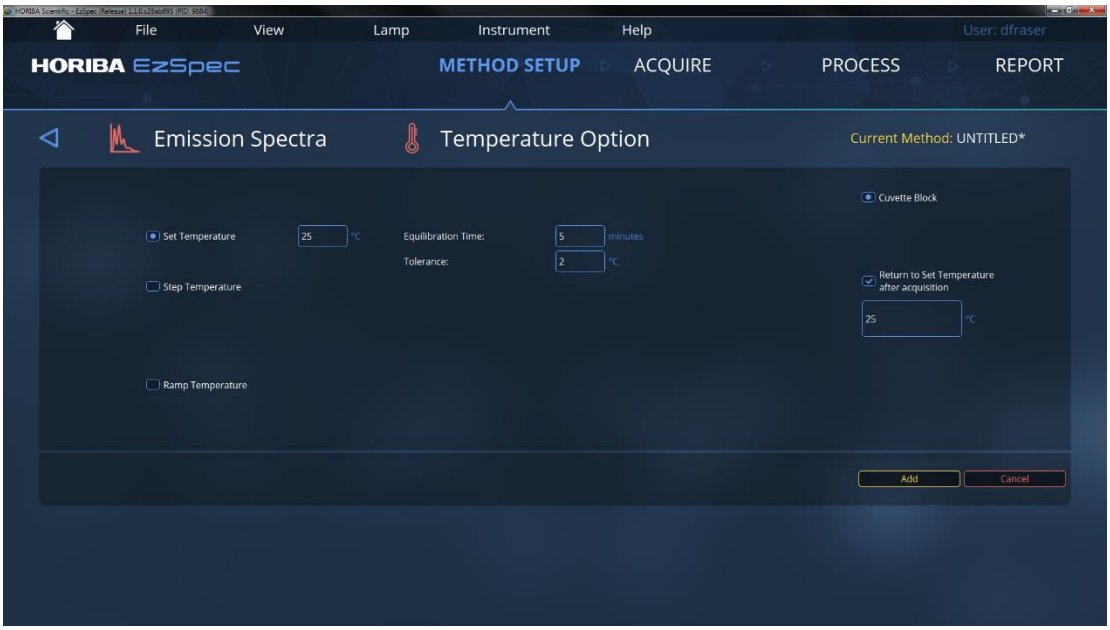
Batch repetition is the final outside loop. It loops the entire experiment setup including options.

6.4.3.1 Temperature Option

Click on the **Temperature** button to show the Temperature Option setup menu.

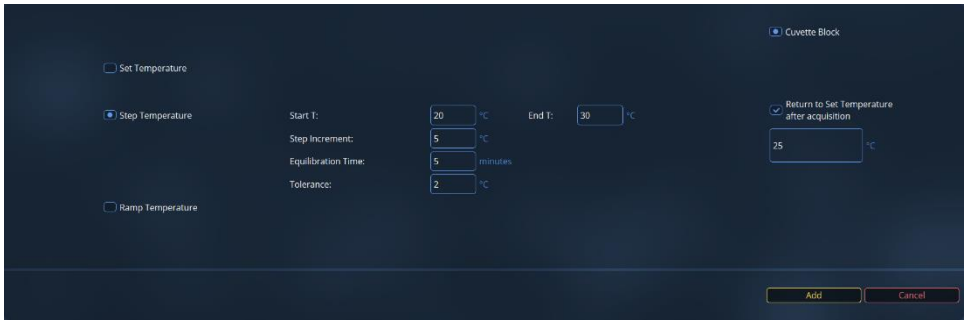
> ⓘ **Note**: The maximum allowed temperature in any temperature field is 105 °C
>
> ⓘ The minimum allowed temperature in any temperature field is -15 °C.

1. On the right, the Return to Set Temperature after acquisition checkbox may be unchecked or checked.



   a. If this box is unchecked, the temperature will hold at the last temperature of the method after the acquisition is completed.
   b. If this box is checked, then an enterable box appears for the user to enter a temperature in °C that the temperature controller will go to after the acquisition is finished.

2. On the left, click one of the radio buttons: **Set Temperature**, **Step Temperature**, or **Ramp Temperature**.
   These temperature options are available depending on the method type
   a. **Set Temperature**
      i. Enter the single temperature in °C in the box.
      ii. Enter the **Equilibration Time** in minutes.
      iii. Enter the **Tolerance** in °C.
      iv. When the user clicks **Acquire** in the Acquire menu, the temperature will begin to go to the **Start T**.
      v. When the temperature controller readout reaches the set temperature ± tolerance, the equilibration time begins counting down.
      vi. After the equilibration time has passed, the acquisition begins.
   b. **Step Temperature**



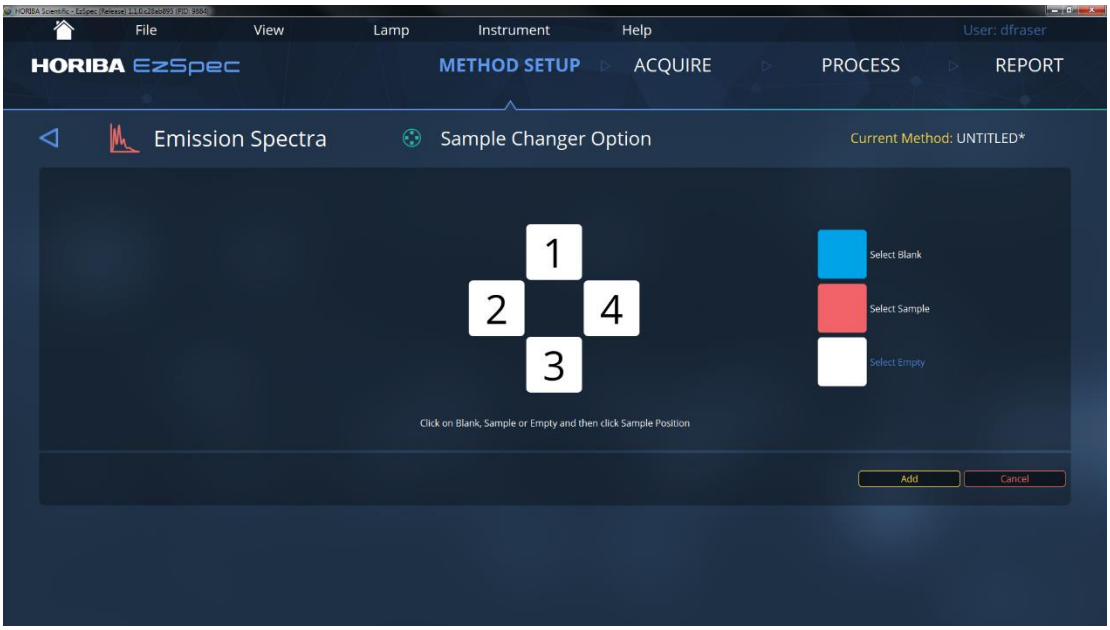
      i. Set the **Start T** in the enterable field.
      ii. Set the **End T** in the enterable field.
      iii. Set the **Step Increment** in the enterable field. The minimum step increment is the tolerance of the SampleSnap-1Pelt or SampleSnap-4Pelt.
      iv. Set the **Equilibration Time** in the enterable field in minutes.
      v. Set the **Tolerance** in the enterable field in °C. The minimum tolerance is 0.2 °C.
      vi. When the user clicks **Acquire** in the Acquire menu, the temperature will begin to go to the **Start T**.
      vii. When the temperature controller readout reaches the set temperature ± tolerance, the equilibration time begins counting down.
      viii. After the equilibration time has passed, the acquisition begins.

c. **Ramp Temperature**

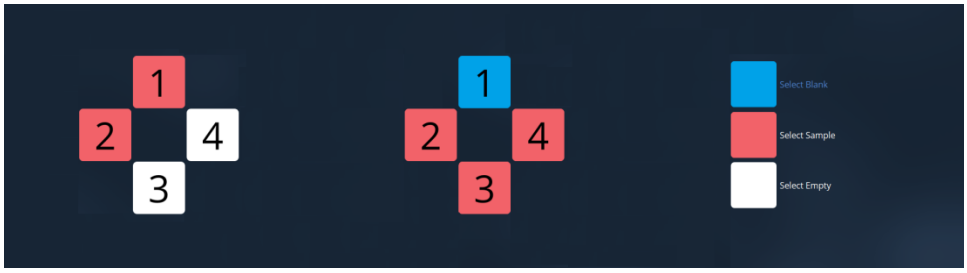
i. Set the **Start T** in the enterable field.
ii. Set the **End T** in the enterable field.
iii. Set the **Ramp Rate** in the enterable field.
iv. Set the **# of Temperature Points** (number value > 2).
    1. The maximum number of temperature points
       = Total Time / Minimum CCD Sampling Time
       where Total time = Absolute value (End T – Start T)/Ramp Rate
       and Minimum CCD Sampling Time = Integration Time set on the Setup Conditions page.
v. Data acquisition happens every Temperature Sampling Increment
   = Absolute Value (End T – Start T) / (# of data acquisitions).
vi. Under the heading **For Start T only**
    1. Set the **Equilibration Time** in the enterable field in minutes.
    2. Set the **Tolerance** in the enterable field in °C.
vii. When the user clicks **Acquire** in the Acquire menu, the temperature will begin to go to the **Start T**.
viii. When the temperature controller readout reaches **Start T ± Tolerance**, the equilibration time begins counting down.
ix. After the equilibration time has passed, the acquisition begins.
    1. Click the **Add** button to add **Temperature** to the **Options Bar**.

6.4.3.2 Sample Changer Option

Click on the **Sample Changer** button to show the Sample Changer Option setup menu.



1. Click on the **Select Blank** button and then click on position **1** if you want to acquire blank data. Only position 1 can contain a blank.
2. Click on the **Select Sample** button and then click on any of positions 1 – 4 to select those positions for a sample.
3. Click on the **Select Empty** button and then click on any of positions 1 – 4 to remove a blank or sample and leave that position unoccupied.
4. The turret runs in order position 1, 2, 3, 4 and then reverses direction to go back to 1. It will not rotate completely around to go from position 4 to 1. Because the turret always goes back to position 1, it saves time if the selected positions start at 1.
5. Examples of selected turret positions:



6. Click the **Add** button to add the **Sample Changer** with selected positions to the **Options Bar.**

6.4.4 Advanced Options

Advanced options consist of **Subtract Blank**, **Batch Requirements**, **Trigger**, and **Other**.

These are seen below the Setup Conditions and Options panel.

> ⓘ **Note:** - Not all advanced options are visible in every Method Setup and the visibility of some options or settings may depend on other settings.

6.4.4.1 Subtract Blank

There are 3 radio buttons: **No Blank**, **Measure Blank**, and **Load previous**.

1. **No Blank** – blank data is not used.
2. **Measure Blank**
    a. In the **Measure the blank** pull down menu, select whether to measure the blank **Before** or **After** the sample. The blank is saved automatically as blank.ezspec_data in the data folder along with the final measured data file.
3. **Load previous**
    a. Click on the **Browse…** button to load and use a previously saved blank data. The blank data path and file name is shown in the box to the right of the Browse button.
    b. The blank method parameters must match the sample method parameters in the Setup Conditions. EzSpec™ gives an error message on loading blank data if this is not the case.

6.4.4.2 Batch Repetitions

1. Batch Repetitions creates the outermost loop with the entire method (including all options, blank measurement, and triggering) inside the loop. **Batch Repetitions** is not used for Kinetics: Emission Spectra and Fixed Wavelength(s).
2. **# of Repetitions**: Enter the number of repetitions in the enterable field. The default value is 1 meaning the entire method runs one time.
3. If the number of repetitions is > 1, then the user can enter the **Time between repetitions** in the enterable field. The minimum value will be the integration time in seconds and the maximum value is 10,000 seconds (around 2.8 hours).

6.4.4.3 Trigger

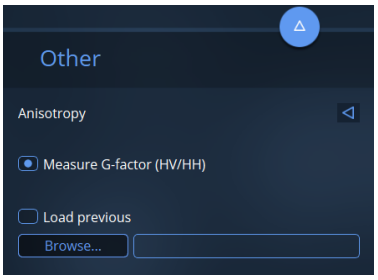
Check Trigger in to begin Acquisition so that an external trigger can be used to begin an acquisition. Trigger connections are for TTL pulses in and out and are BNC connectors located at the back of the Duetta.

6.4.4.4 Other

Up to five radio buttons and a check box.

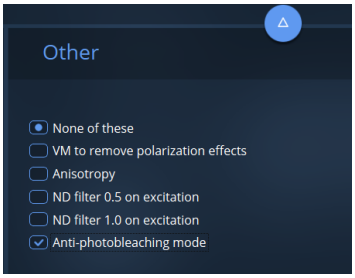
The radio buttons are exclusive - only one can be selected at a time, and one must be selected. The radio buttons include: **None of these**, **VM to remove polarization effects**, **Anisotropy**, **ND filter 0.5 on excitation**, and **ND filter 1.0 on excitation**.

1. **None of these**
   This is the default setting. Neither polarizers nor ND filters will be used. This is the only radio button that will appear for Absorbance Spectra or Absorbance Kinetics.
2. **VM to remove polarization effects**
   This option only applies to the Duetta-Bio version of the instrument. The excitation polarizer is set to vertical (0°) polarization and the emission polarizer is set to magic angle (54.7°) polarization. These polarizer positions cause the emission signal to be isotropic instead of anisotropic, removing any polarized light effects from the emitted light signal.
3. **Anisotropy**
   This option only applies to the Duetta-Bio version of the instrument. If anisotropy is selected, the anisotropy will be measured for that specified method type.
   When the Anisotropy radio button is selected, the other list items disappear and the menu to either **Measure G-factor (HV/HH) or Load previous** G-factor appears.



a. Measure G-factor (HV/HH): Select this radio button to acquire the G-factor along with the anisotropy acquisition. The G-factor is saved automatically as gfactor.ezspec_data in the data folder along with the final measured data file.
b. Load previous
    i. To load a previously acquired G-factor file (.ezspec_data file), click on the Browse… button and select this G-factor file. The G-factor path and file name is shown in the box to the right of the Browse button.
    ii. Here, the method type must be the same as for the anisotropy with the same band pass, integration time, and wavelength parameters.
    iii. Click on the  button to return to the Other menu.
c. The G-factor is measured after the sample acquisition ends (if applicable).
d. While the G-factor is being measured, a popup window will say “Measuring G-factor”.
e. For anisotropy measurements, the G-factor is temperature independent, so the G-factor can still be collected after the rest of the data. If the temperature is set, stepped, or ramped, the G-factor will be collected as soon as the last sample data (VV, VH) is acquired.
f. The G-factor will be calculated and saved with the raw data as well as to the file specified (if the user specifies to save it in the setup).

4. **ND filter 0.5 on excitation**
    a. Uses the ND 0.5 filter on the excitation filter wheel for the duration of the acquisition.
    b. Note that the final processed spectrum with and without using the ND filters will be the same signal, but the raw signals will be proportionally lower. ND filters may be used when too much emission signal is saturating the CCD with the minimum band pass settings and integration time. The ND filter will reduce the amount of excitation light reaching the sample and in turn, reduce the amount of emitted light reaching the CCD detector in proportion. The final spectrum, corrected for the lamp intensity, will be the same corrected intensity as the spectrum acquired without the ND filter. An example is outlined below:
        i. Without an ND filter in place, 10 µamps lamp signal produces 10,000 counts on the CCD, so the corrected signal = 10,000 counts/10 µamps = 1000 counts/µamp.
        ii. With the ND filter of 0.5, 3 µamps lamp signal produces 3000 counts on the CCD, so the corrected signal is 3000 counts/3 µamps=1000 counts/µamp.
5. **ND filter 1.0 on excitation**
    a. Uses the ND 1.0 filter on the excitation filter wheel for the duration of the acquisition.
6. **Anti-photobleaching mode**



a. This checkbox is independent of the above radio buttons in the Other list.
b. The Anti-photobleaching mode checkbox will be visible if the time increment (or the time between repetitions) + total integration time (to include detector accumulations) is greater than the time it takes to open and close the shutter.
c. If checked, the shutter will close after the total integration time and open at the next data point.

6.4.5 Bottom Bar

The Bottom bar shows the **Current Sample Tray** with the identified tray and three buttons: **Next**, **Save Method As…**, and **Cancel**.

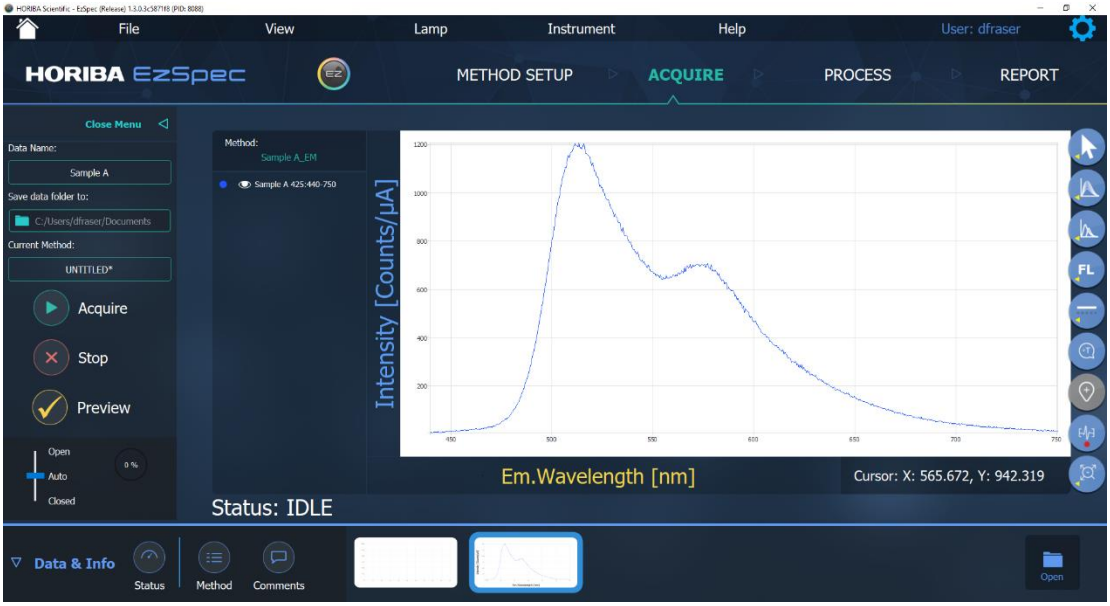
Stir my sample: Checking this checkbox enables the stirrer and shows a slider allowing the user to set the sample stirring speed. The speed range is 0 to 100 rpm (revolutions per minute).

Next: Clicking this **button** changes the Method Setup window to the **Acquire** menu.

Save Method As…: Clicking this button opens a browser window to enter a name for the Method file and select a folder to save it to. This becomes the name, with no suffix, of the **Current Method**. Until a new method has been so named, it uses UNTITLED. You can also use **Save Method As…** to give the current method a new name (creating a copy of a named method that was the current method).

Cancel: Closes the current Method Setup menu and returns to the Main Setup menu. Clicking on the same method type on the Setup menu opens the setup with the most recent settings, even if the setup had not been saved.

6.5 Acquire



6.5.1 Acquisition Toolbar

The Acquisition Toolbar on the left has three different views. The default view shows the Acquisition Toolbar in the center below. Click on the green triangle to the right of **Close Menu** to collapse the toolbar to a thin bar. Click on the green triangle above **Menu** to restore the Acquisition Toolbar. Click on the Preview button to show the Preview Toolbar. Click on the green triangle to the right of **Close Preview** to restore the Acquisition Toolbar.

Collapsed menu | Acquisition Toolbar | Preview Toolbar

6.5.1.1 Acquisition Toolbar

6.5.1.1.1 Data Name

The default Data name is “Sample A”.

Enter the name of the data file you wish to be saved with the measurement in the “Data Name” box. This name will be used for the root name of each trace, and will become the new default data name.

6.5.1.1.2 Suffix

The Data name is also suffixed by a code for the method type and used for the data folder name and the data file name. The suffixed Data Name is also used for the Method name displayed at the top of the legend, unless the method name has been saved prior to acquiring the data.

Method Type	Suffix
Emission spectra	_EM
Excitation spectra	_EX
Capture Value Standard Curve	_StdCur
Capture Value Single Point	_CVSP
EEM or A-TEEM	_EEM
Kinetics Emission Spectra	_KinSpec
Kinetics Fixed Wavelength	_KinFWL
Absorbance Spectra	_AbsTSpec
Absorbance Kinetics	_AbsTKin

6.5.1.1.3 Save data folder to:

Click on the folder icon  and browse to select the location where the acquired data will be saved. A folder will be created with the data name and the timestamp for the acquisition.

6.5.1.1.4 Current Method:

This is the name of the method that is currently in the Method Setup. If the method in the Method Setup has not yet been given a name, the Current Method box will display “UNTITLED*”. An UNTITLED method will be given the name in the **Data Name** box, plus the Method type suffix, when the data is saved. The **Current Method** box shows * if the Method type has not been selected.

6.5.1.1.5 Acquire, Pause and Continue buttons

Click on the **Acquire** button  to start the data acquisition. The **Acquire** button changes to the **Pause** button  when data is being acquired. Click on the **Pause** button to pause acquisition. The **Pause** button changes to **Continue** when acquisition is paused. Click on the **Continue** button to continue acquisition.

6.5.1.1.6 Stop button

Click on the Stop button at any time to stop the acquisition. When data is not being acquired, the Stop button is inactive.

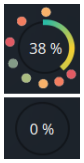
6.5.1.1.7 Preview button

Click on the Preview button to change to the Preview Toolbar. When data is being acquired, the Preview button changes to a grey checkmark and the button is inactive.

6.5.1.1.8 Shutter control

Shutter control is a three stop slide bar with stops: Open, Auto, and Closed. Auto is the default and means that when the instrument is acquiring data, the shutter is open and when it is not acquiring, the shutter is closed. The user can move the slider to the Open or Close position.

6.5.1.1.9 Progress wheel



When acquiring data, the experiment progress is shown as a spinning wheel of dots enclosing the percentage completed.
When not acquiring data, the progress wheel shows “0 %”.

6.5.1.2 Preview Toolbar

The Preview Toolbar stays open until the user hits the **Close Preview** button, which stops the Preview acquisition and returns to the Acquisition Toolbar.

When the Preview Toolbar is open, you cannot click on **Method Setup**, **Process** or **Report** to show those menus.

When the Preview Toolbar is opened, the values show the current setup values.

The user can change any of the values shown in the Preview Toolbar: Excitation wavelength, Excitation and Emission Band Pass, Integration Time, Excitation and Emission Filter Wheel positions, Temperature and Sample Changer position (shown only if present and setup), Detector, and Shutter. The data acquired in Preview shows only one detector at a time (CCD, Ref, or Abs detectors).

There are three buttons at the bottom of the Preview Toolbar: **Run**, **Stop**, and **Update Method**.

6.5.1.2.1 Run

Click the **Run** button to see how the new values affect the data.

The values are not saved to the Method Setup unless the user clicks the **Update Method** button.

6.5.1.2.2 Stop

Click the **Stop** button to stop the current acquisition.

6.5.1.2.3 Update Method

Click the **Update Method** button to save some of the values to the current Method Setup.

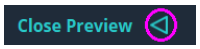
Values that can be saved are: Excitation and Emission Band Pass, Integration Time, and ND Filter status.

Excitation wavelength is updated only if the method is Emission Spectra.

Polarizers, sample position, temperature, shutter will not be updated.

Emission wavelength is not shown in the Preview Toolbar, and is not updated.

6.5.1.3 Close Preview



Click on the arrowhead at the top right of the Preview Toolbar to close Preview and return to the Acquisition Toolbar.

6.5.2 Legend

The legend is shown to the left of the graph or table in the **Acquire**, **Process**, and **Report** menus.

6.5.2.1 Method name

The Method name that appears at the top of the legend is the method that created the data shown in the legend.

If a method type has not been selected and no data has been opened, then the Method name will be blank.

If a method type has been selected, but the method has not been named, and no data has been acquired, then the Method name will be shown as **UNTITLED** until the **Acquire** button is clicked at which point the Current Method and the Method name above the legend will be named as **Data Name** followed by the **Method Type Suffix** (see **Suffix**, page 115).

If the Method was named prior to acquiring the data, then it will keep that name without the Method Type Suffix.

If data is acquired, then both the **Current Method** and the Method name that appears at the top of the legend will be the same. If previously saved data is then opened, the **Current Method** will not change, but the Method name that appears at the top of the legend will change to the method that created the opened data.

6.5.2.2 Trace names

Trace names are set by EzSpec at the time they are created and cannot be changed. The Sample trace names below are based on the default Data Name = Sample A.

Acquisition types	Trace name specification	Sample trace name
Absorbance/Transmission Kinetics	Data Name Ex value "Absorbance"	Sample A 425 Absorbance
Absorbance/Transmission Spectra	Data Name Ex range "Absorbance"	Sample A 300-800 Absorbance
Emission Spectra (EEM, Emission)	Data Name Ex value:Em range	Sample A 450:465-700
Excitation Spectra	Data Name Ex range:Em value	Sample A 300-500:750
Kinetics Emission	Data Name Ex value:Em range,Time value	Sample A 450:450-600,0.756
Timebased (Capture Value Single point, Capture Value Standard Curve, Kinetics Fixed Wavelength)	Data Name Ex value:Em value	Sample A 425:530

6.5.2.3 Trace colors

Trace colors are set by EzSpec. To change the color that a trace shows in a 2D graph, click on the colored dot to the right end of a trace name. A color palette is shown so that you can choose a different color for the trace.

6.5.2.4 Trace visibility

Visible traces are indicated by the trace names being white or cyan. Hidden traces are indicated by the trace names being grey. Trace visibility can be turned off or on by clicking on the eyeball icon between the trace color dot and the trace name.

 This icon indicates the trace is visible in the graph or table. Click on this icon to turn the trace visibility OFF. This will also deselect the trace if it was selected.

 This icon indicates the trace is invisible in the graph or table. Click on this icon to turn the trace visibility ON.

Right clicking in the legend shows the following commands:

6.5.2.5 Legend commands

Select All – Click **Select All** to select all visible traces in the legend. The names of the selected traces will change to cyan color. Hidden traces will not be selected.

Select None – Click **Select None** to deselect all traces in the legend. The names of such visible traces will change to white color. Hidden traces are already deselected.

Hide All – Click **Hide All** to hide all traces in the legend. This will also deselect any selected traces.

Show All – Click **Show All** to show all traces in the legend. This will not change whether already visible traces are selected or not.

Remove Selected – Select traces in the legend and then click **Remove Selected** to delete them from the legend. **Remove Selected** does not change any saved data files. **Warning**: If you use **Remove Selected** to delete traces from the legend and then save the remaining traces using the original file name, the original file will be overwritten and the removed traces will be lost. If you make any changes to EzSpec data and want to save the changes you should save the data with a new name.

6.5.2.6 Detector saturation warning

This warning is flashed above the Graph window if the intensity seen by the CCD detector is too high. To lower the intensity seen by the CCD detector, you can:

- Select narrower **Excitation** and/or **Emission Band Pass** settings
- Decrease the **Integration Time** in the **Method Setup**
- Dilute the sample

6.5.3 Raman Indicator

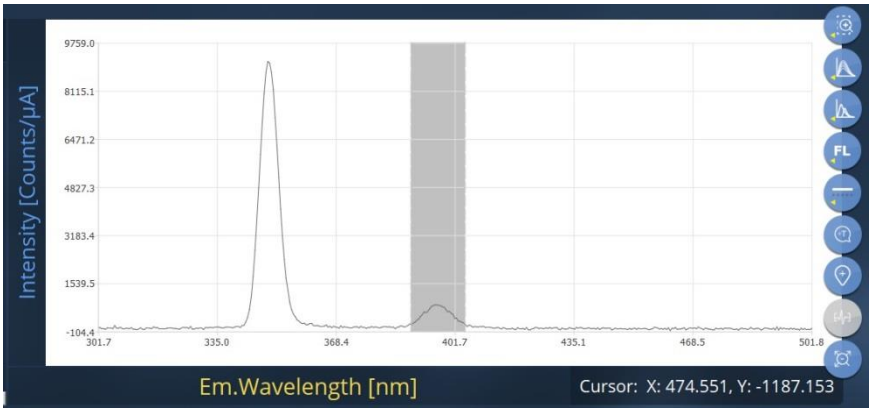
A vertical gray band should appear in the 2D graph where the expected Raman peak(s) from the solvent are located. This is true for any spectral fluorescence graph.

The **View** menu shows **View Raman Peak Indicators**. When this is checked, the 2D Graph will show a vertical gray band and when unchecked will hide the vertical gray band.

The band width is dependent on the band pass used. The range can be calculated by:

Raman peak +/- (sum of band pass)*0.75 ((this is the sum of the band pass * 1.5)/2).

For example, for an emission spectrum in water, at 350 nm excitation, with 5 nm band pass on both slits, the range of the Raman marker would be 397 nm +/- 7.5 nm....389.5 to 404.5 nm.



The Raman indicator should be enabled for the following method types:

- Emission Spectra
- Excitation Spectra
- EEM
- Kinetics (Emission Spectrum)

If the experiment is **Capture Value (Single Point)**, **Capture Value (Standard Curve)**, or **Kinetics (Fixed Wavelengths)**, and the emission wavelength value is set at an expected Raman wavelength for a selected **Solvent** (as selected in the **Method Setup** pull down menu), a popup will occur in the setup as soon as the solvent and emission wavelength are selected and this value can be compared. The Raman Indicator will not work is no solvent (N/A) is selected in the pull down menu.

6.5.4 Status bar

The Status bar is shown below the Acquire Toolbar, the legend and the graph window on the Acquire, Process and Report menus.

When the Duetta is turned off or the computer is not connected to the Duetta, the Status bar will show **No Instrument Detected** in white.

The Status bar will show **Status: IDLE, Instrument warming up…** for 1 hour after the lamp is turned on.

When the Duetta is connected to the computer, and the Duetta is on but not acquiring data, the Status bar will show **Status: IDLE** in white.

If the top cover or the sample cover is open, the Status bar will also show **DOOR OPEN** in red further to the right. This does not apply if the Injection port is open.

When the lamp has been turned off, the status bar will show **Status: IDLE**, and Lamp Locked Out. Wait Until Cool**.** in red further to the right.

When data is being acquired, the Status bar will show **Status: ACQUIRING** in turquoise.

When data acquisition is paused, the Status bar will show **Status: ACQUIRING PAUSED** in red.

6.5.5 Data & Info bar

The **Data & Info** bar is shown at the bottom of the **Acquire**, **Process**, and **Report** menus.

6.5.5.1 Hide and Show buttons

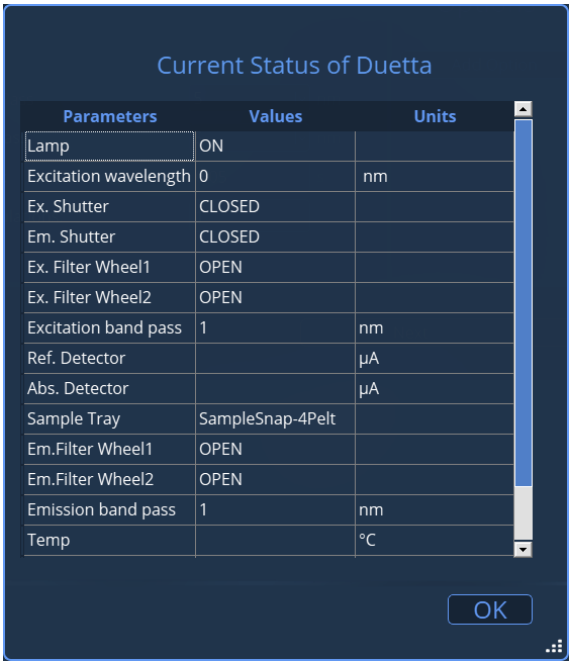
Click on the Hide button  on the left end of the bar to hide the Data & Info bar. When this bar is hidden, click on the Show button  to show the bar.

> **Note**: The **Status**, **Method**, and **Comments** windows here can be open at the same time.

6.5.5.2 Status button 

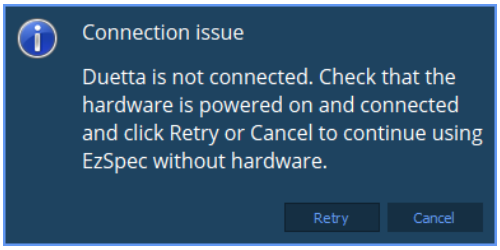
This does the same action as clicking View, Status on the Top Menu. Click the Status button or label to show the current Duetta™ hardware information, or a message if the Duetta™ is not connected. The Status information is not editable but updates with the condition of the instrument hardware.

The Duetta™ must be turned on and connected to the computer to see the current Duetta™ hardware information.



- Lamp ON or OFF
- Excitation wavelength
- Excitation Shutter OPEN or CLOSED
- Emission Shutter OPEN or CLOSED
- Excitation Filter Wheel1 position
- Excitation Filter Wheel2 position
- Excitation band pass
- Ref. Detector intensity
- Abs. Detector intensity
- Sample Tray type
- Emission Filter Wheel1 position
- Emission Filter Wheel2 position
- Emission band pass
- Polarizers in/out (if applicable) and position/orientation
- Current temperature (if applicable)
- Stir Speed (if applicable)
- Sample changer position (if applicable)

If the Duetta™ is not connected to the computer, clicking the Status button shows the message:



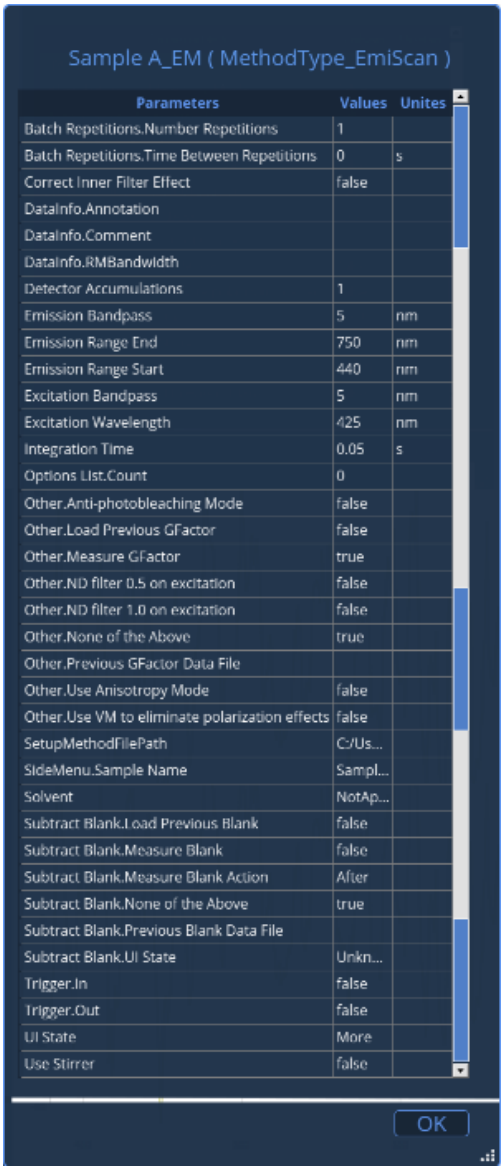
Connect the Duetta ™ to the computer and then click Retry to show the Duetta ™ hardware information. Otherwise, clicking **Cancel** shows a blank Current Status of Duetta™ window.

6.5.5.3 Method button 

This does the same action as clicking the **View, Method** on the Top Menu. Click the **Method** button or label to show a 2 column table with all the method setup information used to acquire the data currently displayed. The information is in alphabetical order of parameter names. The title is created as *Method name* (MethodType_*Method type*). The table is scrollable.

If there is no data in the graph window or table, then **View, Method** will show an empty window.

Method information example: The picture below is stitched from three scrolled views of the **View, Method** window.



6.5.5.4 Comments button

Click the **Comments** button or label to open a window where you can enter or edit comments. Click the **Cancel** button at the bottom to close the window without saving any new or changed text. Click the **Add** button to save the comments and close the Comments window. The saved comments are attached to the data in that thumbnail and can be saved with the data file. If EzSpec is closed without resaving the method or data, the comments will be lost.

When closed, the Comments window can be reopened again by clicking on the button in the bottom toolbar, and the comments can be edited.

Note: Clicking **View, Comments** on the Top Menu opens the Comments window only for viewing.

6.5.5.5 Thumbnails

Thumbnails are smaller versions of the graph view for currently open data files.

The leftmost thumbnail is the live acquisition thumbnail showing the raw detector signals during an acquisition. If no data is being acquired, then this thumbnail is blank or contains the last acquired data set.

When an acquisition is completed, the finished spectrum will appear in the graph and a new blank thumbnail will be inserted at the left end of the **Data & Info** bar.

The blue outline around a thumbnail means it is the graph or table being viewed above.

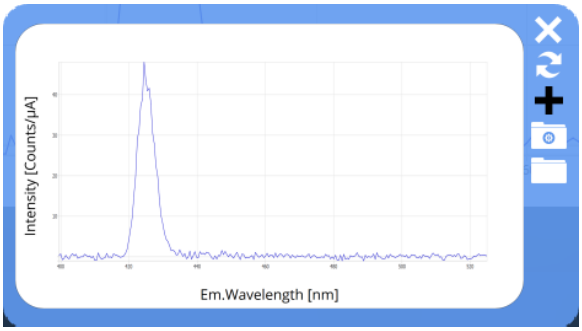
Each thumbnail shows a small version of the graph window displayed the same way it was when last viewed. Changing the data or its display format is reflected in the thumbnail.

Moving the mouse cursor over a thumbnail will show the complete path name for the data file.


If you see this icon when moving the mouse cursor over a thumbnail, it means the data has not yet been saved.

Load Data on the Dashboard, or on the Top Menu **File, Open, Data** opens the data in the graph window, and a thumbnail of the graph is inserted on the bottom bar to the right of the blank thumbnail and to left of all the other open thumbnails so that other thumbnails are shifted to the right.

Right-clicking on a thumbnail shows an enlarged view of the thumbnail window with five buttons in the upper right corner.



Thumbnail icons showing a black symbol on a blue background means that the action cannot be done.

Close - Closes the selected thumbnail and its data. If the leftmost thumbnail is blank it cannot be closed.

Switch - Replaces the current main graph data with the data in the selected thumbnail.

Add - Adds all trace data from the selected thumbnail to the main graph and its associated thumbnail. **Note**: [added or modified] data cannot be deleted, short of closing the data without saving it.

This icon is shown when the thumbnail is of the current graph window – you are not allowed to append the data to itself.

Load This Method - Loads the method that was used to collect the original data in the selected thumbnail, into the **Method Setup** window.

The method cannot be loaded because it has not been saved.

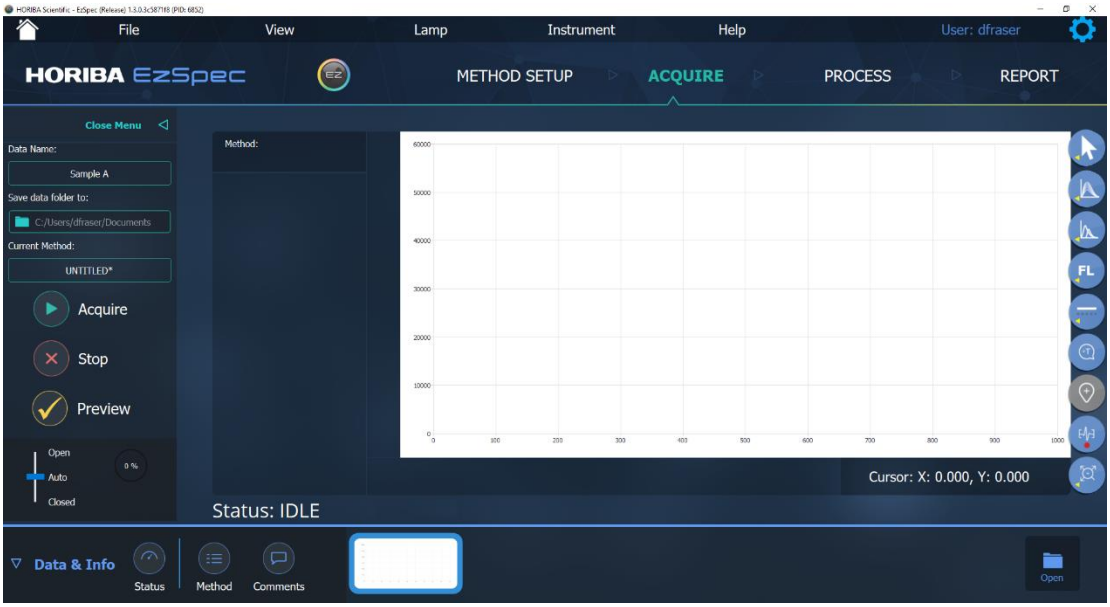
Open – Opens Windows Explorer to the folder file path where the current data is saved. This is the same as **File, Open, Data**.

Closing the active graph also closes the thumbnail, and makes the thumbnail on the leftmost thumbnail the active graph and thumbnail, even if it is blank.

Close All closes the active graph in the graph window and leaves a blank thumbnail.

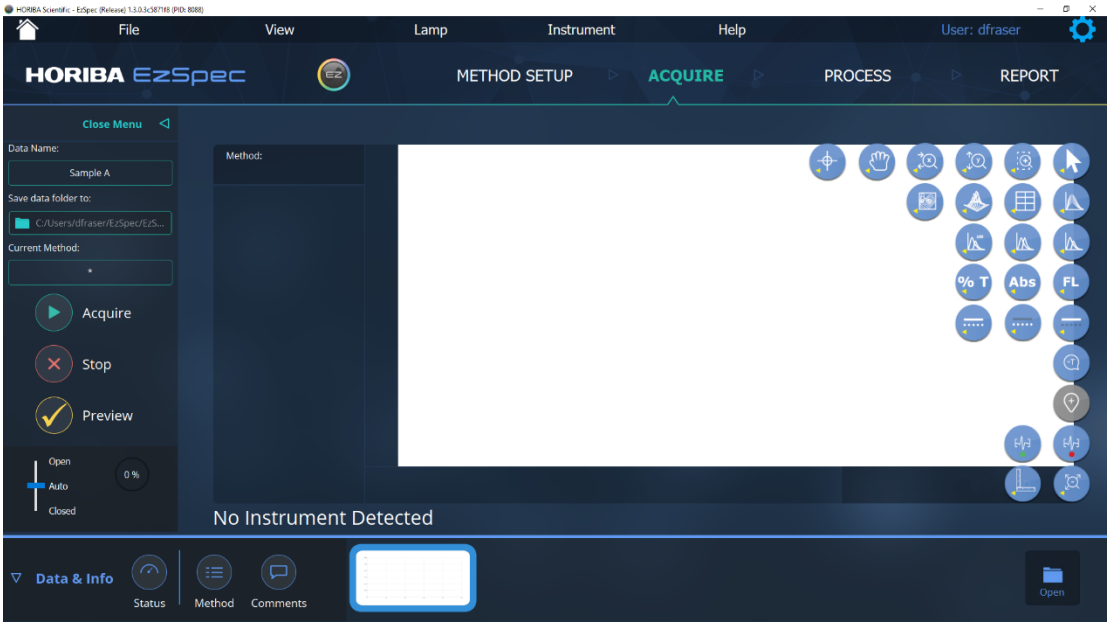
If there are too many thumbnails to fit on the bottom bar, then a scroll bar appears below the thumbnails.

6.5.6 Graph Tool Buttons



The picture above shows Graph Tool buttons on the right side of the graph window. Use these buttons to set or change how data is displayed in the graph window.

The picture below shows the button groups on the right side of the graph window.



6.5.6.1 Using the Graph Tool Buttons

1. The blue background of each button may contain a small yellow arrow in the lower left corner to indicate it is a button group and can be extended.
2. Group buttons remain closed until the button is clicked.
3. When clicked a button changes to a mostly black pattern on a brown background.

4. When you release the button it changes back to a white pattern on a blue background, and if it is part of a group the group extends out to the left. Only one group can be extended at a time.
5. A group stays open until the user clicks on any button or off of the buttons altogether.
6. Click a button in an extended group to select or reselect it. The button changes to a black pattern on a brown background. Only one button in a group can be selected at a time.

7. When you release the button the group slides back in and the only icon shown is the one selected and is shown as a white pattern on a blue background. If a different button has been selected in the group, the other buttons are moved in the group and so the group order will be different the next time it is extended.
8. Buttons that are disabled have a grey or dark grey background, instead of a blue or brown background. For example, the Event Marker button is disabled if the Duetta™ is disconnected from the computer or turned off and EzSpec™ is in offline mode. Clicking on a disabled button with show a message.
9. If a user clicks off the extended group, the group closes and the selection remains the same as before.
10. The graph tool buttons initially displayed along the right edge are the same buttons displayed when the data was saved.

6.5.6.2 Graph Tool Buttons and Button Groups Descriptions

There are 6 groups and 3 single buttons.

1. **Zoom and Cursor mode group**, here showing the Data Cursor, Pan Graph, Zoom X, Zoom Y, Zoom Rectangle, and Arrow (Free) Cursor buttons.

All of these buttons may be used on 2D graphs and 3D Contour plots. None of these buttons can be used on a Table.
The Data Cursor, Pan Graph, and Arrow (Free) Cursor may also be used on 3D Waterfall plots. The Zoom X, Zoom Y, Zoom Rectangle cannot be used on 3D Waterfall plots.
In 3D Waterfall and 3D Contour views you can click on the graph and scroll a mouse wheel to zoom in or out on the view.
Important: If you use the Pan Graph or a Zoom tool to change the display, make sure you change back to the Arrow (Free) Cursor  before doing any data analysis. Several data operations also use the mouse cursor, and the screen may drastically change because the

active mouse tool is still pan or zoom. If the screen was at Full Scale, you may recover by clicking the Full Scale button again.

a. **Data Cursor** 
   Tool tip: “Cursor tracks data”
   Click this button and then put the cursor on the graph. If no data is visible, then no information is displayed. If one or more traces are displayed, then the X, Y, (and Z if applicable) cursor position coordinates are displayed at the bottom of the graph.

b. **Pan Graph** 
   Tool tip: “Pan graph by grab and drag”
   In a 2D graph, click and drag the cursor to move the data around the graph window. If a 3D Waterfall plot is displayed, then clicking and dragging the cursor rotates the Waterfall plot. The drag point is where the cursor touched the graph, and data may even be moved outside the display area. The graph area can be repeatedly panned.

c. **Zoom X** 
   Tool tip: “Zoom X-axis only”
   Click and drag left or right to show a light green band. Release the cursor to expand the band to horizontally fill the graph area. The graph area can be repeatedly zoomed.

d. **Zoom Y** 
   Tool tip: “Zoom Y-axis only”
   Click and drag up or down to show a light green band. Release the cursor to expand the band to vertically fill the graph area. The graph area can be repeatedly zoomed.

e. **Zoom rectangle** 
   Tool tip: “Zoom rectangle”
   Click and drag from one corner to the opposite corner of the rectangle. The rectangle is indicated by a light green color. Release the cursor to expand the selected rectangular area to fill the graph area. The graph area can be repeatedly zoomed.

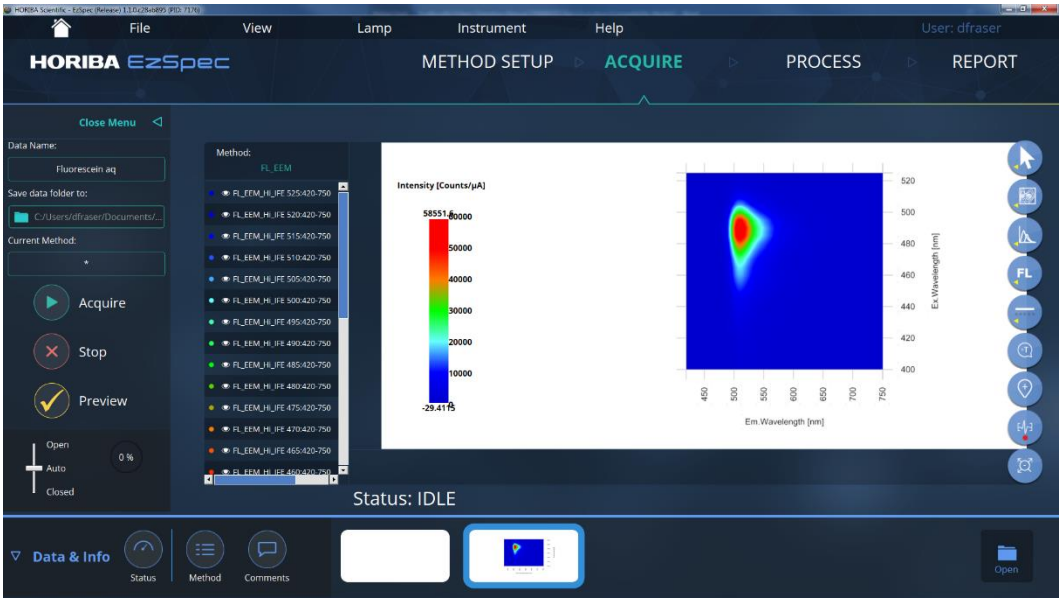
f. **Arrow (Free) Cursor** 
   Tool tip: “Free cursor”
   The coordinates of the arrow cursor are shown below the lower right corner of the graph.

2. **Plot mode group**, here showing the
   2D Graph, Contour Plot View, 3D Waterfall View, and Table View buttons.

The Contour and 3D Waterfall plots require a minimum of 3 visible traces to display data in a graph. Clicking one of these buttons changes the graph view accordingly.

a. **2D Graph View** 
   Tool tip: “View graph as 2D plot”
   Individual traces are displayed as Y vs X data points connected by lines.

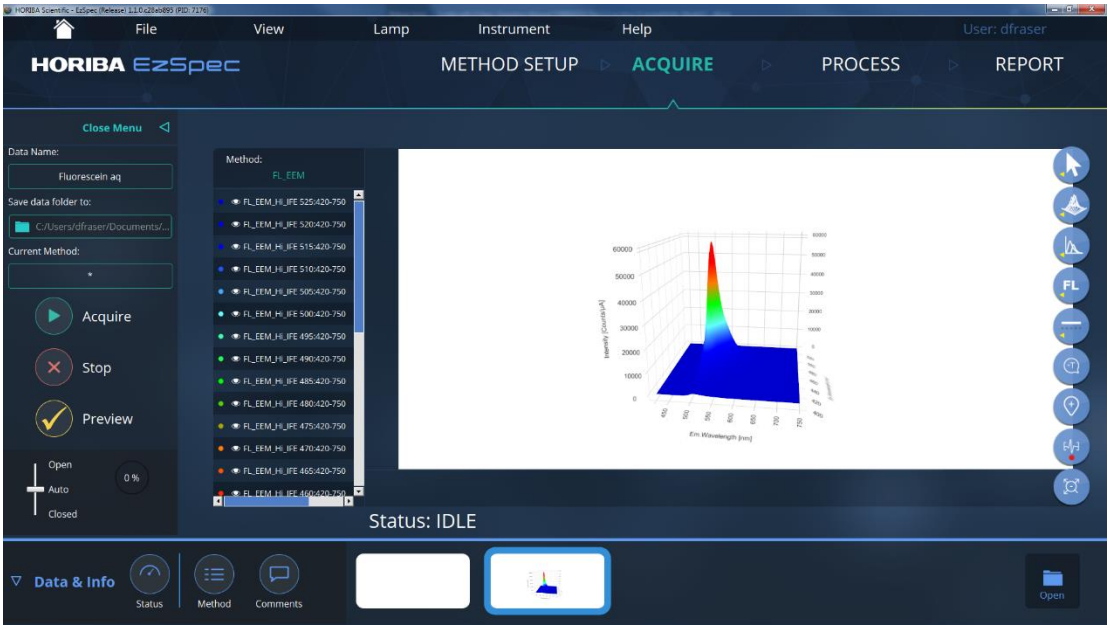
b. **Contour Plot View** 
   Tool tip: “View graph as contour plot”
   This is the default plot type for 3D data. The colors represent the Z axis values as shown on the color scale on the left. The maximum Z-value is shown above the color scale. The Z-value of each pixel on the graph is interpolated from nearby data points. Click on the graph and drag horizontally to rotate the graph. Dragging the graph vertically upwards shows a Z-axis. The tilt of the Z-axis is restricted to the range 0° to +90°. Dragging the graph to the bottom of the graph window returns to the Contour Plot View.



c. **Waterfall View** 

Tool tip: "View graph as waterfall plot"

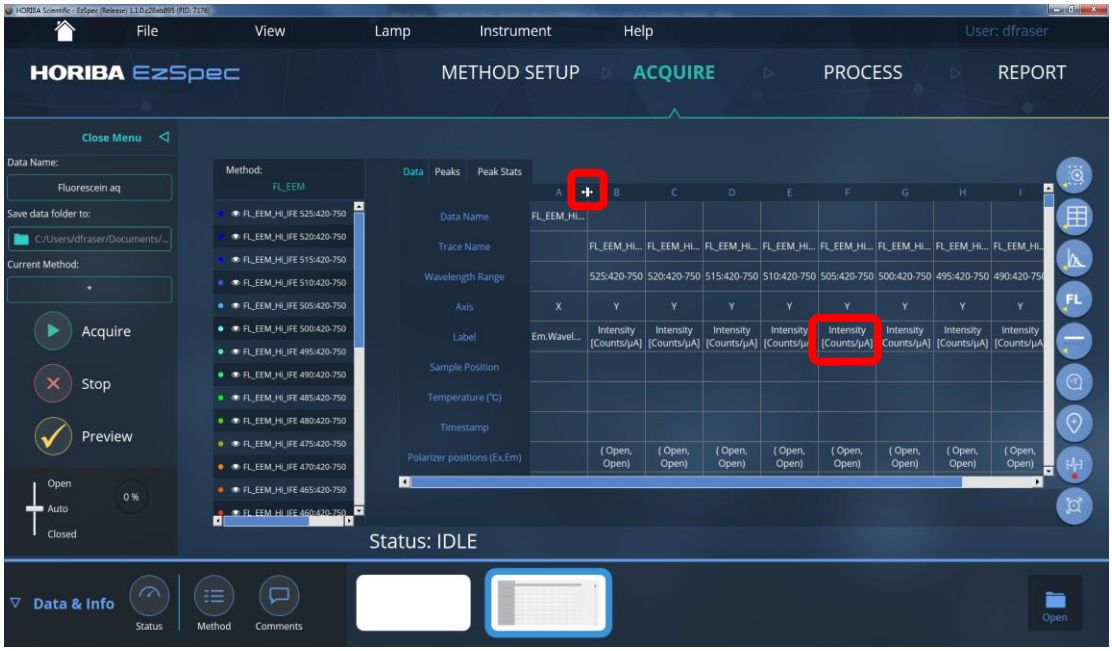
This graph is an alternative to the Contour Plot View, projected in 3D with intensity enumerated along the Z-axis. The colors represent the same intensities as a contour plot of the same data, but the color bar is not shown. Clicking and dragging the graph rotates and tilts the plot. Rolling a mouse scroll bar will zoom the waterfall plot in and out.



d. **Table View** 

Tool tip: “View data table”

The data for visible traces in the legend is presented in a table of n X-Y column pairs for 2D data and/or single value data, or X-nY columns for 3D data, where n is the number of visible data traces in the legend. Additional columns are shown for processed data. The top rows of the table display trace names and Method setup parameter values. If a setup option is not used (such as Rayleigh masking or temperature), then that row will be blank. Hidden traces are not shown in the table. From the table, a user can select data, right click and copy and then paste the data into any spreadsheet or editing program.



To change a column width, place the mouse cursor in the header above the boundary line on the right of the column so that it changes to a boundary cursor , and then click and drag the boundary cursor. If text in a cell includes any spaces, then reducing the column width can wrap the text to a maximum of two lines per row. Double-clicking on the boundary cursor will change the column width to fit the contents to one line per row.

3. **Normalization scaling group**, here showing the Normalize to Highest Trace, Normalize All Traces to 100, and Show Original Scale buttons.

All of these buttons may be used in all plot modes. These buttons do the same actions as the same named Normalization operators in **Process**, **Math**, **Normalization**.

a. **Normalize to maximum peak** 
Tool tip: “Normalize to Maximum Peak”
Click this button to normalize all currently selected traces as a group so the highest point in the group is set to 1.0.

b. **Normalize all traces** 
Tool tip: “Normalize All”
Click this button to normalize all currently selected traces individually to 1.0.

c. **Plot all traces in the original scale** 
Tool tip: “Plot Original Scale”
Click this button to plot all (selected or unselected) visible traces in the original scale.

4. **%**Transmittance, Fluorescence, Absorbance group, here showing the % Transmittance, Fluorescence, and Absorbance buttons.

a. **%Transmittance** 
Tool tip: “Show %T”
Shows the % transmittance data collected.

b. **Fluorescence** 
Tool tip: “Show Fluorescence”
Shows the fluorescence data collected.

c. **Absorbance** 
Tool tip: “Show Absorbance”
Shows the absorbance data collected.

d.

5. **Line/Dot graph group**, here showing the Line and Dot, Dot, and Line buttons

a. **Line and Dot** 
Tool tip: “Show traces as lines and dots”
Each selected trace is shown by dots at the X,Y data point positions connected by lines.

b. **Dot** 
Tool tip: “Show traces as dots”
Each selected trace is shown by dots at the X,Y data point positions.

c. **Line** 

Tool tip: “Show traces as lines”

Each selected trace is shown by the X,Y data point positions connected by lines.

ⓘ **Note**: The default Line/Dot display mode is **Line** (dots not shown). However, the default Axes Scaling is **Full Scale**, so that the graph area includes space where single data point traces occur, but are not visible. To display the single data point traces in the graph, right-click in the legend and select **Select All** so that all trace names change to cyan color. Then, click on the Line/Dot graph group on the right edge of the graph and select **Line and Dot**  . Single data points should now be displayed in the graph. Right-click in the legend and select **Select None** so that the data lines and dots are no longer thick.

---

6. **Add annotation button** 

Tool tip: “Add text to the graph”

Click this button and then click on a 2D or 3D graph to show a cursor where text can be added. When the user clicks off of the annotation, the text is added at that spot. Once text is added to the graph, the user can move the text by clicking and dragging it. To edit the text, you must double-click on the text to make it active (indicated by an outline around the text), and then you can add new text. To delete any part or all of the text, you must make the text active and then use the **Backspace** key. If all of the text is deleted, then clicking outside of the outline deletes it as well.

Annotation is saved with the data.

7. **Add Event Marker button** 

Tool tip: “Add event marker”

Click this button to add an event time stamp during acquisition.

When this button is disabled it shows a grey background.

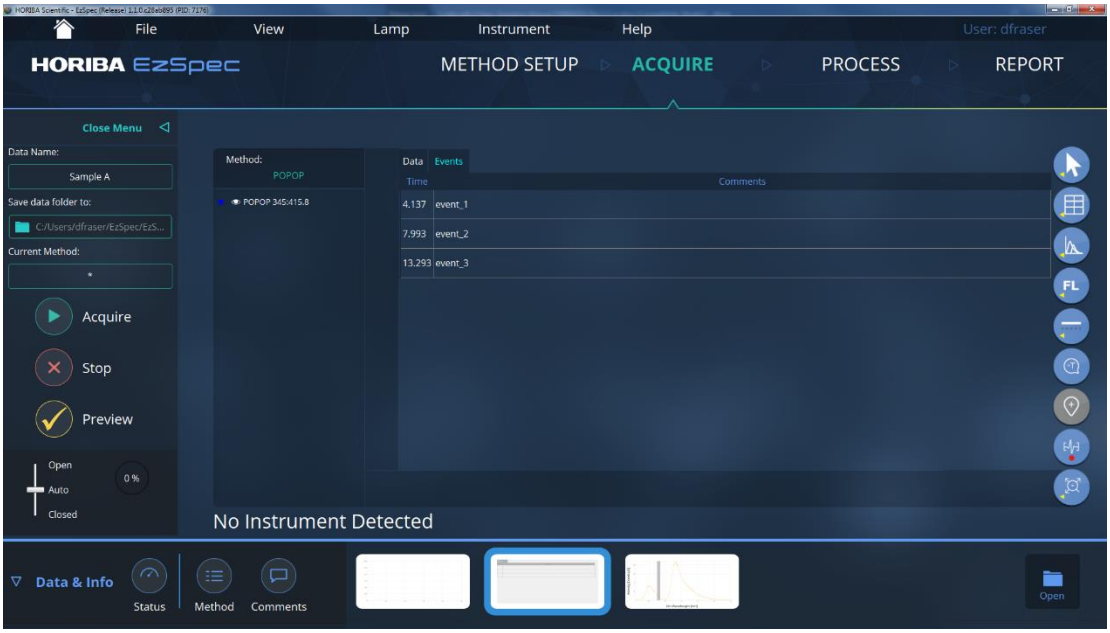
a. Event markers can only be used on time-based data.
   i. Kinetics vs. Emission Spectra
   ii. Kinetics vs. Fixed Wavelength(s)
   iii. Absorbance/%T Kinetics

b. Event markers can be added from the time that the Acquire button is clicked and only during acquisition. When Mark Event is clicked during acquisition, an event is added at that time. The time marked is the time that the event is added - not necessarily on a specific trace if a kinetic emission spectrum is applicable.

The Event Marker button can be used on 2D graphs only.

For 2D graphs of intensity vs. Time, events are added as dashed vertical lines.

Events are recorded in a tab called “Events” in Table View where users can edit and enter text descriptions of each event. These include events recorded during acquisition**.**



8. **Range Selector group**, here showing the Select Range and Disable Range buttons.

a. **Select Range** 
Tool tip: “Select range”

This button is shown when range selection is disabled. Click to enable the selection of the range to be applied to visible selected traces for processing.

b. **Disable Range** 
Tool tip: “Disable range”
This button is shown when range selection is enabled. Click to disable range selection of traces.

9. **Axes Scaling group**, here showing the Full Scale and Set Axes buttons

a. **Full Scale** button 
Tool tip: “Full scale”
Click this button to return all traces to full scale. This button is separated from the Zoom group for accessibility.

b. **Set Axes** button 
Tool tip: “Set axes”
Click this button to bring up a window with X, Y, and Z axes and start, end, and increment values that the user can manually set for the current graph. This would include 2D, contour, and waterfall plots.

6.6 Process

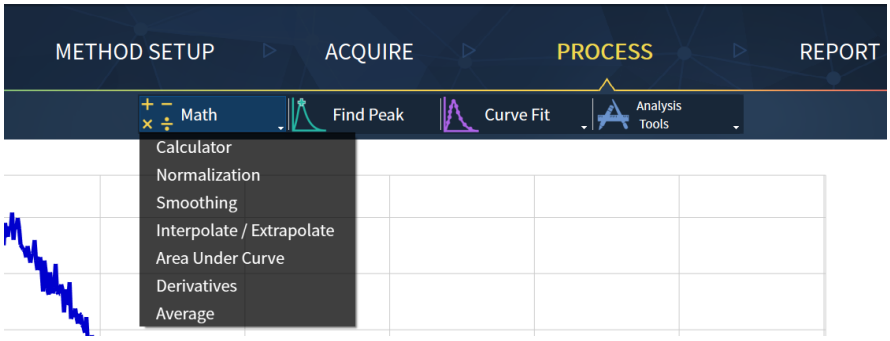
Traces must be visible in the legend to be selected for processing. It doesn't matter if they are completely off scale in the 2D Graph View. Traces can be processed if the Table is the current view.

If more than one trace is selected, some processes will process only the last selected trace, in the order they were selected. For example, if EEM traces with excitation wavelengths 490, 470, 450, and 430 (from top to bottom in the legend) are selected in that order, then Process, Math, Calculation will process only traces 450 and 430 in that order. If the same traces are selected in the order bottom to top in the legend as 430, 450, 470, and 490, then Process, Math, Calculation will process only traces 470 and 490 in that order. Right-clicking in the legend, **Select All** will select all visible traces in the legend in a top to bottom order.

At some steps in Math operations, you can click on the **Back** button to return to the previous step, or click on the **Cancel** button to cancel the operation and return to the main Process menu.

Warning: If you make any changes (including Remove Selected – see page 121) to the data and then save the data using the original file name, the original file will be overwritten and some original data may be lost. If you make any changes to EzSpec data and want to save the changes you should save the data with a new name, using **Save Data As…**.

6.6.1 Math



6.6.1.1 Calculator

The Calculator performs arithmetical operations using a trace as the first operand and another trace and/or a number entered by the user as the second operand. If a trace is selected as the second operand, then entering a number in the enterable box will also be included in the result using the same operator as the first operator. If more than two traces are selected, the Calculator will process only the last two selected traces, in the order they were selected. The result can be:

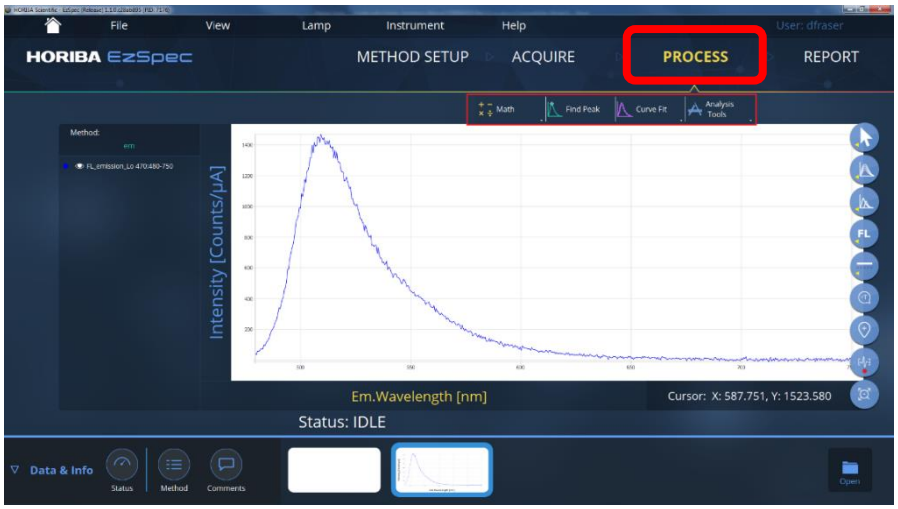
(Trace 1) Operator (Trace 2) Operator (Number)

Or: (Trace 1) Operator (Trace 2)

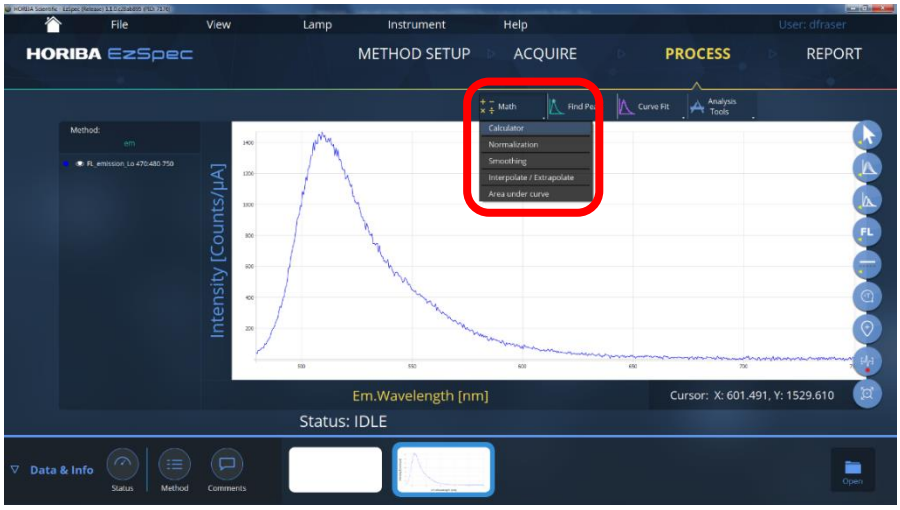
Or: (Trace 1) Operator (Number)

There must be a second operand (Trace or Number) in use.

1. Click on **Process** to see the Process menu.



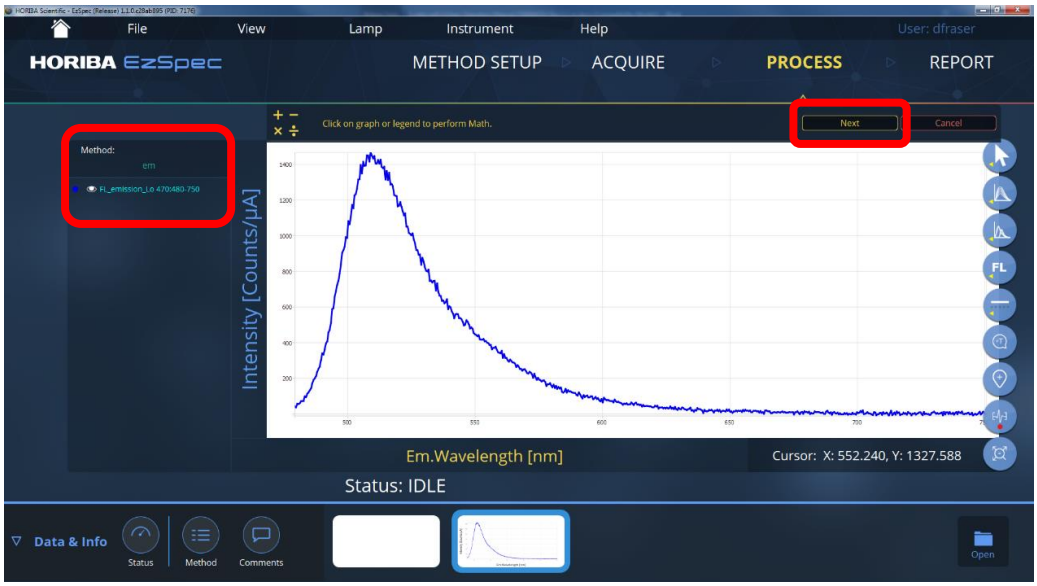
2. Click on **Math**, **Calculator**.



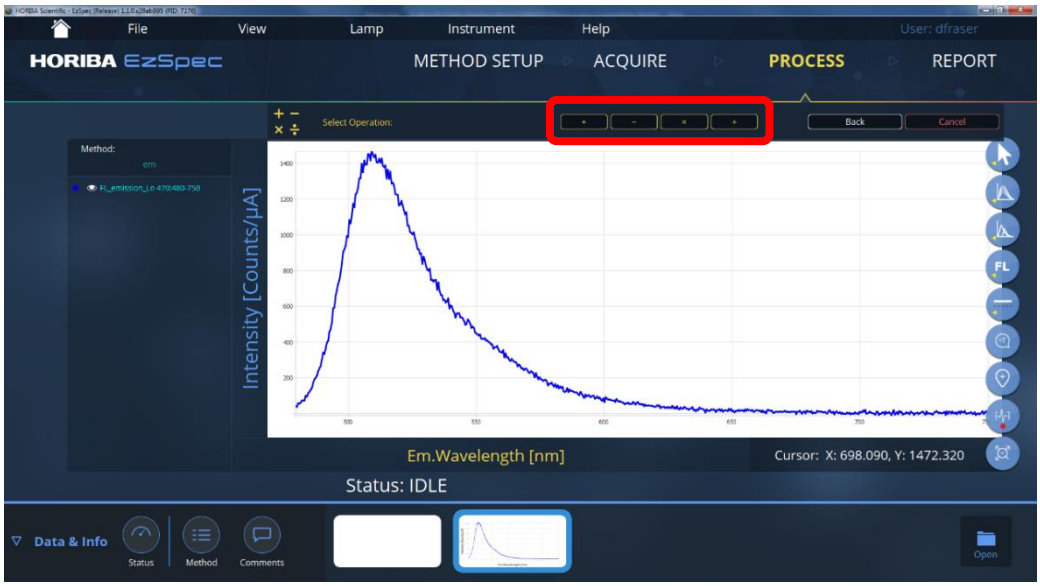
3. Click on one or more traces in the legend or on the graph. Selected traces are indicated when the trace names in the legend turn blue, and when the traces in the graph change to a heavier line.

> ⓘ **Note**: A trace can also be selected before any of steps 1 – 3.

4. Click on the Next button.

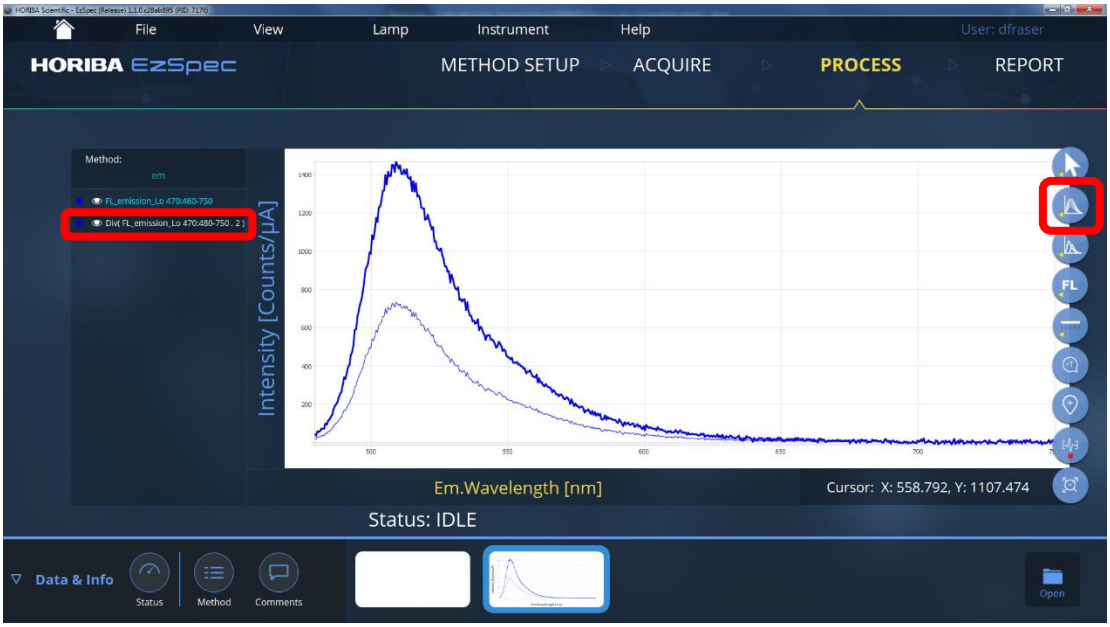


5. Click on one of the arithmetic operators

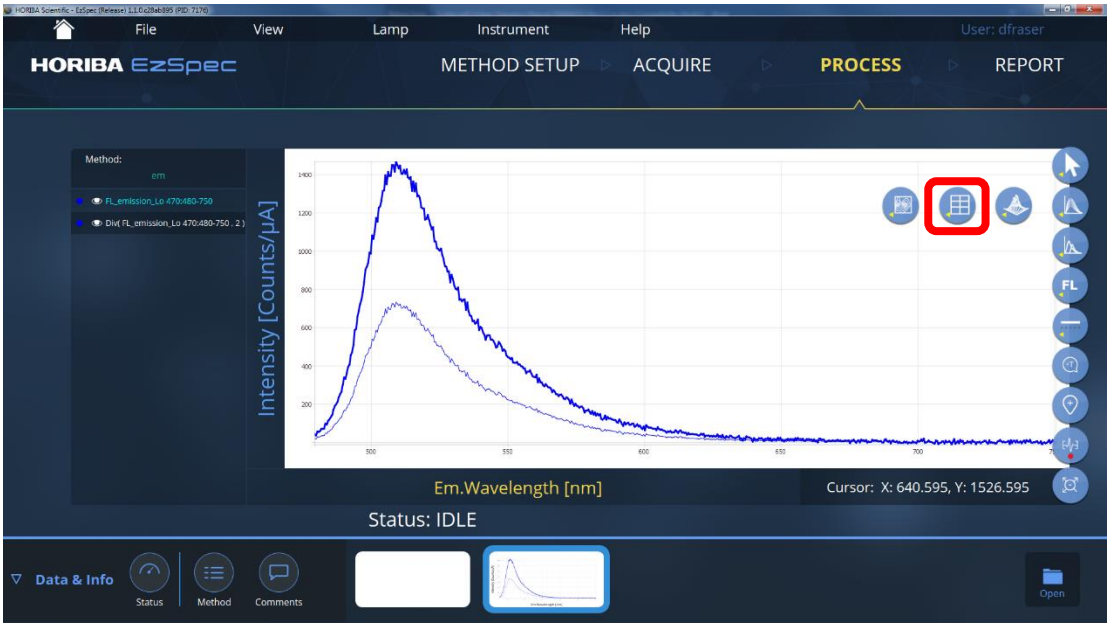


6. You can select the second operand as either a trace in the legend or graph, or enter a numerical value in the box. In this example, the divide (÷) operator has been selected in the previous step, and a value of 2 has been entered for the numerical value in the box.
7. When a second operand trace or value is entered, a Preview plot appears, and it will update if you change the second operand. Click the **Finish** button.

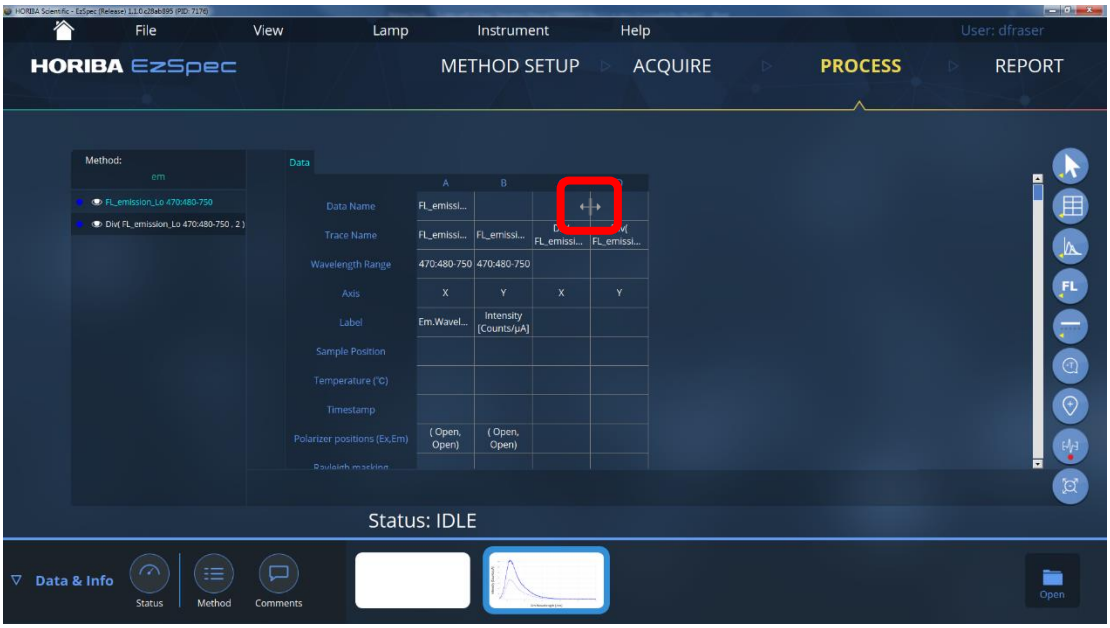
8. A new trace appears in the graph. In the legend the new trace is named by the operation name with the operands inside parentheses.
9. Click on the Plot Mode button to expand the **Plot Mode** group.

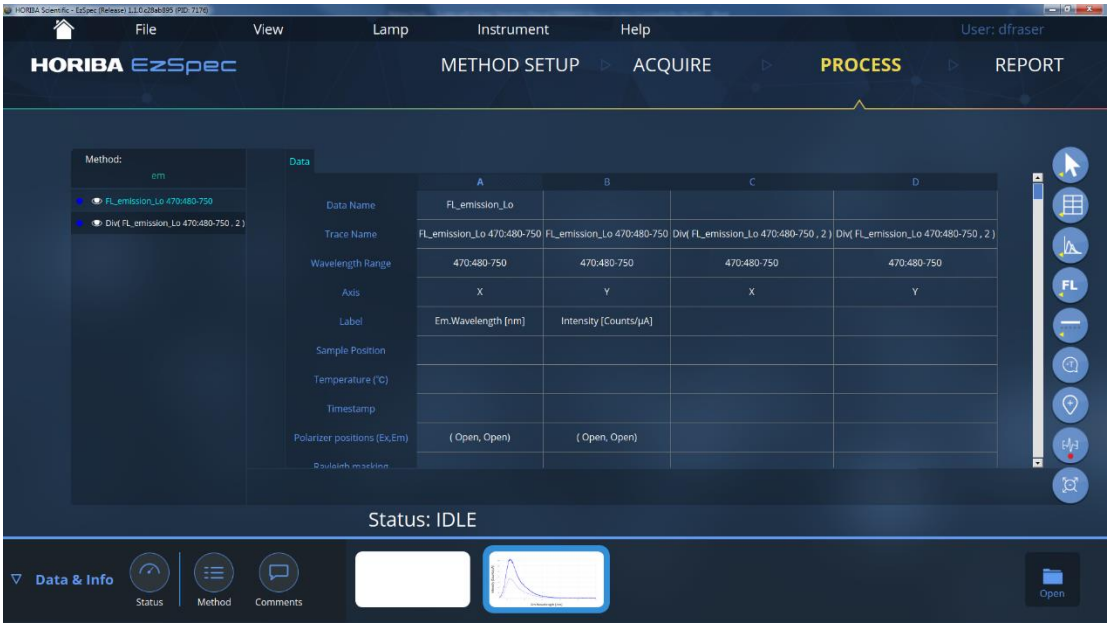


10. Click on the **Table View** button.



11. **Table View** shows X and Y columns for the new trace, with default column widths.
12. Double-click on the column boundary cursors so that the columns show the trace names on one line.

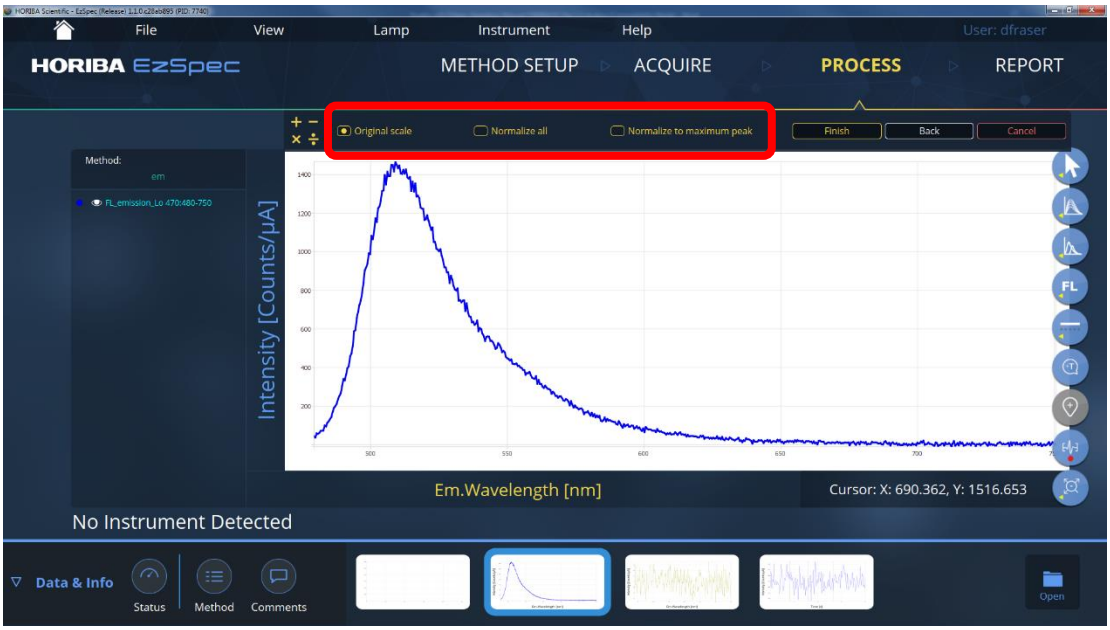




6.6.1.2 Normalization

The operations **Original scale**, **Normalize all**, and **Normalize to maximum peak** do the same actions as the same named Graph Tool buttons.

1. All visible traces in the legend will be processed.
2. Click on **Process**.
3. Click on **Math**, **Normalization**.
4. Click on the **Next** button.
5. Click on **Original scale**, **Normalize all**, or **Normalize to maximum peak**, or a radio button in front of one of these operators.



a. **Original scale** – will plot all visible traces to their original scale.

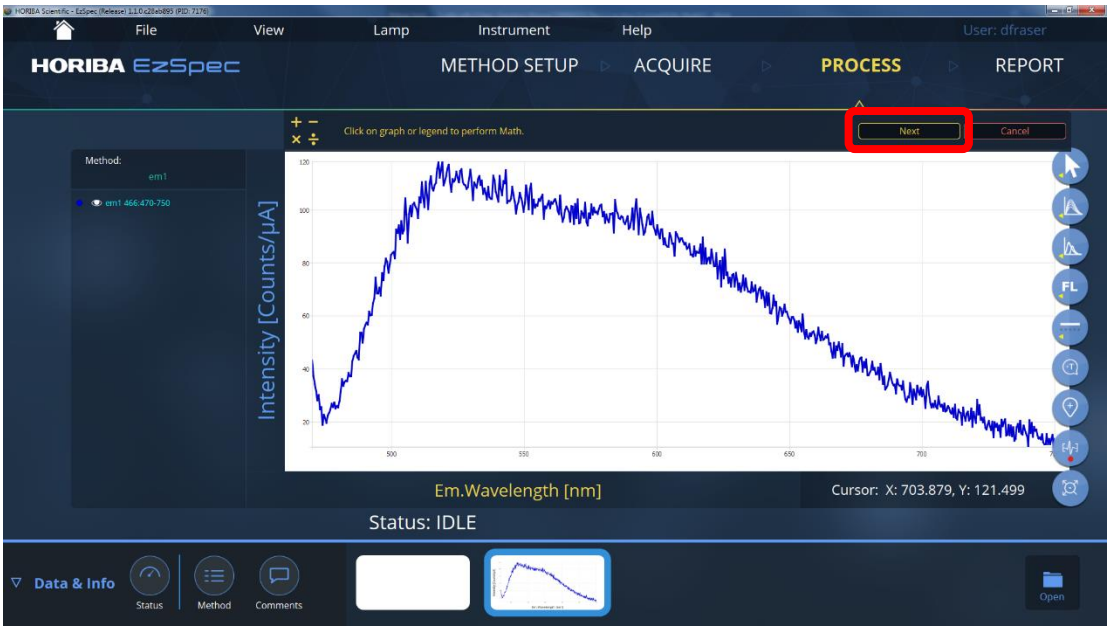
b. **Normalize all** – will normalize all visible traces individually to 1.0.
c. **Normalize to maximum peak** – will normalize all visible traces as a group to 1.0.

6. Click on **Finish**.

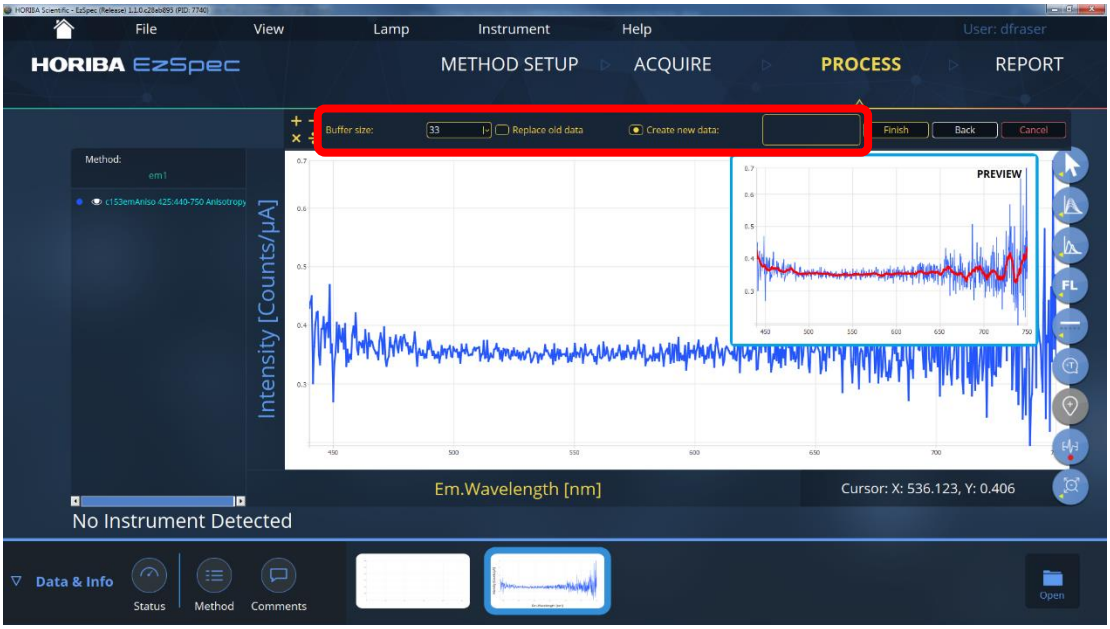
6.6.1.3 Smoothing

This process applies a Savitzky-Golay smoothing to the selected trace, using a 7, 15, 21 or 33-point buffer. A higher buffer value results in greater smoothing.

1. Select a trace in the legend or on the graph. If more than one trace is selected, then only the last selected trace (in the order that they were selected) will be processed.
2. Click on **Process**.
3. Click on **Math**, **Smoothing**.
4. Click on the **Next** button.



5. A Preview plot appears. In it, the smoothed curve will update and change color as you cycle among the different buffer sizes.
   Select a Buffer size from the pull down list. The size options are 7, 15, 21 or 33.

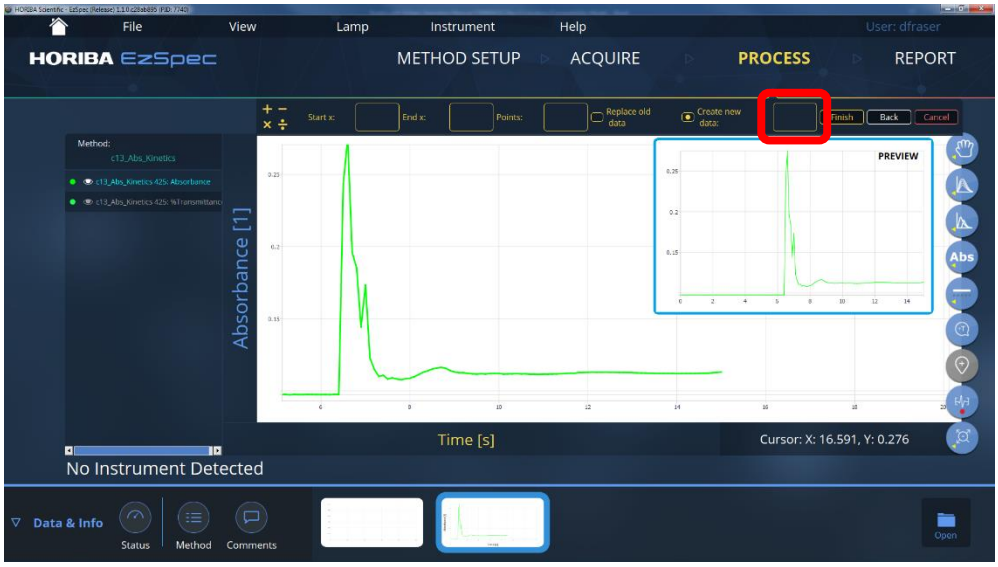


6. Select a radio button for either **Replace old data** or **Create new data**.
7. Enter a a data label in the enterable field.
8. If **Replace old data** is selected, the output will be the smoothed curve using the entered data label, and the original data will be deleted in the legend and graph. If **Create new data** is selected, the output will be the smoothed curve using the entered data label added to the legend and graph.

6.6.1.4 Interpolate / Extrapolate

This process uses linear interpolation which creates connected piece-wise linear fits to the specified data points in the range of the trace data. Beyond the range of the data Extrapolation creates a linear fit to the last two data points in the direction of the extrapolation.

1. Change to **2D Graph View**.
2. Select a trace in the legend or on the graph as the source trace to be processed. If more than one trace is selected only the last selected trace in the order that they were selected will be processed.
3. Click on **Process**, **Math**, **Interpolate** / **Extrapolate**, **Next**. A trace must be selected for the Next button to be active.
4. A Preview inset window appears, as well as boxes in which to enter parameter values.

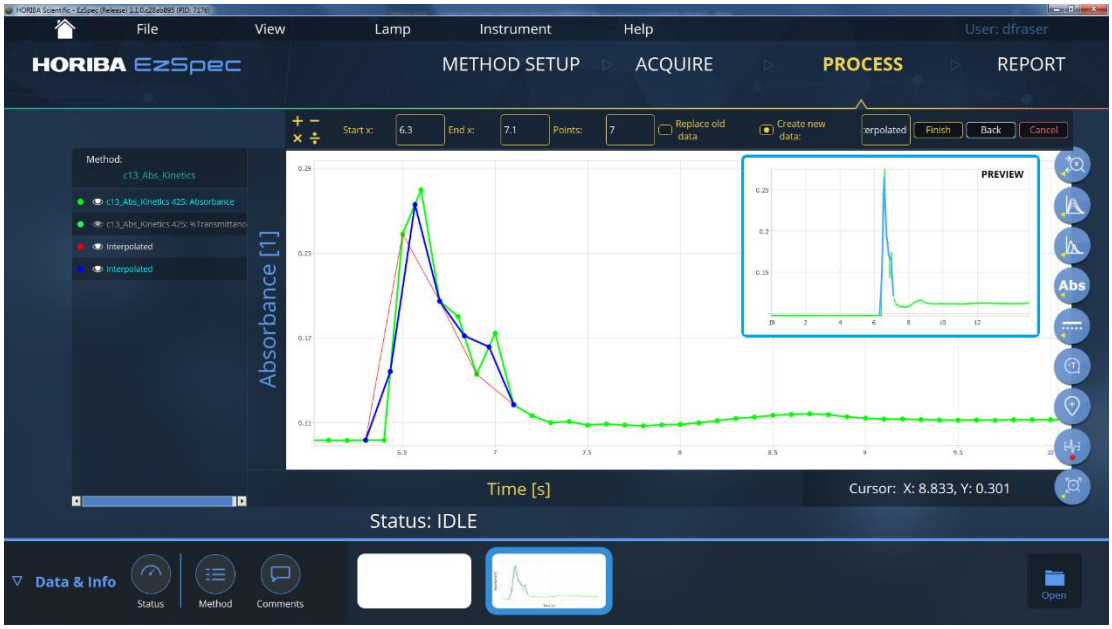


5. Enter **Start x** and **End x** values for the result trace. The values can range inside and/or outside the range of the source trace.
6. Enter the number of **Points** for the result trace. As soon as **Start x**, **End x**, and **Points** have been entered the Preview window shows the results of the process. Changing these values will change the Preview.
7. Click either **Replace old data** or **Create new data**. **Replace old data** will replace the source trace by the result trace, but keeping the source trace name. **Create new data** will create a new trace using the name entered in the enterable box (above figure).
   If no text is entered in this box, a default name will be created as **Interpolated: Source:** source trace name.
8. Click **Finish**. The result trace appears in the legend and graph with the same color as the source trace. Here the result trace was named *Extrapolate*.

9. To show the interpolated / extrapolated result more clearly, the source and result traces are shown with lines and dots, and the result trace color has been changed.

10. The following screenshot shows the piece-wise nature of an interpolated trace. This screenshot was created by processing the interpolation, and then **Process**, **Math**, **Interpolate / Extrapolate** to show the parameters used for the process, but not **Finish**. The result trace was named *Interpolated*. The interpolation is done piece-wise using the specified points in the parameter menu.

11. If **Start x**, **End x**, and **Number of Points** are chosen that do not correspond to the source trace data points, EzSpec™ interpolates to use calculated x, y values as seen in the following with the blue line.



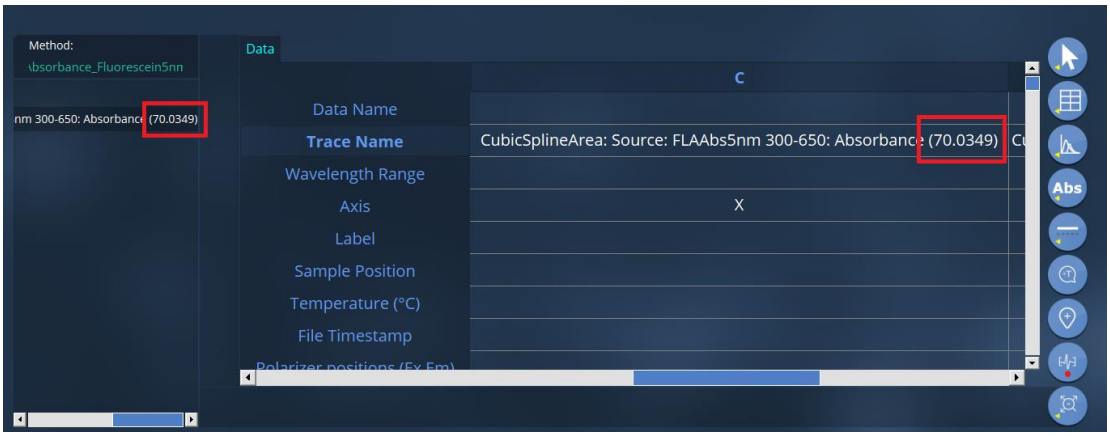
12. To extrapolate from more than the last two points at the end of a trace, you can first use **Process**, **Curve Fit**, **Polynomial**, with a first order (linear) fit to create a straight line, and then extrapolate from the end points of that line.

6.6.1.5 Area under curve

This process fits a cubic spline to the curve and calculates the area under the spline.

1. Change to **2D Graph View**.
2. Select a trace in the legend or on the graph. If more than one trace is selected only the last selected trace in the order that they were selected will be processed.
3. Click on **Process**.
4. Click on **Math**, **Area under curve**.
5. Click **Next**. Preview shows the cubic spline curve and the value of the area under the curve.
6. Click **Finish**. The spline curve appears at the bottom of the legend and in the graph.

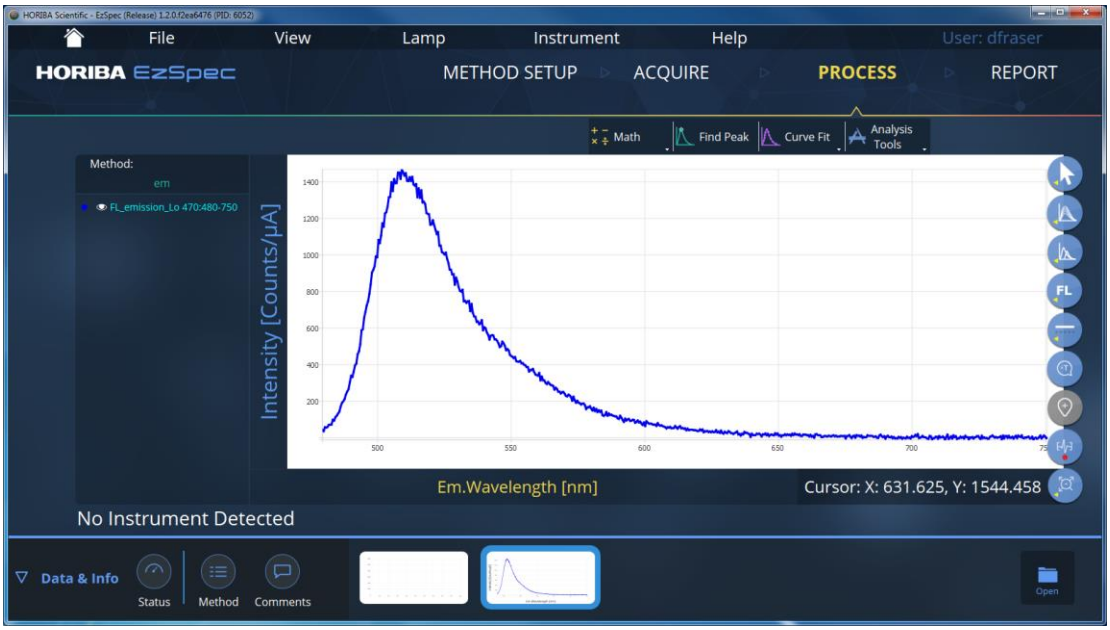
7. The value for the area under the curve is saved in the trace name of the cubic spline curve which can be viewed in the legend and in the trace name shown in the table view.



6.6.1.6 Derivatives

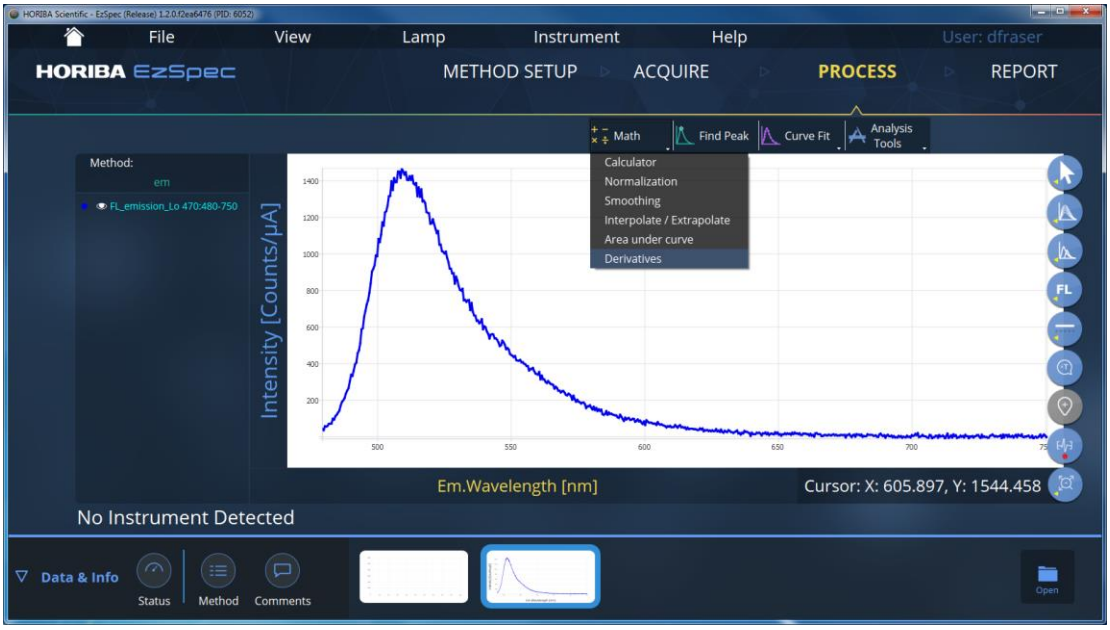
This process calculates the derivatives of the selected traces.

1. Click on **Process** to see the Process menu.

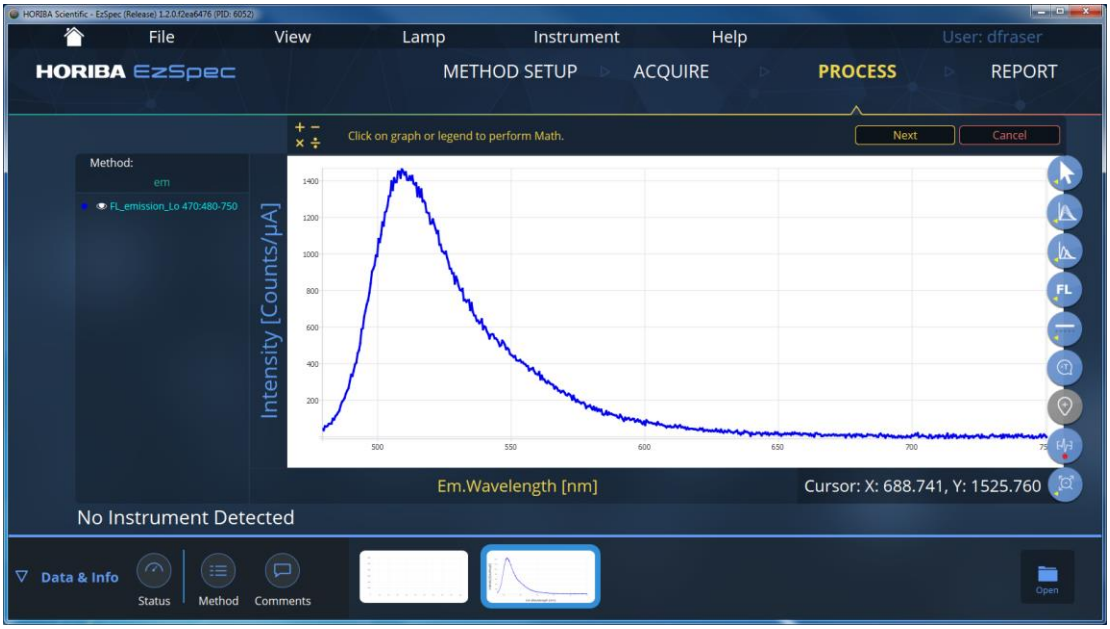


2. Click on **Math.**

3. Click on **Derivatives**.

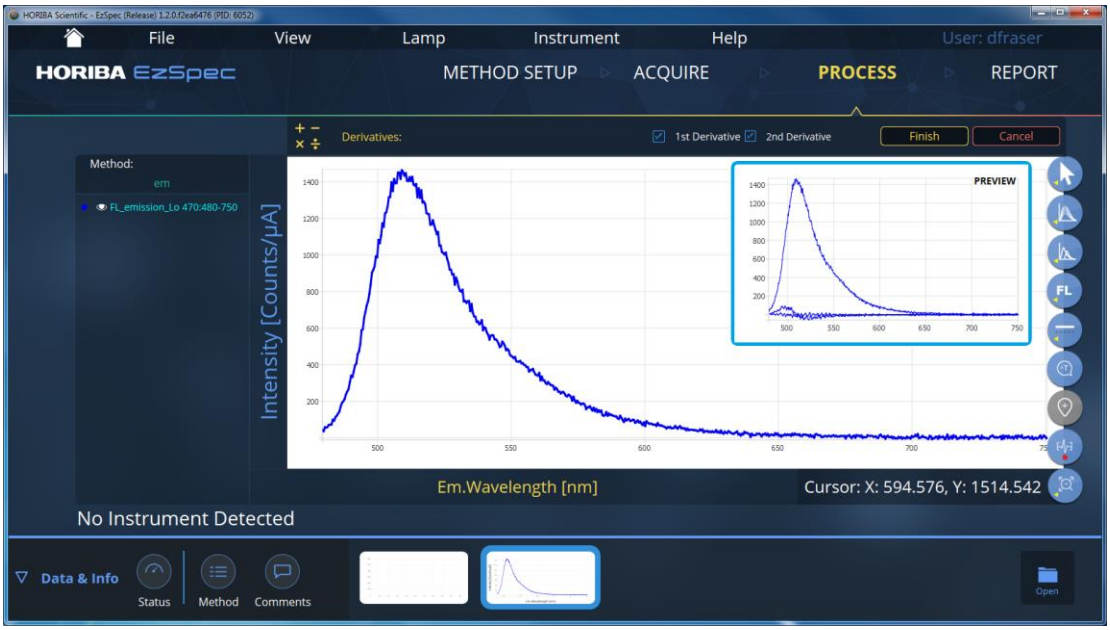


4. Click on **Next** to calculate the derivatives.

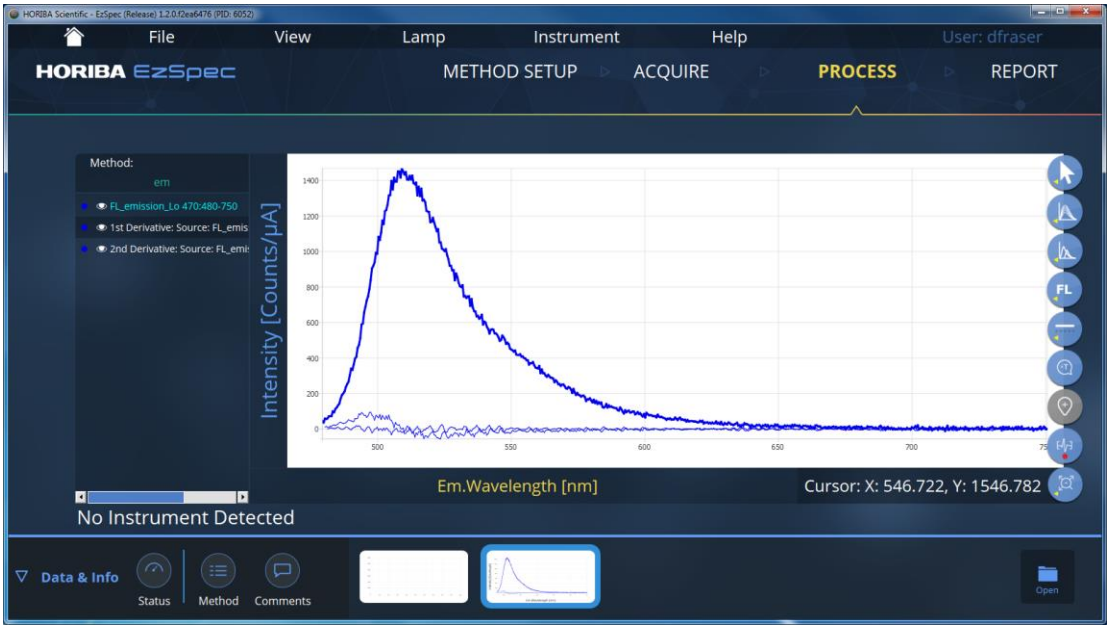


5. The original traces and the 1st and 2nd derivatives are shown in the Preview window.

6. Click on the **1st Derivative** and **2nd Derivative** checkboxes to hide or show the derivative traces.



7. Click **Finish** to show the selected derivative traces in the graph. The Preview window closes.

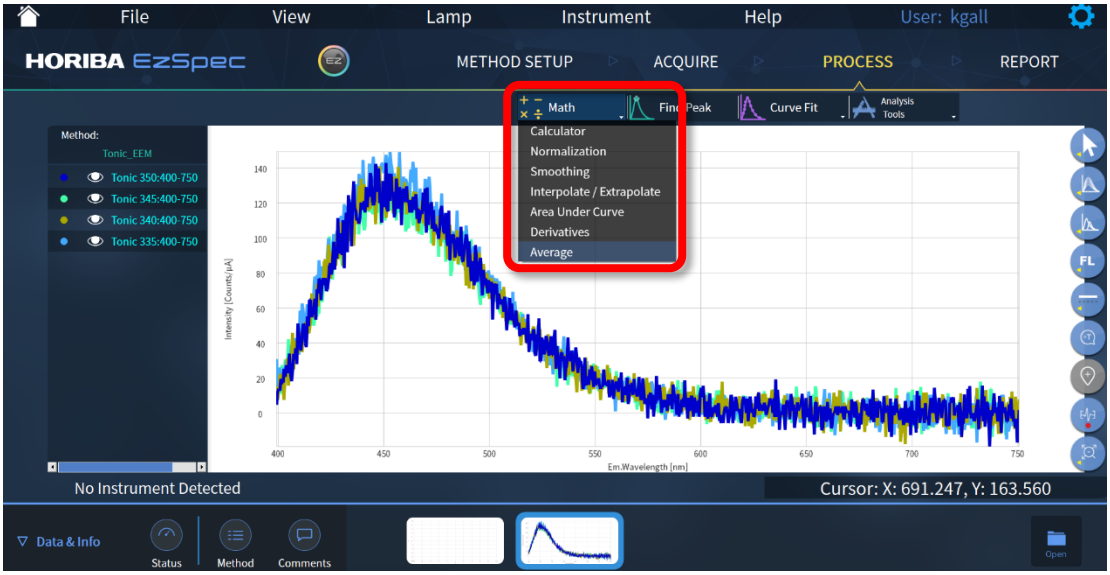


6.6.1.7 Average

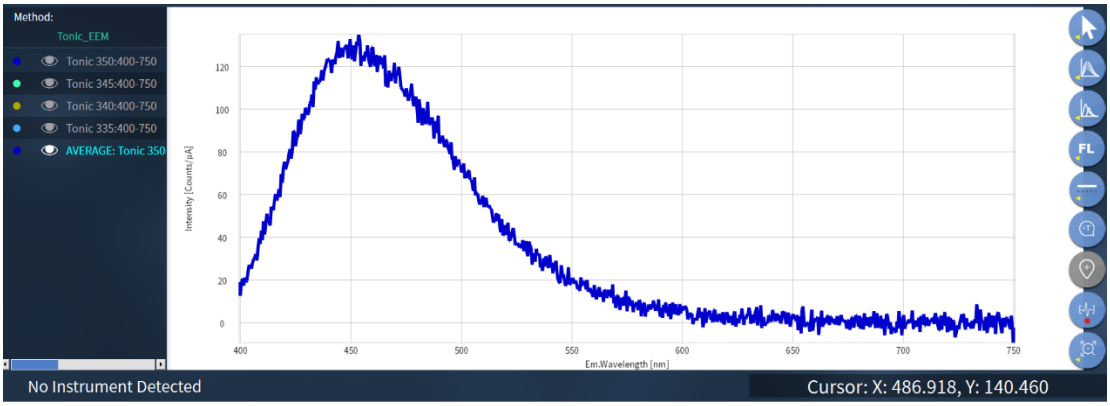
This process calculates the mean average of multiple selected traces.

1. Click on Process, Math, Average
2. Select multiple traces from the graph or legend which are to be averaged
3. Click Next.

4. Select the range cursor locations if the data over only a section of the x-axis is to be averaged. Range cursors are not required.
5. Click Average.



6. The averaged trace will be added to the graph and legend.



6.6.2 Find Peak

This process uses the Local Maximum Method to finds peaks in selected curves. For every point on an input curve, EzSpec™ forms a range with the point in the center of the range, and the length of the range is equal to the **Local Points** value. If the center point Y-value is the maximum Y-value in the range, then the center point is considered to be a candidate peak. Once all points in a curve are checked for being candidate peaks, then the candidate peaks are sorted from highest to lowest Y-value, and the top **# of Peaks** are selected and displayed. This process is applied to each selected curve.

1. Change to **2D Graph View**.
2. Select one or more curves in the legend or on the graph.
3. **Process**, **Find Peak**.
4. **Next**.
5. Click and drag the vertical and horizontal range bars to form a rectangle around the part of the curves where you want to find peaks. The range bars change color from bright blue to purple when selected. If only one range bar of a pair is visible, try clicking and dragging it. The two range bars may initially be overlapped. Only data inside the range bars are used for finding peaks. The range bars can be moved outside the grid so that the entire data range is selected.
6. Enter the **# of Peaks** to be found for each selected curve.
7. Enter the number of **Local Points**.
8. **Find Peak**.

9. Each Peak label shows the curve name and X and Y values of the peak.
10. To move a peak label, click on the label so that it turns blue and drag it.
    To delete a peak label, click and hold a mouse cursor on the label so that it turns blue and press the Delete key.

The above screenshot was created by doing the analysis, moving one of the peak labels, and then repeating the analysis, but not clicking **Find Peak**, to be able to display the result and the parameters selection.

6.6.3 Curve Fit

This process fits an equation to the selected region of a curve. For Polynomial, Decay or Growth fit types the user chooses the order (1, 2, 3, 4) of the fit by a pull down menu. The fit types are:

6.6.3.1 Polynomial (4th order)

$$y(x) = a_0 + a_1 * x + a_2 * x^2 + a_3 * x^3 + a_4 * x^4$$

6.6.3.2 Exponential Decay (4th order)

$$y(x) = a_0 + a_1 * e^{-(x-x_0)/\tau_1} + a_2 * e^{-(x-x_0)/\tau_2} + a_3 * e^{-(x-x_0)/\tau_3} + a_4 * e^{-(x-x_0)/\tau_4}$$

6.6.3.3 Exponential Growth (4th order)

$$y(x) = a_0 + a_1 * e^{(x-x_0)/\tau_1} + a_2 * e^{(x-x_0)/\tau_2} + a_3 * e^{(x-x_0)/\tau_3} + a_4 * e^{(x-x_0)/\tau_4}$$

6.6.3.4 Gaussian

$$y(x;\ y_0, A, x_0, w) = y_0 + A * e^{-((x-x_0)^2)/(2w^2)}$$

where y_0 is the baseline, A is the height of the peak above the baseline, x_0 is the center position of the peak, and w is the standard deviation and controls the width.

6.6.3.5 Lorentzian

$$y(x;\ y_0, A, x_0, w) = y_0 + \frac{A}{\pi w}\left[\frac{w^2}{(x-x_0)^2 + w^2}\right]$$

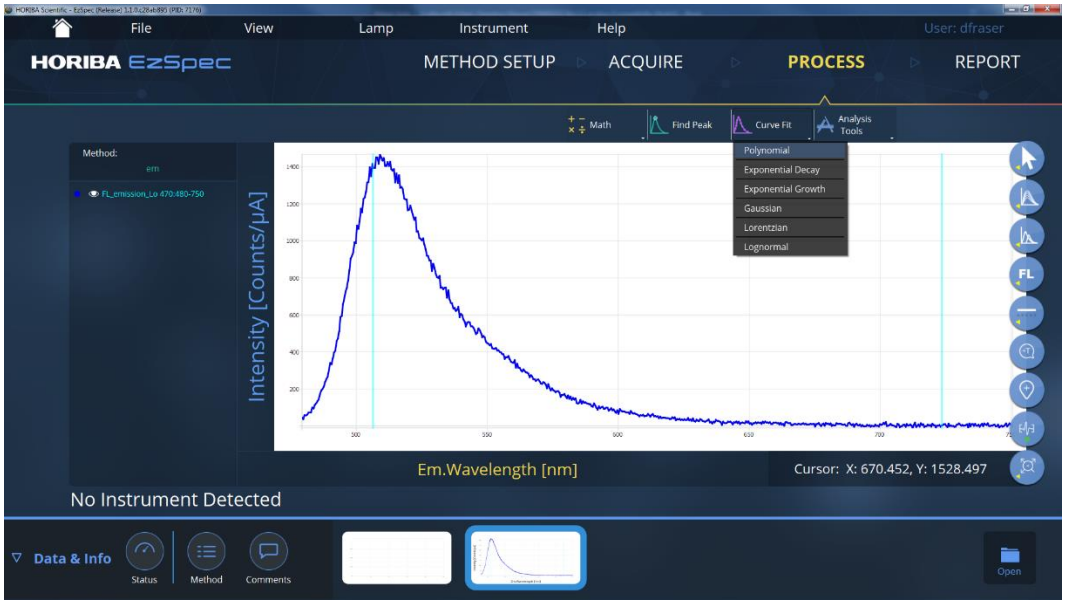
where y_0 is the baseline, $I = \frac{A}{\pi w}$ is the height of the peak above the baseline, x_0 is the center position of the peak, and 2*w is the full width at half maximum (FWHM).

6.6.3.6 LogNormal

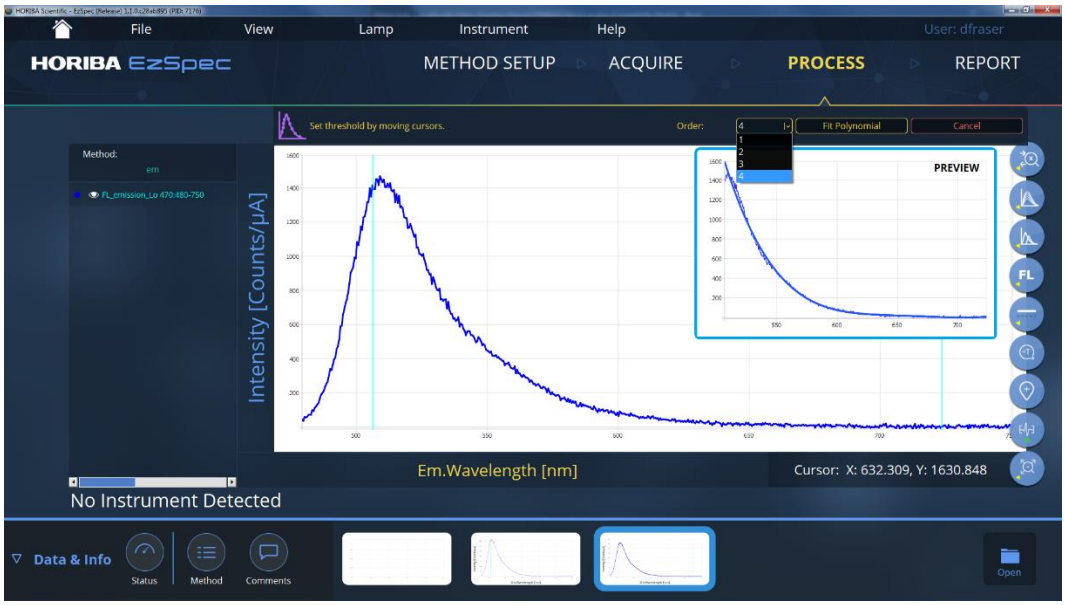
$$y(x;\ y_0, A, x_0, w) = y_0 + A * e^{\left[-\frac{(ln(x/x_0))^2}{w^2}\right]}$$

where y_0 is the baseline, A is the height of the peak above the baseline, x_0 is the center position of the peak, and w is the peak width. Also, $x > 0$.

1. The following steps show an example using polynomial fitting.
2. Change to 2D Graph View.
   Select a trace in the legend or on the graph. If more than one trace is selected only the last selected trace in the order that they were selected will be processed.
3. Click on the **Range Selector group**, and click the **Select Range** button.
4. Click on and move the range bars to select the range of data to be fitted.
5. **Process**, **Curve Fit**, **Polynomial**.

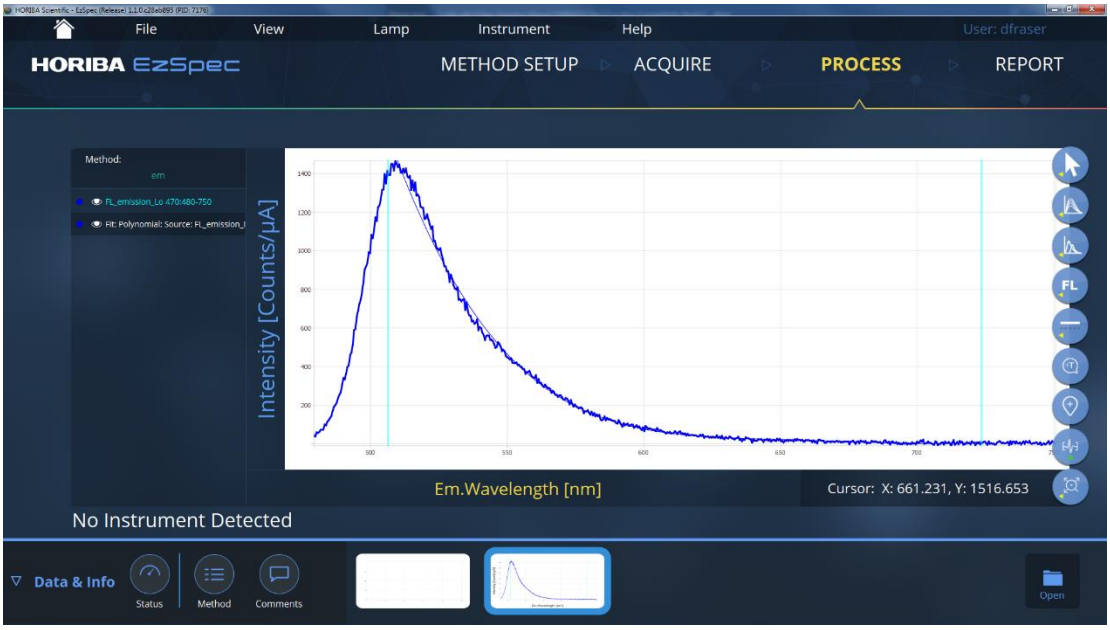


6. Next.

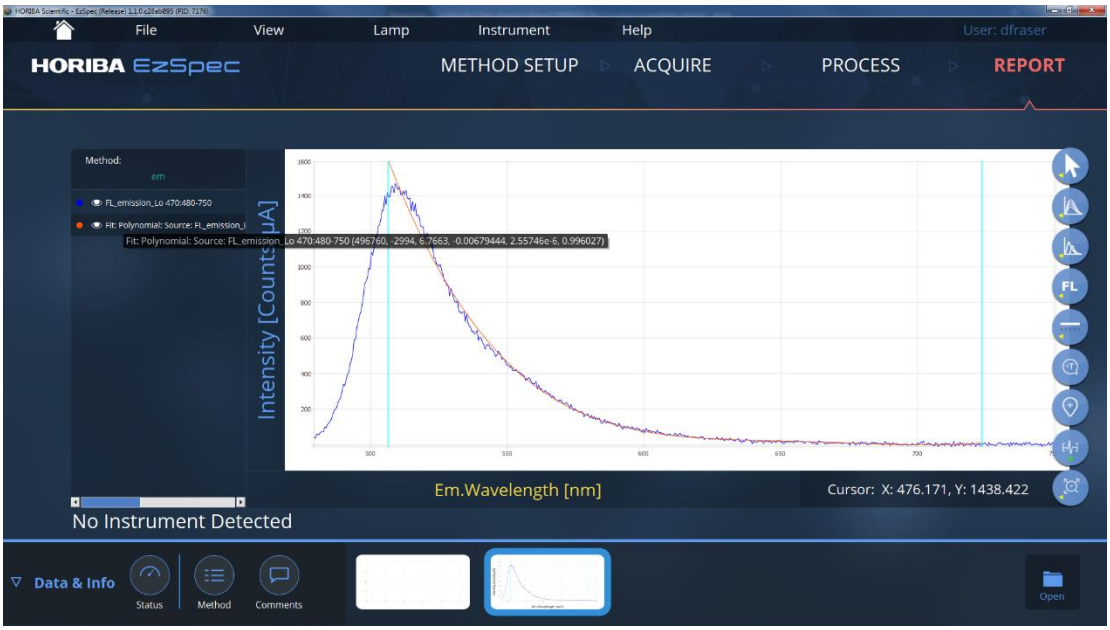


7. Select Order: 4 from the pull down list. The Preview window shows the fit. You can change the fit order.

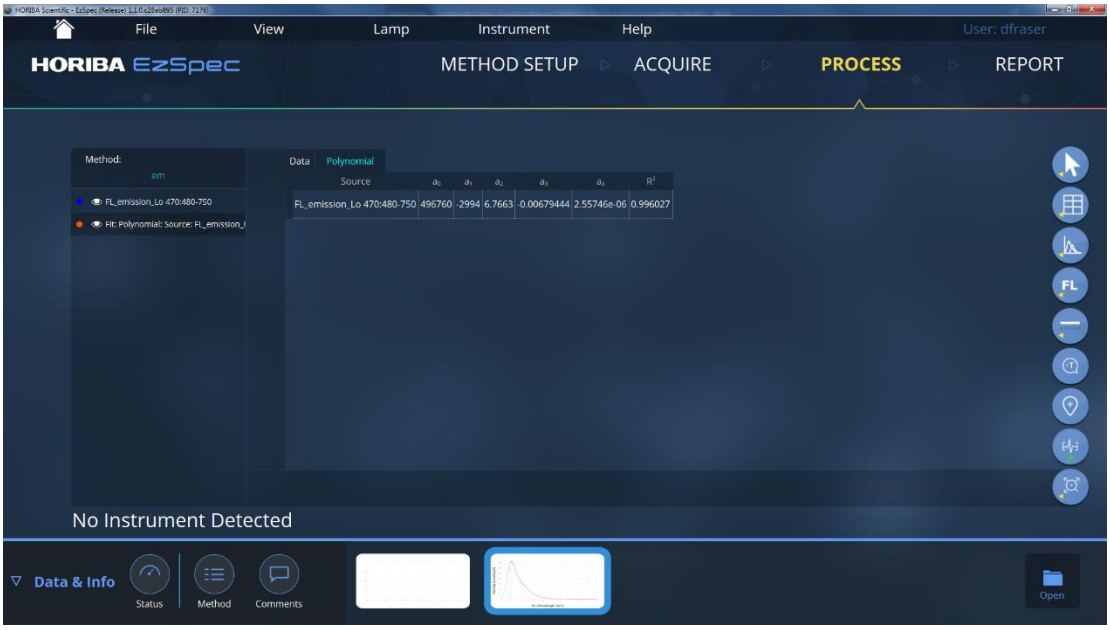
8. Click **Fit Polynomial**. The fitted trace is processed and shown in the legend and graph.



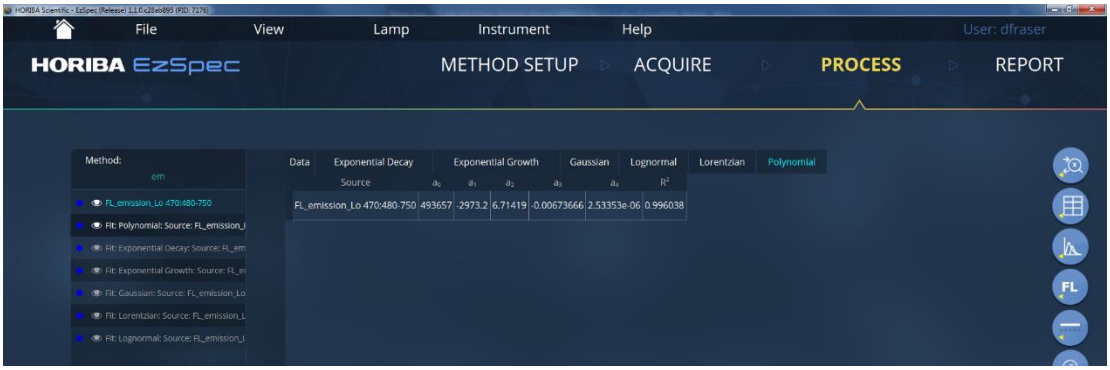
9. Deselect the traces to show thinner lines, change the color of the fitted trace, and hold the cursor over the **Fit Polynomial** trace name in the legend to show the full trace path name. The fit parameter values (a_0 to a_4, and R^2) have been appended to the trace name.



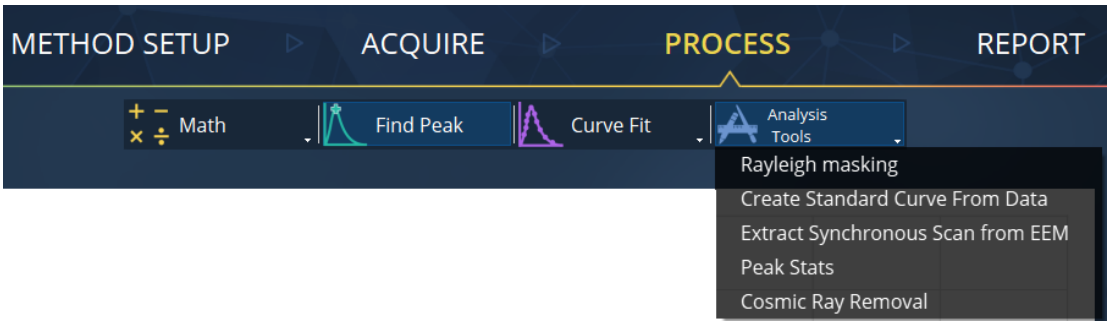
10. Click on the 2D Graph button to expand the **Plot Mode** group and select **Table View**.



11. The Table View defaults to showing the just fit type (here Polynomial) tab with the fit parameters.
12. If more than one fit type is processed, each of the fit types' parameters is shown in a different tab in alphabetical order in Table View. The displayed fit type is indicated by its name in bright blue.



6.6.4 Analysis Tools



6.6.4.1 Rayleigh Masking

This process requires EEM data. Although Rayleigh Masking can be done when the data is open in either 2D Graph, 3D Contour, 3D Waterfall, or Table View, the results are shown in 3D Contour View, and the best effect is to see the data in 3D Contour View before and after processing.

The Band pass sum width = Excitation band pass + Emission Band pass.

The 1st order Rayleigh mask is applied where
(λ_{EX} - Band pass sum width) ≤ λ_{EM} ≤ (λ_{EX} + Band pass sum width)

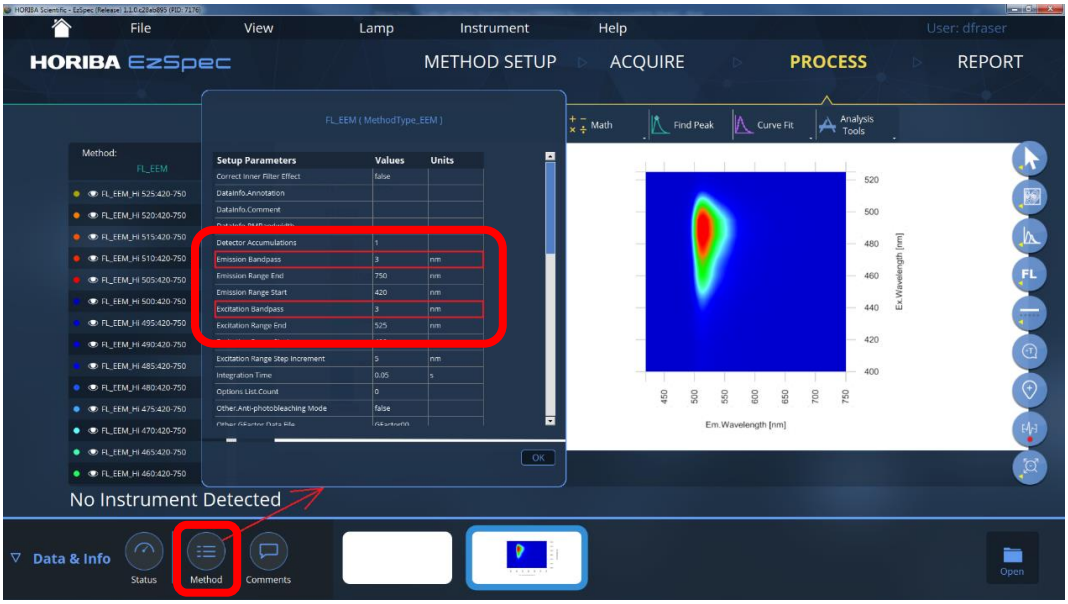
All intensity values in the above masked regions are set to zero.

EzSpec™ then replots and rescales the Rayleigh masked EEM.

A new data file with the same name and extension "_RM.ezspec_data" is automatically created in the same file directory as the original EEM data.

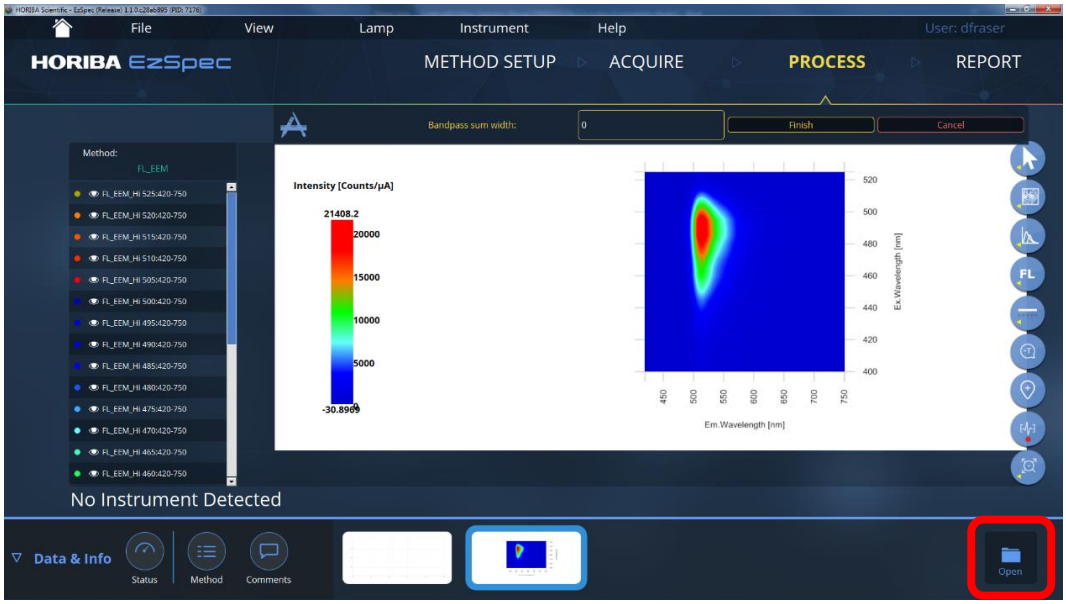
Because the CCD on the Duetta™ has a three-stage order sorting filter on it, the 2nd order Rayleigh scatter peaks will not be present in the spectra.

1. Acquire or open an EEM data file.
2. It may be helpful to check the excitation and emission band passes used to acquire the data.

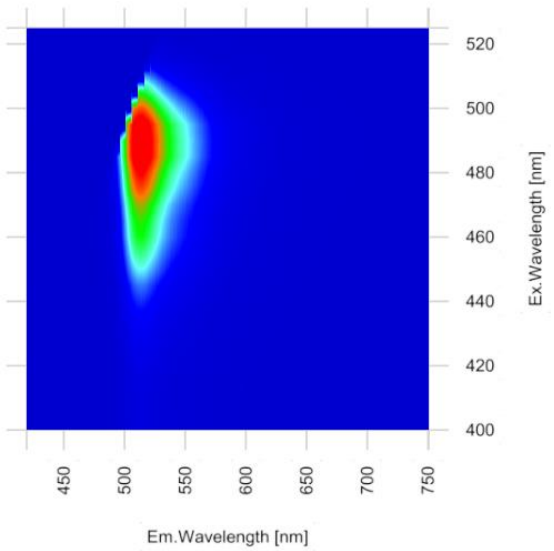


Click on the **Method** button on the **Data & Info** bar to view the band passes in the Method information window.

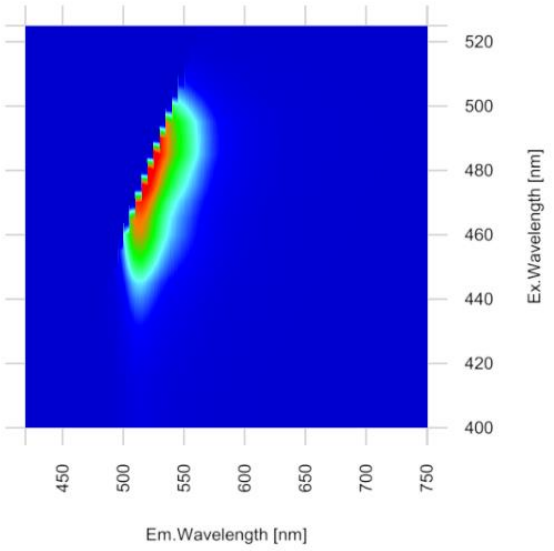
3. Click **OK** to close the Method information window.
4. Process, Analysis Tools, Rayleigh Masking.



5. Enter a **Band pass sum width** (excitation band pass + emission band pass) in the enterable box. If the value is left at 0, then the sum of the excitation and emission band passes in the Method Setup will be used.
6. Click Fi**nish**.



Bandpass sum width: 0 in box, = 6 nm from the Method Setup

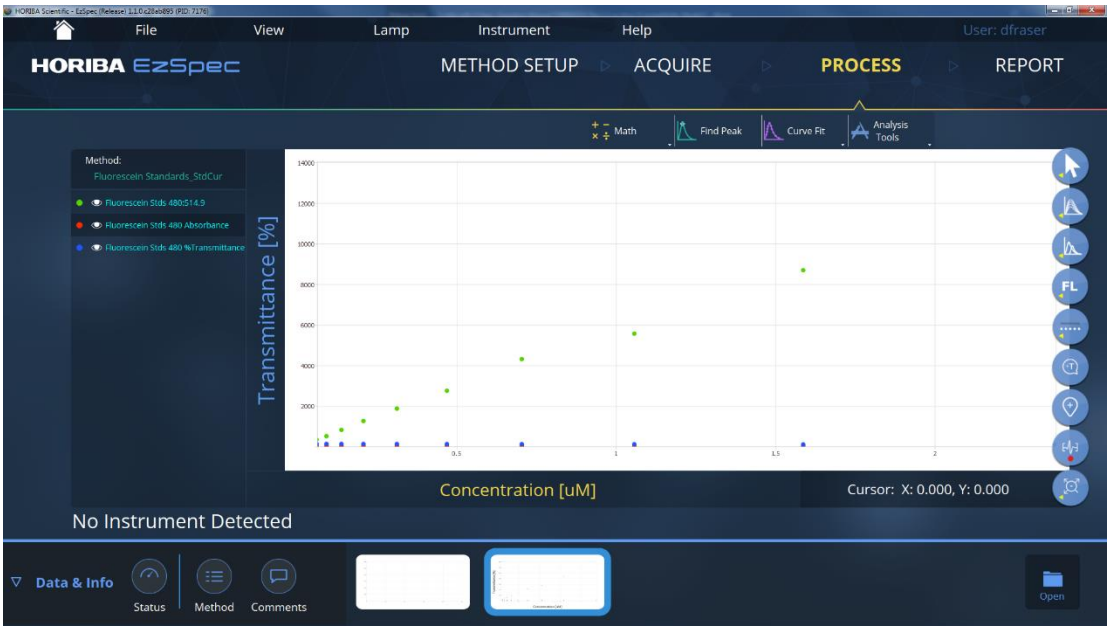


Bandpass sum width 40 nm entered in box

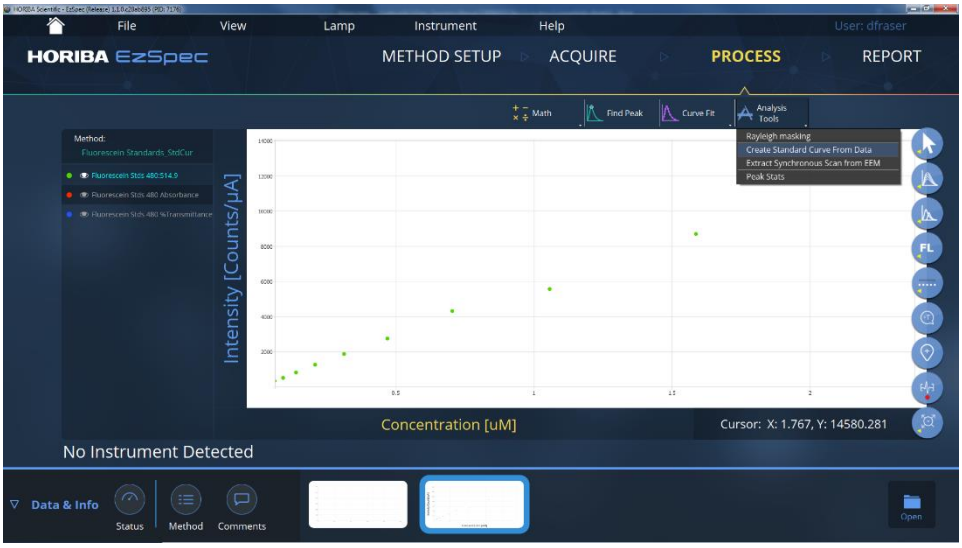
6.6.4.2 Create Standard Curve from Data

The data is acquired using the Capture Value (Standard Curve) method in which a previous blank value is loaded, or a new blank value is measured. For each sample, the user is prompted to enter a concentration for the sample and then acquire the fluorescence intensity.

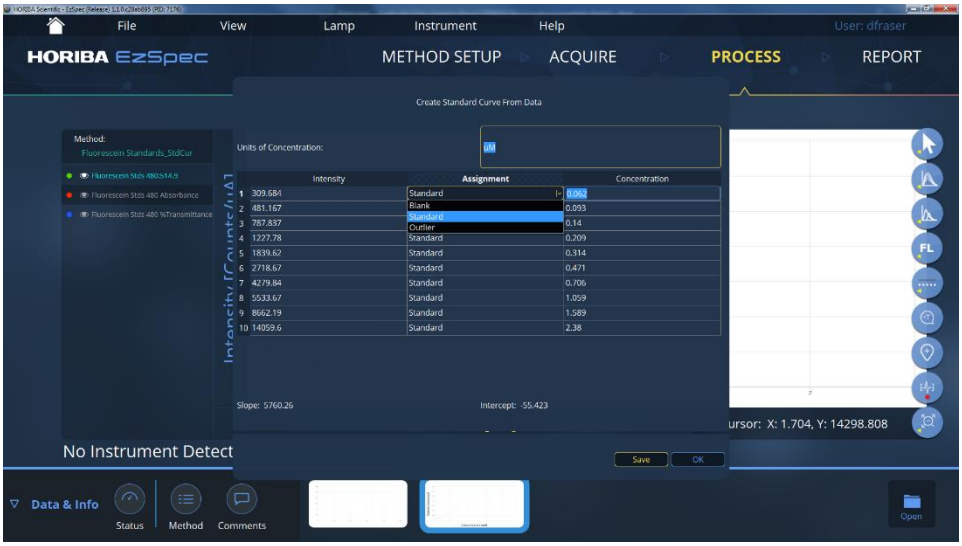
1. Open the data set.



2. Hide the Absorbance and Transmittance curves.
3. **Process**, **Analysis Tools**, **Create Standard Curve From Data**.



4. A table displays the data and the fitted slope and intercept.

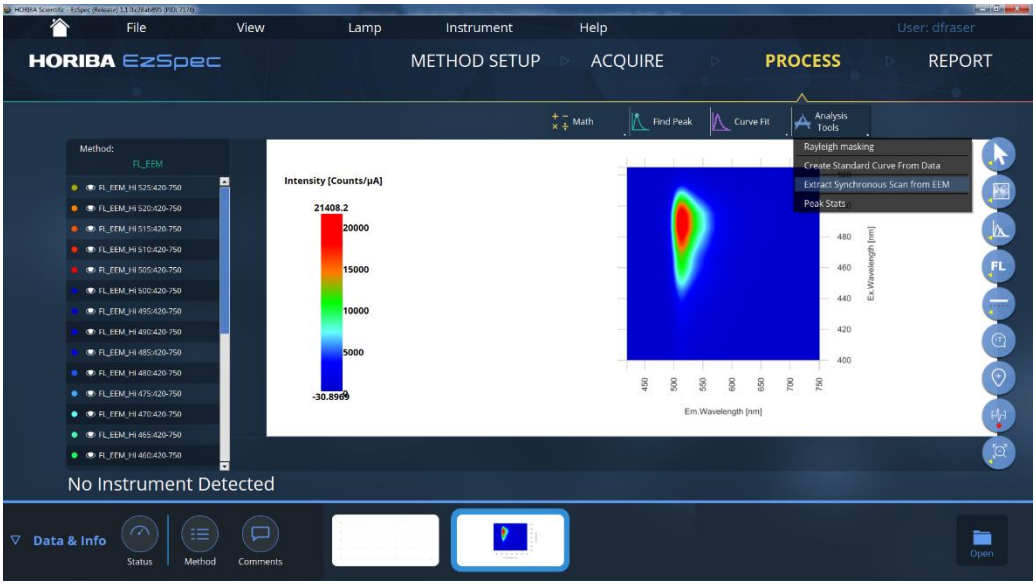


5. The user can change the **Units of Concentration** (for example:.µM or mg/mL).
6. Each data point has a triple-click Assignment pull down list for the user to assign: **Blank**, **Standard**, or **Outlier**
    a. **Blank** = Recognition of a blank data point where the concentration is equal to zero.
    b. **Standard** = Assignment of standards.
    c. **Outlier** = Assignment of any outliers to not be included in the fit.
7. The user can double-click on **Concentration** values to change them.
8. The tool will fit the standard curve of intensity vs. concentration (Blank and Standards only) to a linear equation.
9. The fit is **Intensity = a*Concentration + b** where **a** is the fit slope and **b** is the fit intercept.
10. The intercept **b** should be zero or very close to zero.
11. When **Assignment** or **Concentration** values are changed, clicking outside the changed cell will update the fit results.

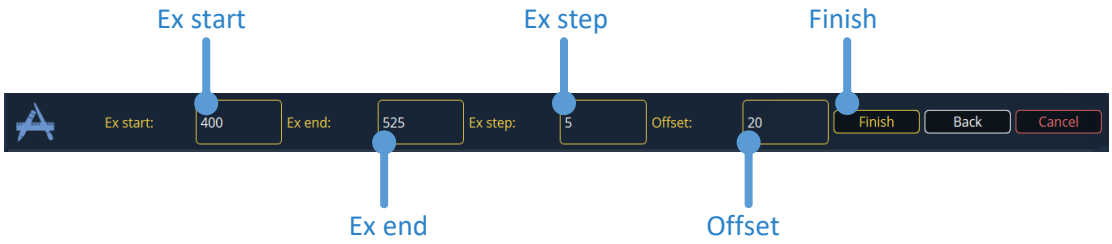
12. Click **Save** to create a file that can be read by the Concentration Curve App.

6.6.4.3 Extract Synchronous Curve from EEM

This process extracts a synchronous scan from EEM data.



1. Acquire or open an EEM data file.
2. Process, Analysis Tools, Extract Synchronous Curve from EEM.
3. Enter the excitation start wavelength in the **Ex start:** box. - This box defaults to the Excitation start wavelength of the EEM data.
4. Enter the excitation end wavelength in the **Ex end:** box – This box defaults to the Excitation end wavelength of the EEM data.



5. Enter the excitation step size in the **Ex step:** box. – This box defaults to the Excitation step size of the EEM data.
6. Enter the offset between the excitation wavelength and emission wavelengths in the **Offset:** box. This box defaults to $\lambda_{Em\ Start}$. - $\lambda_{Ex\ Start}$ of the EEM data.
    a. Zero is allowed
    b. The maximum offset is $\lambda_{Em\ End} - \lambda_{Ex\ Start}$ of the EEM data.
7. **Finish**.
8. The output is a Synchronous scan (Intensity vs. Emission wavelength (nm)) displayed on a new graph while the original EEM is kept as its original graph. This way, the user can go back to the EEM data set and extract another Synchronous Scan if needed. A file with the

name “Synchronous – (original DataName).ezspec_data” is automatically created and saved in the same directory as the original EEM data.

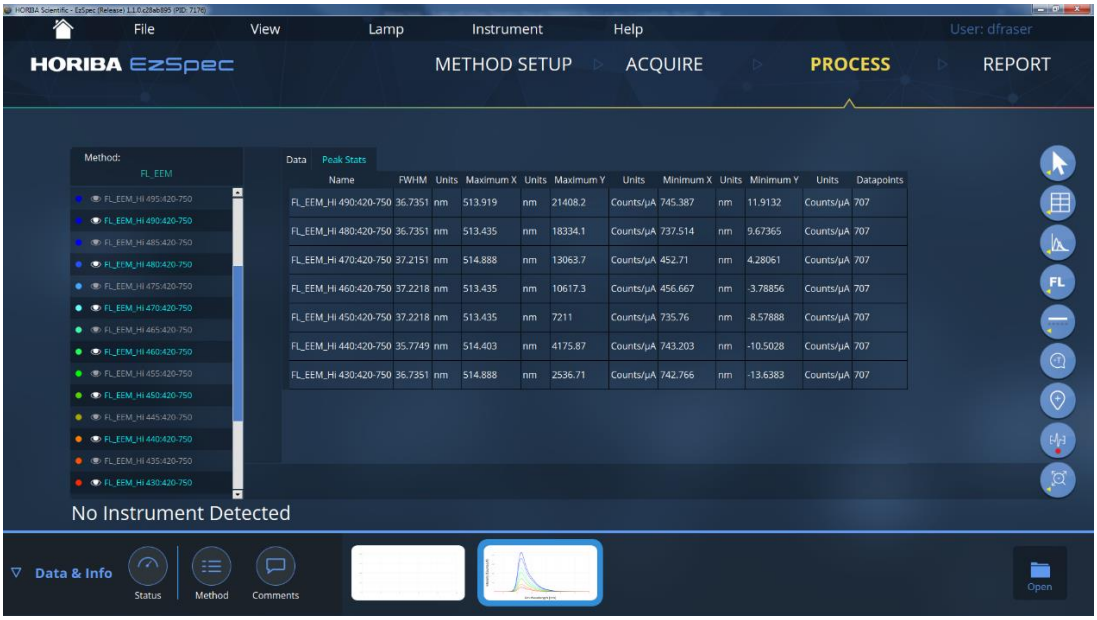
9. You may have to click on the **Axes Scaling** group and then on the **Full Scale** button to rescale the synchronous scan.
10. In the legend the result trace is named “Synchronous spectrum (original trace prefix: Excitation start from step 3 – Excitation End from step 4 above; Offset value)”.

6.6.4.4 Peak Stats

This process uses a Full Width at Half Maximum calculator to analyze the selected curves and display the statistics of the highest and lowest points of each curve. It can be used on any curve type, and in any Graph View.

1. Acquire data or open a data file.
2. Select the desired traces.
3. **Process**, **Analysis Tools**, **Peak Stats**.

4. **Next**. This finds the highest and lowest points of each selected curve and displays the statistics in the **Peak Stats** tab in **Table View**.

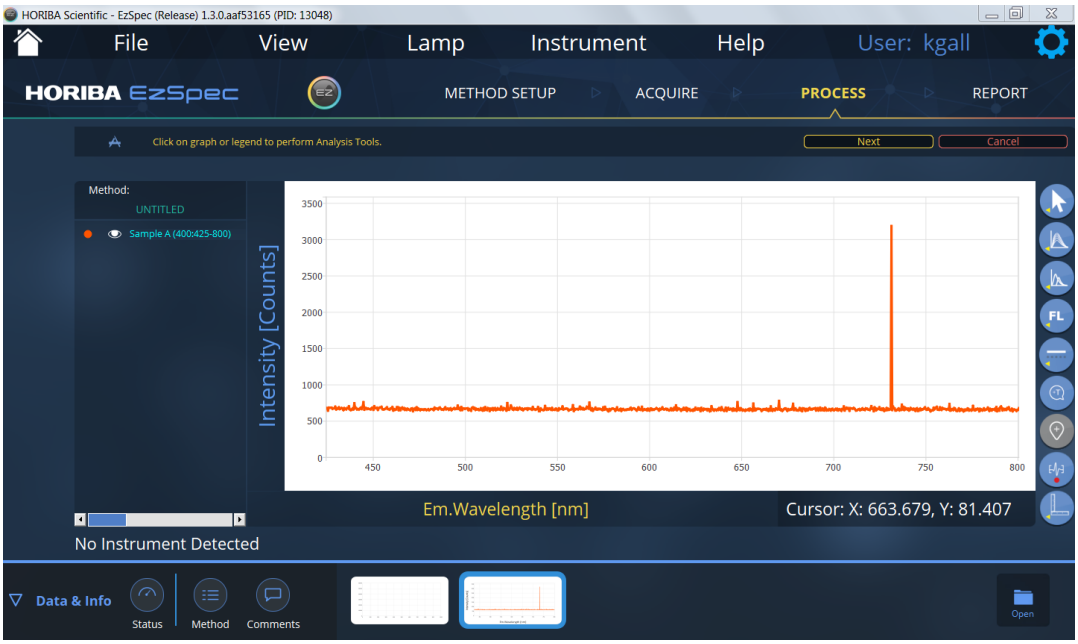


5. **FWHM** – This column shows the Full Width at Half Maximum (FWHM) of the highest point of each selected curve.
6. **Maximum X** - This column shows the X value of the highest point of each selected curve.
7. **Maximum Y** - This column shows the Y value of the highest point of each selected curve.
8. **Minimum X** - This column shows the X value of the lowest point of each selected curve.
9. **Minimum Y** This column shows the Y value of the lowest point of each selected curve.
10. **Datapoints** – This column shows the number of data points of each selected curve.

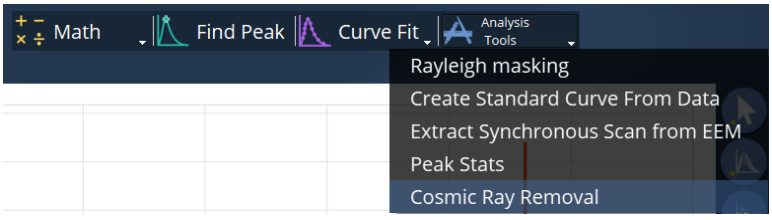
6.6.4.5 Cosmic Ray Removal

This process uses an averaging of points around a single data point to remove a CCD pixel that may have read a cosmic ray or muon. Muons are created when cosmic rays collide with particles in the Earth's atmosphere and are present all around. Since thousands of muons arrive per square meter of Earth's surface every minute, they can occasionally hit the CCD and are detected by single pixels. As these are artifacts, the cosmic ray removal tool will remove the single data point affected in a spectrum.

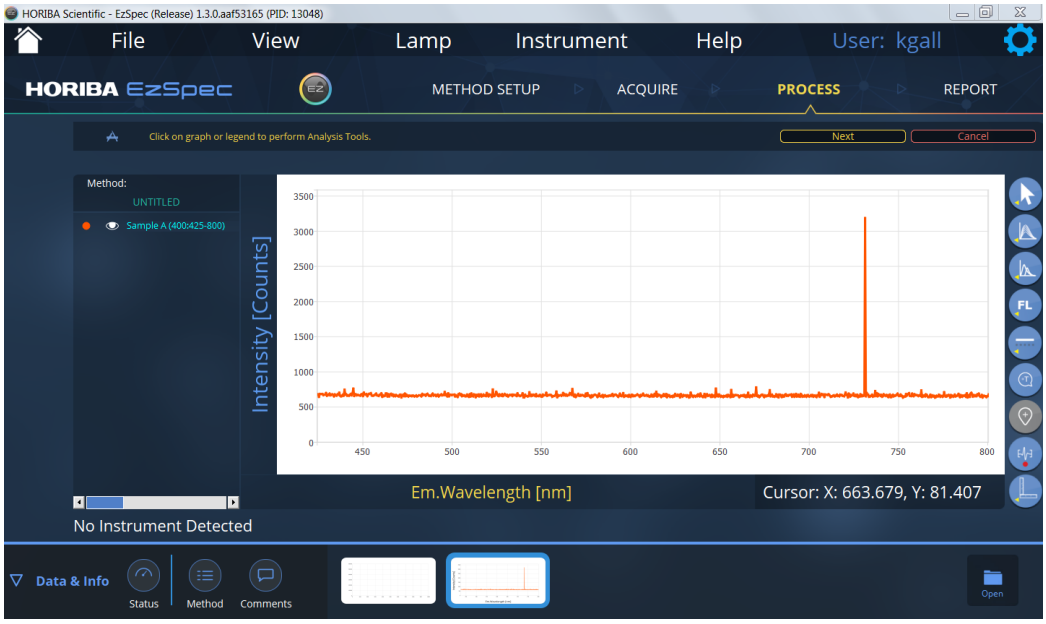
1. Acquire data or open a data file.
2. Select the desired traces in the legend.



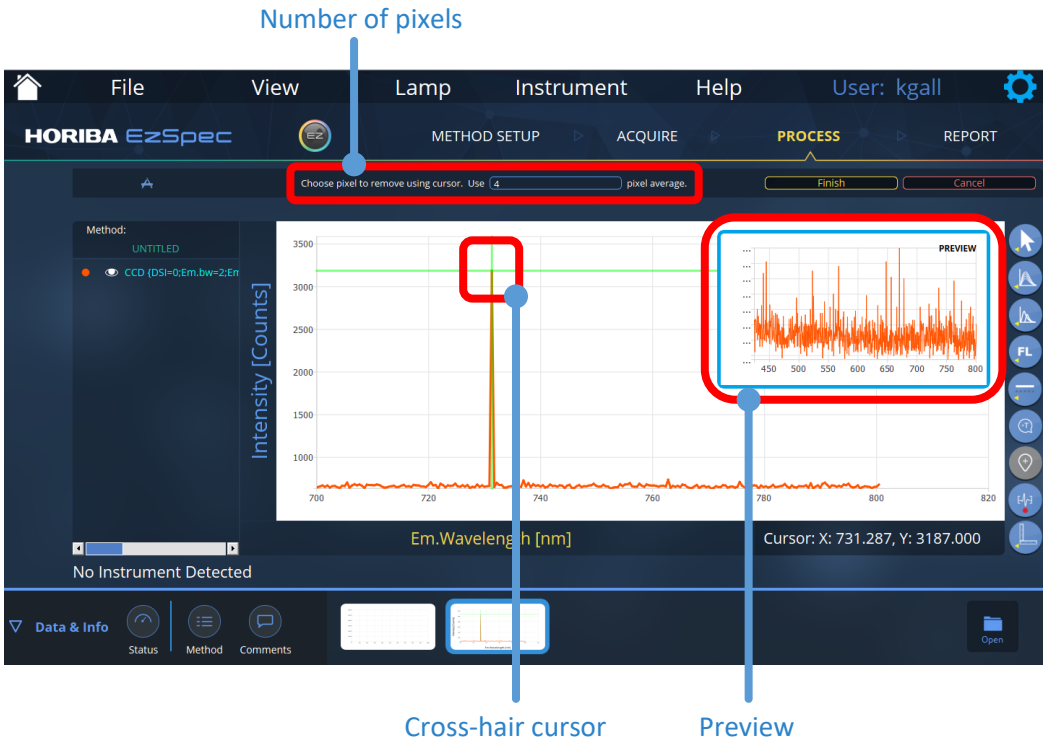
3. Process, Analysis Tools, Cosmic Ray Removal.



4. Click **Next**.

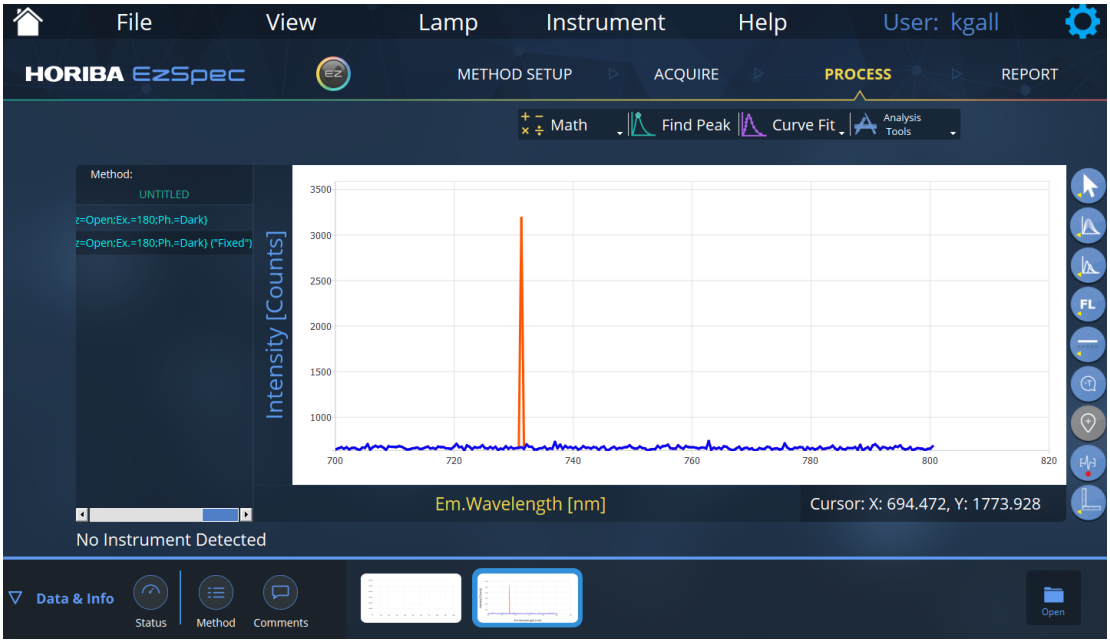


5. Move the cross-hair cursor to the single data point that requires removal and select the number of pixels around the single data point to average.

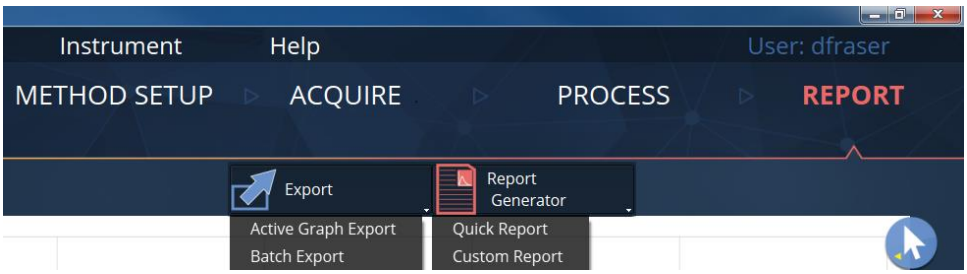


The fixed data will show in the preview window with the chosen settings.

6. Click **Finish** to add the fixed data to the graph.
7. The trace for the fixed data will appear with the same trace name but with “(fixed)” at the end of the trace name.


File
View
Lamp
Instrument
Help
User: kgall
HORIBA EzSpec
METHOD SETUP
ACQUIRE
PROCESS
REPORT
Math
Find Peak
Curve Fit
Analysis Tools
Method:
UNTITLED
z=Open;Ex.=180;Ph.=Dark}
z=Open;Ex.=180;Ph.=Dark} ("Fixed")
Intensity [Counts]
3500
3000
2500
2000
1500
1000
700
720
740
760
780
800
820
Em.Wavelength [nm]
Cursor: X: 694.472, Y: 1773.928
No Instrument Detected
Data & Info
Status
Method
Comments
Open

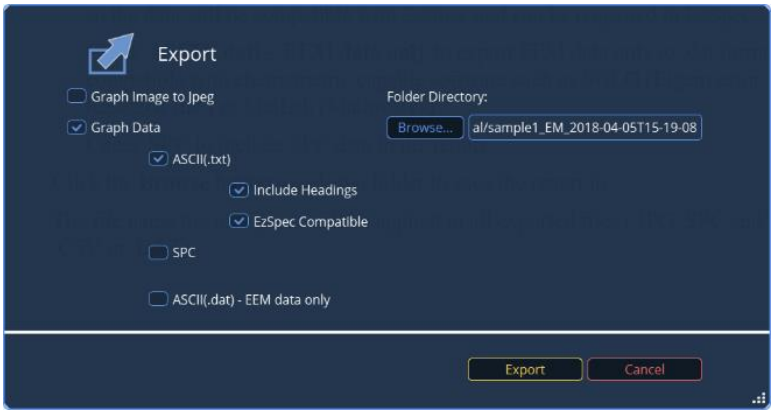
6.7 Report



6.7.1 Export

6.7.1.1 Active Graph Export

Click **Active Graph Export** to export the data set currently shown in the graph window.

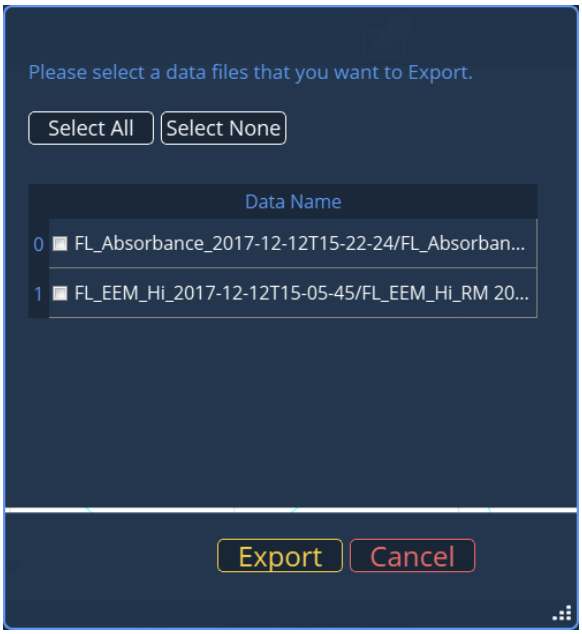


- Check **Graph Image to JPG** to include an image of the currently active graph window in the report.
- Check **Graph Data** and the sub-options to include them in the report.
    - Check **ASCII(.txt)** to export the data as ASCII (.txt format) files. These files are tab delimited. When opening the .txt files created by EzSpec, choose to open them in spreadsheet programs (MS Excel, LibreOffice, etc.) as tab-delimited to be able to view data properly.
        - Check **Include Headings** to include table headings in the currently active graph/table window.
        - Check **EzSpec Compatible** to include EzSpec data headers / footer information so the data will be compatible with EzSpec and can be reopened in EzSpec.
    - Check **SPC** to include SPC data in the report.
    - Check **ASCII(.dat) – EEM data only** to export EEM data only to .dat format, compatible with chemometric capable software such as SOLO (Eigenvector Research Inc.) or MatLab (Mathworks). Consult your third party multivariate

analysis package software company for support on chemometric analysis tools (i.e. PARAFAC, PLS, PCA, etc.).

- Click the **Browse** button to select a folder to save the report in.
- The file name the user gives will be applied to all exported files (.jpg, .spc, .txt or .dat).

6.7.1.2 Batch Export

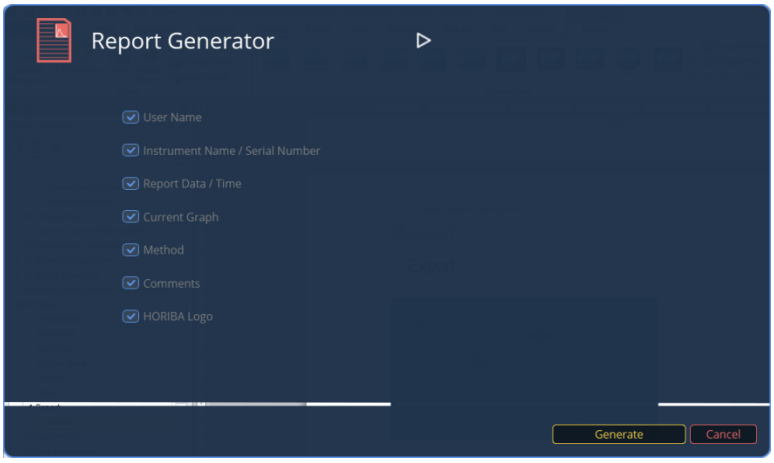


Clicking on **Batch Export** will give a list of all open data sets and the user can **Select All**, **Select None**, or check individual data sets to export. These data sets correspond with open thumbnails. If **Export** is clicked, the above **Export** window will open to allow the user to choose file format options and the file path.

6.7.2 Report Generator

6.7.2.1 Quick Report

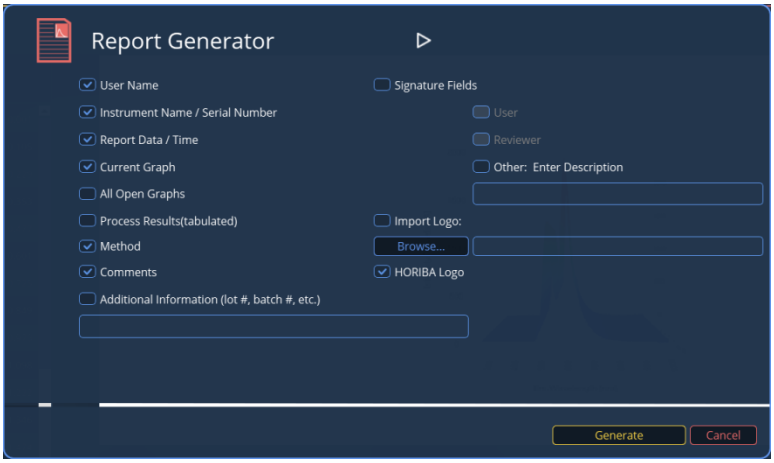
Quick Report first shows the **Report Generator** window.



Click **Generate**. All items will be generated (not optional). Both the Method name and details will be included.

6.7.2.2 Custom Report

Custom Report first shows the Report Generator window, similar to the Quick Report, but with more items. All fields are optional.



7 Measuring Fluorescence Anisotropy on the Duetta-Bio

Fluorescence anisotropy is a measurement of the changing orientation of a molecule in space, with respect to the time between the absorption and emission events. Absorption and emission indicate the spatial alignment of the molecule's dipoles relative to the electric vector of the electromagnetic wave of excitation and emitted light, respectively.

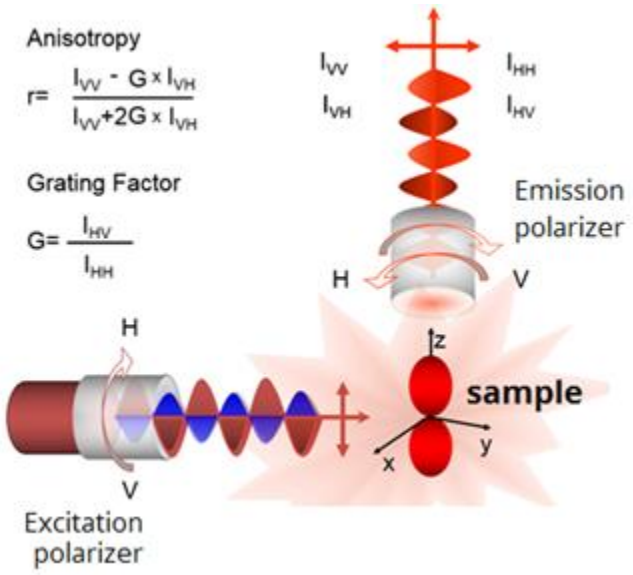
In other words, if the fluorophore population is excited with vertically polarized light, the emitted light will retain some of that polarization based on how fast it is rotating or moving in solution. The faster the reorientational motion, the more depolarized the emitted light will be (and anisotropy values will be lower). The slower the motion, the more the emitted light retains the polarization (and the anisotropy values will be higher).

Anisotropy, r $$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

- **VV denotes vertical excitation, vertical emission**
- **VH denotes vertical excitation, horizontal emission**

To use this information, polarizers are placed in the excitation light path and the emission light path of a fluorometer. The anisotropy is calculated by taking the ratio of the intensities in the above equation, where I_{VV} indicates the intensity with a polarizer set to vertical orientation on the excitation light path and a polarizer set to vertical orientation on the detected emission. I_{VH} indicates the intensity when using a vertical polarizer on the excitation and horizontal polarizer on the emission. *G* is a grating factor used as a correction for the instrument's differential transmission of the two orthogonal vector orientations. I_{HV}/I_{HH} is used to calculate the G-factor and *G* is put into the numerator and denominator of the anisotropy equation as shown in the figure below.

The Duetta-Bio has the capability to measure fluorescence anisotropy with the use of polarizers installed on the excitation and emission filter wheels. There are polarizers set to the Vertical (0°) and Horizontal (90°) orientation on the excitation light path before the reference photodiode. There are polarizers which can be set to the Vertical (0°), Horizontal (90°), and Magic Angle (54.7°) on the emission light path on the way into the emission spectrograph.



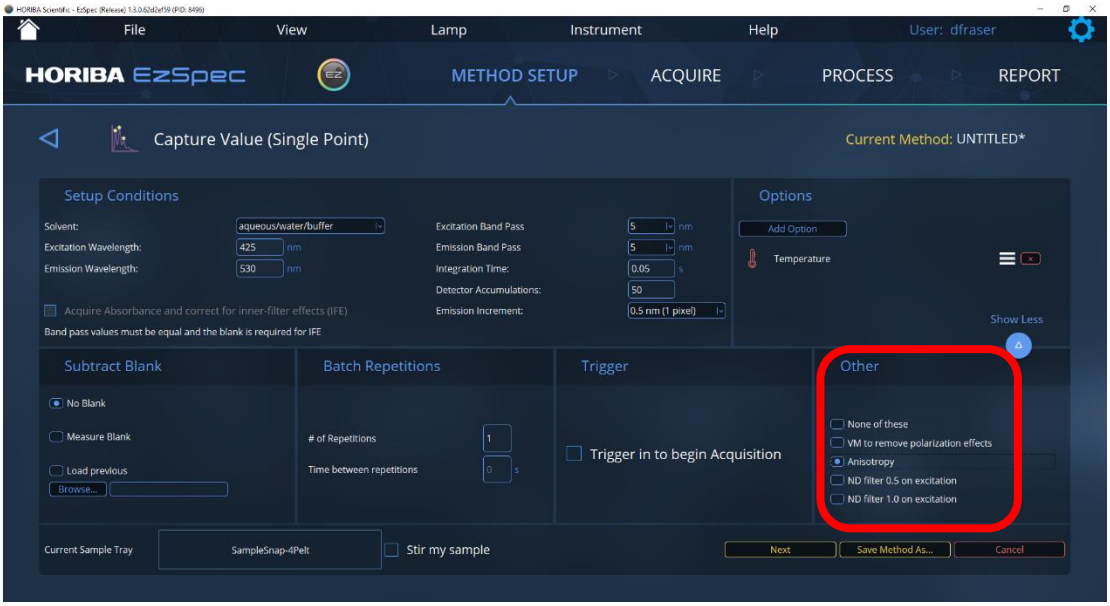
The above drawing depicts a fluorescence anisotropy experiment using excitation and emission polarizers rotated to 0 degrees (Vertical, V) and 90 degrees (Horizontal, H) orientations.

How does the experiment work? First the fluorescence is measured with the excitation polarizer set at vertical and the emission polarizer also set at vertical orientation. The intensity is plugged into the anisotropy equation as I_{VV}.

Then, the measurement is repeated with the emission polarizer set at horizontal orientation and the intensity is put into the equation as I_{VH}. The anisotropy formula contains a factor of 2 because there are two orthogonal orientations that the deflection from the VV vector can be projected onto, H_x and H_y, resulting in two I_{VH} components. A related expression, degree of polarization *p,* is often used to describe a two dimensional polarization parameter with only one horizontal component accounted for. In this latter case the formula would lack the multiplier *2* for I_{VH} with *p* taking the place of *r*.

Next, the G-factor is calculated by measuring the intensity at HH and HV and inserting I_{HV} and I_{HH} into the equation for *G*. Anisotropy, denoted by lower case "*r*" is often used as an indicator of molecular size, diffusion, and viscosity.

In EzSpec software, for any of the fluorescence methods (Emission Spectra, Excitation Spectra, Capture Value, EEM, and Kinetics), the option to measure anisotropy can be found in the "Other" section within each method setup page.



The above is an example of the EzSpec method setup for Duetta-Bio to measure Anisotropy for a Capture Value (Single Point) acquisition with changing temperature.

If, for example, Anisotropy is checked for an Emission Spectrum method setup, the emission spectrum will be measured with polarizers in the positions of VV, VH, HV, and HH (with the first letter denoting the excitation polarizer position and the second letter denoting the emission polarizer position) and the anisotropy will be plotted versus emission wavelength. If Anisotropy is selected for EEM method setup, the anisotropy will be plotted as Excitation vs. Emission vs. Anisotropy, and so on. The G-factor will be measured at the end of every acquisition if "Measure G-factor" is selected. Alternatively, a G-factor measured using the same experiment parameters can be loaded into the Load G-factor option. For example, this may be useful for shortening a long experiment with either multiple accumulations or long acquisition or integration times. For loading a premeasured G-factor, use the file saved in the previously measured data folder called "gfactor.ezspec_data". The G-factor must have been measured using the same method setup (i.e. excitation wavelength(s), emission wavelength(s), integration time, band pass, etc.).

Fluorescence anisotropy can also be measured in time-resolved mode using an instrument with fluorescence lifetime capabilities. Time-resolved anisotropy has the capability to measure reorientational time constants that can give information on diffusion, molecular size/volume, and local viscosity and is also very useful for binding/aggregation and/or dissociation experiments. As Duetta has a CCD emission detector, it only has the capability of measuring steady state anisotropy. For instruments that can perform time-resolved anisotropy, consider DeltaFlex

dedicated fluorescence lifetime instruments or DeltaTime additions to any steady state PMT-based HORIBA fluorometer. Some useful equations for using both steady state anisotropy *r* and time-resolved anisotropy *r(t)* measurements are shown below.

Anisotropy

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

VV denotes vertical excitation, vertical emission
VH denotes vertical excitation, horizontal emission

The grating factor, G is an instrumental correction factor.

Time-resolved Anisotropy

$$r(t) = \frac{I_{VV}(t) - GI_{VH}(t)}{I_{VV}(t) + 2GI_{VH}(t)}$$

Fundamental anisotropy

$$r_0 = \frac{2}{5}\left(\frac{3\cos^2\beta - 1}{2}\right)$$

$-0.2 \le r_0 \le 0.4$

β is the angle between absorption and emission transitions

Perrin Equation

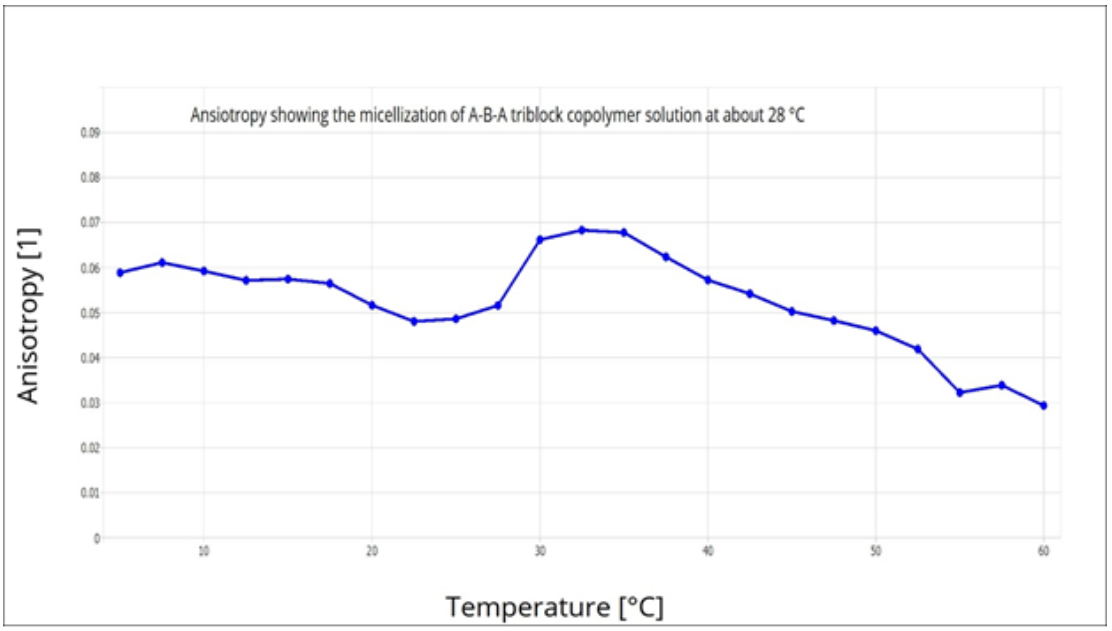
$$\frac{r_0}{r} = 1 + \frac{\tau}{\theta_{rot}} = 1 + 6D_\tau$$

θ_{rot}= reorientation time constant
D_τ=diffusion coefficient

Stokes-Einstein-Debye Equation

$$\theta_{rot} = \frac{\eta V}{k_B T}$$

η is viscosity
V is molecular (rotor) volume
k_b is Boltzmann's constant
T is temperature



Above is an example of steady state anisotropy of coumarin 153 measured with changing temperature. The hydrophobic coumarin was dissolved in an aqueous solution of A-B-A triblock copolymer with amphiphilic properties. At temperatures above 28 °C, for this particular polymer concentration, the polymer forms micelle aggregates in solution and the coumarin experiences a change in local viscosity by moving from a fluid-like environment to the more preferable hydrophobic core of the micelles at higher temperatures, in a more confined environment. The change in fluorescence anisotropy of the coumarin as related to reorientational motion of the dye can be used to detect the temperature at which this polymer forms micelle aggregates.

For more information on Duetta-Bio or fluorescence anisotropy methods and instrumentation, contact HORIBA Scientific or go to the website:

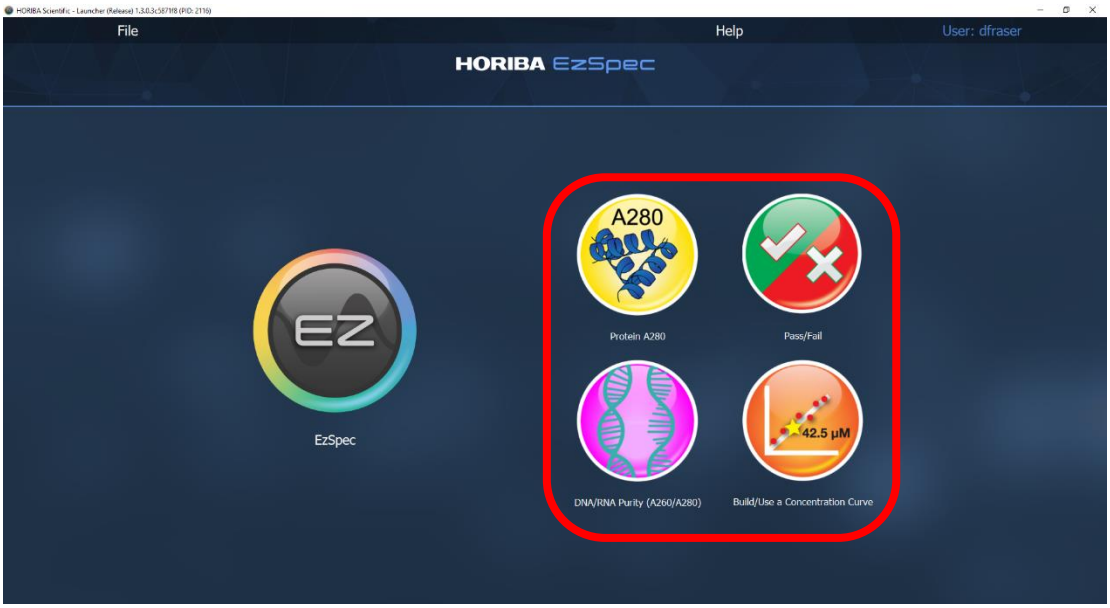
https://www.horiba.com/en_en/technology/measurement-and-control-techniques/molecular-spectroscopy/fluorescence-spectroscopy/what-is-fluorescence-anisotropy-or-fluorescence-polarization/

8 Applications or “Apps”

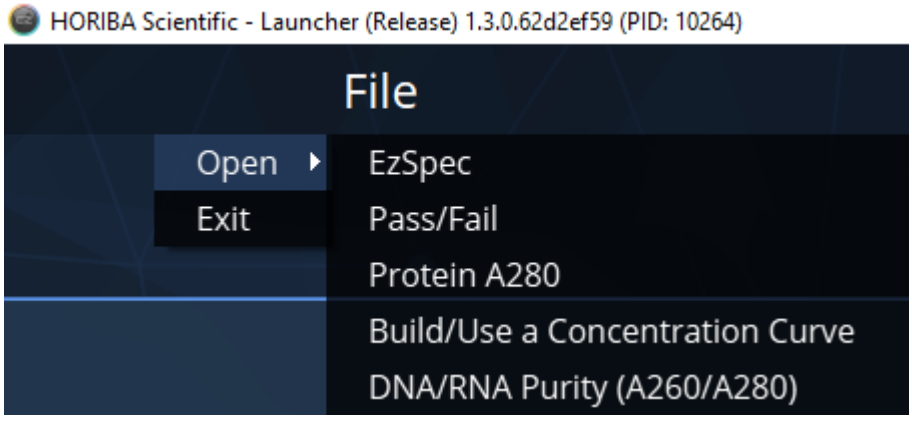
Applications or “Apps” are methods developed by HORIBA for specific fluorescence or absorbance applications and are opened from the Launcher Window.

Apps are very specific methods focused on a single measurement or series of measurements to answer a specific question.

Click on one of the App icons on the right side of the Launcher window to open the App.



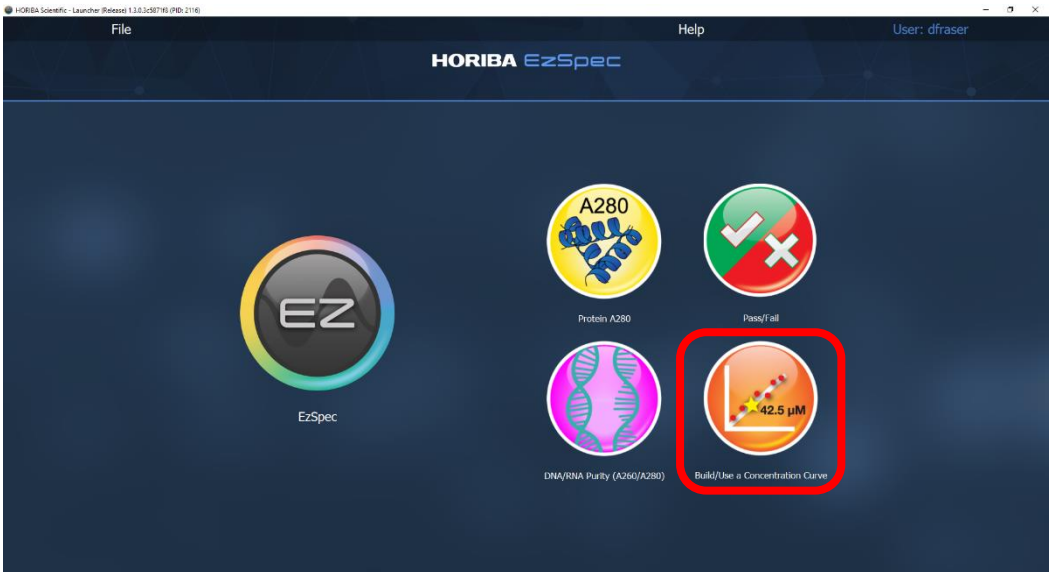
Or, on the Launcher window, Top Menu, click on **File**, **Open** to show the menu where you can click on **EzSpec** to go to the EzSpec module, or click on an App name to open it.



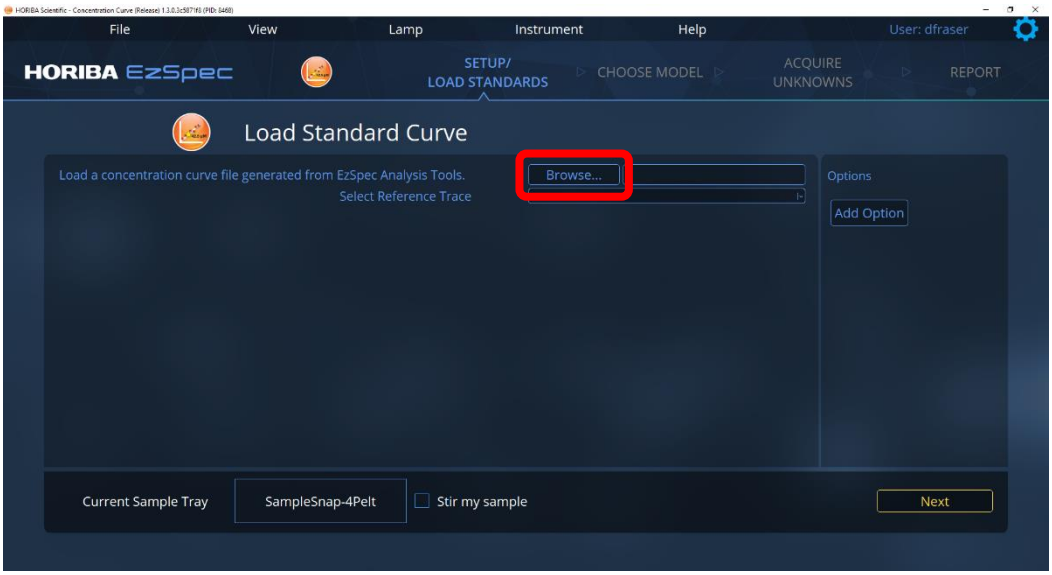
8.1 Concentration Curve

The Concentration Curve App uses a calibration curve created in Developer Mode to acquire the spectral intensity of an unknown sample or set of samples and use the calibration curve to calculate the concentrations of unknown samples.

1. On the Launcher Window click on the **Build/Use a Concentration Curve** button to open the App.

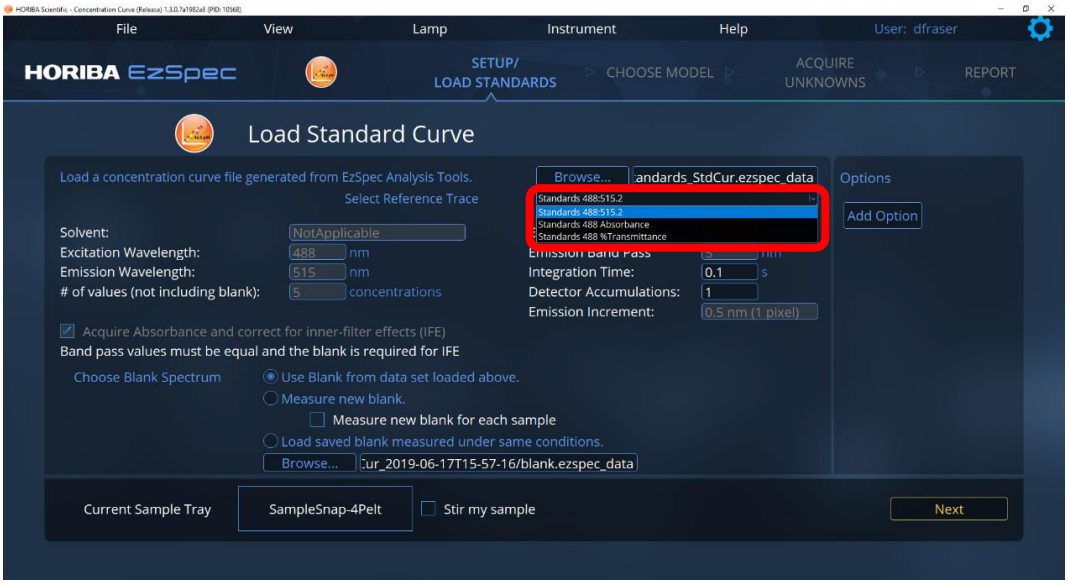


The **Load Standard Curve** window appears.



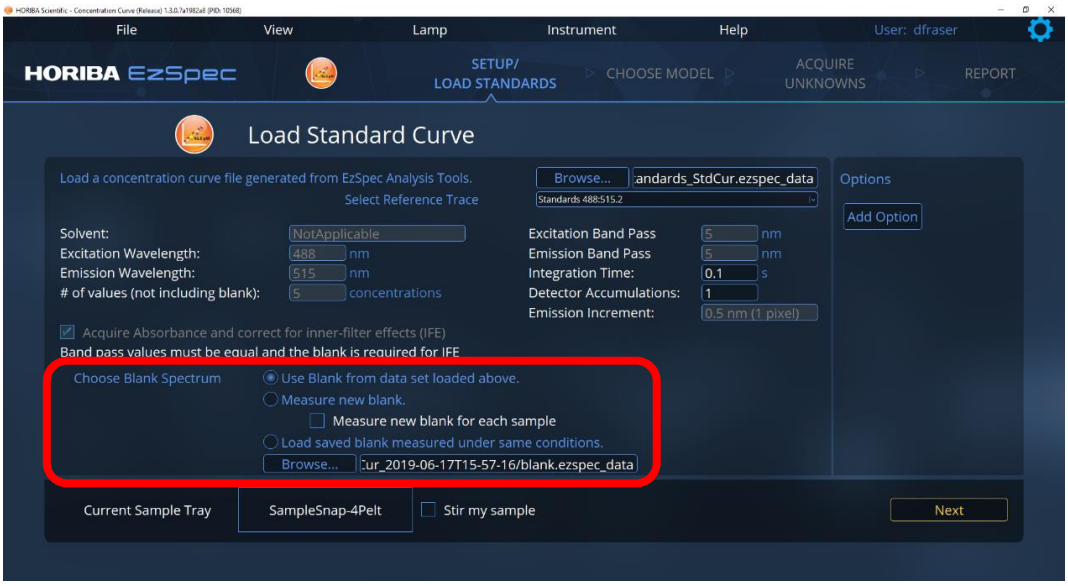
2. Click the **Browse** button to load a concentration curve data set containing measured concentration standards, collected from EzSpec using the Capture Value Standard Curve method. This file must be a .ezspec_data file. The method from that data set containing the concentration standards will be loaded into the application.

3. Click on the pull down menu to select the desired Reference Trace.



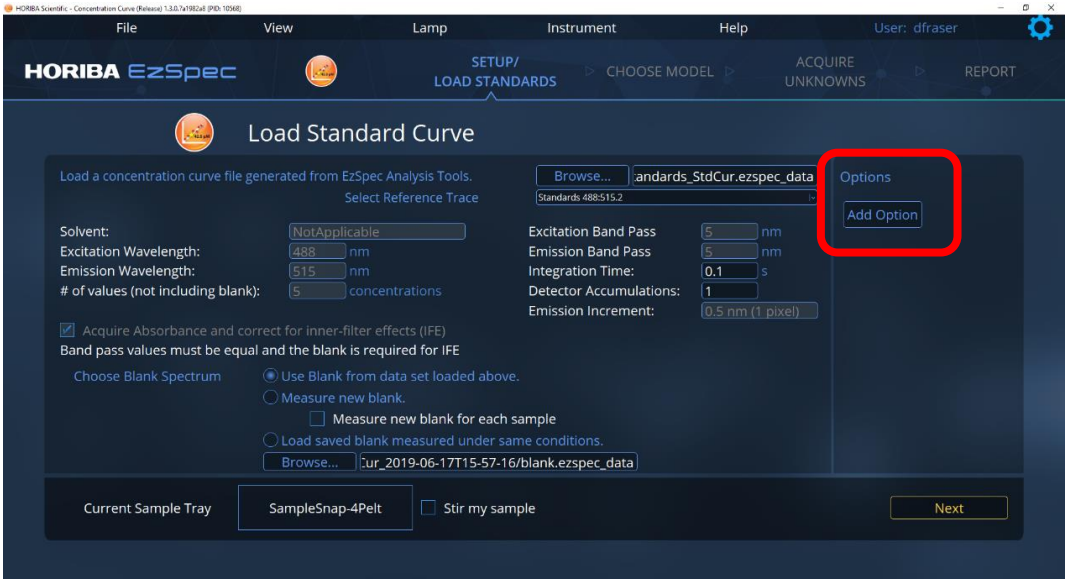
The selected Reference trace must be the same data type (Fluorescence, Absorbance, or %Transmittance) as the unknowns to be determined. In this example, the trace and corresponding method selected is Fluorescence.

4. Choose the **Blank Spectrum** source:

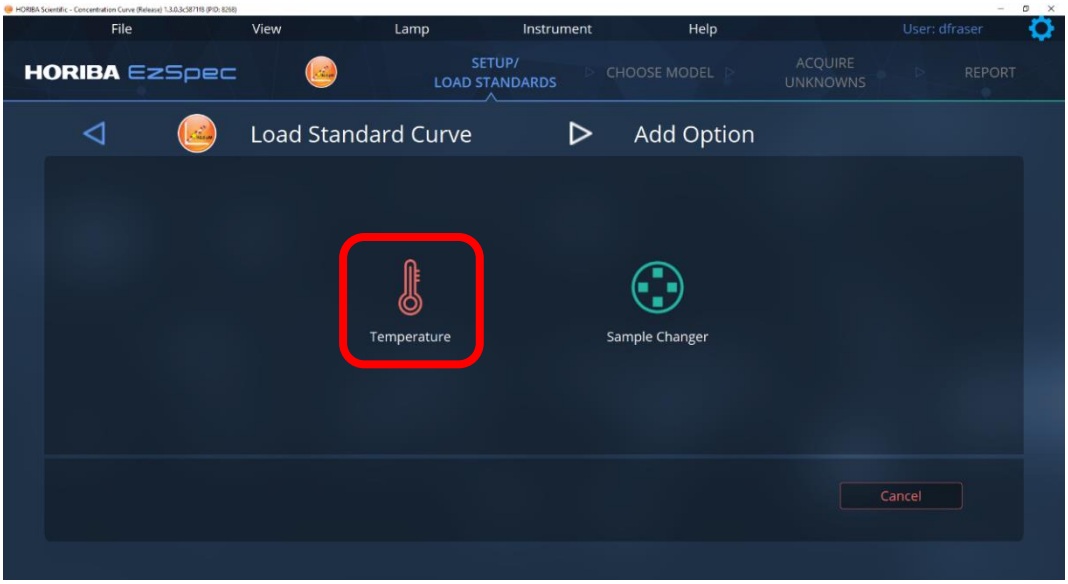


a. If using the blank acquired with the Standard Curve, click **Use Blank from data set loaded above**. This is the option chosen for this case.
b. If loading a different blank but measured under the same conditions as the Reference Trace, click **Load saved blank measured under same conditions**, click on the **Browse** button and select the blank file.
c. If the **Integration Time**, **Detector Accumulations**, or **Emission Increment** are different than the method used for the loaded standard curve, the blank cannot be loaded from the standard curve data set and must be remeasured (click **Measure new blank**).
d. The default is **Measure new blank**.
    i. If **the user wants a different matching blank measurement for each sample** , check the **Measure new blank for each sample** checkbox.

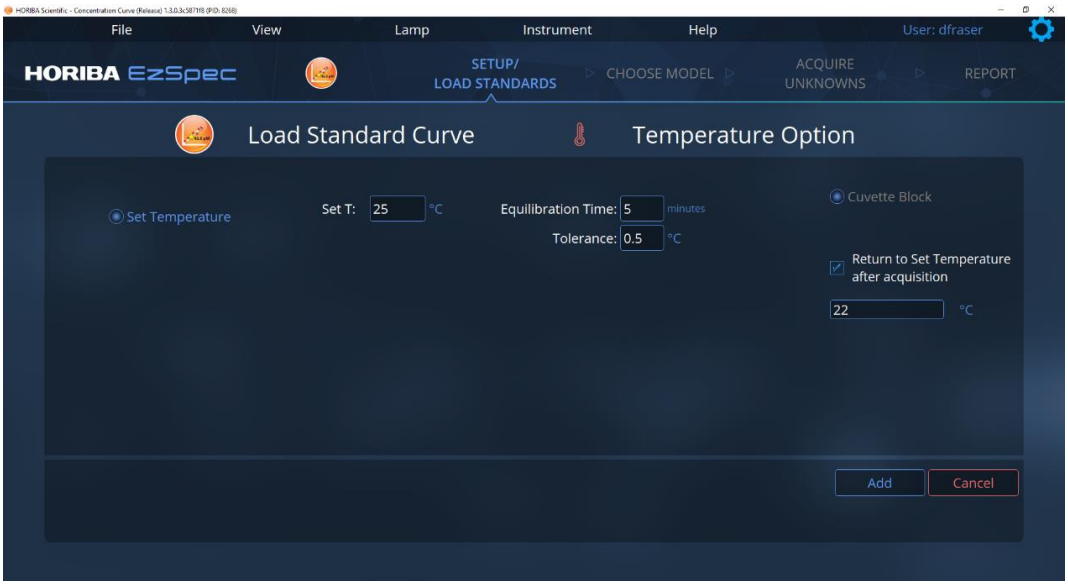
5. Click on the **Add Option** button to open a menu of available options.



a. The **Add Optio**n window appears. If an option is not available on the tray in place, the option will be grayed out.

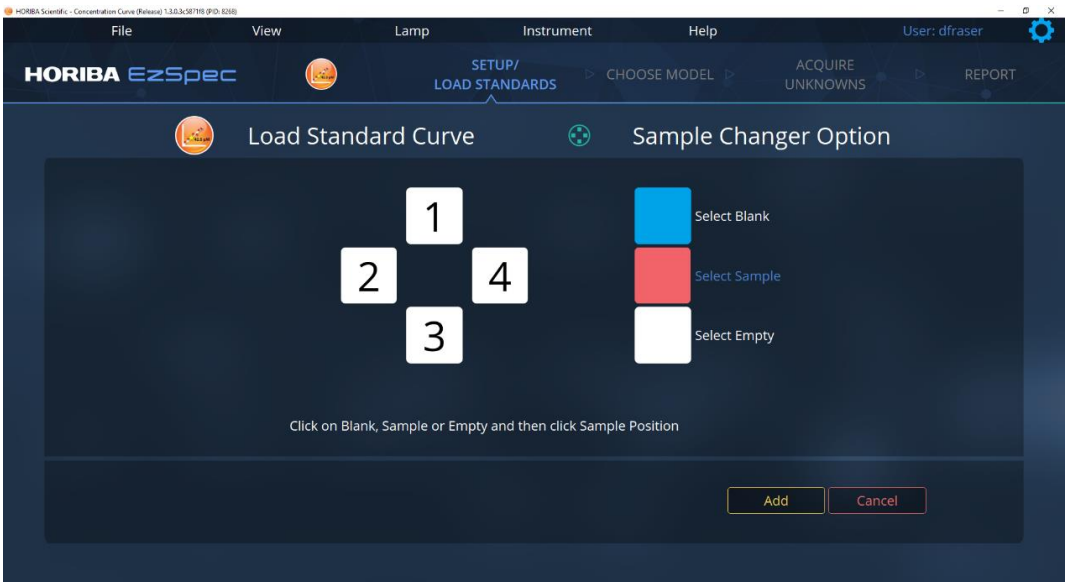


b. Click on the **Temperature** icon to open the **Temperature Option** window. If both Temperature and Sample Changer are added, Temperature will always be listed and executed before Sample Changer.



i. Enter a temperature in °C in the **Set T** box. The allowed temperature range is -15 °C to 105 °C.
ii. Enter the **Equilibration Time** in minutes.
iii. Enter the **Tolerance** in °C.
iv. The **Return to Set Temperature after acquisition** checkbox may be unchecked or checked. It is initially checked by default.
    1. If this checkbox is unchecked, the enterable box below it disappears, and the temperature will hold at the last temperature of the method after the acquisition is completed.
    2. If this box is checked, then an enterable box appears for the user to enter a temperature in °C that the temperature controller will go to after the acquisition is finished.
v. Click the **Add** button to add **Temperature** to the **Options** list and close the Temperature Option window.

c. Click on the **Sample Changer** icon to open the **Sample Changer** window.



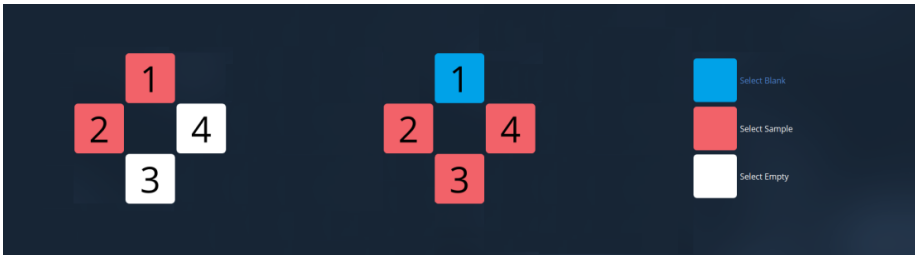
i. Click on the **Select Blank** button and then click on position **1** if you want to acquire blank data. Only position 1 can contain a blank.

ii. Click on the **Select Sample** button and then click on any of positions 1 to 4 to select those positions for a sample.

iii. Click on the **Select Empty** button and then click on any of positions 1 to 4 to remove a blank or sample and leave that position unoccupied.

iv. The turret runs in order of selected positions 1, 2, 3, 4 and then reverses direction to go back to 1. It will not rotate completely around to go from position 4 to 1. Because the turret always goes back to position 1, it saves time if the selected positions start at 1 and are consecutive.

v. Examples of selected turret positions:

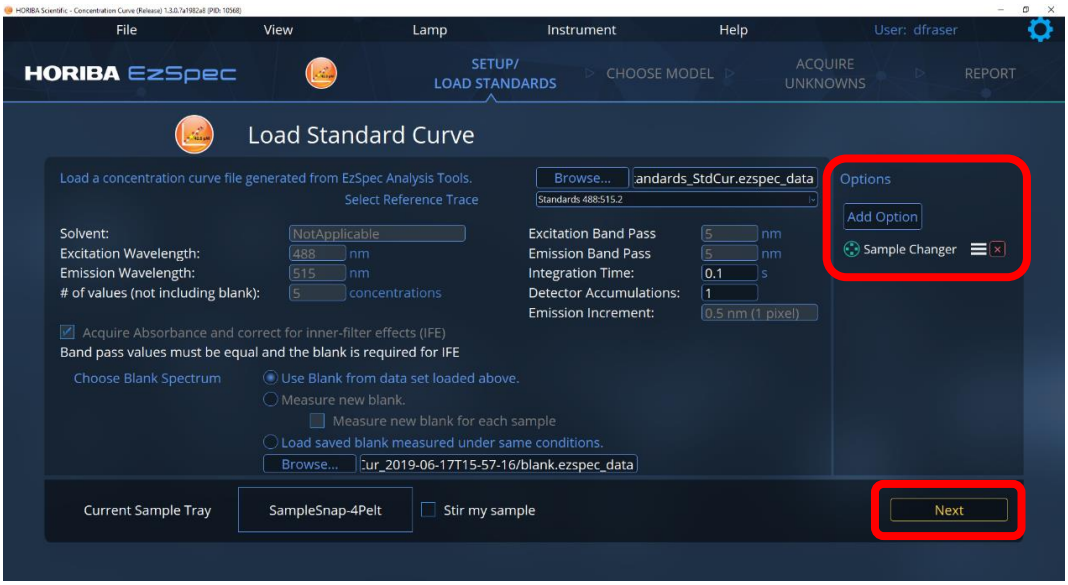


vi. Click the **Add** button on the Sample Changer Option window to add the Sample Changer with selected positions to the Options Bar and close the Sample Changer Option window.

When options have been added to the Options Bar, each option has **Edit** and **Delete** buttons.

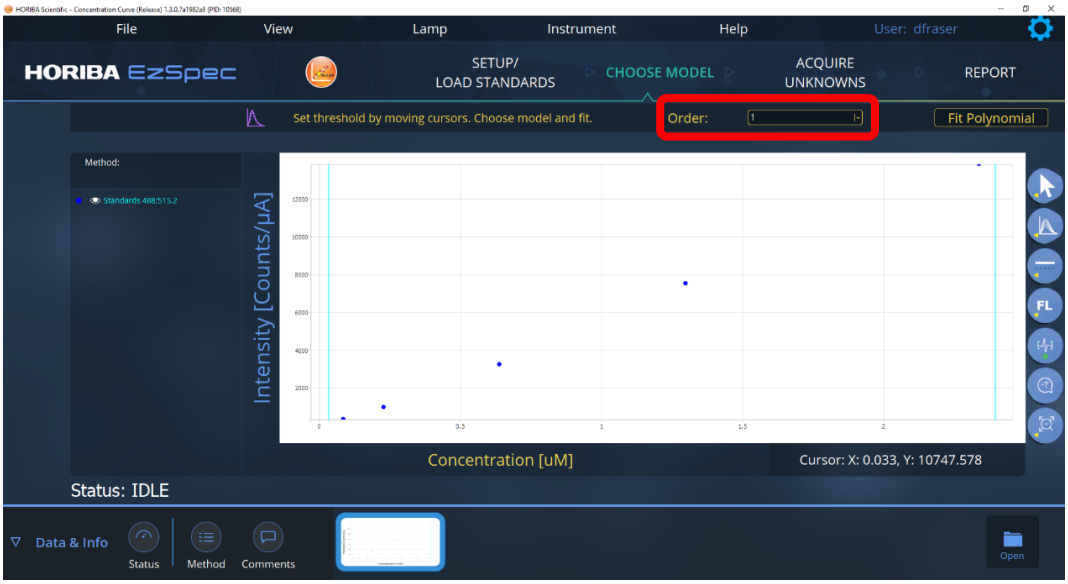
Click the **Delete** button to delete the option from the Options Bar.

Click the **Edit** button to open the option menu for that option.



In this example, the temperature option was not used as the blank file did not have any temperature information.

6. Click the **Next** button to show the **Choose Model** window.
7. The Reference trace appears in the graph.
8. Choose the polynomial order to fit the standard curve data. Set the range of data to be used in the fit by clicking and dragging the cursors in light blue. Here, the data is fitted to a linear (order 1 polynomial) equation using all of the standard curve (reference trace) data points.

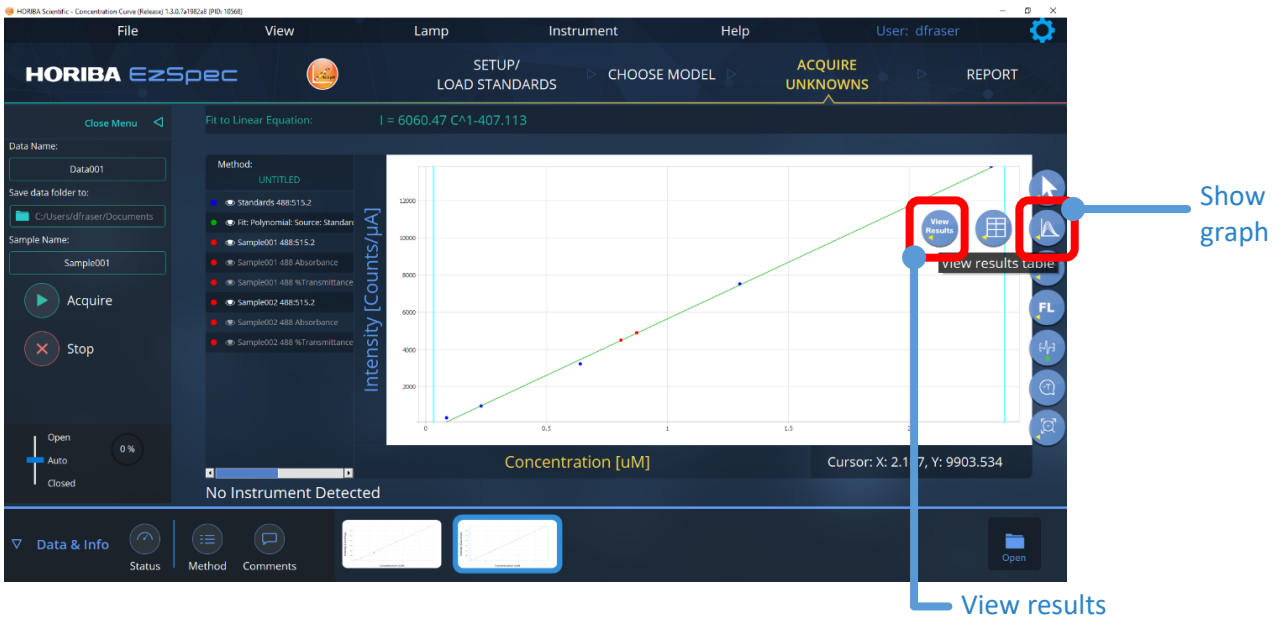


9. Click **Accept** to use the resulting fit for the calculation of unknown concentrations going forward. The **Refit** button allows the user to change the fit parameters and/or model used.

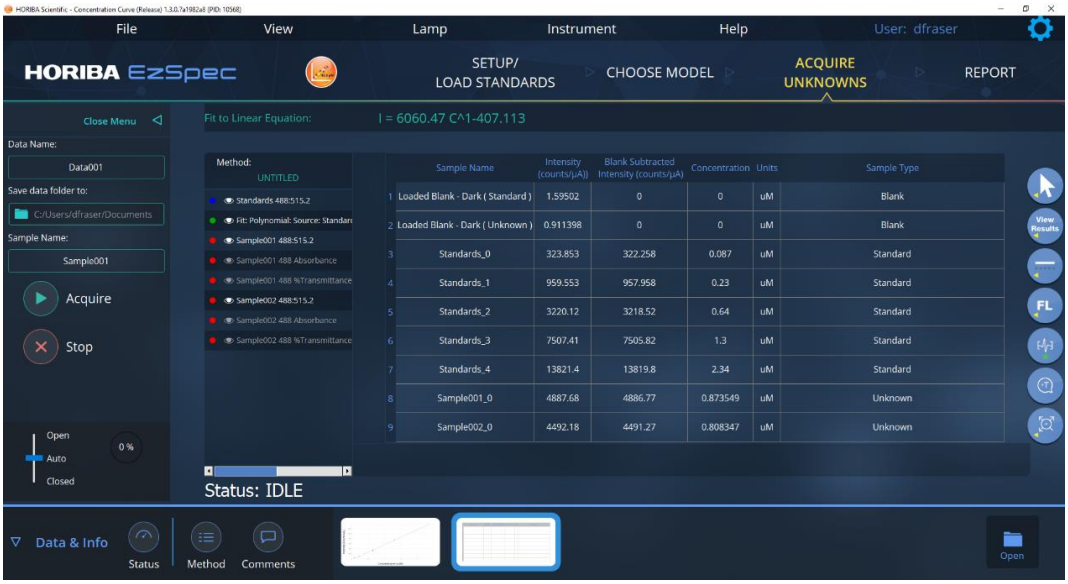
After accepting the fit, the window changes to the **Acquire Unknowns** menu.

10. Give the data a name under “Data Name”.
11. Click on the file folder icon  under “Save data folder to” and browse to choose a folder location for the resulting data folder to be created and to contain all of the resulting processed and raw data files.
12. Give the first unknown sample a name under “Sample Name”.
13. Click **Acquire** to measure an unknown sample.

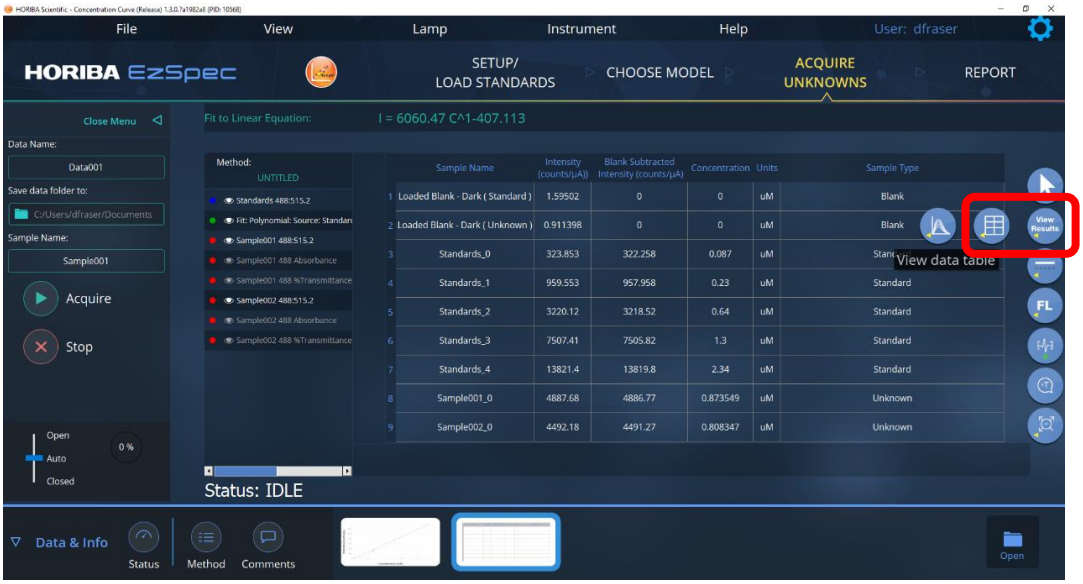
14. The unknowns data appear in the graph along the fitted curve. Click on the **Show Graph** button to expand the Plot Mode group.



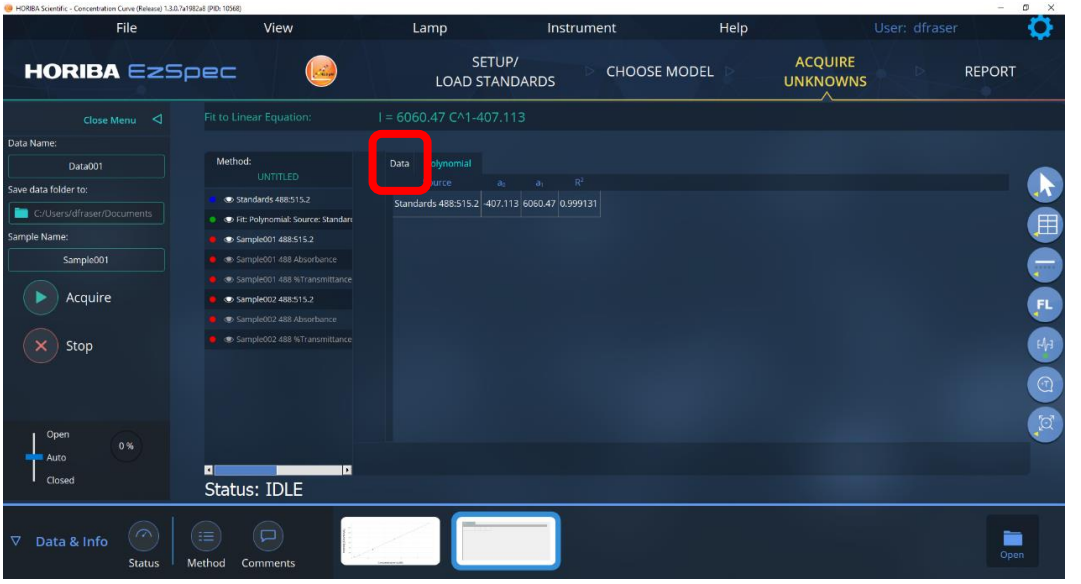
15. Click on the **View results table** button.
16. The Standard Calibration Curve and the Unknown data appears in the table.



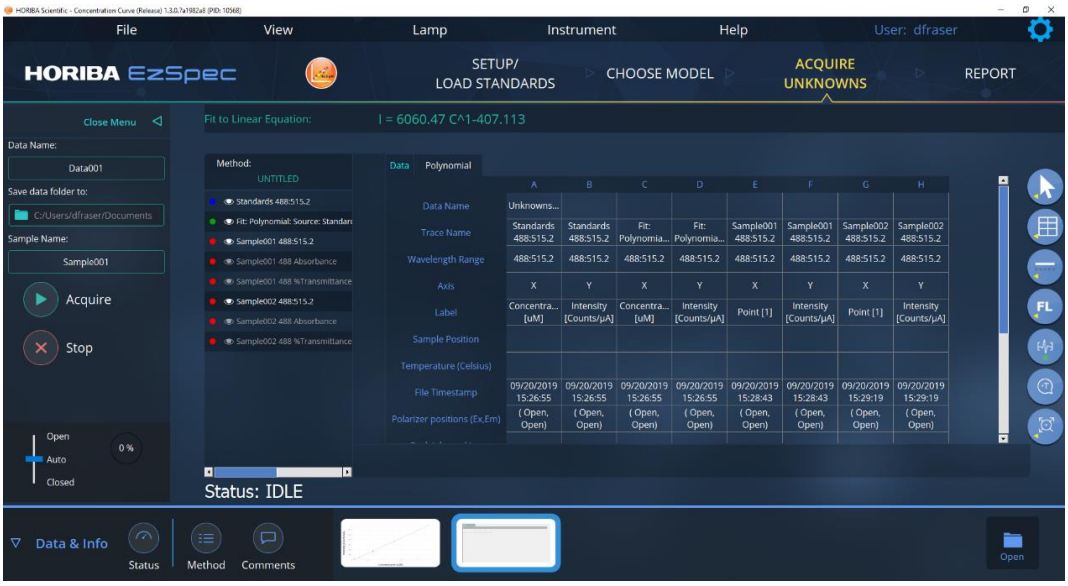
17. Click on the **View Results** button to expand the Plot Mode group. Select the **View data table** button.



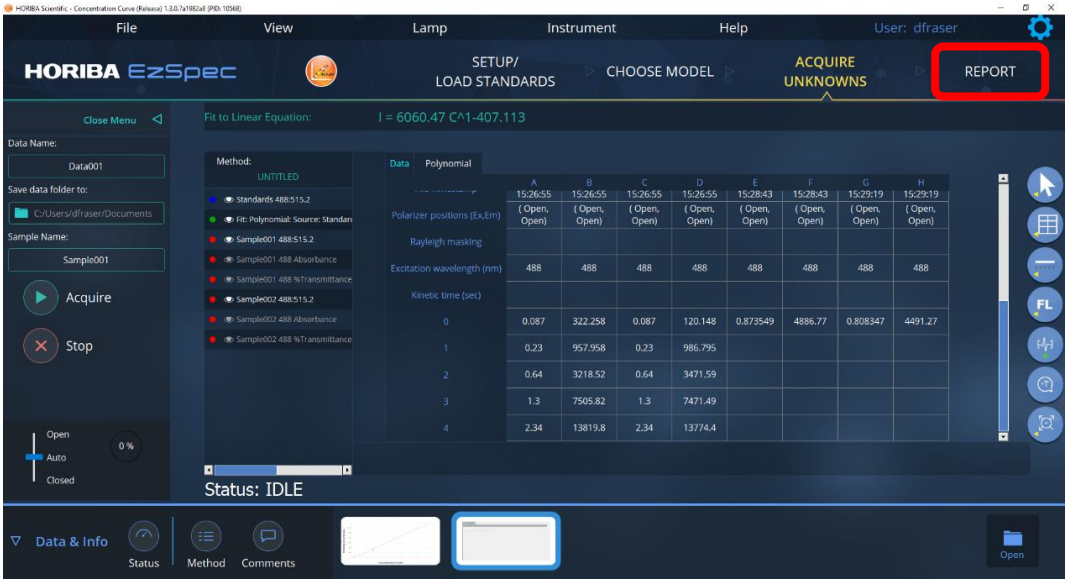
18. The View data table appears showing the **Polynomial** tab.
19. Click the **Data** tab.



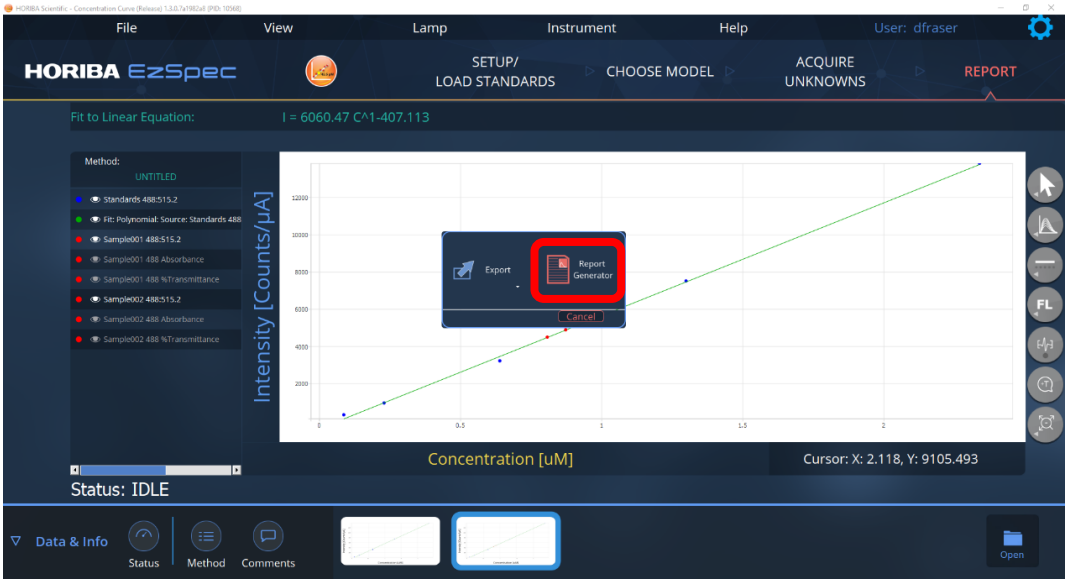
20. The **Data** tab appears showing the measured fluorescence intensity and/or absorbance and/or %transmittance values for each sample along with header labels for each column. Scroll down to see the measured and concentration values.



21. Click on **Report** to see the Report options.

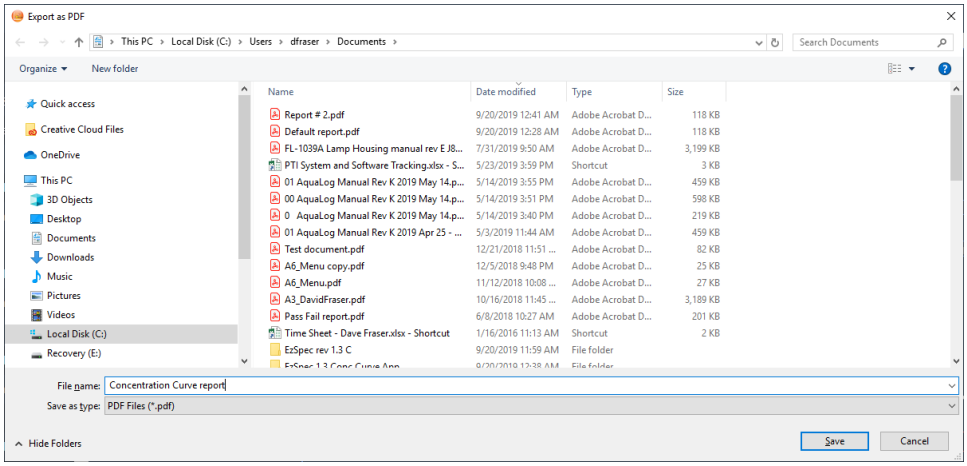


22. Click on **Report Generator**.



23. In the Report Generator choose the options required in the report and Click **Generate**.

24. An Export to PDF window appears. Enter a file name and click **Save**.

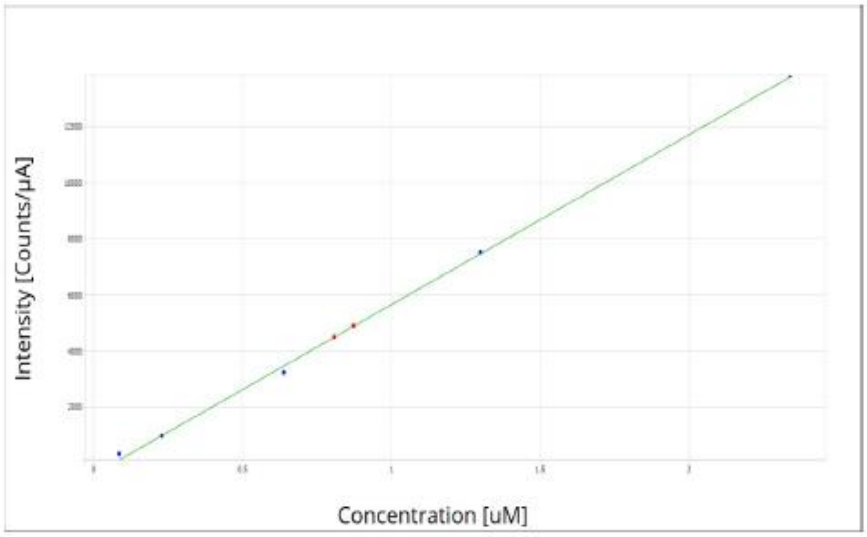


The report appears.

HORIBA Scientific

Instrument Name:
Serial Number: Emulating
Date of Report: Sep 23, 2019 15:15:15

User Name: dfraser



Legend:

1. Standards 488:515.2
2. Fit: Polynomial: Source: Standards 488:515.2 (-407.113, 6060.47, 0.999131)
3. Sample001 488:515.2
4. Sample001 488 Absorbance
5. Sample001 488 %Transmittance
6. Sample002 488:515.2
7. Sample002 488 Absorbance
8. Sample002 488 %Transmittance

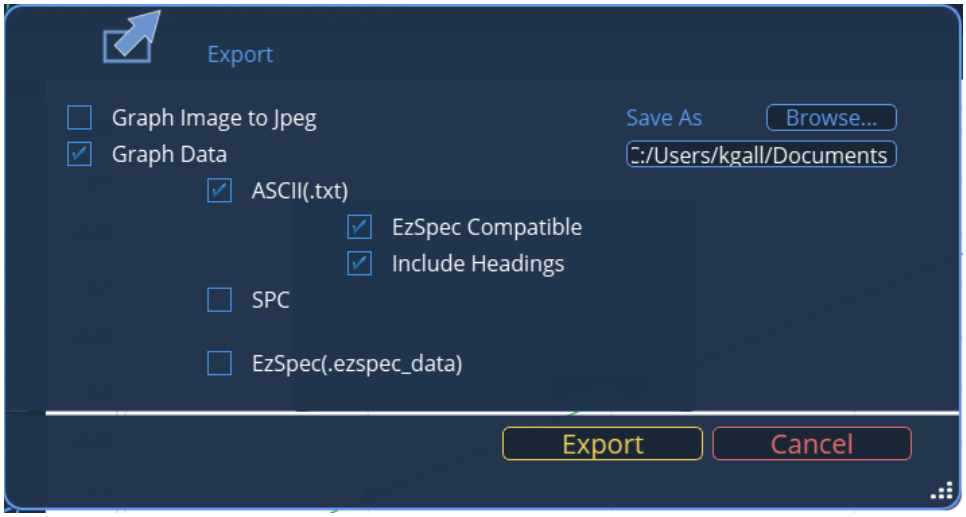
Method Name:

Comment:

25. **Report**, **Export** menu:

The data created in the Concentration Curve App is saved as a file with extension .ezspec_CC. This file can only be opened within the Concentration Curve application unless exported as an .ezspec_data file. In that case, it may be opened in the EzSpec main application for further advanced analysis. The data can also be exported to image file (graph) or ASCII or SPC files. To access the export menu, go to the **Report** menu and choose **Export**. Select the formats for which export is required and choose the filepath to which the files will be saved. Then click **Export** and the files will be exported as specified.

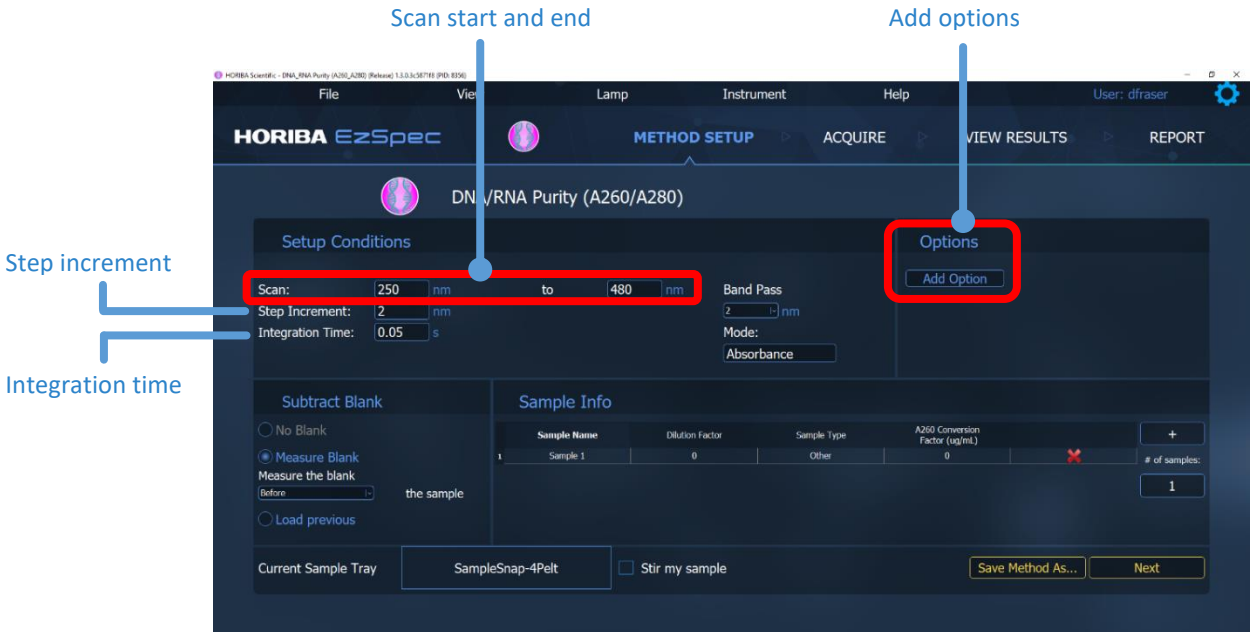
See also the procedure for the DNA RNA and Pass Fail apps.



8.2 DNA/RNA Purity (A260/A280) App

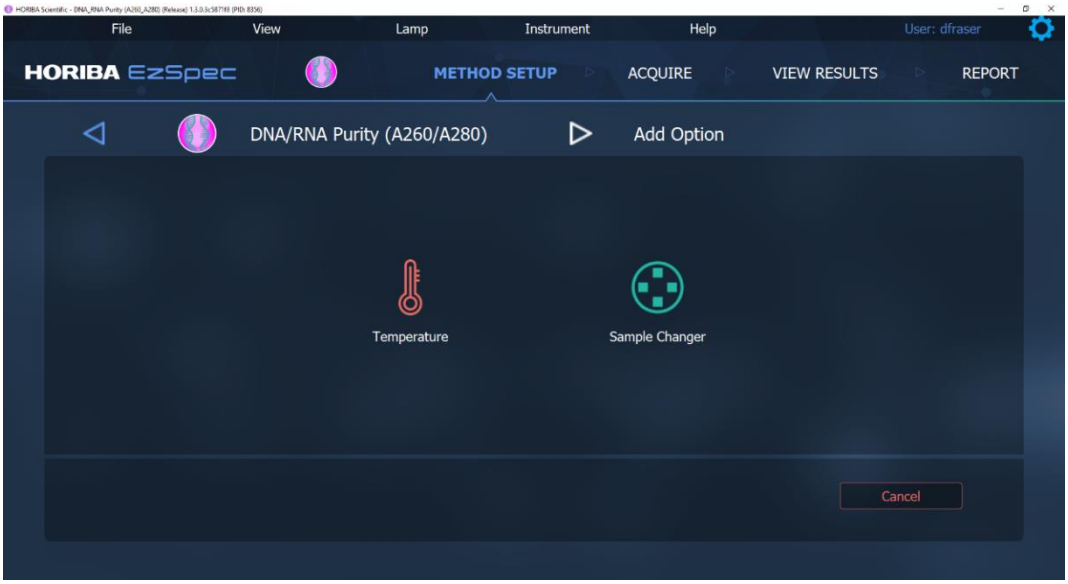
The DNA/RNA Purity (A260/A280) App is an application for measuring the absorbance ratio at 260 nm and 280 nm of a DNA or RNA sample and calculating the RNA or DNA purity of that sample. The ratio of the Absorbance at 260 nm to the Absorbance at 280 nm, using the absorbance at 330 nm as a background subtraction for turbidity, gives a value. This value, if between 1.7 and 2.0, shows good purity for DNA or RNA. If the value is lower than 1.7, the sample is likely contaminated. The concentration of the DNA or RNA can also be found from the absorbance at 260 nm, using a given conversion factor and a dilution factor. The app automatically measures the absorbance spectrum and gives the ratio for purity (A260/A280) and the concentration of DNA or RNA in a table.

1. On the Launcher Window click on the **DNA/RNA Purity** button to open the App.
2. METHOD SETUP window
    a. **Absorbance** is the only option in the Mode list.
    b. Enter a value for the **Integration Time** in seconds. Longer integration time will result in lower noise in the final absorbance spectra.
    c. Enter a value for the **Step Increment** in nm.
    d. Select a **Band Pass** value (2, 3, or 5 nm) from the pull down menu.
    e. Enter the **Scan** Start and End wavelengths (nm). The scan must start at or below 260 nm and end at or after 330 nm.

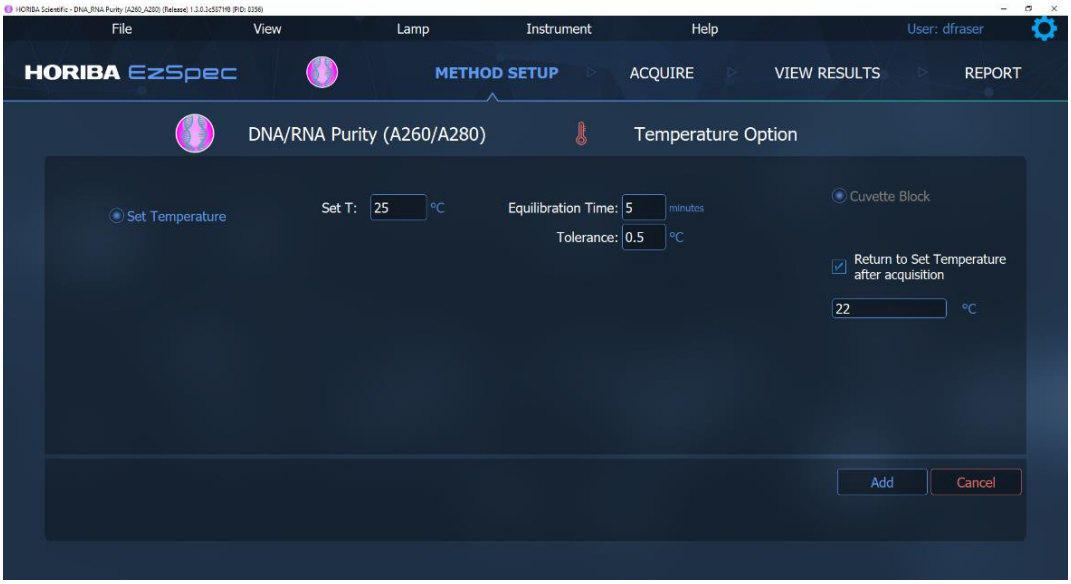


3. Click the **Add Option** button to open a menu of available options.

a. The **Add Option** window appears. If an option is not available on the tray in place, the option will be grayed out.



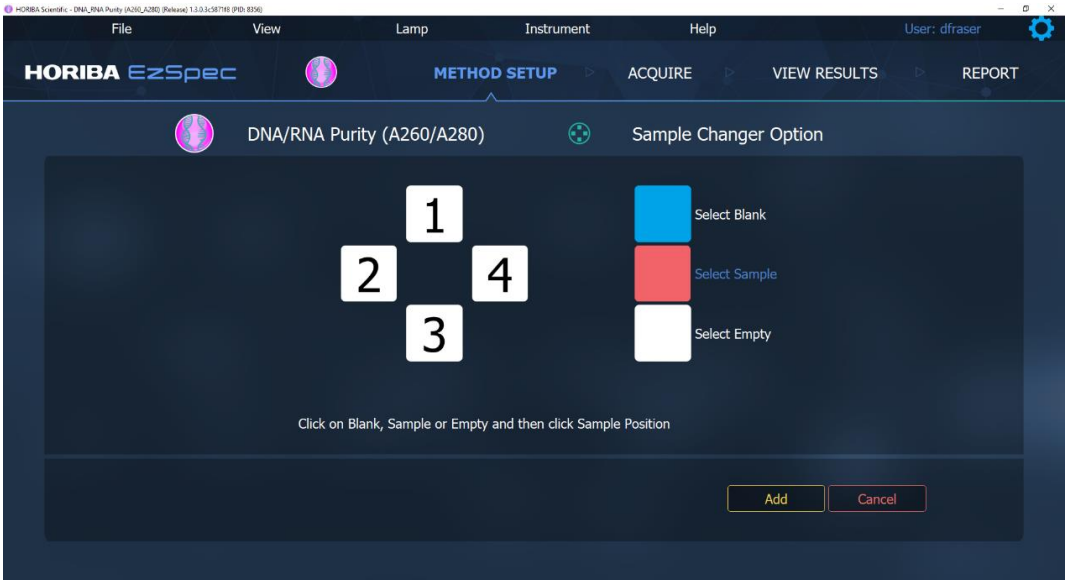
b. Click on the **Temperature** icon to open the **Temperature Option** window. If both Temperature and Sample Changer are added, Temperature will always be listed and executed before Sample Changer.
    i. Enter a temperature in °C in the **Set T** box. The allowed temperature range is -15 °C to 105 °C.
    ii. Enter the **Equilibration Time** in minutes. 5 minutes is a normal time.
    iii. Enter the **Tolerance** in °C.
    iv. The **Return to Set Temperature after acquisition** checkbox may be unchecked or checked. It is initially checked by default.



1. If this checkbox is unchecked, the enterable box below it disappears, and the temperature will hold at the last temperature of the method after the acquisition is completed.
2. If this check box is checked, then an enterable box appears for the user to enter a temperature in °C that the temperature controller will go to after the acquisition is finished.

v. Click the **Add** button to add **Temperature** to the Options Bar and close the **Temperature Option** window.

c. On the **Add Option** window click on the Sample Changer icon to open the **Sample Changer Option** window.



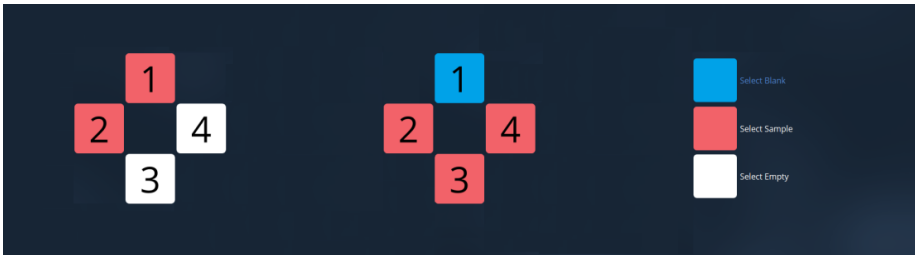
i. Click on the **Select Blank** button and then click on position **1** if you want to acquire blank data. Only position 1 can contain a blank.

ii. Click on the **Select Sample** button and then click on any unoccupied positions to select those positions for samples.

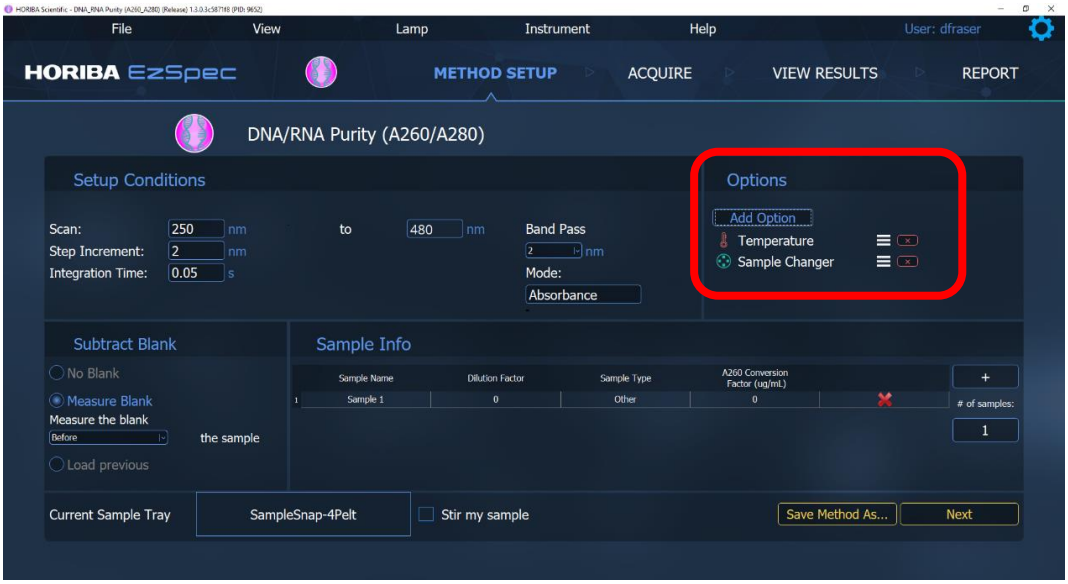
iii. Click on the **Select Empty** button and then click on any of positions 1 to 4 to remove a blank or sample and leave that position unoccupied.

iv. The turret runs in order of selected positions 1, 2, 3, 4 and then reverses direction to go back to 1. It will not rotate completely around to go from position 4 to 1. Because the turret always goes back to position 1, it saves time if the selected positions start at 1 and are consecutive.

v. Examples of selected turret positions:

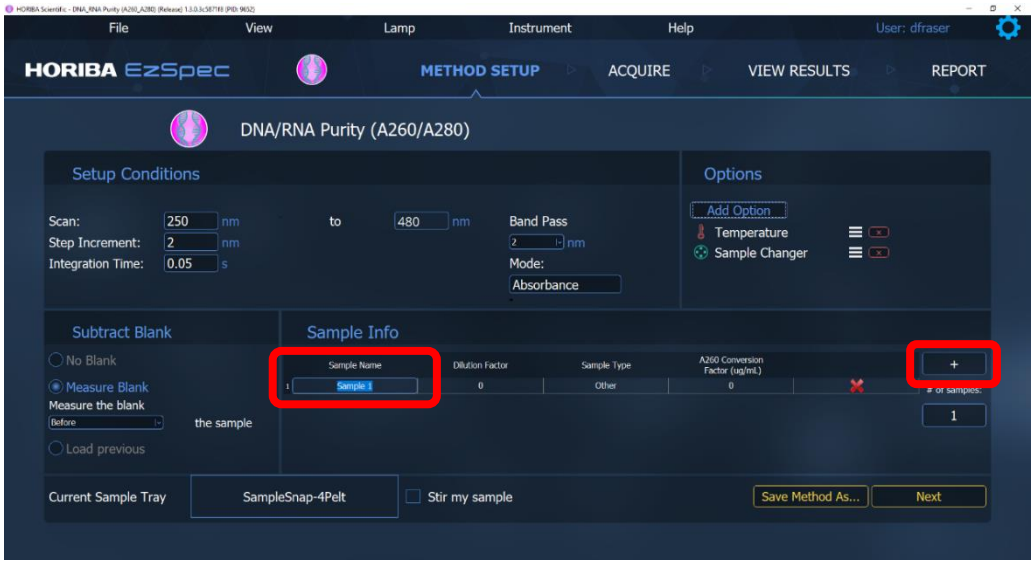


vi. Click the **Add** button to add the **Sample Changer** with selected positions to the Options Bar and close the Sample Changer Option window. When options have been added to the Options Bar, each option has Edit and Delete buttons. Click the **Delete** button to delete that option from the Options Bar. Click an **Edit** button to open the option menu for that option.

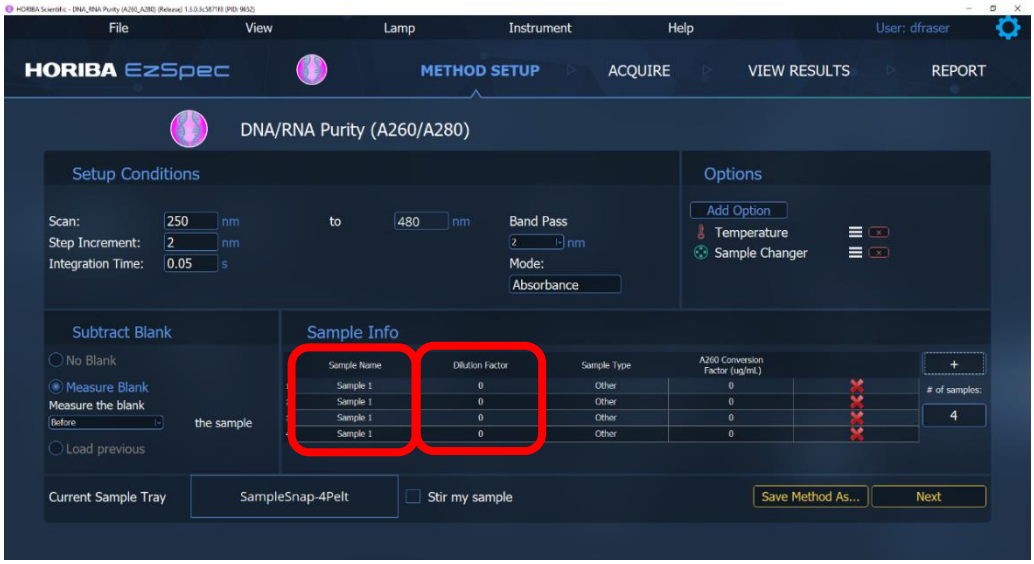


4. A Blank measurement is required. Select either Measure Blank, or Load previous.
   a. For the **Measure Blank** option, select whether to measure the blank **Before** or **After** the sample(s). If a sample changer is used, the blank will always be measured first. If the blank is loaded, the blank.ezspec_file should be used from a previously measured Absorbance Spectrum scan using the same input parameters as the current method.

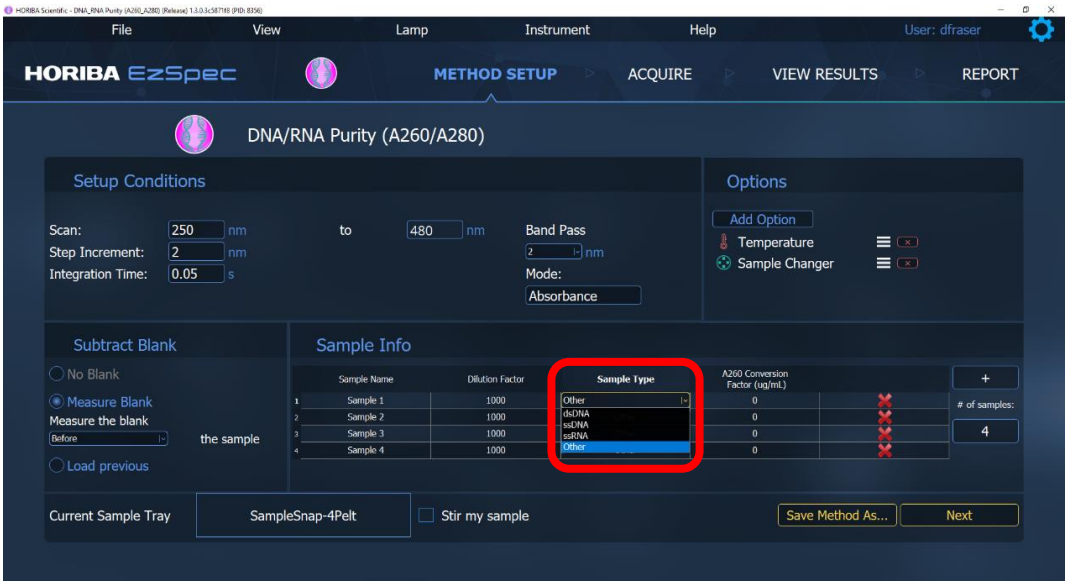
5. Enter the Sample Information
    a. If you want to change the Sample name, double-click on **Sample** in the first row to enter a new sample name. In this case the name was not changed. The sample name is user defined.
    b. Click the **+** button the number of times as you want additional samples in the table.



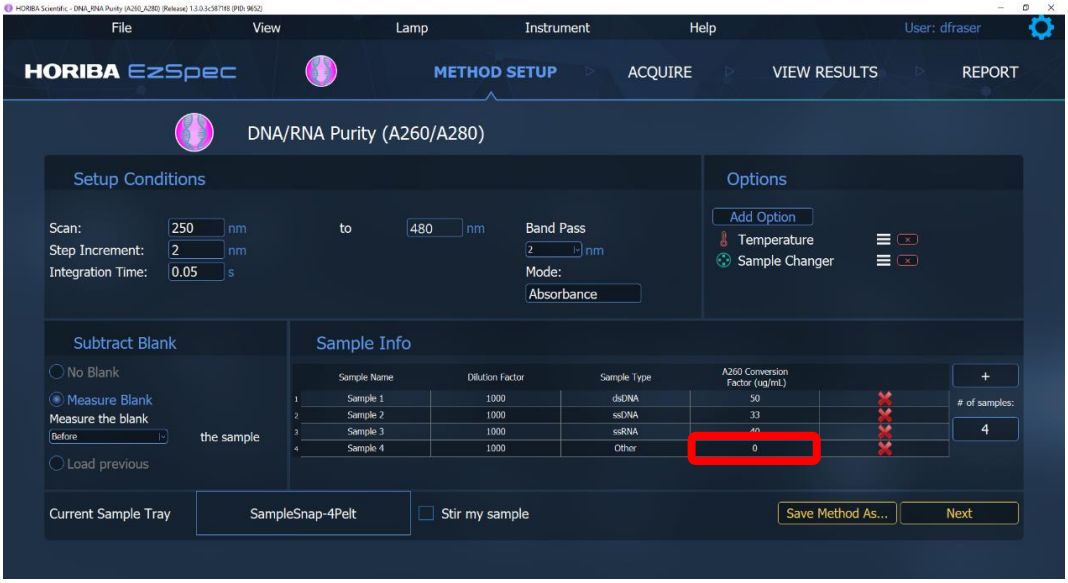
    c. Click on the first **Sample Name** cell, press Ctrl-C to copy the name and then paste the name in each Sample Name cell below, also incrementing the numbers as you go or changing the name to the desired sample description.
    d. Click on each of the **Dilution Factor** values to enter new values. The dilution factor will account for dilution of the measured sample from the real DNA or RNA sample of interest. If the measured solution is a solution 100th the concentration of the solution of interest, enter 100 for the dilution factor.



    e. Click in each **Sample Type** cell and select the type from the drop down list.

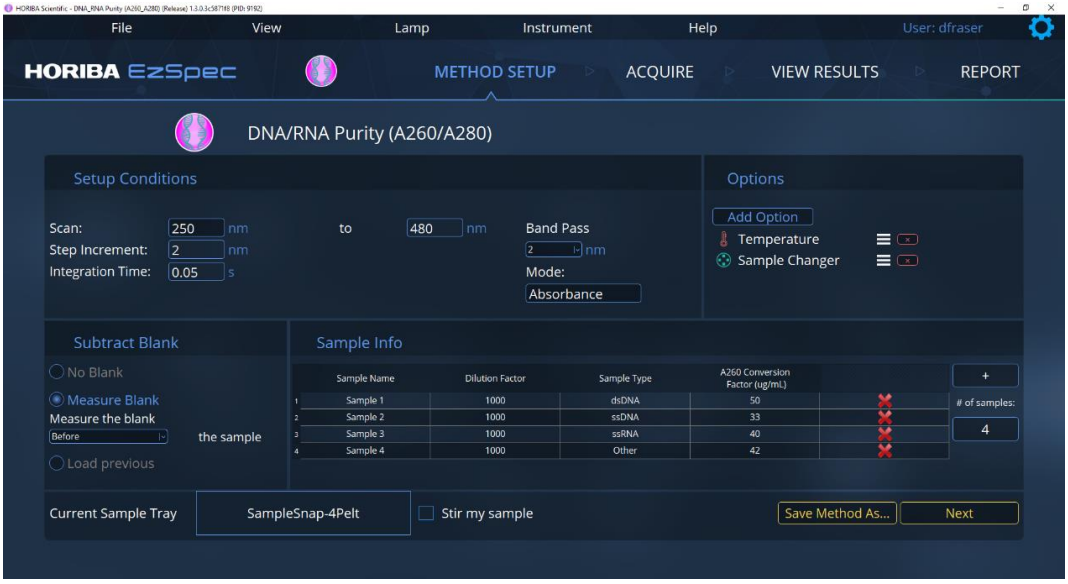


Selecting **dsDNA** (double stranded DNA), **ssDNA** (single stranded DNA), or **ssRNA** (single stranded RNA) will automatically fill in the Conversion Factor (50, 33, and 40 µg/mL, respectively), but these values can be edited. If **Other** is selected, the **Conversion Factor** is left at 0 and should be edited to a user-specified value.

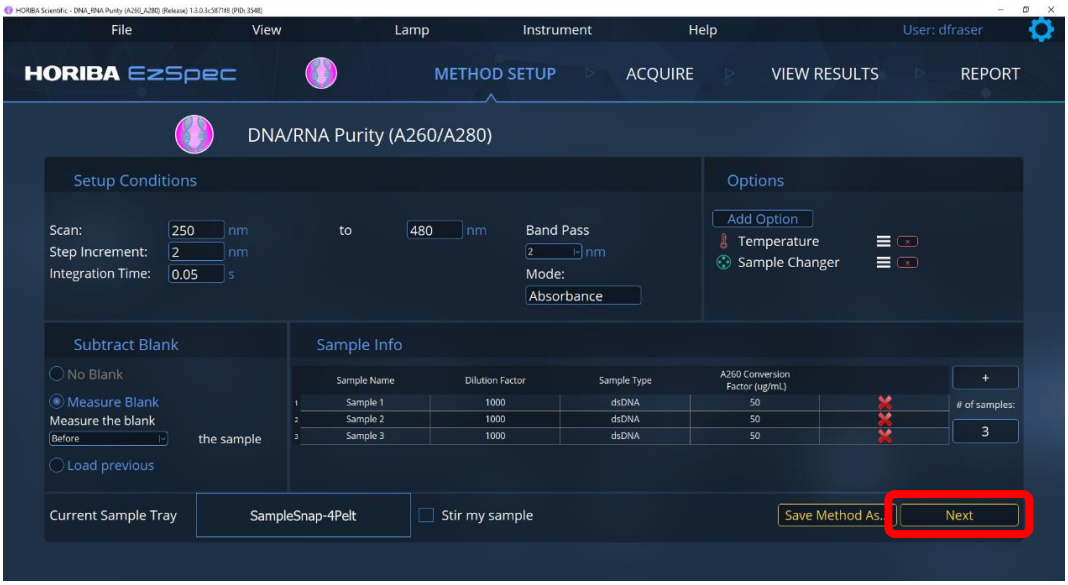


f. A positive non-zero value for the **Conversion Factor** must be entered.

For example:

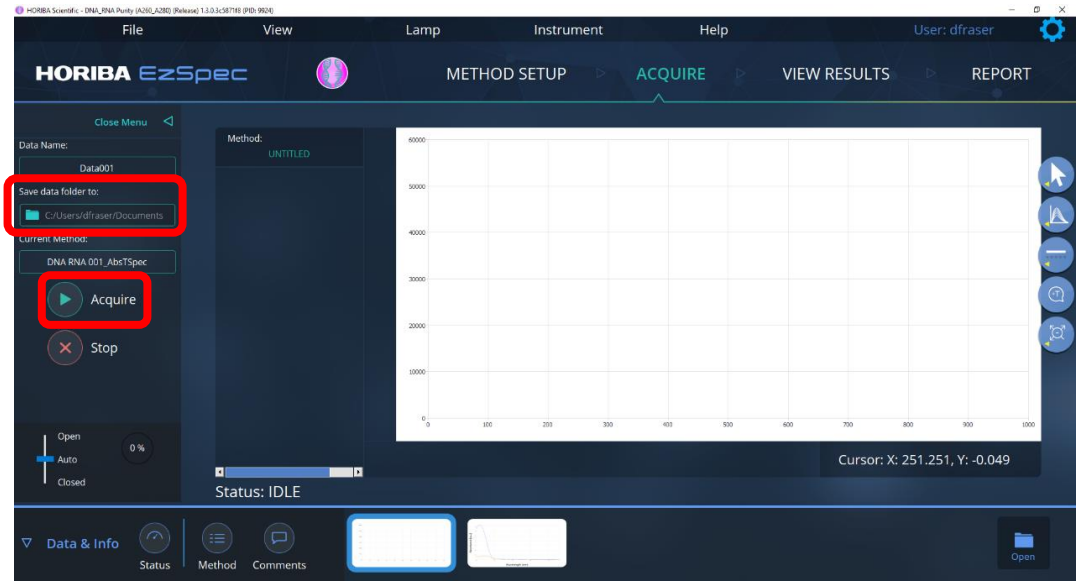


In the following method, only 3 samples are to be acquired.



6. Click **Next** to show the Acquire menu.

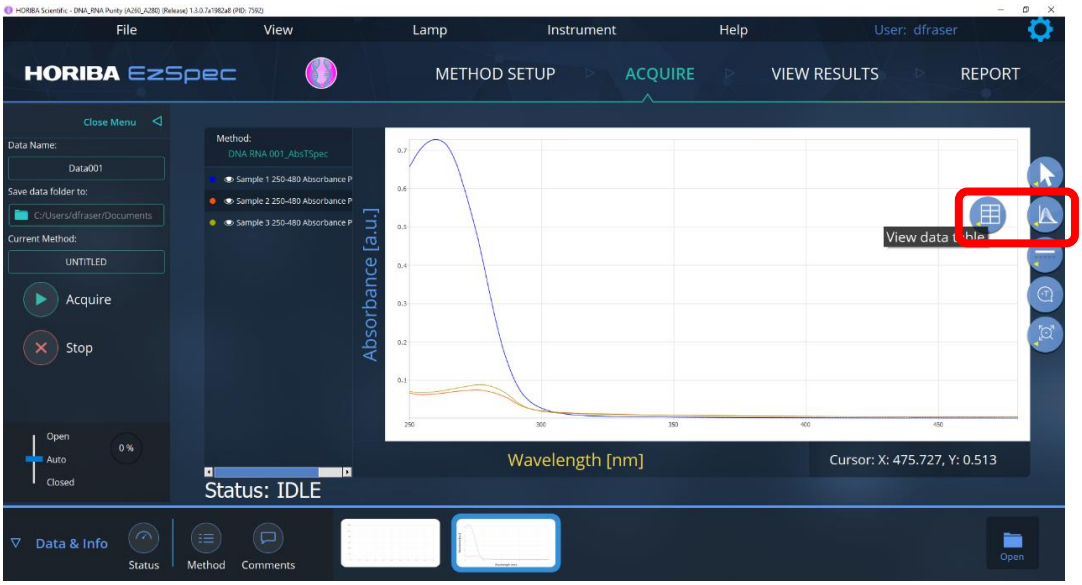
7. Enter a data folder pathname so that all of the acquired data will be saved into the created folder with that given name and a timestamp.
8. Click **Acquire** to acquire each sample trace.



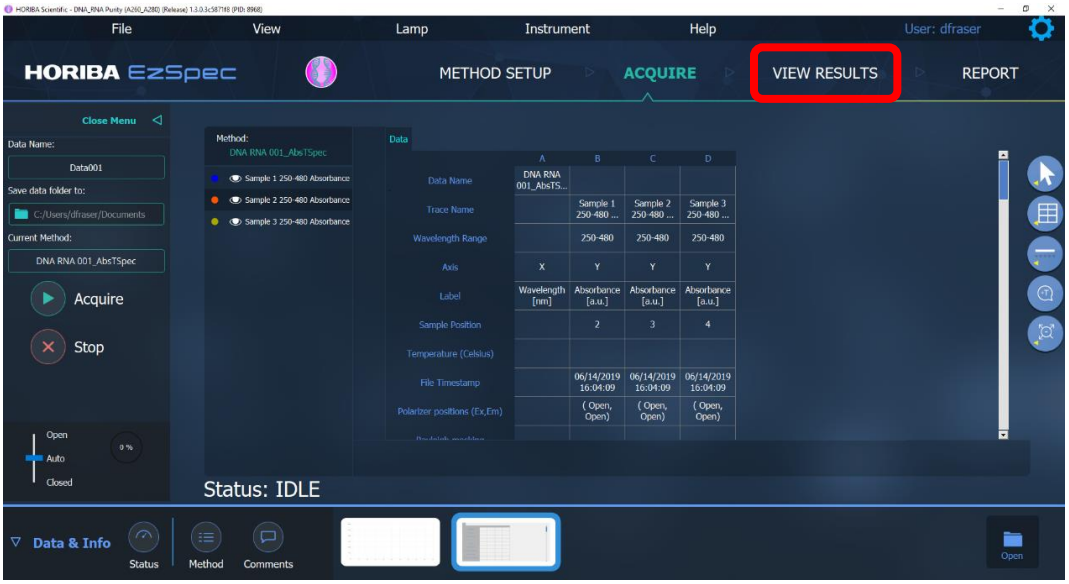
If there is only a single cuvette holder, then you will be prompted to change the sample, and the Duetta will reequilibrate if **Temperature** is enabled.

If there are more than 4 samples (including a blank) to be acquired when using a Sample Changer, then you will be prompted to change the samples, and the Duetta will reequilibrate if **Temperature** is enabled.

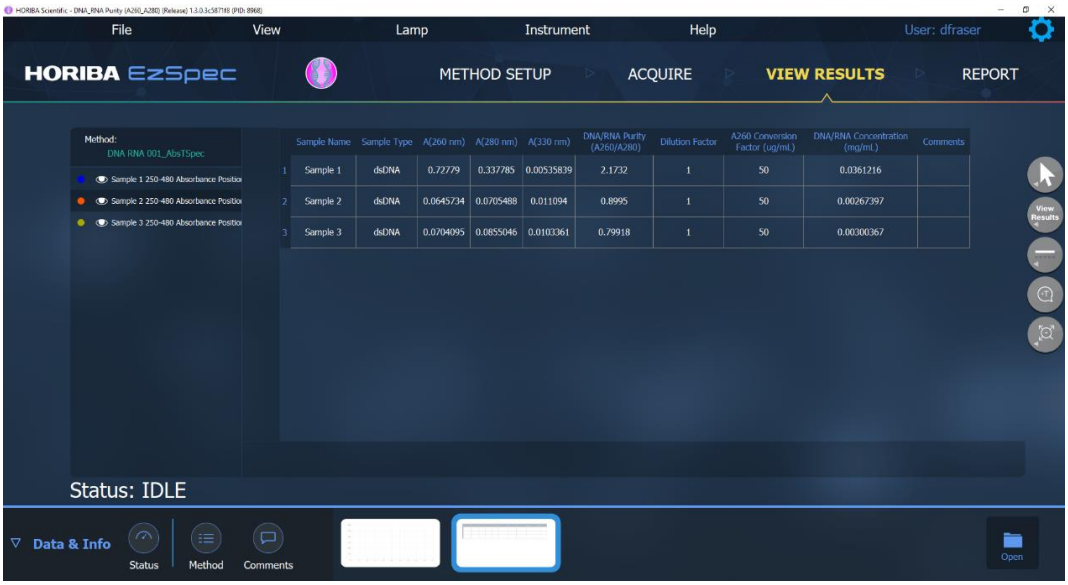
9. When the acquisition is finished, click on the **2D Graph** button  to expand the Plot mode group.



10. Then click on the **View data table** button  to show the data table.
11. Click on **VIEW RESULTS** to see the View Results window.



The **VIEW RESULTS** window will give the absorbance values at 260, 280, and 330 nm, the A260/A280 ratio, as well as the concentration of DNA or RNA as applicable, based on the absorbance at 260 nm, the given dilution factor, and the A260 conversion factor.



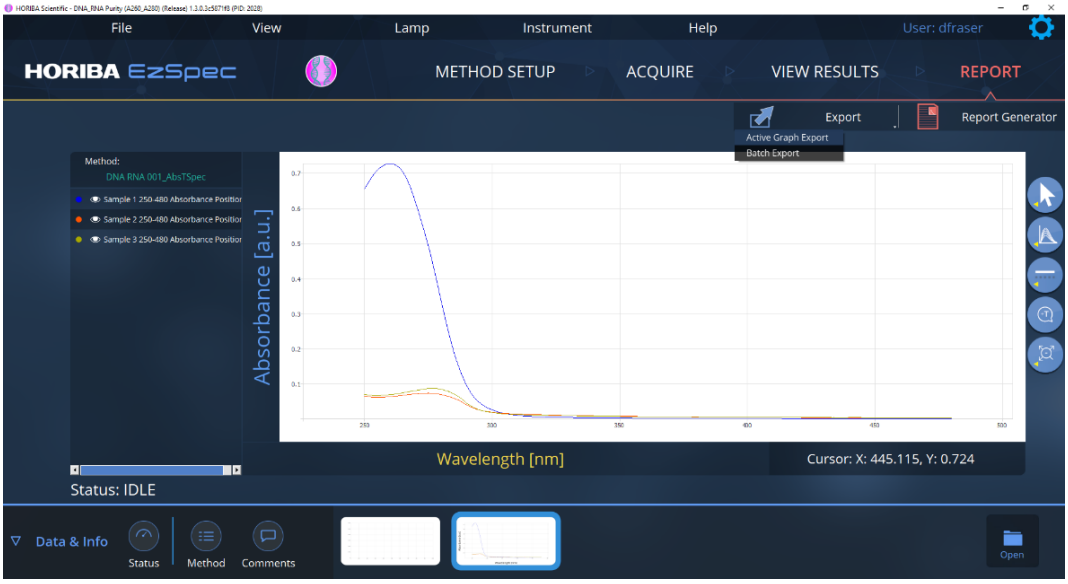
$$DNA\ or\ RNA\ purity\ = \frac{A260 - A330}{A280 - A330}$$

$DNA\ or\ RNA\ concentration$ (in mg/mL)

$$= \left(\left((A260 - A330) * A260\ conversion\ factor\ \left(\frac{\mu g}{mL}\right)\right) * dilution\ factor\right) * \left(\frac{1\ mg}{1000\ \mu g}\right)$$

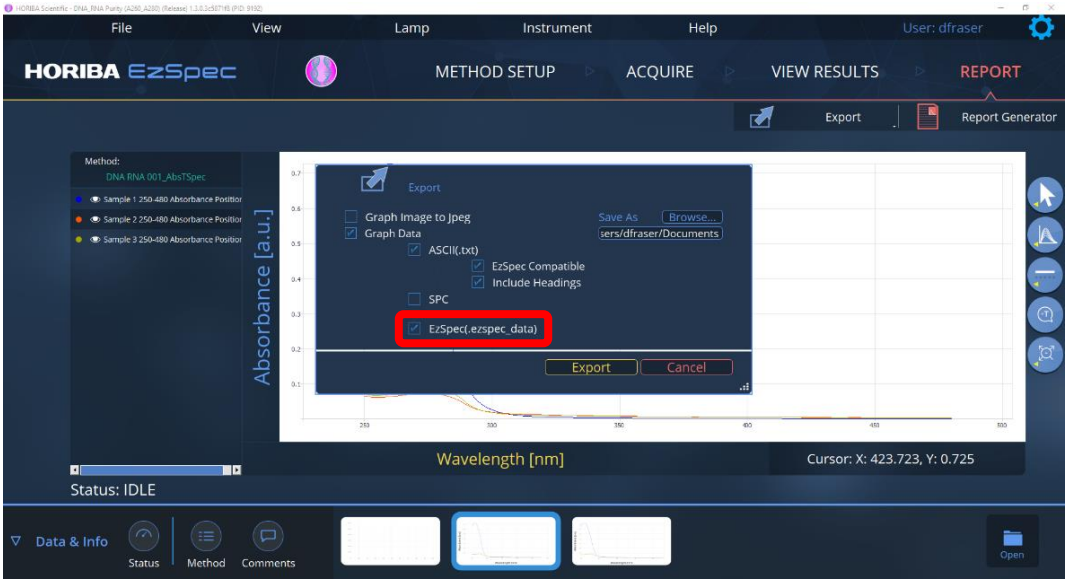
The dilution factor (no units), and the A260 Conversion factor (in µg/mL) are editable in the results table and the concentration calculation will update when these values are updated.

12. Click on **Report**, **Export**, **Active Graph Export** to see the Active Graph Export window.



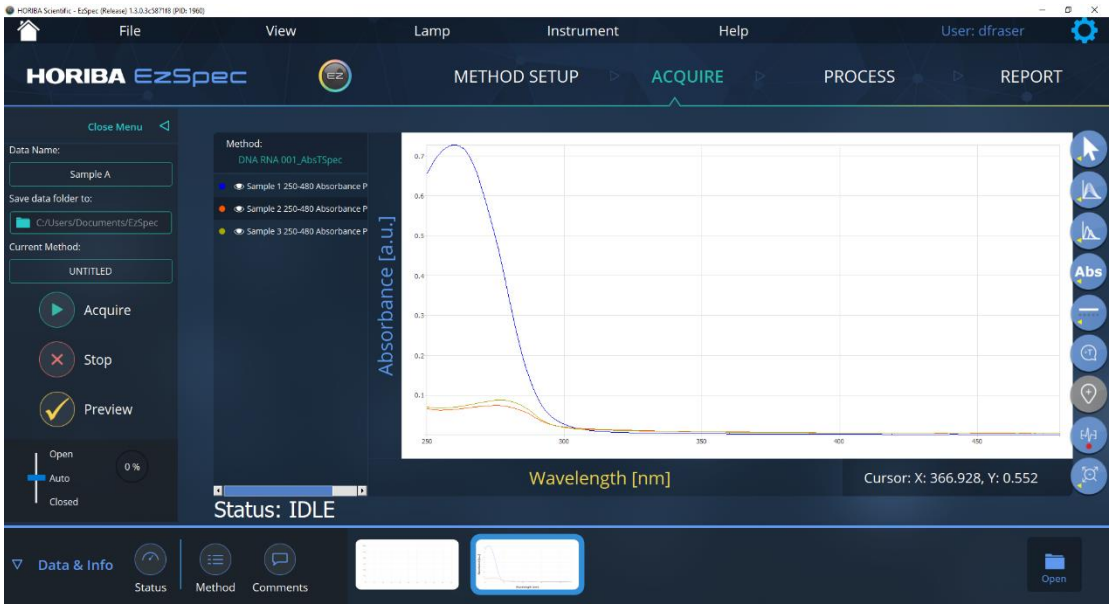
This opens the Active Graph Export window with the default settings.
Exporting the results includes enabling options to save the **View Results** window as a jpeg image, and exporting the data as an EzSpec data file and as SPC data. The default setup is with Export(.ezspec_data) checkbox unchecked.

13. Check the EzSpec(.ezspec_data) checkbox so that EzSpec is able to show the colors in the exported data.



14. Click the Export button 

.
15. Click File, Exit to close the DNA/RNA app.
16. On the Launcher window, click the EzSpec button to open EzSpec.
17. On the EzSpec Top menu, click File, Open, Data to see the Open EzSpec Data File window.
18. Find and open the export folder selected in step 12 and click on the data file.
    The data opens in EzSpec.

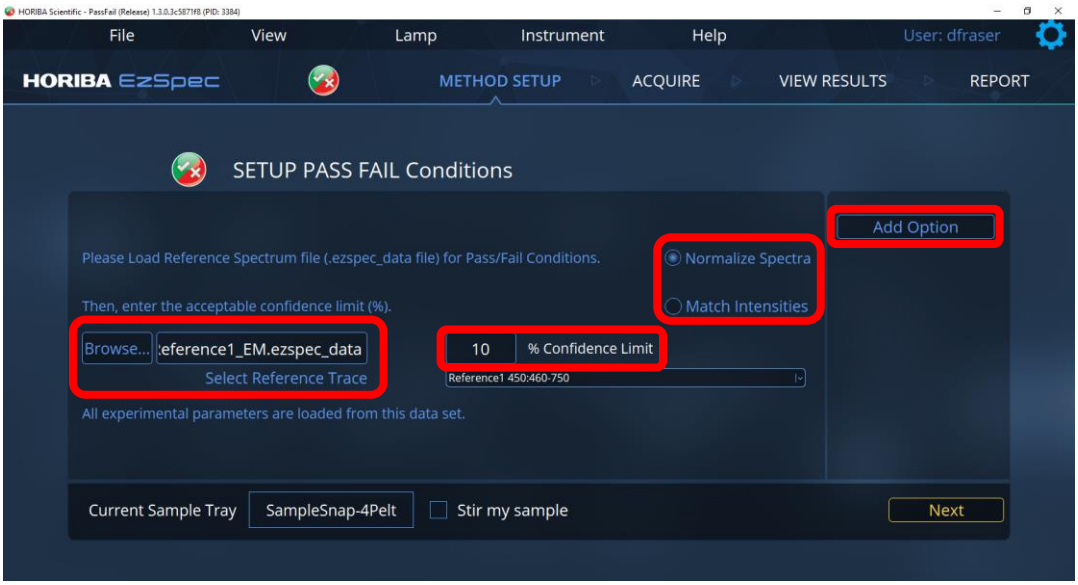


19. The **Report**, **Generate Report** procedure in the Protein A280 app is the same as described in Concentration Curve.

8.3 Pass/Fail App

The Pass/Fail App tests that a sample trace data matches a reference trace within a defined confidence limit at every point. If it does the sample is listed as Pass. If the sample trace falls outside of the confidence limit at any point in the trace, then the sample is listed as Fail. The correlation coefficient for each sample tested is also shown in the results. The confidence limit and correlation coefficient calculations are explained at the end of this section.

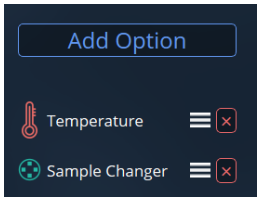
1. On the Launcher Window click on the **Pass/Fail** button to open the App.
2. **SETUP PASS FAIL Conditions**
    a. Click **Browse**. Load the Reference Trace file (absorbance, transmittance, excitation or emission spectrum).



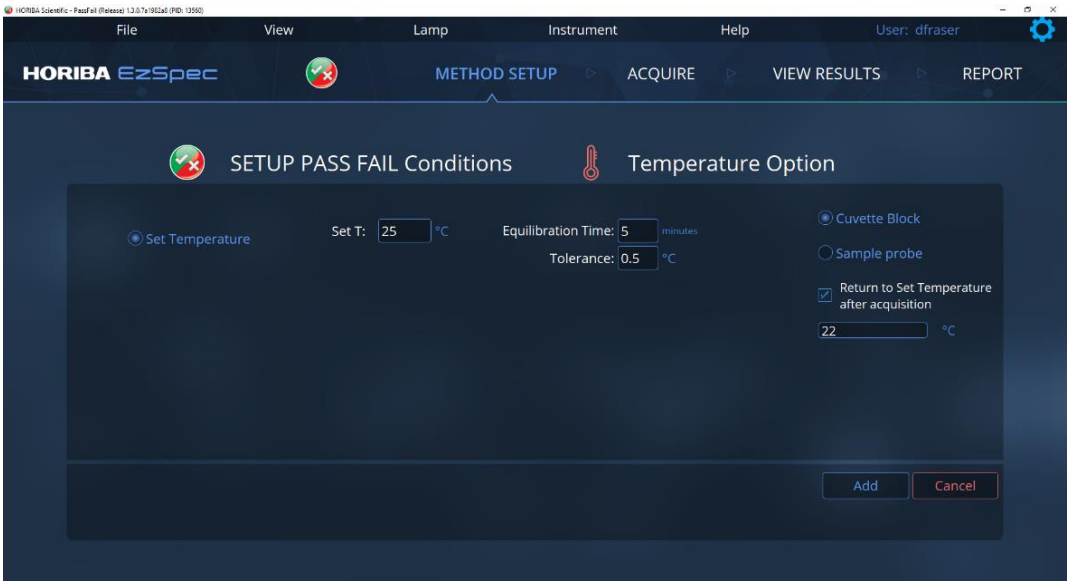
    b. Enter a value (to 1 decimal place) in the **% Confidence Limit** box.
    c. There are two radio buttons on the right.
        i. Click **Normalize Spectra** to normalize the reference and sample traces to the maximum intensity and compare the normalized spectra for spectral shape.
        ii. Click **Match Intensities** to use the reference and sample intensities as they are and compare the traces for both spectral shape and intensity.
3. Click on **Add Option** to open a menu of available options.

a. The **SETUP PASS FAIL Conditions – Add Options** window appears. These buttons are shown in color if the Duetta detects them, but greyed out if not detected.

When options have been added to the Option Bar, each option has Edit and Delete buttons. Click the **Edit** button to open the option menu for that option. The **Delete** button deletes the option from the Options Bar.



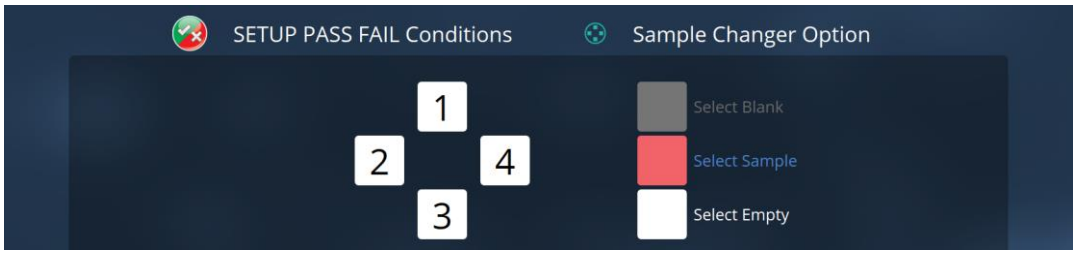
b. Click on the **Temperature** icon to open the **Temperature Option** window. If both Temperature and Sample Changer are added, Temperature will always be listed and executed before Sample Changer.



i. Enter a temperature in °C in the **Set T** box. The allowed temperature range is -15 °C to 105 °C.
ii. Enter the **Equilibration Time** in minutes. 5 minutes is a normal time.
iii. Enter the Tolerance in °C.
iv. Enter a Set Temperature after Acquisition in the box.
v. The Return to Set Temperature after acquisition checkbox may be unchecked or checked.
    1. If this box is unchecked, the temperature will hold at the last temperature of the method after the acquisition is completed.
    2. If this box is checked, then an enterable box appears for the user to enter a temperature in °C that the temperature controller will go to after the acquisition is finished.
vi. Click the **Add** button to add **Temperature** to the **Options Bar** and close the **Temperature Option** window.

c. Click on the **Sample Changer** icon to open the **Sample Changer** window. The Reference file loaded in step 2 above must include a blank trace for the **Select Blank** button to be active.

i. If the **Reference file** does not include a blank, then the **Select Blank** button is inactive.



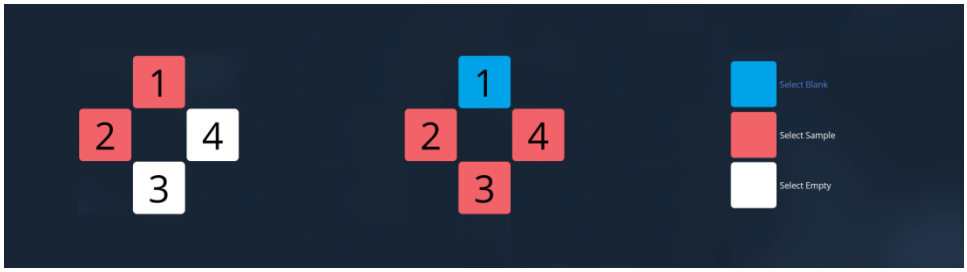
ii. If the **Select Blank** button is active, click on the **Select Blank** button and then click on position **1** if you want to acquire blank data. Only position 1 can contain a blank.

iii. Click on the **Select Sample** button and then click on any of positions 1 to 4 to select those positions for a sample.

iv. Click on the **Select Empty** button and then click on any of positions 1 to 4 to remove a blank or sample and leave that position unoccupied.

v. The turret runs in order of selected positions 1, 2, 3, 4 and then reverses direction to go back to 1. It will not rotate completely around to go from position 4 to 1. Because the turret always goes back to position 1, it saves time if the selected positions start at 1.

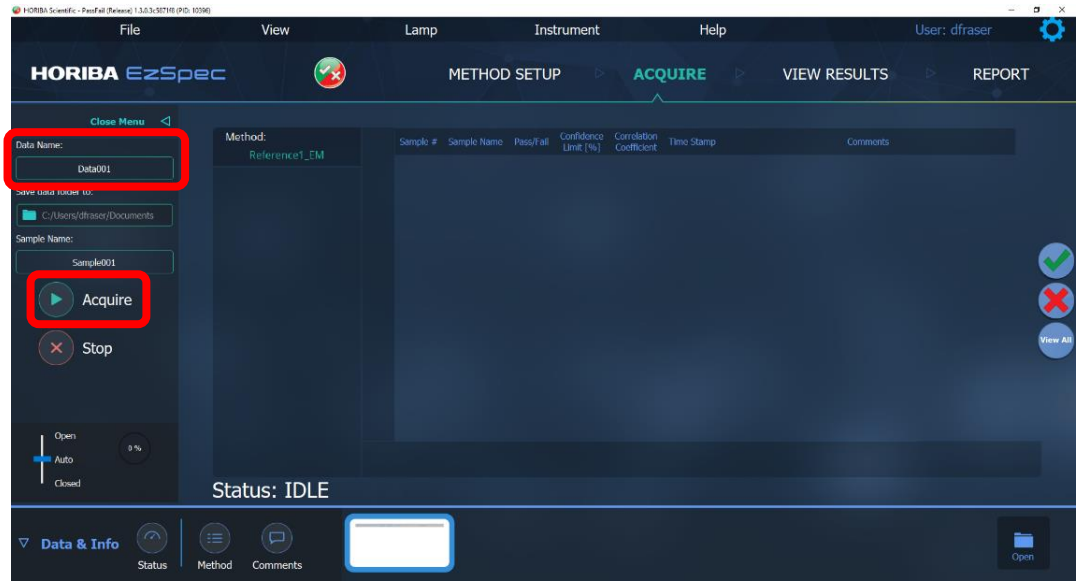
vi. Examples of selected turret positions:



vii. Click the **Add** button to add the **Sample Changer** with selected positions to the **Options Bar** and close the Sample Changer Option window.

4. Click **Next** to show the Acquire menu.

5. Enter a name of the data file you wish to be saved for the measurement under “Data Name”. This name will also be used as the first part of the file folder name. The default Data Name is “Data001”.

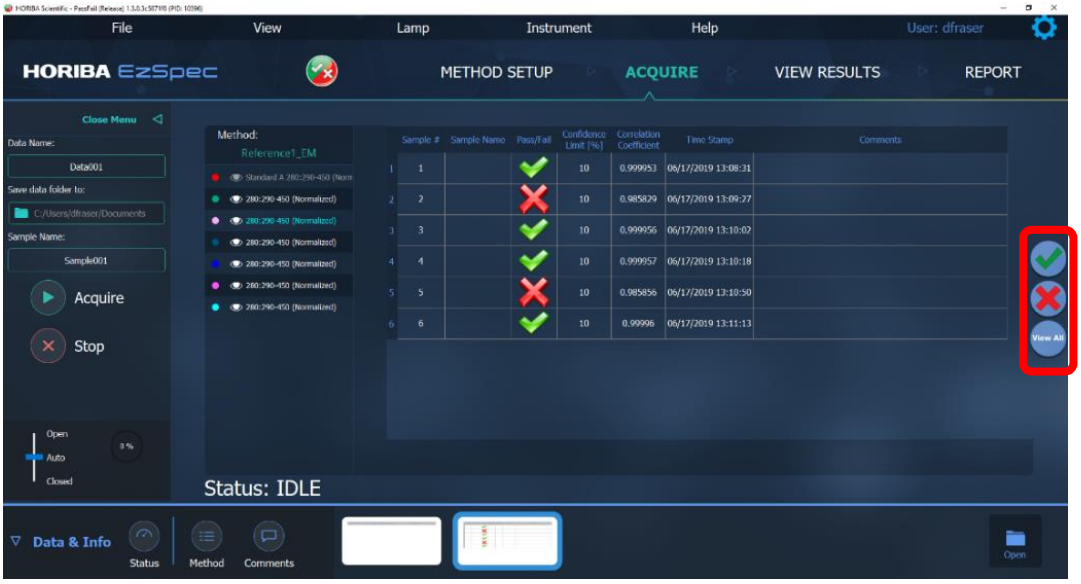


The default sample name is Sample001. Subsequent sample names will autoincrement as Sample002, etc. The correlation coefficient and the timestamp are shown for each trace.

6. Click **Acquire** to acquire each sample trace.
   If there is only a single cuvette holder, then you will be prompted to change the sample, and the Duetta will reequilibrate if Temperature is enabled.

   If there are more than 4 samples (including a blank) to be acquired when using a Sample Changer, then you will be prompted to change the samples, and the Duetta will reequilibrate if Temperature is enabled.

7. Trace viewing buttons



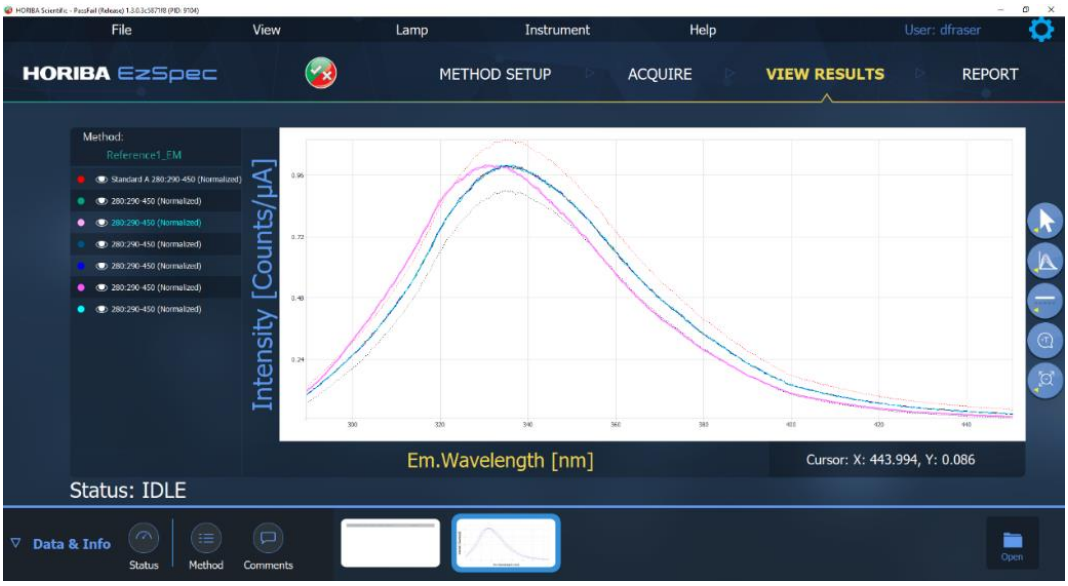
a. These viewing choices on the right side of the **Acquire** window determine which traces are visible in both the **Acquire** window and the **VIEW SPECTRA** window.

b. Clicking the **Show All Passed** button  will show only the traces that passed the current Confidence Limit test.

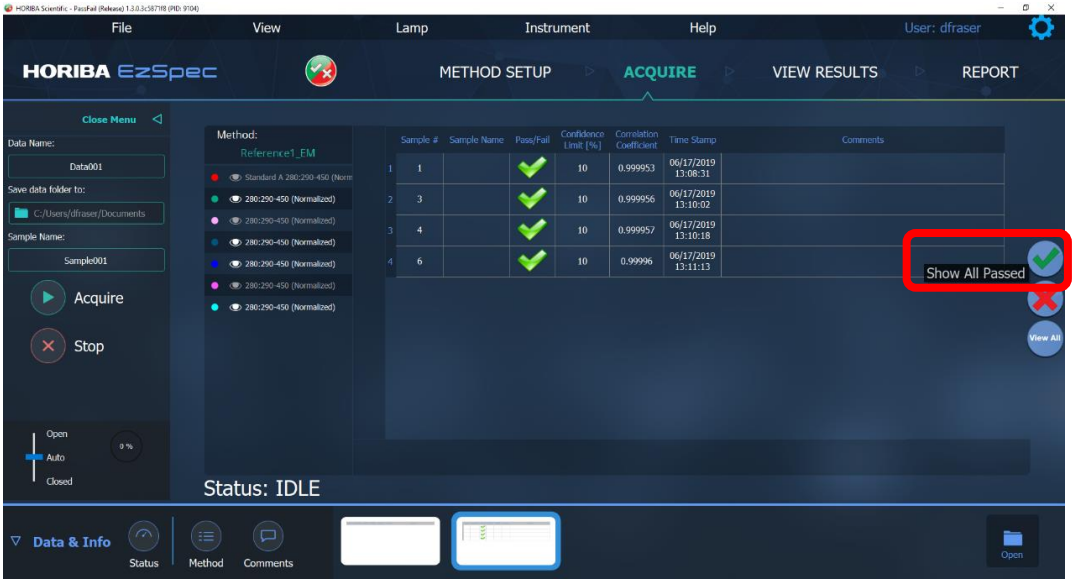
c. Clicking the **Show All Failed** button  will show only the traces that failed the current Confidence Limit test.

d. Clicking the **View All** button  will show all the traces for the current Confidence Limit test. **View All** is the default selection.

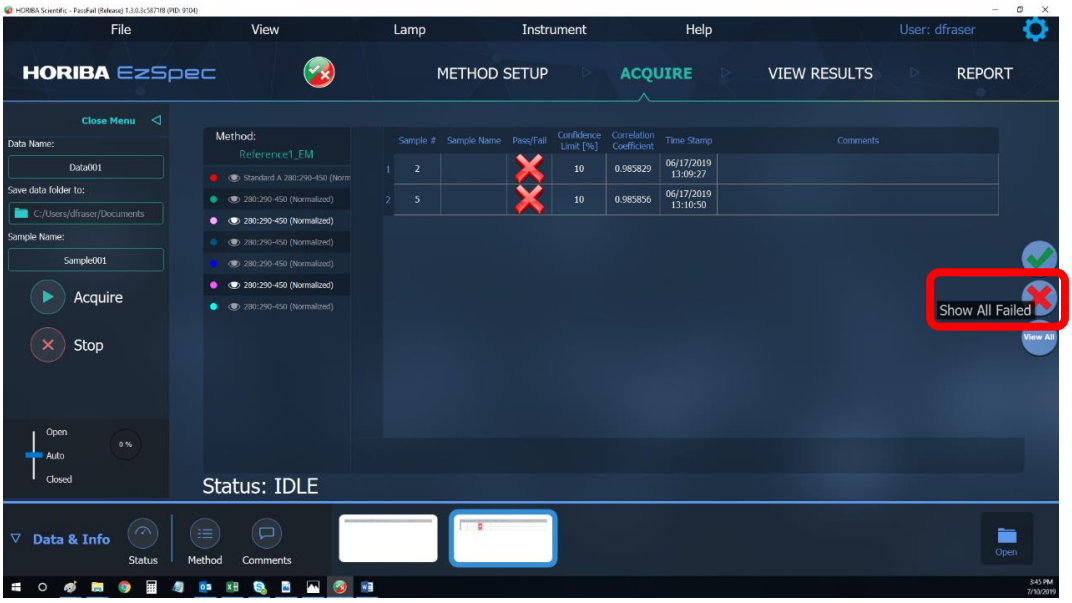
8. Click on **VIEW RESULTS** to see the 2D Graph with the upper and lower confidence limit traces as red dotted lines, and the Reference and Sample traces that are currently visible in the Acquire menu.



9. In the **Acquire** window click the **Show All Passed** button.



10. In the **Acquire** window click the **Show All Failed** button.



11. In the **Acquire** window click the **View All** button, then in the **View Results** window click on the **2D Graph** button to expand the **Plot Mode** group, and then click on the **Table View** button to show the table of the data.


File
View
Lamp
Instrument
Help
User: dfraser
HORIBA EzSpec
METHOD SETUP
ACQUIRE
VIEW RESULTS
REPORT
Method:
Reference1_EM
Data
Data Name
Trace Name
Wavelength Range
Axis
Label
Sample Position
Temperature (Celsius)
File Timestamp
Polarizer positions (Ex,Em)
Status: IDLE
Data & Info
Status
Method
Comments
Open

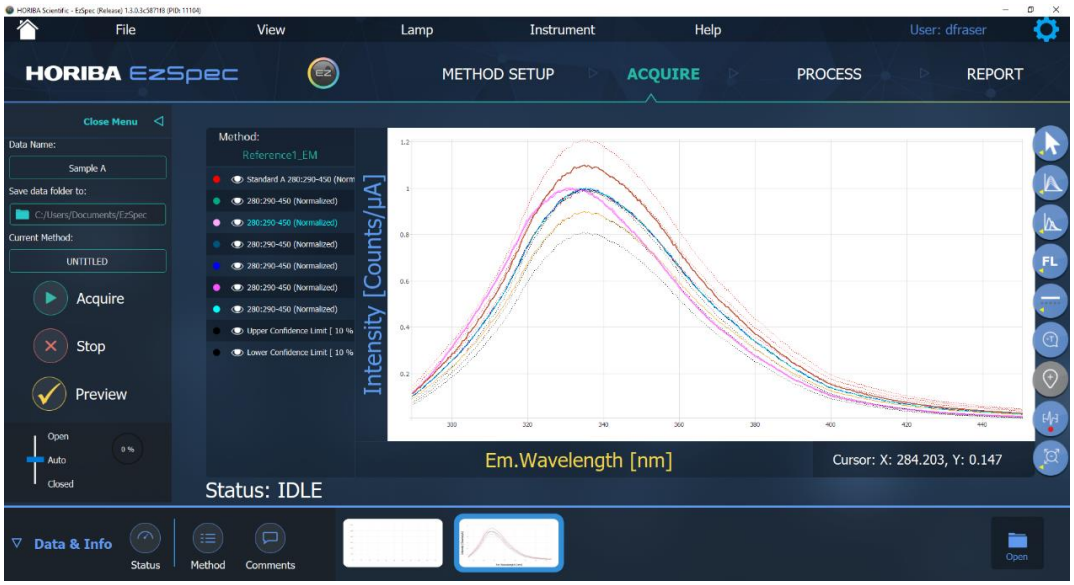
12. Click **Report**, **Export**, **Active Graph Export**.

This opens the Active Graph Export window with the default settings. Exporting the results includes enabling options to save the View Results window to a jpeg image, and exporting the data as an EzSpec data file and as SPC data.

13. Click on the **Browse** button to select the folder where you want to save the report and data.
14. Check the **EzSpec(.ezspec_data)** checkbox to be able to show the colors and pass/fail information in the exported data.
15. Click the **Export** button 

.
16. Click **File**, **Exit** to close the Pass/Fail app.
17. On the Launcher window, click the **EzSpec** button to open EzSpec.

18. On the EzSpec Dashboard, click **File**, **Open**, **Data** to see the Open EzSpec Data File window.
19. Open the export folder selected in step 13 and select the exported file. The exported data is opened in EzSpec.



20. Hide the last two trace names in the legend:
    Upper Confidence Limit [10 %] and
    Lower Confidence Limit [10 %].

21. The **Report**, **Generate Report** procedure in the Pass/Fail app is the same as described in Concentration Curve.

8.3.1 Confidence Limits

The confidence limit here is a weighted confidence limit, scaling each limit value to the peak intensity of the reference spectrum. The confidence limit is calculated by the equation below, where N_i is the signal intensity at wavelength, i. N_{max} is the maximum intensity of the entire spectrum. CL+ is the upper confidence limit and CL− is the lower confidence limit.

$$\Delta N_i = 0.1 * \sqrt{N_i * N_{max}}$$

$$CL+ = N_i + \Delta N_i$$

$$CL- = N_i - \Delta N_i$$

8.3.2 Correlation Coefficient

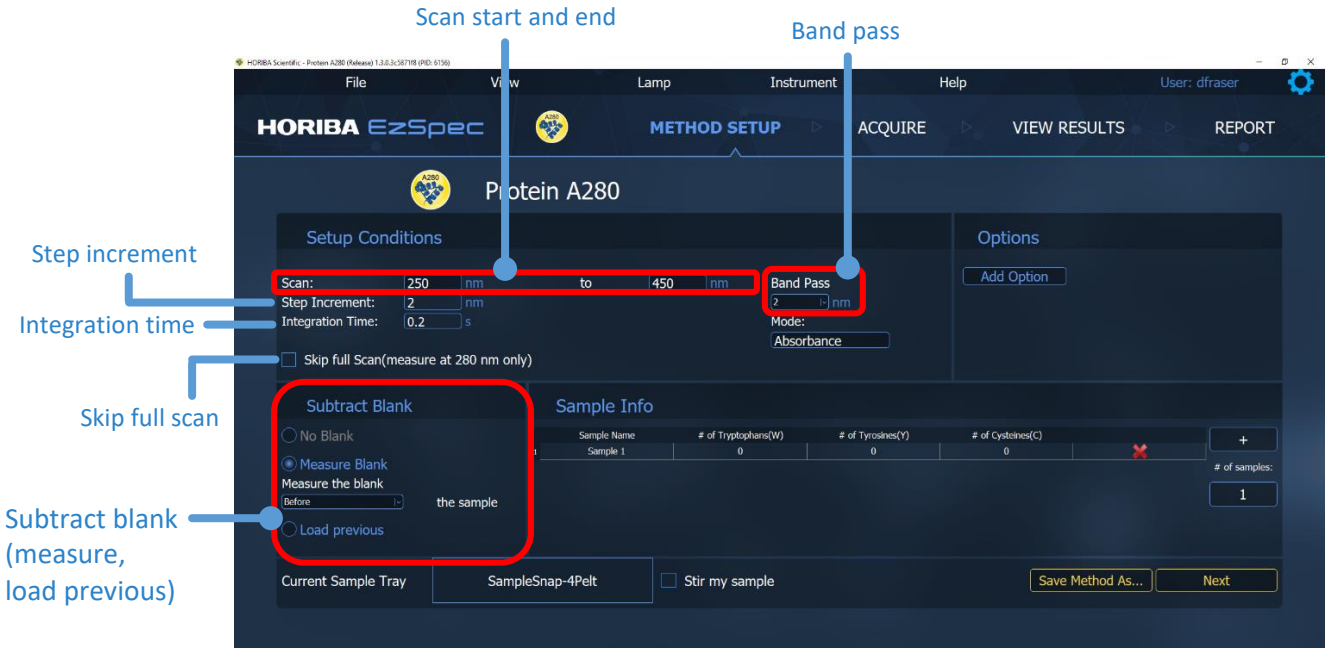
The correlation coefficient is a measure of how well the measured sample spectrum matches the reference spectrum. A maximum value of 1 is equal to an spectrum that exactly matches the values of the reference spectrum. The equation for the correlation coefficient (cc) is below where x is the intensity value of the reference spectrum and $\bar{x}$is the mean average of intensities (average x) for the reference spectrum, y is the y intensity value of the measured sample (unknown sample) and $\bar{y}$ is the mean average of intensities (average y) for the measured sample.

$$CC = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sqrt{\sum(x - \bar{x})^2\left((y - \bar{y})\right)^2}}$$

8.4 Protein A280 App

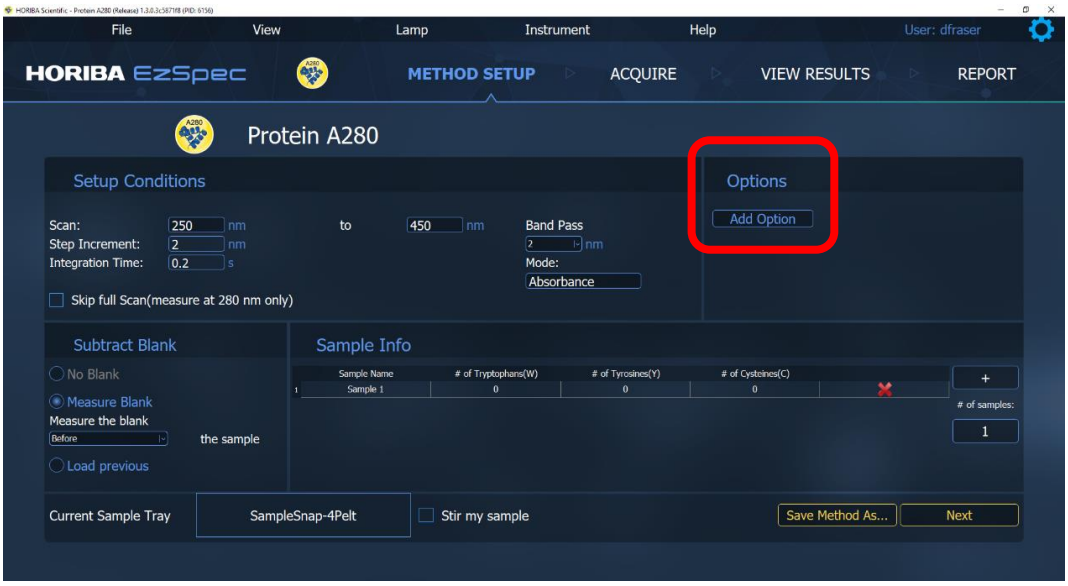
The Protein A280 App measures the absorbance value at 280 nm of a known protein and calculates the concentration of that protein. The user inputs the known number of tryptophan, tyrosine, and cysteine residues to get a calculated absorptivity at 280 nm for the total protein. Using Beer's law (c=A/ε*b), the concentration is calculated.

1. On the Launcher Window click on the **Protein A280** button to open the App.
2. **METHOD SETUP window**
    a. **Absorbance** is the only option in the Mode list.
    b. Enter a value for the **Integration Time**.
    c. Enter a value for the **Step Increment**.
    d. Enter the **Scan** Start and End wavelengths (nm).

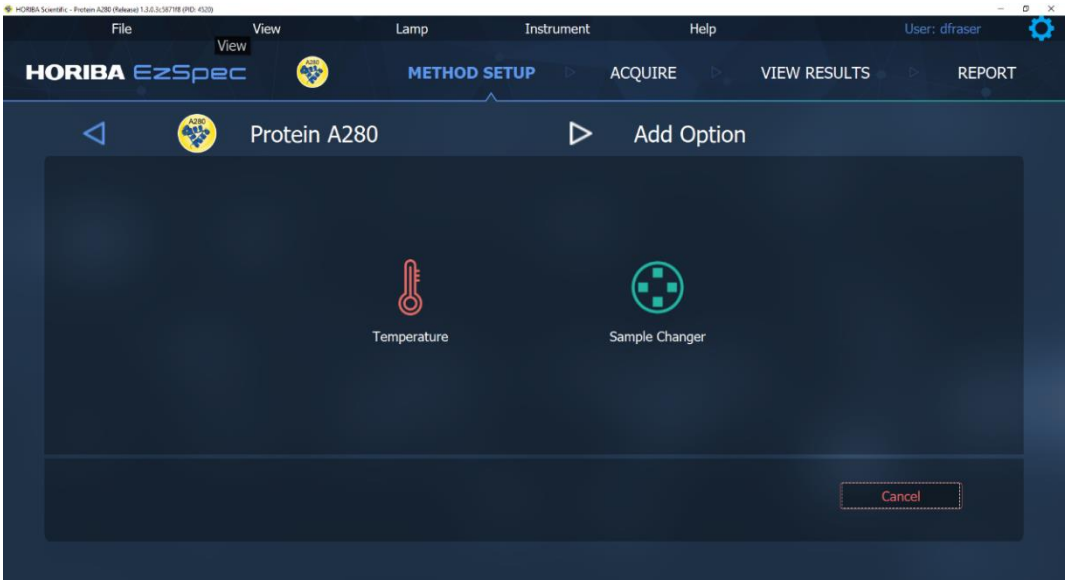


    e. Select a **Band Pass** value from the pull down menu. Only 2, 3, or 5 nm are available.
    f. Either check the check box “**Skip full Scan (measure at 280 nm only)**” or not.
        i. If this box is checked, then EzSpec™ will measure the absorbance at 280 nm only at 1 second integration time.
        ii. If this box is unchecked, then EzSpec™ will measure the absorbance spectrum as specified in the Setup Conditions.
3. A Blank measurement is required. Select either **Measure Blank**, or **Load previous**.

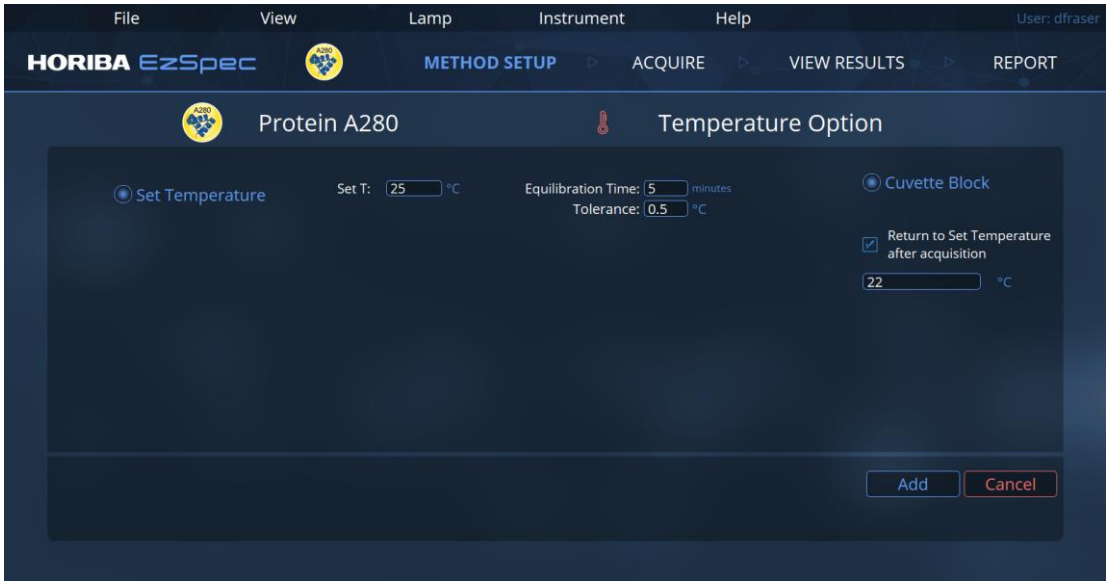
4. Click on **Add Option** to open a menu of available options.



a. The **Add Option** window appears. If an option is not available on the tray in place, the option will be grayed out.

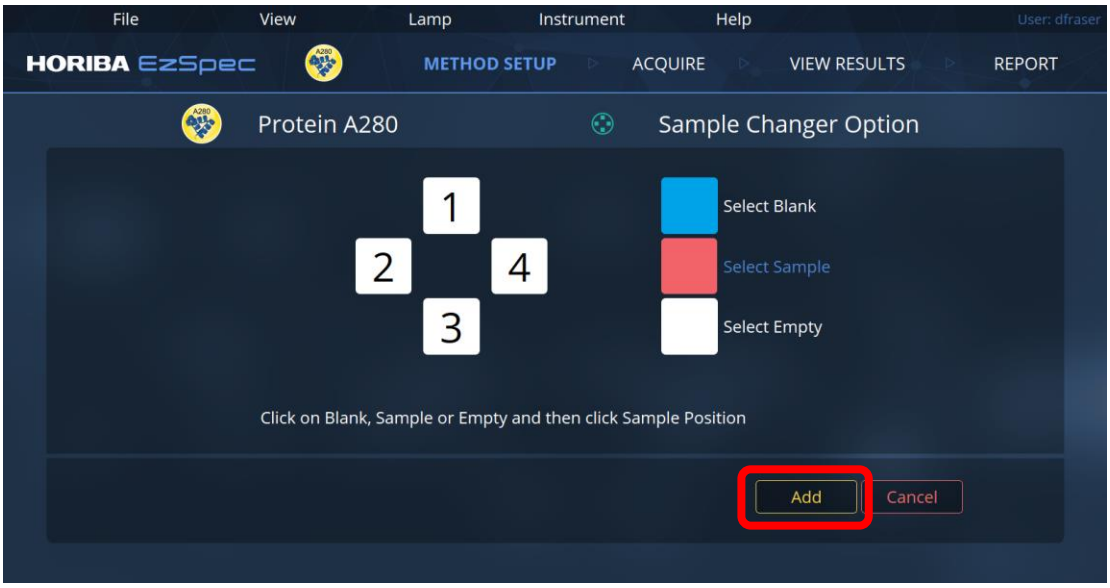


b. Click on the **Temperature** icon to open the **Temperature Option** window. If both Temperature and Sample Changer are added, Temperature will always be listed and executed before Sample Changer.



i. Enter a temperature in °C in the **Set T** box. The allowed temperature range is -15 °C to 105 °C.
ii. Enter the **Equilibration Time** in minutes.
iii. Enter the **Tolerance** in °C.
iv. The **Return to Set Temperature after acquisition** checkbox may be unchecked or checked. It is initially checked by default.
    1. If this checkbox is unchecked, the enterable box below it disappears, and the temperature will hold at the last temperature of the method after the acquisition is completed.
    2. If this box is checked, then an enterable box appears for the user to enter a temperature in °C that the temperature controller will go to after the acquisition is finished.
v. Click the **Add** button to add **Temperature** to the **Options** list and close the Temperature Option window.

c. Click on the **Sample Changer** icon to open the **Sample Changer** window.



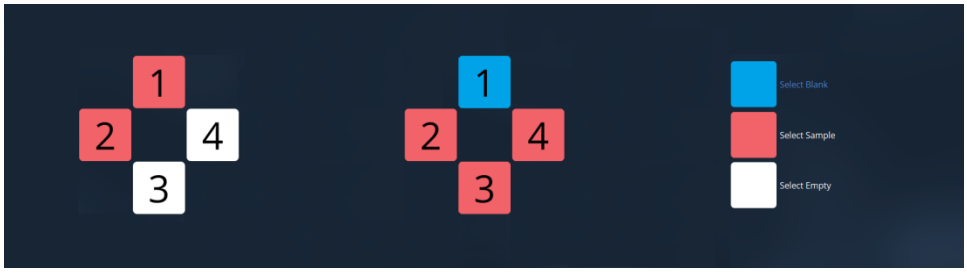
i. Click on the **Select Blank** button and then click on position **1** if you want to acquire blank data. Only position 1 can contain a blank.

ii. Click on the **Select Sample** button and then click on any of positions 1 to 4 to select those positions for a sample.

iii. Click on the **Select Empty** button and then click on any of positions 1 to 4 to remove a blank or sample and leave that position unoccupied.

iv. The turret runs in order of selected positions 1, 2, 3, 4 and then reverses direction to go back to 1. It will not rotate completely around to go from position 4 to 1. Because the turret always goes back to position 1, it saves time if the selected positions start at 1 and are consecutive.

v. Examples of selected turret positions:

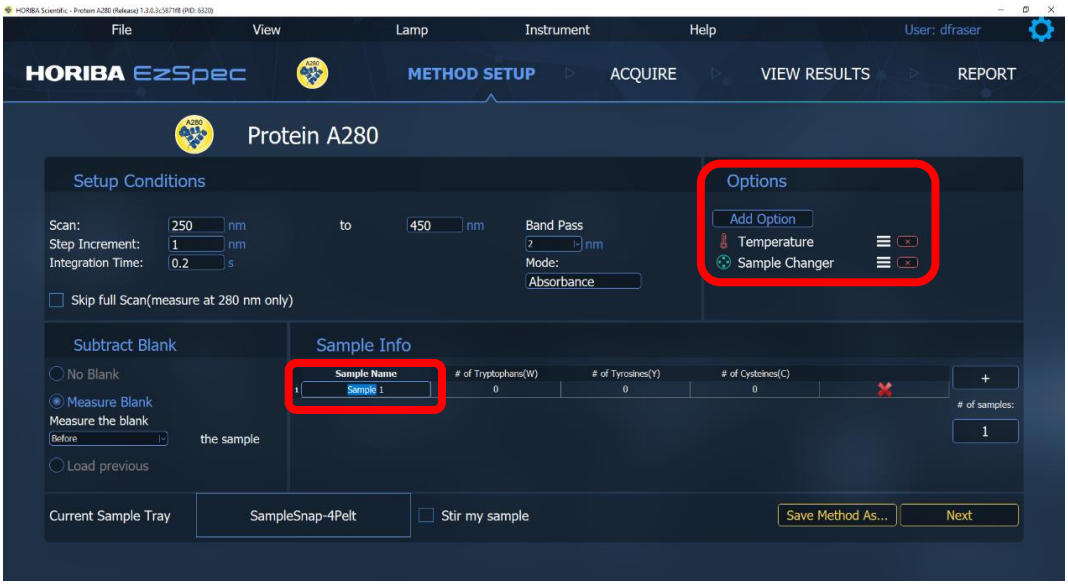


vi. Click the **Add** button to add the Sample Changer with selected positions to the Options Bar and close the Sample Changer Option window.

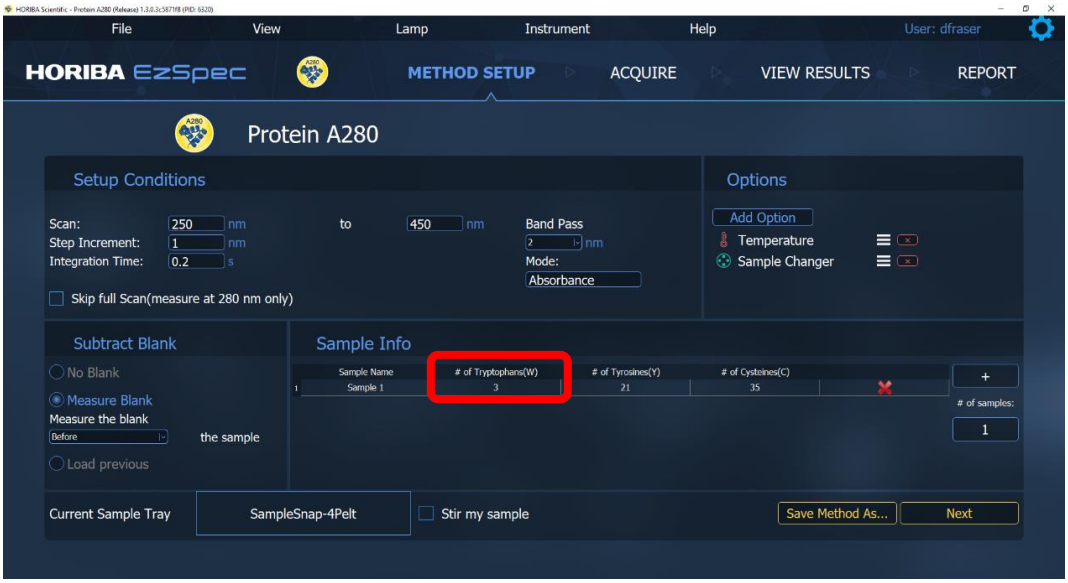
When options have been added to the Options Bar, each option has **Edit** and **Delete** buttons.

Click the **Delete** button  to delete the option from the Options Bar.

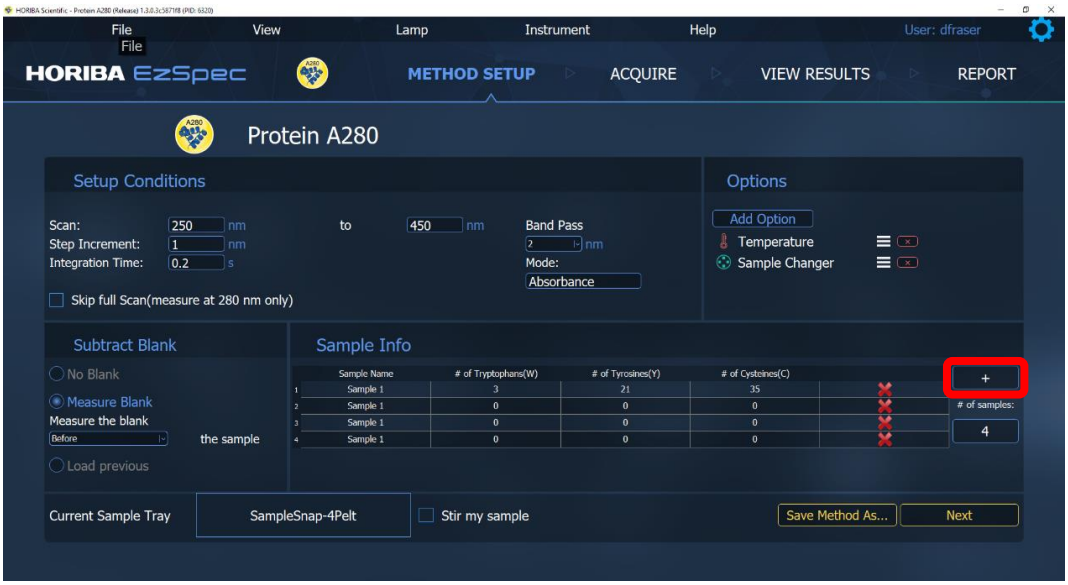
Click the **Edit** button  to open the option menu for that option.



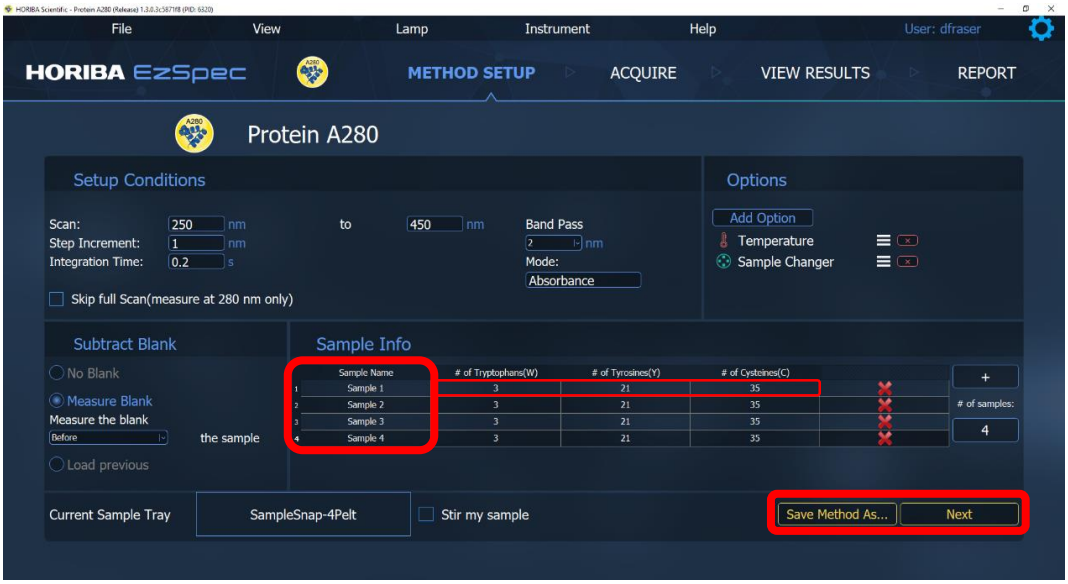
5. Enter the Sample Information
    a. If you want to change the Sample name, double-click on **Sample** in the first row to enter a new sample name. Do not change the number 1. In this case the name was not changed.
    b. Click In each residue cell on the first row and enter the number of residues. You can press the tab key to move to the next cell.



c. Click the **+** button the number of times as you want additional samples in the table.
This produces new rows, each with the default name Sample 1, and 0 residues in each cell.

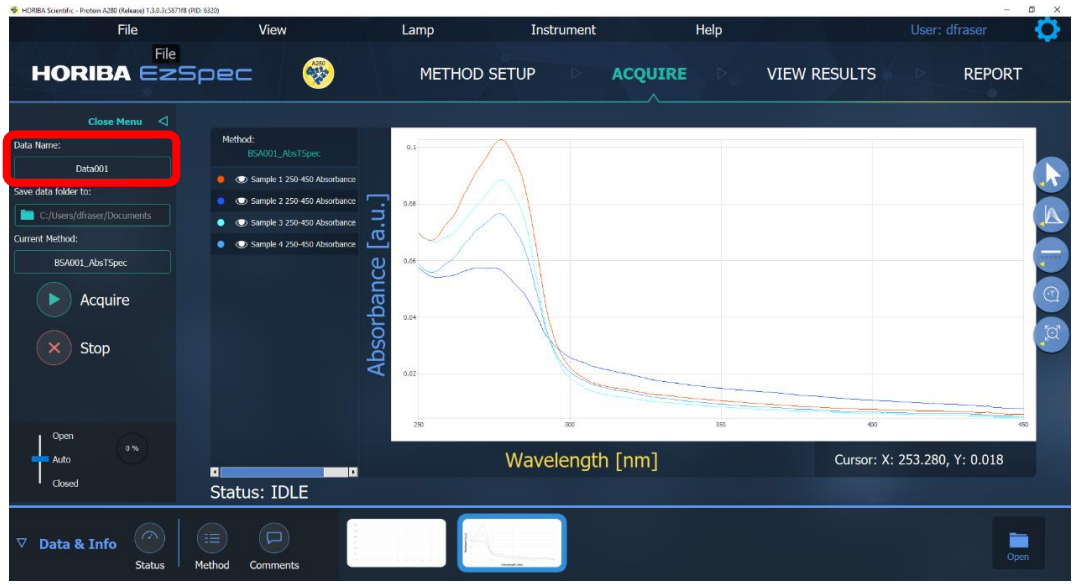


d. Click on the first **Sample Name** cell, press Ctrl-C to copy the name and then paste the name in each Sample Name cell below, also incrementing the numbers as you go.



e. Copy the number of residues in each of the first row residue cells in the first row and paste them into the rows below.
f. Click on **Save Method As** and enter a name for the method. Click **Save**.
g. Click **Next** to show the **Acquire** menu.

6. Enter the name of the data file you wish to be saved for the measurement in the **Data Name** box. This name will also be used as the first part of the file folder names. The default Data Name is “Data001”.



7. Acquire procedure (for a single cuvette holder)
    a. The **Acquire** and **Stop** buttons and the Shutter control are the same as in EzSpec.
    b. If a blank measurement is not required because a previous blank has been loaded in the **Setup**, then clicking the **Acquire** button will show a prompt **Please insert sample**. Insert the first sample and click **Next**. Continue to remove the sample, insert the next sample, and click **Next** until all the measurements have been completed.
    c. If a blank is to be acquired, then clicking the **Acquire** button will show a prompt **Please insert blank** or **Please insert sample** depending on the order specified in the Setup. Insert the blank or sample accordingly and click **Next** to make the measurement.

    d. At the next prompt **Please insert sample** or **Please insert blank**, insert the sample or blank accordingly and click **Next** to make the measurement. The blank is only measured once per sample set.
    e. Thereafter, clicking the **Acquire** button will show a prompt **Please insert sample**. Insert the second sample and click **Next**. Continue to remove the sample, insert the next sample, and click **Next** until all the measurements have been completed.
8. Acquire procedure (for a sample changer):
    Click **Acquire** to acquire the blank measurement (if required) and other samples as

inserted in the sample changer.

If there are more than 4 samples (including a possible blank) to be acquired, then you will be prompted to change the samples, and the Duetta will reequilibrate if Temperature is enabled.

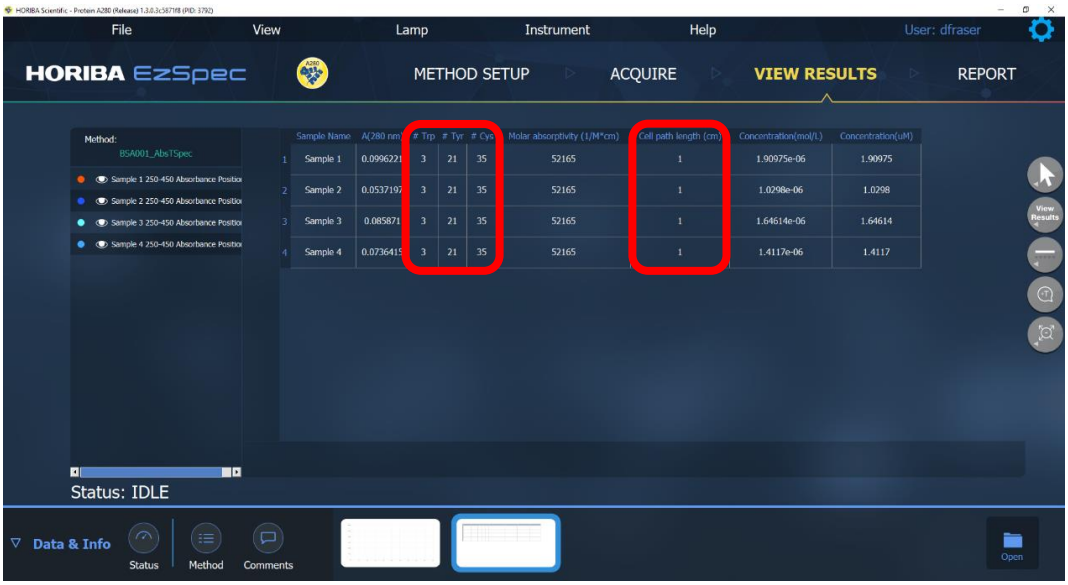
9. View Results
    a. Processing is done automatically as the samples are measured.
    b. The concentration is calculated as:

$$c = Absorbance_{280}/\epsilon_{280} * cell\ path\ length$$

    c. The molar absorptivity at 280 nm is calculated as:

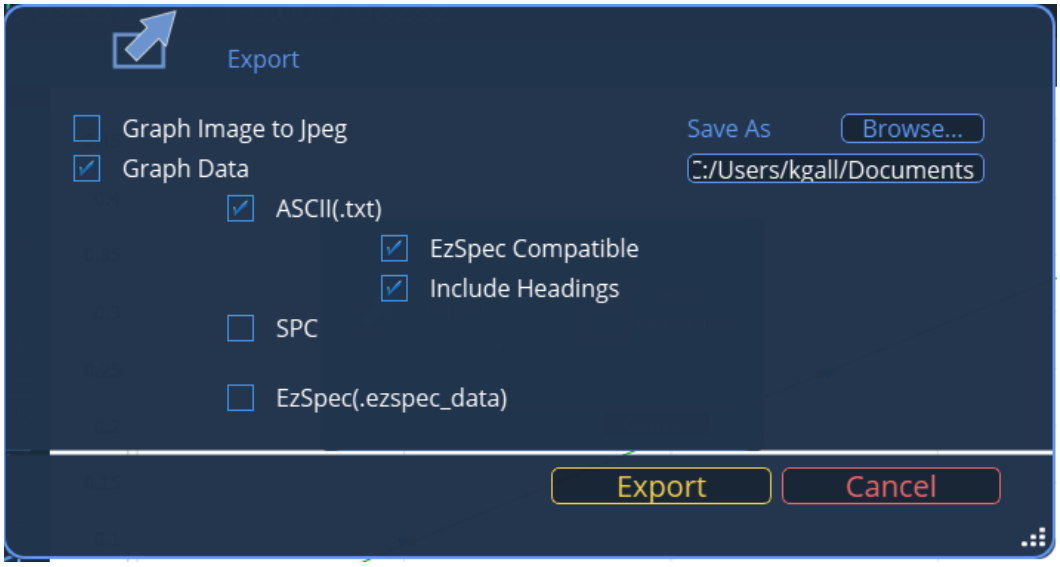
$$\epsilon_{280} = (\#W*5500) + (\#Y*1490) + (\#C*125)$$

    where W = tryptophan, Y = tyrosine, and C = cysteine amino acid residues.
    d. The concentrations are shown in M (mol/L) and µM (1×10^{-6} M).
    e. In the table the user can change the numbers of Trp, Tyr, and Cys residues, and the cell path lengths, and the table will update the concentration values.



10. The **Report**, **Generate Report** procedure in the Protein A280 app is the same as described in Concentration Curve.

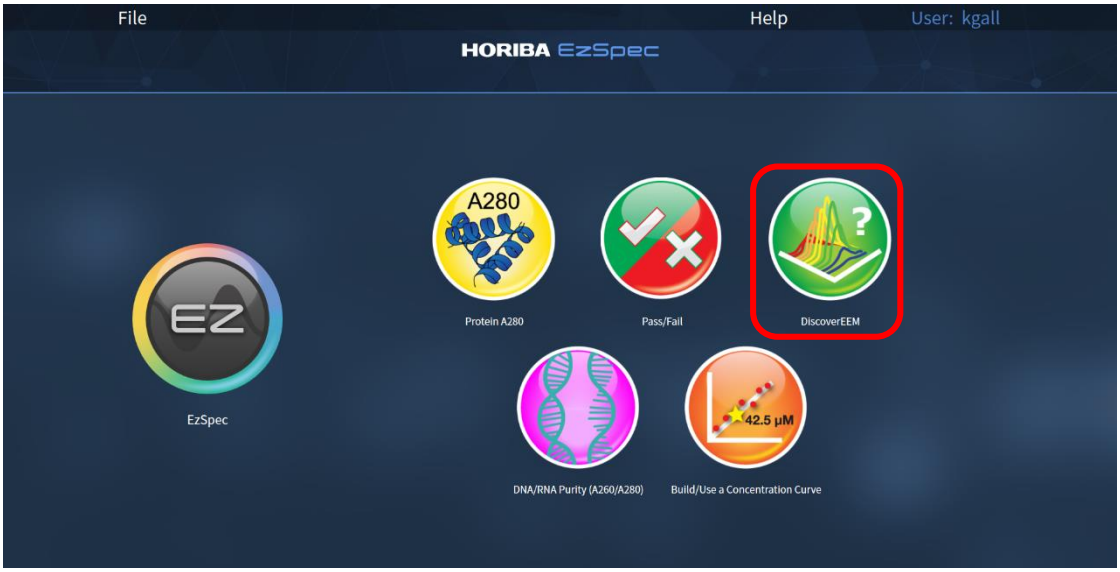
11. **Report**, **Export** menu:
    The data created in the Protein A280 App is saved as a file with extension .ezspec_A280. This file can only be opened within the Protein A280 application unless exported to an .ezspec_data file. In that case, it may be opened in the EzSpec main application for further advanced analysis. The data can also be exported to image file (graph) or ASCII or SPC files. To access the export menu, go to the **Report** menu and choose **Export**. Select the formats for which export is required and choose the filepath to which the files will be saved. Then click **Export** and the files will be exported as specified.
    See also the procedures for DNA RNA and Pass Fail.



8.5 DiscoverEEM App

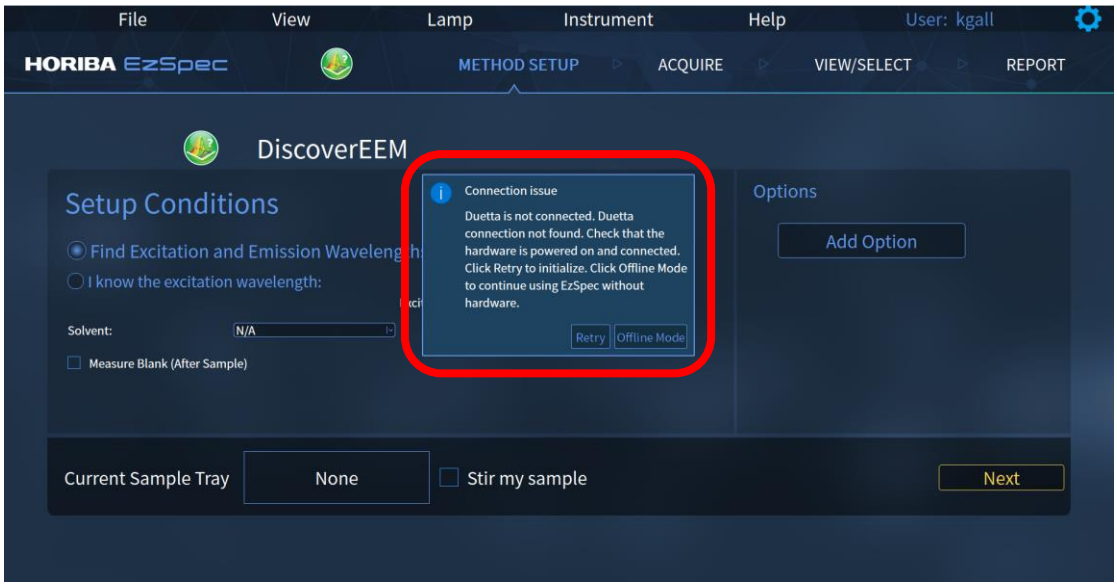
The DiscoverEEM application is a useful tool that allows the Duetta to automatically scan the excitation emission matrix (EEM) of an unknown sample while automatically tuning the method parameters, and provide a molecular fingerprint of such a sample to the user. From the EEM contour plot, the excitation spectrum and emission spectrum can be extracted to give the 2D fluorescence profile of a sample with optimized signal. If a sample absorbance peak is known, a user can also input that into the method and the DiscoverEEM app will tune the emission spectrum acquisition, find the emission peak, and then scan the excitation spectrum to give the profile. The autonomous acquisition used in the DiscoverEEM app is a great way to go through the trial and error of selecting the optimal scan parameters, but by letting the software do it for you.

1. From the launcher screen, click on the DiscoverEEM app icon.

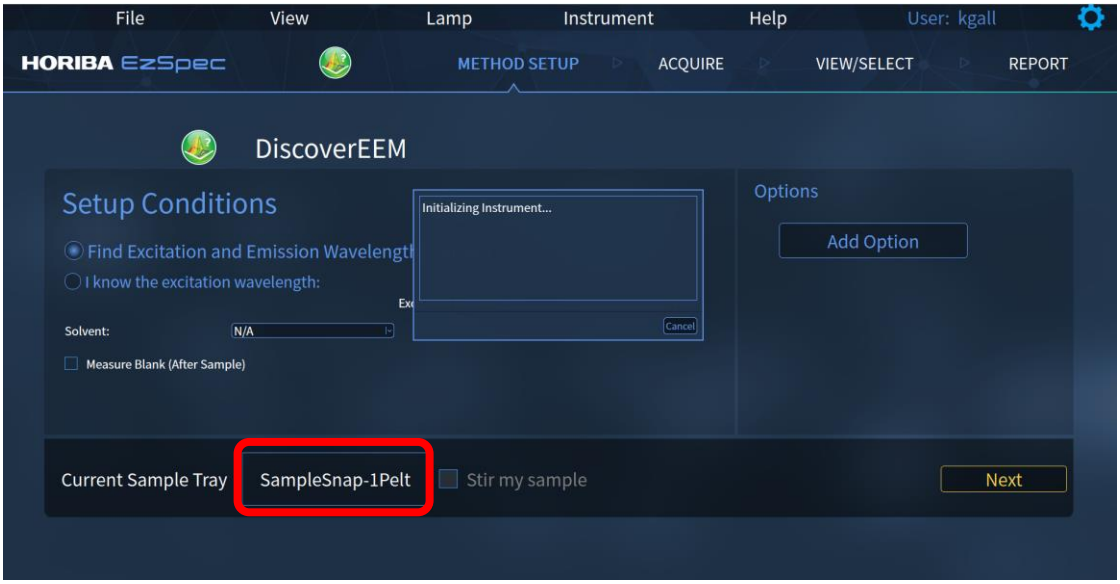


The launcher will close, and after a second or two, the DiscoverEEM application will open. The software will first check to see if there is a Duetta connected and if so, the instrument will go through initialization. If not, the message below will appear.

2. Click Offline Mode to work offline or check that the Duetta is powered on, the USB cable is connected to the instrument and the computer, and then click Retry.

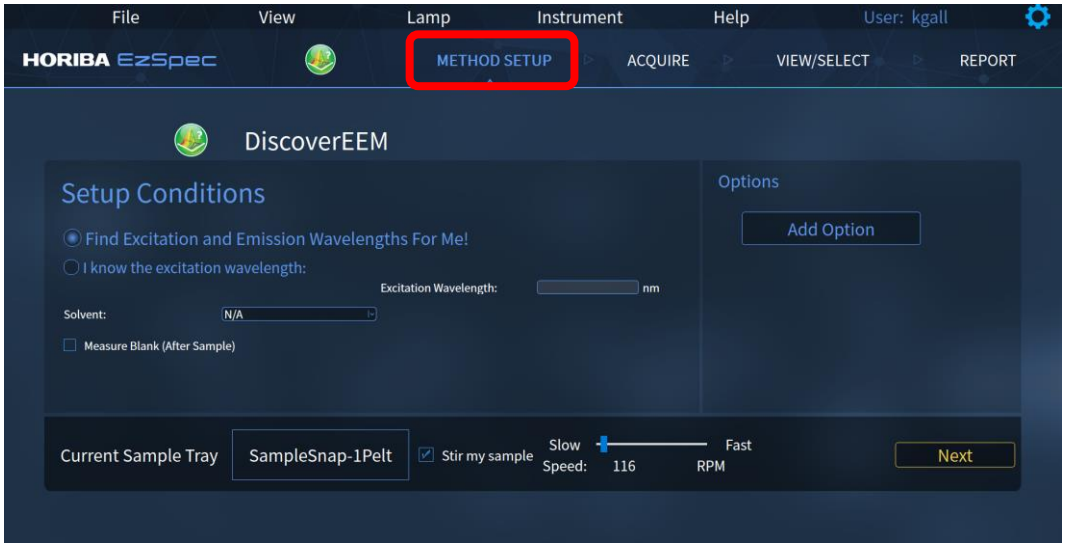


If instrument connected:



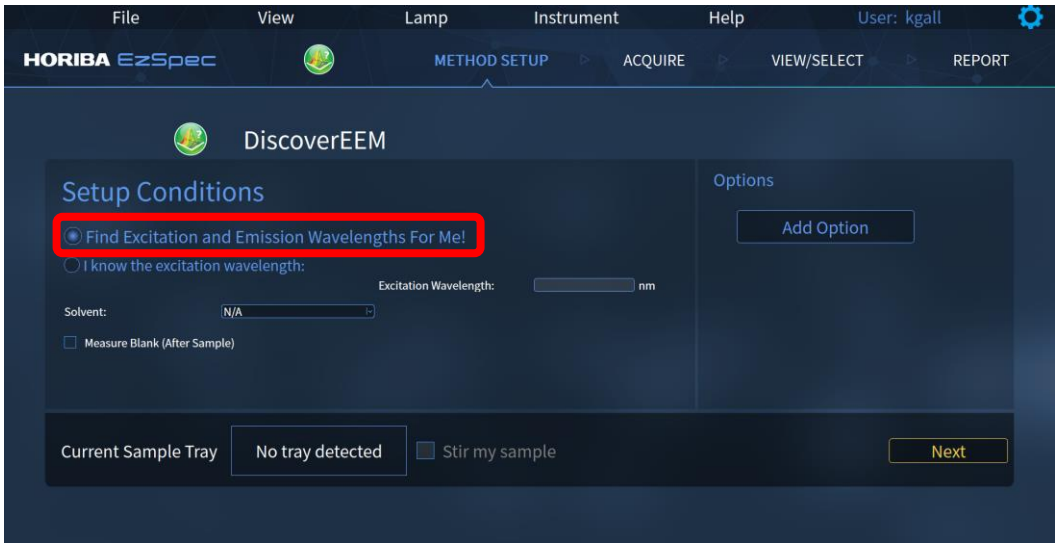
Once initialized, Method Setup menu will appear and the Sample Tray will be identified at the bottom of the page.

3. Method Setup Menu:



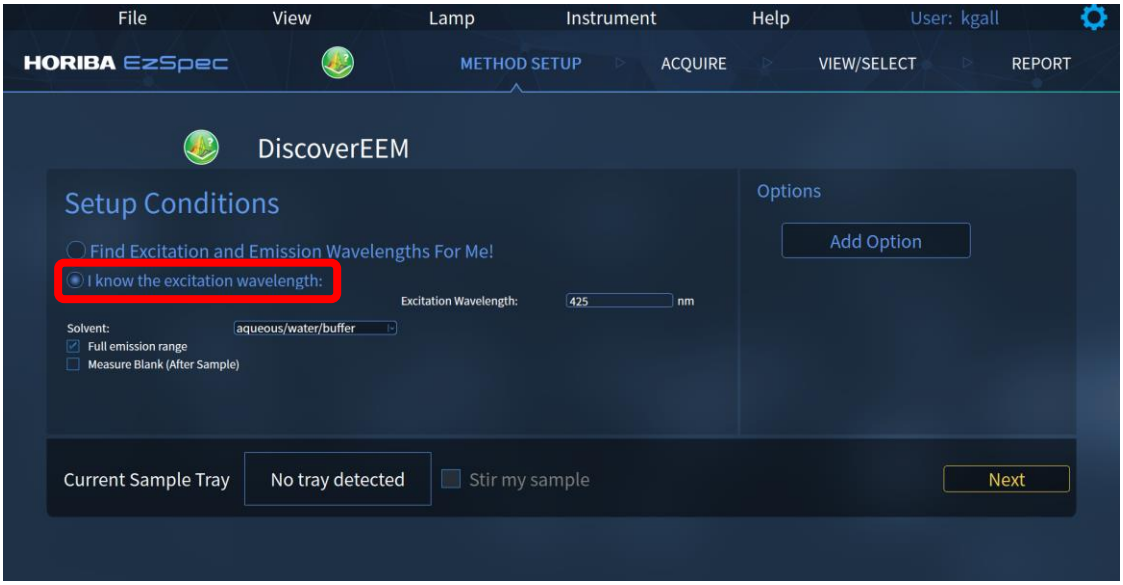
There are two options in the Method Setup: “Find Excitation and Emission Wavelength For Me!” and “I know the excitation wavelength.”

a. Find Excitation and Emission Wavelengths For Me!



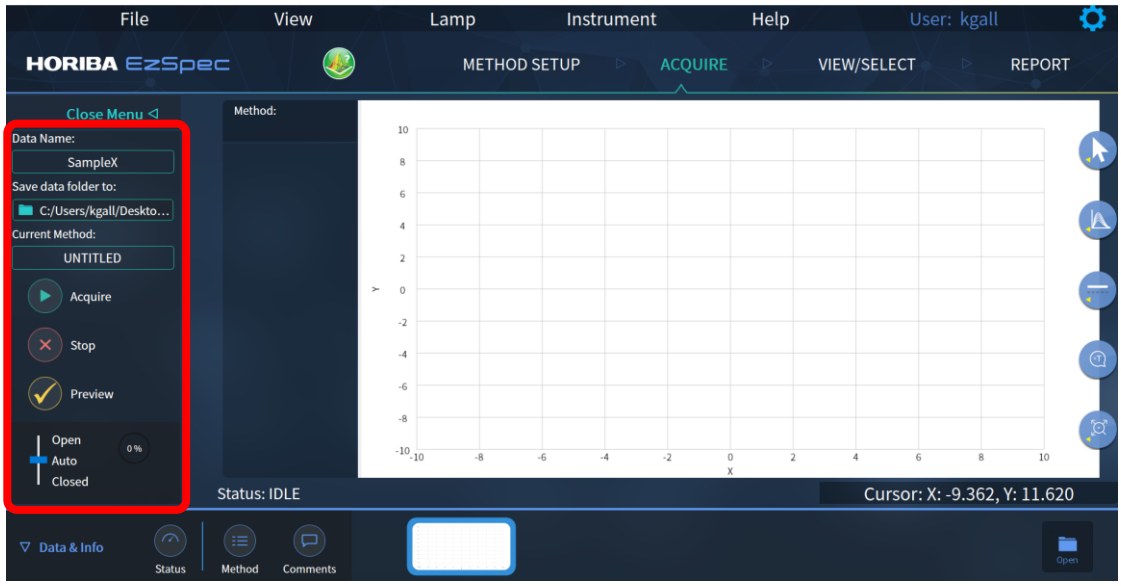
The Find Excitation and Emission Wavelengths For Me! option will scan the entire excitation spectrum from 1000 nm to 250 nm, every 10 nm, and measure the emission spectra from 250-1100 nm at each of those excitation wavelengths. For this option, you can choose the solvent and opt to measure a blank so that any blank signal can be acquired and subtracted after the sample acquisition. The temperature-controlled trays and 4-position sample changer can be added to the method setup using the “Add Option” button.

b. I know the excitation wavelength:



The “I know the excitation wavelength” option is a shortcut from the method above. In this option, the excitation wavelength, if known, can be entered into the method so that the emission spectrum is measured using only that excitation wavelength. After the method parameters are tuned by the software, the peak of the emission spectrum will be found, and can be used to scan the excitation spectrum after that. In the end, both the excitation spectrum and the emission spectrum are acquired with optimal acquisition parameters. The solvent can also be selected so that the software calculates and shows the expected Raman scattering peak from the input solvent after a profile is collected and graphed.

4. Acquire Menu



a. Input a descriptor of the sample into the Data Name. When the data is acquired, a folder with this name and the timestamp will be created in the directory, which is specified in the space below. Click Browse to change the directory to which the data folder(s) will be created.

b. Acquire button will start acquisition. If a blank is selected to be measured, the software will prompt the user to Insert Sample or Insert Blank into the sample tray before the appropriate acquisitions. The software will automatically acquire the emission spectrum at the excitation wavelength entered into the method and check the peak maximum. From this signal, the integration time, band pass, and neutral density filter settings will be autonomously tuned to reach a peak signal of between 5000 and 40,000 counts on the CCD detector. If the method chosen was “I know the excitation” then the emission peak will be found and the excitation spectrum collected using that wavelength. If “Find it for me” was selected, the entire EEM will then be acquired with the optimal method settings and the final graph will be displayed with the peak wavelengths found and displayed. Final graphs will have corrections for dark, Ref signal, spectral correction factors, and blank (if applicable).

   Here, the raw data is shown in the live acquisition. Each sharp peak seen here is the scatter peak from the excitation wavelength used for that particular emission spectrum acquisition. The broader peak is fluorescence from the sample being acquired, and this peak is used for tuning the method parameters.

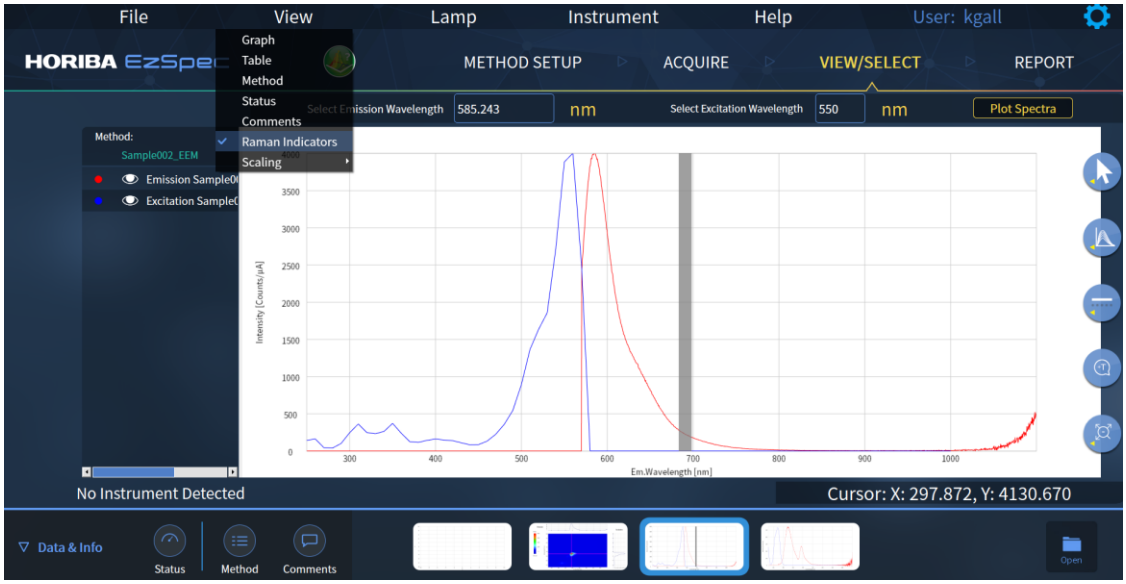
c. The status bar below the graph will show what the autotuning step of the method acquisition is doing during the data collection. Here, the integration time has been decreased because the CCD signal from the first acquisition was too high.
d. Stop button will stop the acquisition in progress.
e. Preview will open the Preview mode, where the user is able to view the raw CCD, Ref or Abs detector signal with control over the excitation wavelength, band pass values, integration time, shutter, and filter wheels.
f. Click Run to acquire raw detector signal and Stop to stop the Preview acquisition.
g. Click Close Preview (at top of the Preview menu) to go back to the Acquire menu screen.

h. Files saved on Acquire:

DiscoverEEM methods are automatically saved as a .ezspec_method_disc file within the data folder, along with the data file EEM, any saved Profiles, the raw data, the log files (disc_log.txt and icl_log.txt) and the

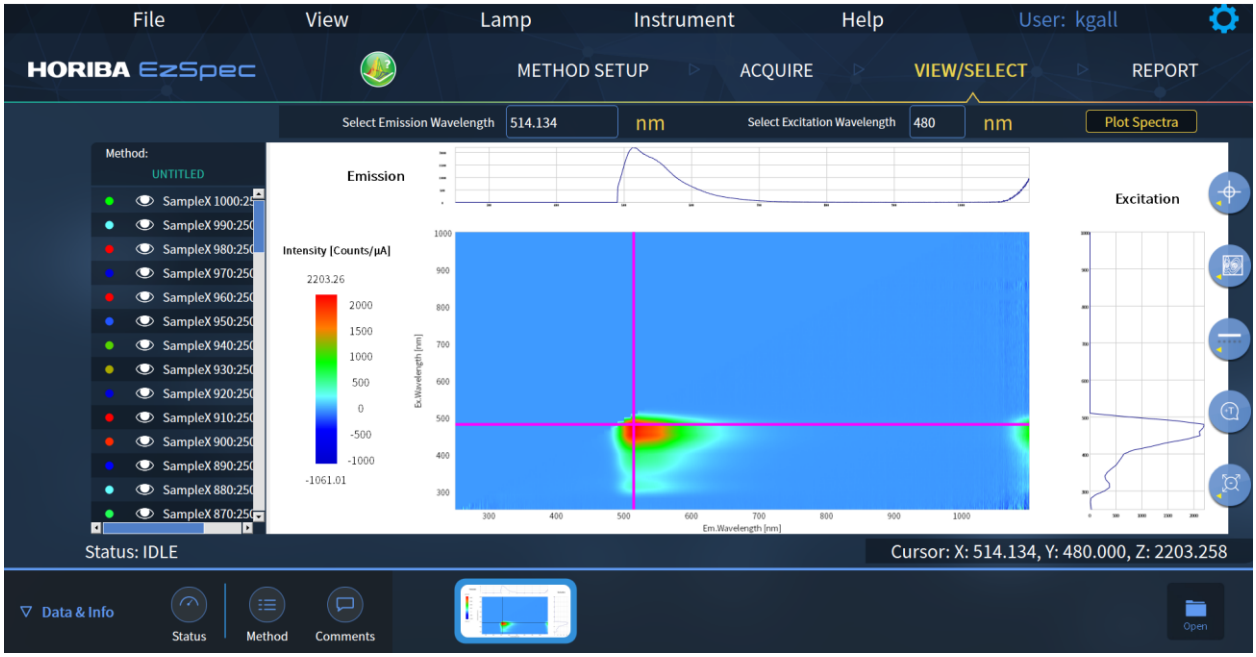
File type	Extension	Description
Data Name EEM file	*Data Name*_EEM.ezspec_disc	Final processed data from DiscoverEEM app. This data can only be opened within the DiscoverEEM application
Profile data file	Profile_XXXex_YYYem.espec_disc	
Method file	*.ezspec_method_disc	
Raw data	*.ezspec_raw	
Correction factors	Corrections.ezspec_data	
Icl log file	Icl_log.txt	
Application log file	Disc_log.txt	

Raman Indicators: Raman band indicators can be viewed or hidden on the graph from the View men, by checking or unchecking Raman Indicators. The Raman peak wavelength location(s) are calculated by the solvent input into the method and the excitation wavelength used for a particular emission spectrum.



5. After the EEM is collected (in the case of “Find it for me”), the software will automatically find the highest peak intensity and report the excitation and emission wavelength values for that peak.

   a. Click OK and the full EEM can be viewed. Move the cursors or type in the excitation and emission wavelength values, which will move the cursors to show the excitation spectrum profile (graph to the right) and emission spectrum profile (graph above the EEM contour plot).



   b. When the peak values and profiles are set, click Plot Profile. The excitation and emission spectra will be plotted and overlaid in a separate 2D graph and thumbnail.

c. This profile data will be saved as Profile_XXXem_XXXex within the data folder and can be recalled and opened by the DiscoverEEM application or exported to another format, including the ezspec_data file format to be used within the EzSpec main application.

d. Clicking on the thumbnail containing the EEM within the View/Select menu enables the user to move the cursors and plot/save more than one Profile of the excitation and emission spectra if needed.

The Report menu enables the user to export data, graphs, and create PDF reports as in other apps within EzSpec software.

9 Maintenance

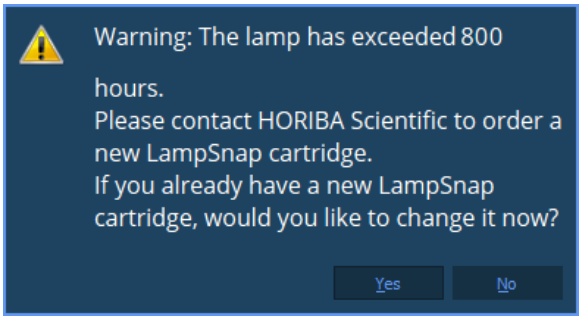
9.1 Introduction

The Duetta™ requires little maintenance. To remove dust and fingerprints, wipe the outside panels with a clean, damp cloth. The lamp and air filter are the only components that must be replaced routinely, generally at the same time. The xenon lamp is held in the pre-aligned LampSnap module for ease of replacement. Regular examination of the xenon lamp scan and water Raman spectrum performed as part of the Service Report serve as indicators of the system's integrity.

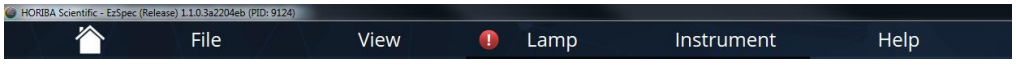
9.2 Lamp replacement

9.2.1 When to replace the lamp

Obtaining good spectral results depends on the xenon lamp. The Duetta™ keeps track of lamp usage automatically. After 800 hours of use, a **Lamp hours** warning notice appears on the host computer's monitor every time you initialize the Duetta™:

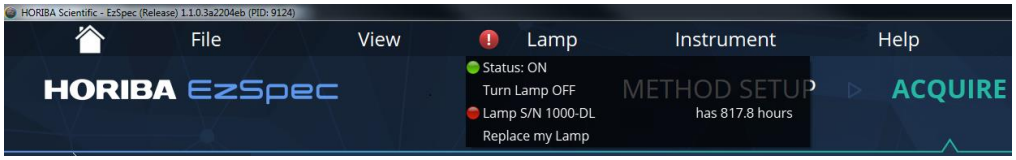


Clicking **No** will close the prompt.

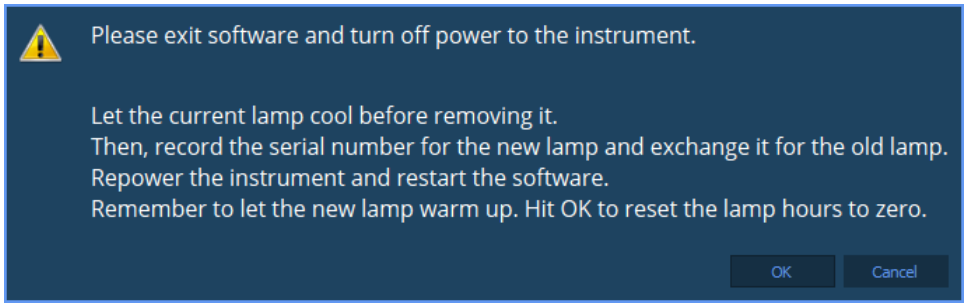


If the user ignores the warning, the Top Menu shows a notification until the lamp hours are reset:

Clicking on **Lamp** will show the following Lamp menu:



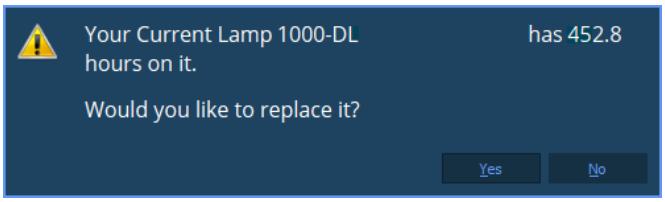
Clicking **Yes** will display the following prompt:



Clicking **Cancel** will close the prompt.

Click the **OK** button to start the lamp replacement procedure (next pages). Replacing the lamp within the recommended time may prevent a catastrophic failure. Each time the lamp is turned on constitutes one full hour of use. Therefore, HORIBA Scientific suggests leaving the lamp on during brief periods of inactivity.

If you want to replace the lamp before it has had 800 hours of use, then on the Lamp menu, click **Replace My lamp** to see the prompt:



Clicking **No** will close the prompt.

Clicking **Yes** will display the **Please exit software and turn off power to the instrument** prompt above.

9.2.2 Hazards

⚠ **Caution: The LampSnap is delicate. Mishandling may seriously damage it. Exercise extreme caution while installing or removing the lamp to avoid bumping the lamp against anything (otherwise it could cause lamp breakage or damage to the equipment).**

⚠ **Caution: Compact arc lamps contain highly pressurized gas, and present an explosion hazard even when cold. Wear face protection, such as a protective face shield, and gloves whenever handling lamps.**

⚠ **Caution: Do not operate the Duetta™ where volatiles are present (otherwise it could cause fire or explosion).**

⚠ **Caution: Never touch the quartz envelope with bare hands. Such handling may lead to deterioration and premature lamp failure. If the quartz envelope is dirty or has been accidentally handled with bare hands, then clean the quartz envelope with an alcohol swab to remove any residue.**

⚠ **Caution: The lamp remains extremely hot after it has been turned off. The lamp fans will run for 5 min after the unit is powered off. The lamp may still be hot to the touch. Let the lamp sit an additional 25 minutes prior to replacement.**

⚠ **Caution: Do not operate the Duetta™ with any other lamp. Only the LampSnap is approved for installation in the Duetta™.**

⚠ **Caution: Use the LampSnap only in the Duetta™. Do not make any other use of the LampSnap (otherwise it could cause lamp breakage or damage to the equipment).**

9.2.3 Replacing the lamp

1. Switch off and prepare the Duetta™.
    a. On the front of the Duetta™ press and hold the power button for more than 3 seconds to turn the lamp off. The power button should then be flashing to indicate the lamp power is off.
    b. The fans run for 5 minutes after turning the Duetta™ off to cool the lamp. Wait until the fans have stopped running, and then remove the AC (mains) power cord from the Duetta™.
    c. Disconnect the USB cable, and any other cables attached to the Duetta™.
2. Open the lamp housing door.
   On the lower right side of the Duetta™ open the lamp housing door by pushing down on the latch at the top of the door.

3. Pull the lamp access panel up and away from the Duetta™.

4. Unlock the blue lamp latch by rotating it 90° left or right.

5. Grasp the tab on the LampSnap module and pull it straight out of the Duetta™. Set it aside.

⚠ **Caution: Never touch the quartz envelope with bare hands. Such handling may lead to deterioration and premature lamp failure.**

6. Remove the LampSnap box from the shipping carton and place it on a table near the Duetta™.

7. Open the box and lift out the top foam layer to show the LampSnap module in its plastic bag.

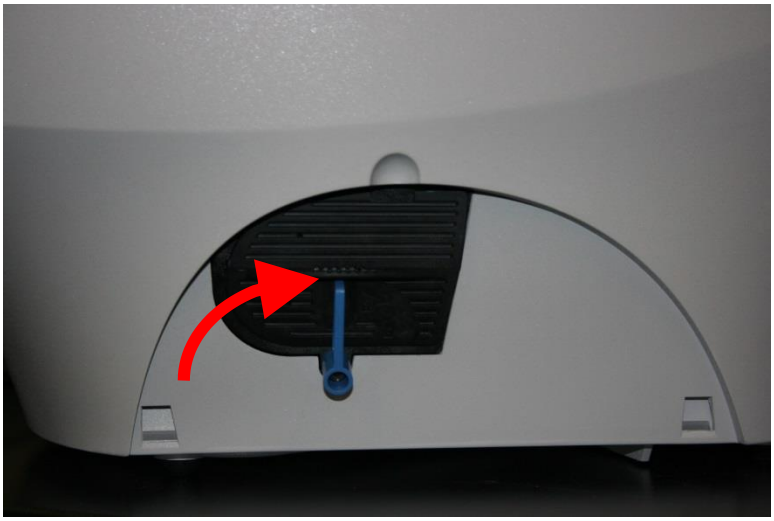
⚠ **Caution: Never touch the quartz envelope with bare hands. Such handling may lead to deterioration and premature lamp failure. If the quartz envelope is dirty or has been accidentally handled with bare hands, then clean the quartz envelope with an alcohol swab to remove any residue.**

8. Remove the plastic bag with the LampSnap from the box, open the bag, remove the LampSnap and place it on the table. Do NOT lay the LampSnap on the foam as this will cause static to attract dust and other particles to the lamp surface which will burn on the surface and shorten the life of the lamp.
9. Orient the front bottom edges of the replacement LampSnap module with the top and bottom grooves in the lamp housing.

10. Slide the LampSnap module into the lamp housing.

11. Push on the tab on the end of the LampSnap module to make sure it is fully seated.

12. Lock the LampSnap module in place by rotating the blue latch 90° clockwise to an upright position.



13. Re-install the lamp access panel.

9.3 Disposal of old lamps

Place the old lamp in the LampSnap box until disposal. Otherwise it could cause injury by exploding resulting in flying glass shards.

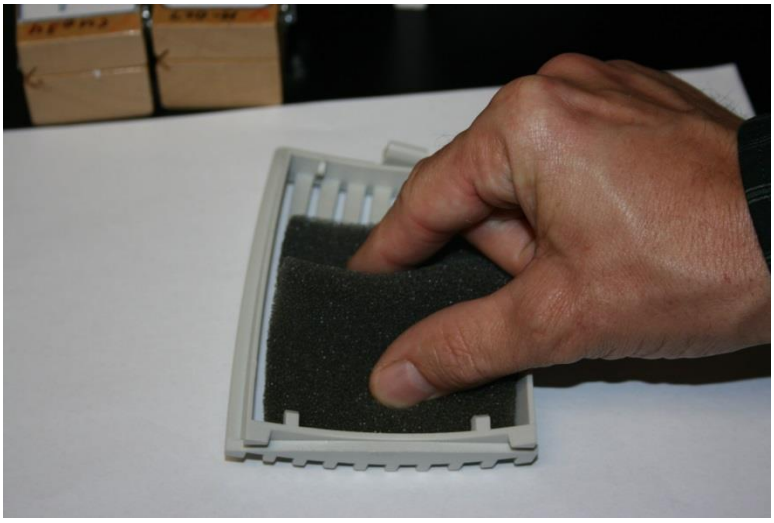
Wrap the LampSnap module in 5 to 6 layers of heavy paper towel, newsprint or heavy cloth, fold the ends of the paper over, and hit the center bulb with a hammer so that the bulb is broken. The Xenon gas that escapes is not poisonous and returns to the atmosphere. The remains of the LampSnap can be disposed of as broken glass, metal and plastic.

9.4 Replacing the air filter

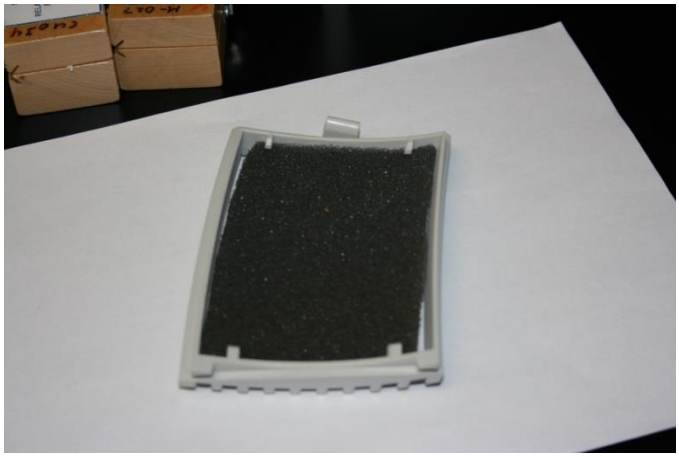
Replace the air filter once per year.

1. Press down on the latch at the top of the air filter cover and lift the cover away from the Duetta™.

2. Remove the old filter from the cover.



3. Install a new filter (PN 5700006697) using the tabs.



4. Reinstall the air filter cover onto the Duetta™.

5. Secure the cover by pushing the top back in.

6. The old air filter can be disposed as normal trash.

10 Frequently Asked Questions

What is A-TEEM™?

A-TEEM™ stands for Absorbance-Transmittance Excitation Emission Matrix. A fluorescence EEM (excitation emission matrix) scans the wavelength range at which a solution or sample is excited and measures the emission spectrum at each scanned excitation, giving a 3D plot of excitation vs. emission vs. intensity. An A-TEEM™ is the method for which the absorbance/%transmittance spectra are also collected for the same sample and these are applied to the EEM to correct for inner-filter effects (IFE) to give a more accurate 3D contour of the fluorescence profile. Inner-filter effects are explained further in Section 1 of the Duetta manual (Introduction), but without IFE correction, spectra, including fluorescence EEMs may be inaccurate due to reabsorption from the sample and inadequate illumination of the sample due to high concentrations. Duetta is unique in that the absorbance and fluorescence EEM can be measured on the same instrument without moving the sample, and IFE corrections are automatically applied with EzSpec software.

Is EzSpec software compatible with Windows 10?

Yes. EzSpec is recommended for use with Windows10 operating systems.

Do spectral correction factors have to be loaded into the software?

No. Correction files are already saved on the instrument in the Duetta USB drive internal to the instrument. These correction factors are measured at the factory, in production of the instrument and saved on the instrument memory itself. The file is saved as corrections.ezspec_data and this file is also saved within every data folder created for measured data.

How do I know if the Duetta is wavelength calibrated?

When in the Method Setup, in the Help menu, Service Report, Run Test Report. This will lead you through a series of two tests: 1.) first check the excitation calibration using the Autocal xenon lamp output on the Reference detector (after 1 hour of instrument warm up) and 2.) to check the emission calibration and throughput (water Raman test).

At what wavelength should I expect the xenon lamp peak as shown on the Ref detector?

Xenon lines will depend on temperature and pressure. Not all xenon lamp bulbs are the same. Different internal temperature and pressure in different types of xenon arc lamp bulbs will cause some of the xenon line peak locations to vary slightly. The peak at 473.4 nm is used because this peak does not change significantly with temperature or pressure {Ref: M. Kettlitz, et.al. J. Quant. Spectrosc. Radiat. Transfer (1985) Vol 34, No. 3, pp 275-282.}. The peak at around 467-469 nm will also appear to shift with increased band pass because multiple lines from the xenon lamp are not resolved well, causing multiple lines to lump together into one peak. In the Autocal test, a 2 nm excitation band pass is used to resolve the peak at 473.4 nm properly. On some other HORIBA fluorometers such as the FluoroMax and Fluorolog instruments, the xenon lamp is a different bulb design, so the 467 peak is routinely at 467.1 nm while on the Duetta, it is usually closer to 468.5 nm. This is the reason we use the 473.4 nm peak to calibrate correctly instead of the peak close to 467-469 nm.

What do I do if the water Raman peak is incorrect?

First, calibrate the excitation monochromator using the Autocal function in the Service Report. After the excitation monochromator is calibrated using the 473.4 nm xenon peak, the water Raman peak should appear in the correct wavelength location. Remember that the emission optics on Duetta are a fixed spectrograph with CCD so if the wavelength has moved, there is most likely a mechanical issue. In this case, please contact your local HORIBA representative and/or Service department.

How can I see if the excitation light is hitting my sample?

Preview mode is accessible in the Acquire menu. Go into Preview by clicking the Preview button. Set the excitation wavelength to 550 nm (visible green light) and move the shutter to the open position. Open the Duetta lid to view if the green light is aligned to the sample position. Use caution when opening the Duetta lid so as not to shine or reflect light into your eyes. The use of laser safety goggles is recommended. If using a SampleSnap-UNI, adjust the sample angle and optimize to maximize emission signal.

Can I run the instrument with the lid open?

Yes, any method can be acquired with the lid open. The status at the bottom of the graph (in the Acquire menu) will indicate “Door Open” to notify any user, but will not prevent a method from successfully acquiring data.

At what signal will the CCD detector reach saturation?

The CCD detector saturates at signals higher than 65535 counts. While the CCD is linear up to this value, EzSpec software will give the user a warning about detector saturation if signals of 50,000 counts or higher are measured in an acquisition. To reduce risk of CCD detector saturation, use shorter integration time, smaller slit width (band pass) or neutral density filters which are available in the “Other” section of each method setup.

Why does the use of the neutral density (ND) filter in Duetta not reduce the final corrected spectral intensity?

The neutral density filters in the excitation optical path are reducing the amount of excitation light reaching the sample in the sample compartment, which in turn, reduces the number of photons that reach the emission detector (CCD). The final corrected spectrum that is shown in the data is corrected for the signal measured on the Reference detector (Ref). The Ref detector is a photodiode that measures the signal output from the xenon lamp and is used to correct for any fluctuations in lamp intensity. The signal on the Ref detector is directly related to the intensity of light reaching the sample. Each detector is also multiplied by correction factors, and dark noise is subtracted, but these have little effect between use of ND filters and not. So, for the sake of simplicity, the signal in the final graph is CCD/Ref. If, for example, there are 10 photons detected on the Ref detector coming from the xenon lamp, and this in turn gives a value of 10,000 counts on the CCD from the sample emission, the final graph would show 10,000 counts/10 µamp = 1,000 counts/µamp.

Now, when there is a neutral density filter with OD 1.0 (10% transmission) in the excitation light path, the value on the Ref detector (related directly to the excitation light hitting the sample) would be 1 µamp and the CCD would measure only 1,000 counts. With less light reaching the sample, there are less photons emitted, and reaching the CCD. The final graph would then show 1,000 counts/1 µamp = 1,000 counts/µamp. Because of this ratio, the signal on the graph remains linear with the use of ND filters.

(For more information on complete detector correction, see the Duetta manual – section 2.5, Overview of Analysis of Samples)

How can I overlay two or more spectra in the same plot?

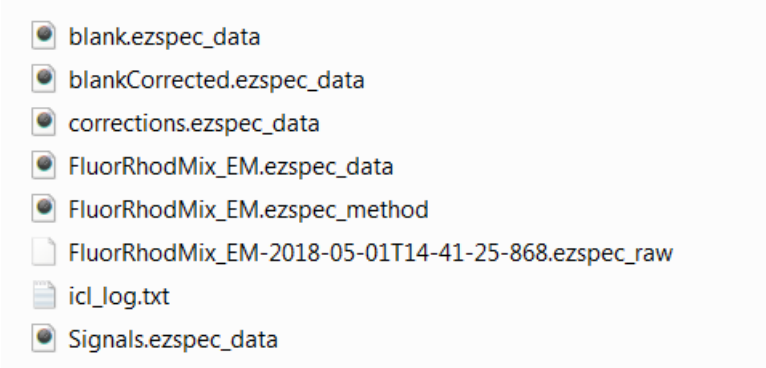
Click on the thumbnail of the graph to which traces will be added. This is now the active graph. Then, right click in the graph containing the traces that will be added to the active graph. Click on the "+" icon to add those traces to the active graph. Use the legend to hide or view traces of interest.

Why do I see so many different traces in the live display while the data is being acquired?

In the live acquisition, the graph will show all of the raw detector signals including the CCD, Ref, Abs detectors. The traces will be separate for blank/sample and if scanning an excitation/absorbance range, there will be different traces for each order-sorting filter position. Order-sorting filters switch positions/filters at 700 nm and at 440 nm. If, for example, you are acquiring an excitation spectrum and scanning the entire wavelength range (250-1000 nm), there will be three traces each in the live acquisition graph (CCD, Ref, Abs). Each of these detectors will have a separate trace for each filter position: one without an order-sorting filter in the range 250-440 nm, one with a 410LP filter in the range 440-700 nm and one with a 525LP filter in the range 700-1000 nm. These traces will be doubled if there is a blank measured (sample plus blank).

There are many files saved with my data. What are they?

There are several files always saved with the final data file (.ezspec_data). Below is a description of what each file contains for a data set called by the user “FluorRhodMix.” This data is an emission spectrum with blank subtraction enabled. FluorRhodMix_EM.ezspec_data is the fully corrected and blank-subtracted spectrum shown in EzSpec software at the end of the acquisition. Descriptions for the other files are in detail below:



blank.ezspec_data: Individual detector signal of the blank measured (or loaded blank file). Use this file for loading a blank into another method.

blankCorrected.ezspec_data: The corrected spectrum of the blank for dark noise and detector efficiencies. Open to compare the blank spectrum to a sample spectrum, inspect for contamination of a blank, or do further analysis (i.e. manual blank subtraction from a sample measured without a blank).

corrections.ezspec_data: Spectral correction factors for both excitation detector and optics (XCorrect) and emission detector and optics (MCorrect). These are measured in the production of the instrument and saved on the Duetta internal USB drive.

FluorRhodMix_EM.ezspec_data: This is the fully corrected data from the acquisition and the data displayed in the graph in EzSpec software at the end of the acquisition. The file is named the “Data Name” with an extension indicating the method type (i.e. EM for emission spectrum).

FluorRhodMix_EM.ezspec_method: The method file used to acquire the data in the same folder. This file can be loaded into EzSpec to repeat the method acquisition with the same parameters.

FluorRhodMix_EM-2018-05-01T14-41-25-868.ezspec_raw: This is the raw detector signal file that contains a single trace for all detectors with variations of dark, blank, sample, temperature, sample position, and excitation order sorting filter position. Open this file from the File, Open, Raw Data menu. The file is timestamped for data traceability.

icl_log.txt: This is the log file recording every step of the acquisition by the hardware. It is used for troubleshooting in the event of an error.

Signals.ezspec_data: This is the file showing each detector signal over the entire spectral range of the acquisition, as well as the correction factor values used for that particular acquisition. There are not separate traces for each filter position as in the .ezspec_raw data file, so the Signals file is a copy of the raw data, only easier to view.

How can I see the raw detector signals from an experiment measured on Duetta?

For every data set that is collected, there is a file saved called “Signals.ezspec_data” in the time-stamped data folder. EzSpec can open this file and each signal is plotted/reported separately for Sample/Blank/Dark/polarization angle/etc. for each detector CCD/Ref/Abs. For more detailed detector signal information for methods that scan multiple excitation/absorbance wavelengths (or data from any method), the “raw” data file can be opened from the File, Open, Data, Raw Data. The difference in this file is that each signal as above is reported separately if a different excitation filter position is used. This is typically not useful except for specifying data traceability to meet compliance with some regulatory agencies. It is easier to view individual detector signals using the Signals.ezspec_data file. See the section on “Overview of analysis of samples” in Section 2.5 of the manual for more information on how the detector signals are processed to give final, corrected spectra and results.

Is Duetta compatible with microcuvettes?

Yes. Duetta will work with microcuvettes or cuvettes with small windows (3 mm or larger). To improve reproducibility in the light path when working with microcuvettes, be sure to push the tray all the way forward towards the back of Duetta (towards the emission optics) when changing the cuvette so that the tray and cuvette positions are set the same each time. When ordering/using any cuvettes or microcuvettes, make sure that the Z-height is 15 mm to match the optical path of Duetta. Microcuvettes may not be compatible with the 4-position turret.

Can I upgrade my Duetta to a Duetta-Bio?

No. The Duetta-Bio, which has polarizers in the excitation and emission filter wheels, must be ordered as a separate instrument. A Duetta without polarizers cannot be updated to be a Duetta-Bio (with polarizers).

Can I add another light source to Duetta?

The LampSnap xenon lamp cartridge is specifically designed and pre-aligned to fit the Duetta and Duetta-Bio. There are currently no accessories to add a third-party light source for use in Duetta or Duetta-Bio.

What are Detector Accumulations?

Detector accumulations is a setting in each method setup to measure the fluorescence signal on the CCD detector multiple times and sum them together to get better signal to noise ratio. This is especially useful for measurement of samples with low fluorescence signal, requiring longer integration times. An integration time can be set and then multiple accumulations measures the signal with longer total effective integration without saturating the CCD detector.

How can I improve the sensitivity of my fluorescence spectrum measured by Duetta?

There are several ways to improve sensitivity, or limit of detection for fluorescence (and absorbance) measurements. When measuring a sample with low concentration or low intensity

signal, the background of the spectrum must be subtracted as cleanly and completely as possible. Using a closely matching blank (clean solvent and clean cuvette) to perform a blank subtraction, helps remove background immensely. For dilutions, it is a good idea to measure the blank first, and then pipette the sample into the blank to create the fluorescent molecule solution concentration of interest. Then, measure the sample spectrum. Using clean solvent, clean cuvette, the same cuvette, and not moving the cuvette between measurements, all help create a better match between a sample and a blank.

Another way to measure lower concentration samples and improve performance of the instrument is to use optical filters. While Duetta does have order sorting filters on the excitation light path and on the emission light path (to eliminate effects from 2nd order excitation light), the use of additional filters can improve stray light rejection of the instrument.

For example, if the fluorescence emission spectrum is measured using 400 nm excitation light, and the emission peak is around 500 nm, using a 400 nm band pass filter on the excitation light path will help reduce the amount of stray light coming from the excitation monochromator (e.g. other wavelengths getting through with the 400 nm light exciting the sample). This will reduce the background in the emission spectrum due to that stray light. Similarly, a 450 nm long pass filter on the emission side will reduce stray light getting to the emission monochromator and also reduce the background, making it easier to resolve peaks close to the baseline. While the stray light from 400 nm excitation will be blocked in this case, the emission at wavelengths longer than 450 nm will be let through, to allow measurement of the 500 nm fluorescence emission peak.

To reduce noise in such a low signal measurement, the use of longer integration times on the CCD detector (or absorbance detector for absorbance measurements) and summed accumulations of the CCD detector (effectively increasing the integration time as well) will help reduce noise and give a cleaner spectrum when signal intensity is low.

For highly scattering samples, such as solids (films, powders, etc.), optical filters are helpful as well as changing the angle of the sample in the SampleSnap-UNI holder to 30 or 60 degrees. This method allows reflection of the direct 45-degree excitation light away from the emission path, but allows detection of photoluminescence, which is emitted in all directions. Playing with the sample angle may improve the resolution of the spectrum and reduce background from the excitation light in this way.

In what absorbance range is the inner-filter effect correction valid?

The concentration range for which it is valid to use IFE correction on a fluorescence spectrum/intensity values is dependent on the sample itself. At high concentrations and absorbance, the fluorescence from the sample may interfere in the absorbance measurement and the absorbance values acquired will be less accurate. The absorbance and concentration at which this occurs very much depends on the extinction coefficient and the quantum yield of the sample itself. At lower concentrations, a fluorescent molecule with high extinction and high quantum yield will emit more fluorescence and interfere with the absorbance values than a molecule or sample with low extinction and low fluorescence quantum yield. For a high extinction/high quantum yield molecule such as fluorescein, the upper limit of absorbance is approximately 0.8-1.0 absorbance units depending on the solvent and temperature of the solution.

For some more useful information on Fluorescence Spectroscopy, please refer to the tutorial on the HORIBA website:

https://www.horiba.com/en_en/technology/measurement-and-control-techniques/molecular-spectroscopy/what-is-fluorescence-spectroscopy/

11 Troubleshooting

11.1 Lamp will not ignite

If the lamp will not ignite (button on front of the Duetta™ remains flashing instead of steady), there are several possible reasons.

First, there is a magnetic safety interlock on the lamp cartridge panel. Please check that the panel door is closed properly before trying to ignite the lamp.

Second, the front button on the Duetta™ should not be powered on for at least 20 seconds after the main power switch on the back of the Duetta™ is turned on. Turn the back of the Duetta™ off. Wait at least 20 seconds. Then, try igniting the lamp by powering the front button (hold for 1-3 seconds) once more.

Another reason that the lamp may not ignite properly is that there is a 5-minute time out to prevent the instrument from powering up after the lamp has been powered off. The Duetta™ will not be able to be powered back on and the lamp will not be able to reignite for 5 minutes after powering off for protection of the lamp and instrument. If the lamp was only just powered off, wait at least 5 minutes for the lamp to cool and then retry powering the Duetta™ which will ignite the lamp again.

⚠ Warning: Igniting a hot lamp can cause severe damage to the instrument!

Finally, a lamp may not ignite if there is an issue with the lamp or if the lamp has been used for many hours past the recommended usage. The xenon lamp on the Duetta™ has recommended use for 800 hours or less. If the lamp has more than 800 hours of use (as indicated by the Lamp menu in EzSpec™ software), replace the lamp cartridge with a new one. If the lamp is broken or there is an issue with the lamp, replace the lamp cartridge with a new one. New lamp cartridges can be ordered from your local HORIBA representative.

11.2 Duetta will not turn on

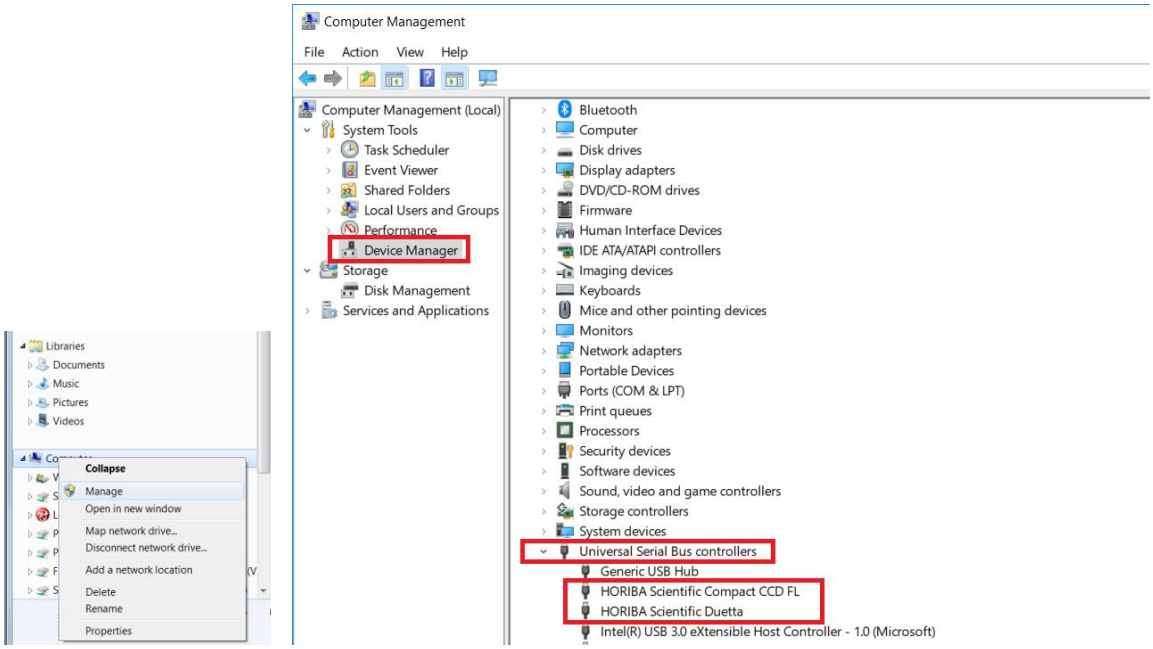
If the Duetta™ will not power on properly, first check that the power cable on the back of the instrument is plugged in properly and the power button on the back is turned on. Wait 20 seconds after powering this main power switch on before pressing the power button on the front of the Duetta™. Hold the button for 1-3 seconds. If the button is held for less than 1 second or more than 3 seconds, the Duetta™ will not power on. Try again for between 1-3 seconds. If the Duetta™ has only recently been powered down (less than five minutes before turning the power back on), see “Lamp will not ignite” section above.

If the Duetta™ will still not power on properly, turn off the mains power switch on the back of the Duetta™. Wait at least 30 seconds and power it back on. Wait at least 20 more seconds and then power the Duetta™ from the front switch.

If the Duetta™ will still not power on properly, please contact your HORIBA service representative.

11.3 Software will not initialize the Duetta™

If the USB cable is not plugged into the Duetta™ and/or the software is not installed properly, the power button on the front of the Duetta™ will be orange instead of blue. Orange means that the Duetta™ is not connected to a PC properly. To connect properly and change the light to blue, first check that the USB cable is secure on both the PC or laptop and the Duetta™ itself. Then, check that the Duetta™ is a recognized device in the PC device manager. To see this, go to Windows Explorer. Right click on Computer (Win7) or My PC (Win10) and go to Manage. Then, click on Device Manager in the left toolbar. There should be two devices in the USB controller section: HORIBA Scientific Compact CCD FL and HORIBA Scientific Duetta™. If both of these are not recognized or there is a warning for any of them, please reinstall the software using the setup.exe installer program found on the USB stick provided with the instrument. The installer will reinstall both the software and the device drivers if not done properly before.



After the software is installed and the USB cable is secure, note the recognition of the devices and that the front button is powered on and blue. If not, please contact your HORIBA service representative.

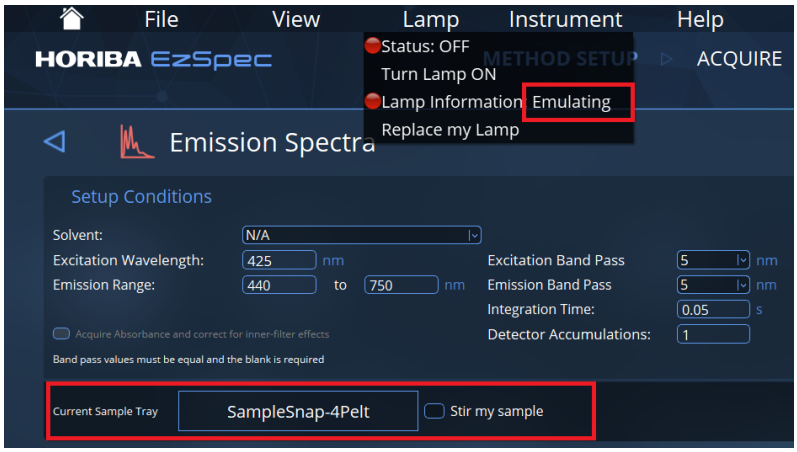
11.4 Power Cycling Duetta from Back Panel

When power cycling the Duetta using the back panel power switch, be sure to leave the switch in the OFF position for at least 20s before switching it back to the ON position. If the internal processor is not properly powered OFF, it will not boot up properly. The instrument will not operate if the processor does not boot up correctly. The ONLY way to recover from this failed operational state is to properly power cycle the instrument.

11.5 Sample Tray is not recognized by EzSpec

The SampleSnap sample trays are recognized by EzSpec™ software using an electronic board on the tray itself. The contacts on the bottom of the tray should connect to the contacts on the inside back of the Duetta™ sample compartment (see the photographs on the next page). If the SampleSnap is not recognized by the software, in the Method Setup window of EzSpec™, the "Current Sample Tray" will indicate "No tray detected."

If the Duetta™ is not connected and EzSpec™ does not recognize an instrument at all, the Current Sample Tray will run in emulation and indicate "SampleSnap-4Pelt" and the Lamp Information will indicate: Emulating.

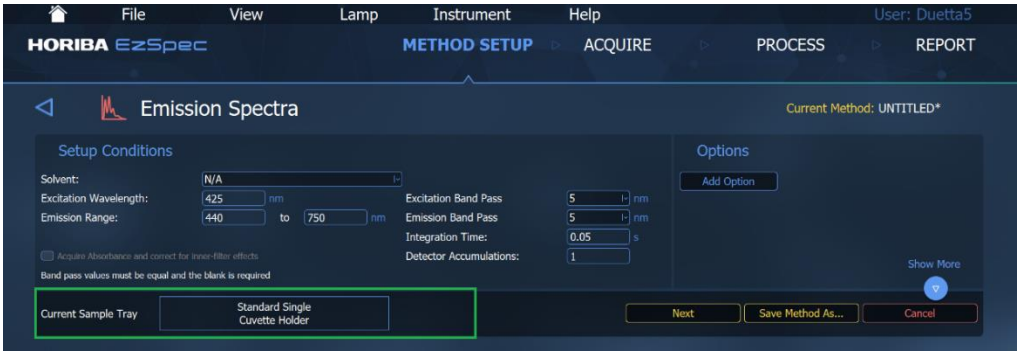


Check that the tray is seated properly in the sample compartment, pushing the tab with the electrical contacts as far in as possible so that the contacts on the tray are touching the contacts on the inside of the Duetta™.

Bottom view of a gap bed drawer. On the bottom of each gap bed drawer there is a row of electronic contacts.

These contacts fit into a socket with complementary contacts in the back of the sample compartment gap-bed.

The image below is the connected SampleSnap standard Cuvette holder.



11.6 Wavelength Calibration is not correct

To test the wavelength calibration and optical throughput of the Duetta™, the Service Report can be run through the Help menu in EzSpec™ software. (See sections 6.3.8, 6.3.8.5). If the water Raman is not within 1 nm of 397 nm, please make sure that the Duetta™ has been turned on and warmed up for at least 1 hour before performing this test. The calibration may shift 1-2 nm during this warm up period. Also, make sure to use a clean quartz cuvette (not plastic) with ultrapure water. A scratched or contaminated cuvette and/or dirty water will cause the water Raman peak to shift in both wavelength and intensity. In this case, the calibration may appear to be incorrect, but a clean quartz cuvette and ultrapure water should be used to ensure proper measurement of the calibration. Clean the quartz cuvette with dilute nitric acid (anywhere from 10% to fuming nitric acid (50% in water)) and then with soapy water. After this, rinse the cuvette with ultrapure

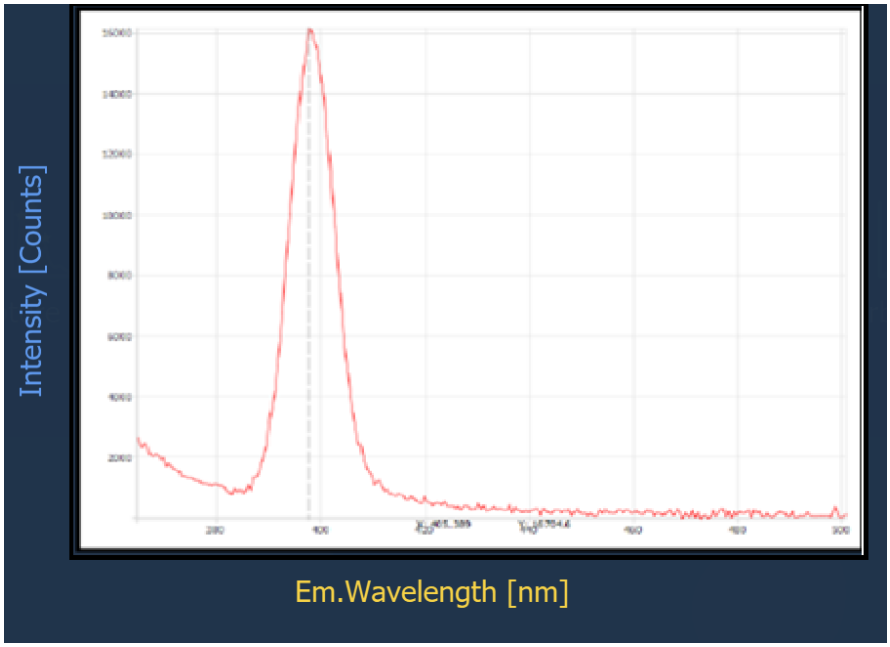
water several times before filling the cuvette with ultrapure water for the water Raman measurement.



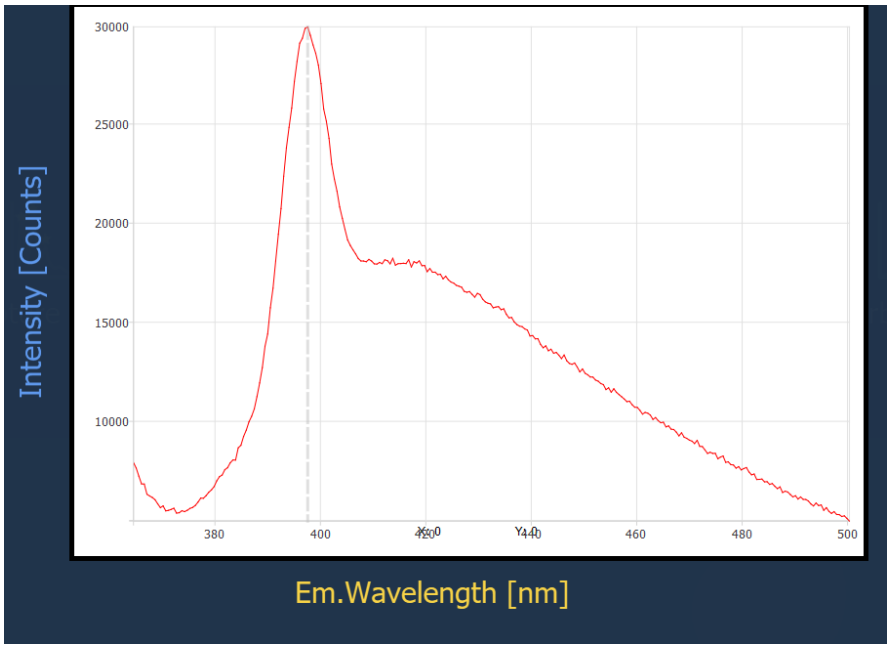
⚠ Warning: Use personal protective equipment and caution when handling nitric acid. Refer to the MSDS sheet.

If the water Raman peak is more than 10 nm away from 397 nm, there is most likely a mechanical issue with the grating, which will warrant repair. Wavelength calibration is stored on the firmware, in a permanent configuration file on the Duetta™ itself. If the Duetta™ main board internal to the instrument has been corrupted (whether electronically or physically damaged), the calibration may be incorrect as well. In either of these cases, please contact your local HORIBA service representative to further investigate.

Clean water/clean cuvette:



Dirty water/scratched cuvette:



11.7 Software closes when opening Data or a Method

OpenGL graphics must be installed and compatible on the PC using EzSpec software. Make sure that the OpenGL graphics drivers are the most up-to-date version of OpenGL. If more than one graphics card is installed on the computer, make sure that the one using OpenGL is being used to run the EzSpec software application.

11.8 Correction files not found

The Correction factor files must be on the USB drive in an unhidden folder and in the correct format. If the correction factor file (corrections.ezspec_data) is not found on the USB drive internal to Duetta, it can be found 1.) on the USB thumb drive that came with Duetta and 2.) in any previously measured data folder. A backup copy of the most recent corrections.ezspec_data file used is always saved to the PC in the directory C:\Program Data\HORIBA Scientific\EzSpec\instrument\corrections.ezspec_data along with a file called id.txt which can be read and used in case there isn't a corrections file properly found on the USB drive internal to Duetta. For additional help in finding/using the corrections file, please contact the HORIBA Service department.

12 Components & Accessories

Accessories for the Duetta™ can be added to obtain optimum results for a variety of applications. The following list represents all the accessories and components, in alphabetical order, available for the Duetta™ spectrofluorometers. A brief description of each is included. Like the list presented below, the descriptions that follow are alphabetized, except where logical order dictates otherwise.

For additional information or product literature on any of these items, contact your local Sales Representative.

Itemized List of Duetta™ Accessories

12.1 SampleSnap-1Pelt Single Position Peltier

The SampleSnap-1Pelt is a Peltier temperature-controlled, single-position cuvette holder. It contains a variable speed magnetic stirrer for the cuvette, and dry gas ports under each of the 4 exposed cuvette windows. An opaque cover is provided to permit control of the gaseous environment above the cuvette and to prevent light leakage into the spectrometer. A variety of optical slits are provided to limit excitation or emitted light.

The yellow pin at the back right of the cuvette holder is the cuvette lifter. When you place a standard cuvette in the cuvette holder, you cannot remove it with your fingers. You need to pull the yellow pin up and then you can grab the cuvette.

12.1.1 Installation and removal

See the instructions for the SampleSnap-4Pelt on the next three pages for those parts that apply to the SampleSnap-1Pelt.

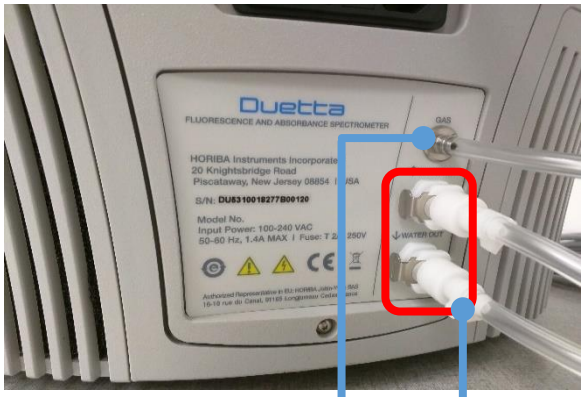
12.2 SampleSnap-4Pelt 4 Position Turret Peltier

The SampleSnap-4Pelt is a Peltier temperature-controlled, four-position cuvette holder. It contains a stepping motor drive for rapid position changes, variable speed magnetic stirring for each cuvette, and dry gas ports under each of the 12 exposed cuvette windows. An opaque cover is provided to permit control of the gaseous environment above the cuvettes and to prevent light leakage into the spectrometer. A variety of optical slits are provided to limit excitation or emitted light. Because of the thicker block on the turret cuvette holders, microcuvettes can be used but may show some signal variability between positions. It is not recommended to use microcells with windows smaller than 5 mm in the SampleSnap-4Pelt turret.

12.2.1 Installation

Internal coolant line quick fit connectors

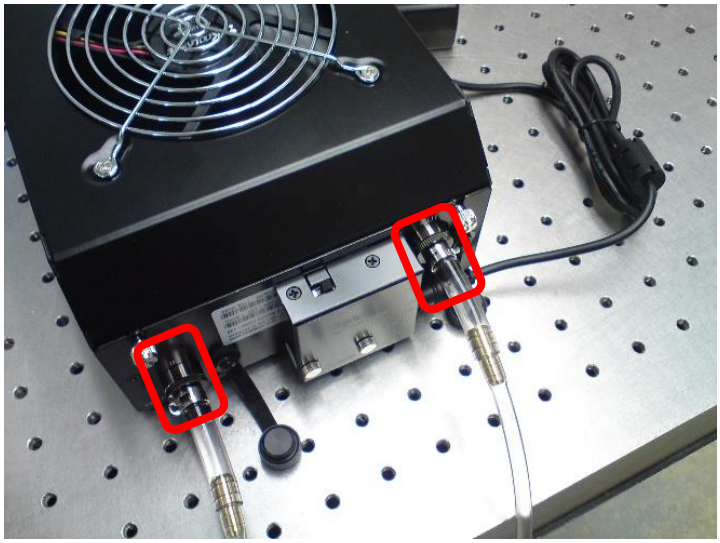
Gas hose barb



External GAS connector

WATER IN and WATER OUT quick connect connectors

1. Connect the internal coolant lines on the left side of the sample compartment by pushing the tubing connectors into the quick fit connectors. It does not matter which line is In or Out.
2. If dry gas is required, push the plastic hose onto the Gas hose barb inside the Duetta™ and an external gas hose to the external GAS connector.
3. The external coolant hoses are cut to length and connected to white adapters at one end and to black threaded adapters on the other end. Insert and push the white adapters into the white WATER IN and WATER OUT quick-connect connectors on the back panel of the Duetta™. It does not matter which line is IN or OUT.
4. Screw the black threaded adapters into the IN and OUT connectors on the back of the Koolance EXT-440CU. See pages 6 – 7 of the 18 of the EXT-440 User's Manual.



5. Align the red dot on the single round end of the cable with the red dot on the upper edge of the 26-pin AUX 3 connector on the back panel of the Duetta and then push the cable end in until it stops.

6. Connect the other end(s) of the cable to the connector(s) on the back of the TC1 Temperature Controller (one connector for a SampleSnap-1Pelt, and two connectors for a SampleSnap-4Pelt).

12.2.2 Filling the Koolance cooling system reservoir

The supplied Koolance cooling system EXT-440CU is a different model than described in the manufacturer's EXT-440 manual. The supplied cooling system does not come with temperature sensors. Please skip the parts in the manual pertaining to them.

1. Once all the coolant lines have been connected, the cooling system can be filled with coolant. The fill port is located on top of the reservoir.
2. Place the cooling system above the level of the Duetta.
3. Remove the large slot-headed screw with a screwdriver or large coin.
4. Slowly pour the coolant into the reservoir. The coolant should be filled to about 6 mm (1/4 inch) from the top of the reservoir. **Do not overfill**.
5. Replace the fill port on the reservoir. **Do not overtighten the fill port**.

6. The Koolance cooling system needs to be powered ON to assist in the filling process. Press the SET button on the front panel of the EXT-440CU cooling system to display “P” mode above it. Press the Decrease or Increase buttons to set the pump speed to display “4”. During this process, the cooling system often needs to be tilted gently to allow air to escape from the pump. Until the pump is “burped” in this manner, it can cavitate and there will be no circulation. The reservoir level will decrease during this process. Remove the port cap and add more liquid. Refer to the EXT-440 User’s Manual, page 19.

For more information on using the Koolance cooling system, see the EXT-440 User’s Manual, particularly pages 6-7, 15, 17-18, and 20-23.

12.2.3 Operation

Start the Koolance circulator, then turn on the TC-1 controller with USB connected to the laptop or PC. Initialize the software after the tray, circulator, and TC-1 controller are all connected and powered on.

If the SampleSnap-4Pelt, SampleSnap-1Pelt, or SampleSnap-WJ trays are removed from Duetta™ and then reinserted after the software is already running, use the following procedure: First reinsert/reconnect the tray. Go to the **Instrument** menu and click on **Initialize**, then check the **Method Setup** windows to see that the tray is correctly connected and identified by EzSpec. If it is not, check the connections and retry this procedure.

12.2.4 Removal

Turn off the power to the TC1 Temperature Controller, Duetta, and Koolance cooling system before removing any cables, water or gas lines.

To disconnect the water lines push in the metal tabs on the sides of the quick fit connectors.

To disconnect the gas lines, pull the plastic hose off the hose barb.

To remove the cable from the AUX 3 connector on the back panel of the Duetta, pull the grooved slider back and then pull the cable away from the Duetta.

12.3 SampleSnap-WJ Stirrer and Water Cooled Sample Holder

The SampleSnap-WJ has a water jacket with external hose connections that can be connected to an external water bath for temperature control. Gas purging can be used to keep the cuvette windows from fogging when circulating low temperature water. It also contains a magnetic stirrer to stir a flea in the cuvette.

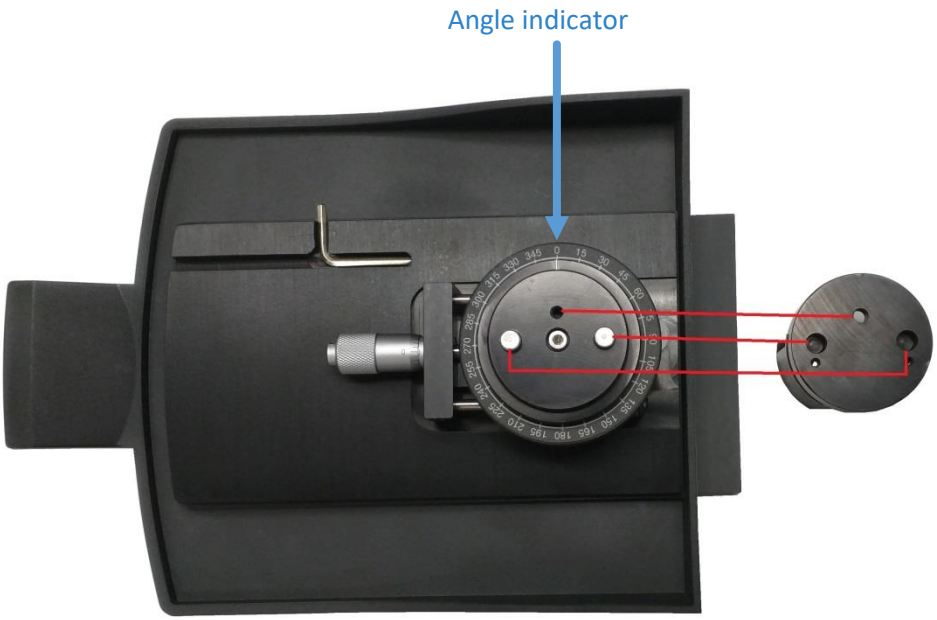
12.3.1 Installation

See the instructions for the SampleSnap-4Pelt on the previous three pages for those parts that apply to the connection and removal of coolant and dry gas lines.

12.4 SampleSnap-UNI Universal Sample Holder Tray

The Universal Sample Holder tray has a continuous 360° rotation base on a single axis translation stage. The translation axis is perpendicular to the excitation beam. This tray and the sample holders that mount on top of it allow front face illumination of solid, powder, and liquid samples. The sample holder can rotated to any angle to avoid direct reflection of the excitation beam onto the CCD detector. The front surface of the sample is aligned with the rotation axis.

The picture below has a SampleSnap-FF Front Face Cuvette Holder upside down to show the alignment of the holes in the base of a sample holder with the two pins on the Universal Sample Holder. Place a sample holder over the Universal Sample Holder so the holes in the sample holder base fits over the pins. Then use the Allen key to screw the supplied cap socket screw through the hole in the sample holder base plate into the Universal Sample Holder.



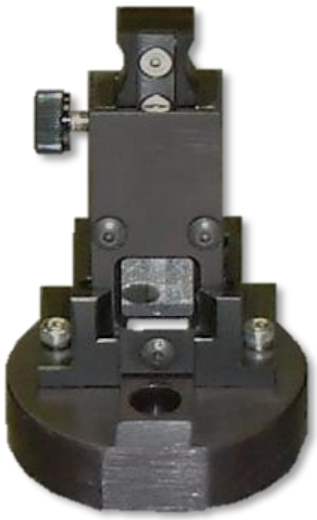
To rotate a sample, turn the sample holder base plate by hand. In this picture the angle indicator points to 0°.

When the dial on the translation stage handle reads 0.0, the center of the sample holder front face and the rotation axis are in line with the center of the excitation beam. Rotate the translation stage handle to move the center of the front face away from the axis of the excitation beam.

12.5 SampleSnap-SS Solid Sample Holder (films or slides)

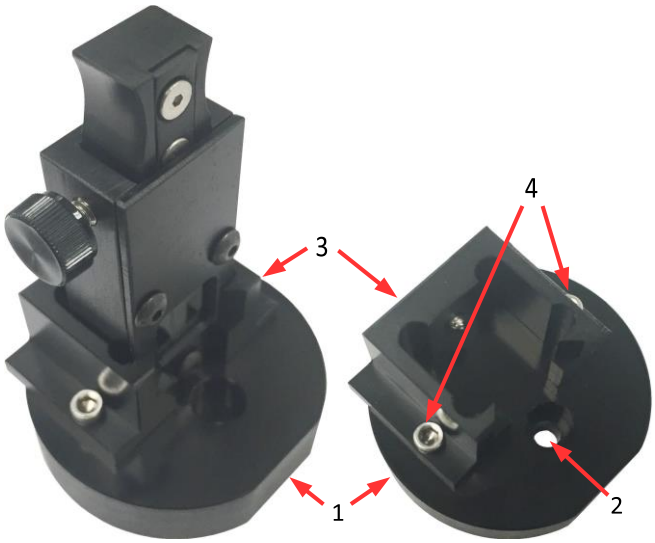
The SampleSnap-SS is an accessory that mounts on top of the SampleSnap-UNI Universal Sample Holder tray. The Solid Sample Holder can hold films or slides between the two plates with openings in both plates to allow transmission through the sample. Turn the thumbscrew on the back of the Solid Sample Holder to move the back plate and open the gap between the plates to allow a sample to be inserted. The front surface of the sample should be placed against the farther plate from the thumbscrew. Then turn the thumbscrew to close the gap and hold the sample securely.

12.6 SampleSnap-PWD Powdered Sample Holder



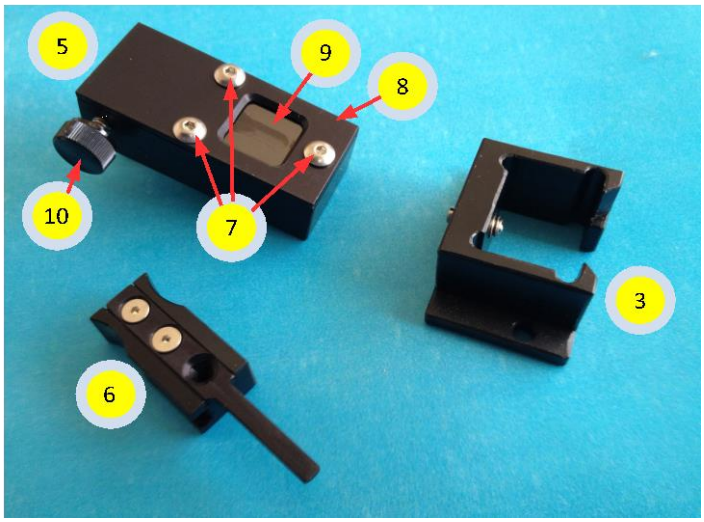
The SampleSnap-PWD is an accessory that mounts on top of the SampleSnap-UNI Universal Sample Holder tray. The Powdered Sample Holder holds powder against a glass cover slip window. It must be handled with care so to not break the window and spill the powder contents. Cover slips are 12 mm x 12 mm square with 0.16 mm thickness and a tolerance of ±0.01mm to fit into the window slot of the powder holder

The powder holder subassembly is made of Delrin®. See https://www.calpaclab.com/acetal-polyoxymethylene-chemical-compatibility-chart/ for compounds or solvents that may react with Delrin® during use or cleaning.



12.6.1 Installation and use instructions

The powder holder base (1) is mounted with one screw through the hole (2) on top of the universal base. The powder holder mount (3) is mounted with two screws (4) on top of the powder holder base (1).



The powder holder subassembly (5) is an insert that slides into the holder mount (3).

The packing insert (6) slides into the inside of the powder holder subassembly (5) to pack and keep the powder in place. Use a 0.050 inch Allen wrench to remove the screws to change the length of the tamping rod, then put the screws back in the respective holes.

Three screws (7) hold the cover plate (8) in place to hold the cover slip window (9) (Windows For SampleSnap-PWD, K-210) in place. Underneath the window there is a groove / slot to put the powder in.

A thumbscrew (10) is used to hold the packing insert (6) in place inside the powder holder subassembly (5).

You should remove the powder holder subassembly (5) from the holder mount (3) when installing or removing the Powdered Sample Holder from the Universal Sample Holder tray. With the packing insert (6) in the powder holder subassembly (5), the top of the packing insert (6) may be too high to use the supplied Allen key to tighten the screw through the hole (2) in the powder holder base (1) into the universal base.

12.6.1.1 To load a powder sample:

1. Remove the powder holder subassembly (5) from the holder mount (3). This is to prevent powder from spilling on the rest of the sample holder when loading powder.
2. Loosen the thumbscrew (10).
3. Remove the tamping insert (6) from the powder holder subassembly (5).

4. Use a curled spatula to load powder into the throat of the powder holder subassembly (5). Use at least the minimum powder volume of 22 mm^3. Otherwise the powder may be out of the excitation light beam.
5. Carefully insert the tamping insert (6) into the throat of the powder holder subassembly (5).
6. Gently tap on the top of the tamping insert (6) to pack the powder. Do NOT use force to push the tamping insert onto the powder, as this will likely break the window.
7. Remove the tamping insert (6) from the throat of the powder holder subassembly (5).
8. Repeat steps 4 to 7 above as necessary to load more powder.
9. Insert the powder holder subassembly (5) into the holder mount (3).

12.6.1.2 Cleaning a powdered sample holder

1. If the powder sample is 'non-sticky', remove the container part, just pour the powder out and while inverted, and tap it against the bench to get rid of residual powder particles.
2. If the holder requires a more thorough cleaning, do the following:
3. With a 1/16 inch Allen key (hex wrench) remove the 3 screws (7) that hold the cover plate (8) in place.
4. Be very careful with the quartz cover slip window (9), which is fragile. Place the window in a small glass container and soak in solvent appropriate to cleaning the powder (e.g. water, alcohol, acetone). After soaking, gently remove the window and place on a soft tissue paper to dry.
5. The Delrin® parts of the powder container can be washed with a solvent of choice and dried.
6. When all parts are dry, reassemble the container part by gently placing the quartz window (9) in the square groove around the top edge of the open part of the container and attaching the cover plate (8) with the three screws (7).

12.6.1.3 Specifications of the powdered sample holder

Powder Sample Holder component	Dimension/volume
Quartz window	12.7 × 12.7 mm (0.50 × 0.50 in)
Cover plate opening	10.0 × 10.0 mm (0.39 × 0.39 in)
Powder cavity (visible straight portion) (W × D × H)	2.90 × 1.23 × 6.20 mm (0.114 × 0.048 × 0.244 in)
Minimum powder	22 mm^3

12.7 SampleSnap-FF Front Face Cuvette Holder

The SampleSnap-FF is an accessory that mounts on top of the SampleSnap-UNI Universal Sample Holder tray. The SampleSnap-FF holds a standard cuvette for front face illumination of liquid samples.

12.8 Stop Flow Accessory

Complete stop flow accessory. The stopped flow accessory is used to rapidly mix small volumes of two (or more) different chemicals in a cuvette, quickly stop the flow of chemicals to the cuvette and monitor the resulting chemical reaction via optical means. In some instances, the chemical reaction will affect the luminescence and this optical signal can be monitored using a fluorometer. The primary experimental interest is in the rate of the chemical reaction following the mixing in the cuvette, in addition to the spectral changes of luminescence [455-0048]. Compressor not included.

12.9 Cuvettes and Cells

- Microsense Microliter Fluorescence Sample Volume Accessory – Measures 1-5 µL of sample. Fits into standard 1 cm cuvette holders. Can be used for both fluorescence spectra and TCSPC fluorescence lifetimes measurements. Operational over full spectral range of instruments. Includes one reflector cap. Not suitable for absorbance.
- 10mm Quartz Cuvette 10mm quartz cuvette.
- Micro Cuvette 100-130µL (requires K-153 or K-153A).
- Semi Micro Cuvette Max volume 1.4 ml, min volume 400µL with Peltier sample holder. No adaptor required.
- Micro Cuvette Insert (012-0001) For single cuvette holder.
- Micro Cuvette Insert (012-0001) For Peltier holder.

12.10 Temperature Bath

External circulating temperature control, sample chamber mounted. Range -25 to 150 C. Sensor and cables included. Requires a thermostated cuvette holder (SampleSnap-WJ).

12.11 Other Parts and Accessories

- Custom filter insert 2” square
- Adapters for 1 inch round filters
- ND Filters
- Laptop
- Windows For SampleSnap-PWD
- Replacement LampSnap
- Replacement Dust Filter
- Standard SampleSnap Cuvette Holder
- Replacement Injection Port Insert

13 Technical Specifications

13.1 Introduction

Each Duetta™ system consists of:

- An excitation source
- An excitation monochromator
- A sampling module with reference detector
- An absorption detector
- An emission spectrograph with CCD.

Each system is controlled by a PC-compatible computer.

The details and specifications for each component of the Duetta™ spectrometer follow.

13.2 Spectrofluorometer system

13.2.1 Excitation channel

Excitation source	75 W xenon arc lamp, continuous output, ozone-free. Dedicated cartridge for snap-in replacement
Lamp stability	1% per hour
Dispersion	Single-grating scanning monochromator
Slit band pass	AutoSlit wheel (Band pass: 1, 2, 3, 5, 10, 20 nm)
Filters	Five position filter wheel 1: one 410 nm LP, one 525 nm LP, two closed (Shutter), one open. The 410 LP and 525 LP filters are used for automatic order sorting.
Excitation range	250 - 1000 nm
Excitation value	0, or 250 – 1000 nm
Wavelength accuracy	±1 nm
Maximum scan speed	500 nm s^{-1}
Stray light rejection	< 10^{-4} at 350 nm
Polarizers (Optional - 0nly with Duetta-Bio)	Five position filter wheel 2: V (optional), H (optional), ND 0.5, ND 1.0, Open

13.2.2 Sample compartment

Sample module	The sample module has a removable gap-bed assembly for sampling accessory replacement.
Z-height for cuvettes	15 mm
Reference detector	Si detector for excitation reference correction from 250–1000 nm.
Cooling water for accessories	Flow rate: 100 – 300 mL/min Pressure: 0.3 bar (5 psi) maximum. Do not exceed input water pressure of 1.7 bar (25 psi) as damage may occur.
Temperature range with the SampleSnap-1Pelt or SampleSnap-4Pelt accessory	-15 to +105 °C (SampleSnap-1Pelt) -15 to 110 °C (SampleSnap-4Pelt) Dry nitrogen gas may be needed to prevent condensation
Temperature precision	±0.02 °C

13.2.3 Emission channel

Detector	Spectrograph with thermoelectrically-cooled CCD
Readout time	130 ms
Detector range	250 – 1100 nm
Emission grating	365 gr/mm
Sensitivity	Double-distilled, de-ionized, ICP-grade water Raman scan 6,000:1 signal-to-noise ratio at 397 nm, 5 nm band pass, 40 s integration time, background noise first standard deviation at 450 nm
Excitation and emission shutters	Computer-controlled open and closed apertures on filter wheels
Integration time	0.05 – 600 s
Emission band pass	AutoSlit (Band pass: 1, 2, 3, 5, 10, 20 nm)
Polarizers (0nly with Duetta-Bio)	Five position filter wheel 1: V, H, Magic (54.7°), Closed (shutter), Open
Aperture	Five position filter wheel 2: Open, 15 mm aperture for 1, 2 mm band pass

13.2.4 Absorbance

Detector range	Silicon photodiode 250 – 1000 nm
Bandwidth Slew speed	1, 2, 3, 5, 10, 20 nm Up to 500 nm s^{-1}
Integration time	0.001 – 600 s
Wavelength accuracy	±1 nm
Absorbance range	0 – 2 A
Absorbance accuracy	±0.02 A
Absorbance stability	<0.002 A h^{-1}
Absorbance repeatability	±0.002 A from 0 to 1 A
Stray light in absorbance path	<1% at 260 nm

13.2.5 Total system

Duetta™ Dimensions (W × D × H)	43 cm × 51 cm × 39 cm 17 in × 20 in × 15.4 in Height needed to open the top cover: 76 cm, 30 in
Dimensions (sample compartment only) (W × D × H)	14.0 cm × 17.8 cm × 17.8 cm 5.5 in × 7 in × 7 in
Weight	20.4 kg (45 lb.)
Ambient temperature range	15 – 30 °C 59 – 86 °F
Maximum relative humidity	80 %
Power	Universal AC single-phase input power; 90 – 240 V AC; line frequency 50 – 60 Hz.
Fuses	Two 5 × 20 mm IEC approved, 2.0 A, 250 V, Time Delay fuses (Cooper Bussman part number GDC-4A or equivalent)

13.3 Minimum host computer requirements

13.3.1 Software

EzSpec v1.4 is Windows® 10, or 11 Touch screen compatible, 64-bit OS.

13.3.2 Hardware

- 4 GB RAM (16 GB RAM recommended)
- 64 GB hard-disk space
- One available USB port for Duetta connection
- Minimum screen resolution: 1024 × 768
- Touch screen compatible (optional)
- Internet connection recommended to allow remote HORIBA Service, including EzSpec™ updates

> ⓘ **Note**: Additional USB ports may be required to control accessories such as the temperature controller.

13.4 EzSpec™ software

- Font: Open Sans
- Touch screen compatible
- Available languages: English.

14 Service Information

14.1 Service Policy

If you need assistance in resolving a problem with your instrument, contact our Customer Service Department directly, or if outside the United States, through our representative or affiliate covering your location.

Often it is possible to correct, reduce, or localize the problem through discussion with our Customer Service Engineers.

All instruments are covered by warranty. The warranty statement is printed inside of this manual. Service for out-of-warranty instruments is also available, for a fee. Contact HORIBA Instruments Incorporated or your local representative for details and cost estimates.

If your problem relates to software, please verify your computer's operation by running any diagnostic routines that were provided with it. Please refer to the software documentation for troubleshooting procedures. If you must call for Technical Support, please be ready to provide the software serial number, as well as the software version and firmware version of any controller or interface options in your system. The software version can be determined by selecting the software name at the right end of the menu bar and clicking on “About.” Also knowing the memory type and allocation, and other computer hardware configuration data from the PC’s CMOS Setup utility may be useful.

In the United States, customers may contact the Customer Service department directly. From other locations worldwide, contact the representative or affiliate for your location.

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If an instrument or component must be returned, the method described on the following page should be followed to expedite servicing and reduce your downtime.

14.2 Return authorization

All instruments and components returned to the factory must be accompanied by a Return Authorization Number issued by our Service Department.

To issue a Return Authorization number, we require:

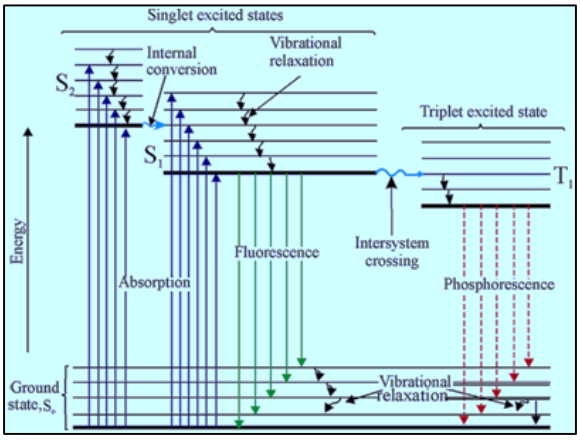
- The model and serial number of the instrument
- Alist of items and/or components to be returned
- A description of the problem, including operating settings
- The instrument user's name, mailing address, telephone, and fax numbers
- The shipping address for shipment of the instrument to you after service
- Your Purchase Order number and billing information for non-warranty services
- Our original Sales Order number, if known
- Your Customer Account number, if known
- Any special instructions

15 Glossary

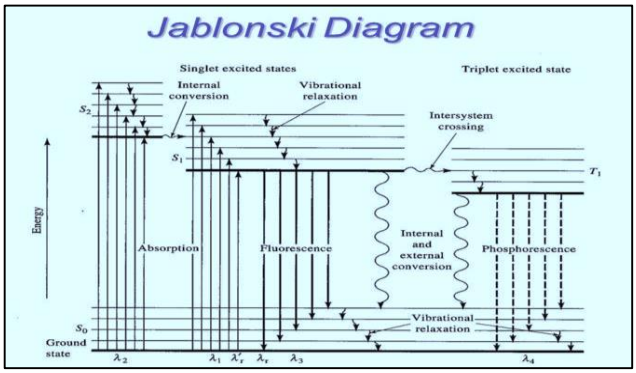
Term	Definition
Absorbance	The extent of light absorption by a substance. Absorbance, $A = -\log T$, where T is the transmittance of the sample. Absorbance is also synonymous with optical density, OD. Absorbance can be calculated using the Beer-Lambert Law: $A = \varepsilon c l = \text{OD} = -\log T$ ε = extinction coefficient (M^{-1} cm^{-1}) c = sample concentration (M) l = path length (cm).
Absorption	Process in which atoms absorb light and their electrons are excited to higher energy levels. This process typically occurs in ~10^{-15} s.
Acquisition modes (Ref, CCD, Abs channels)	The logical input channels used on the spectrofluorometer to input collected signal from the detectors present on the system. The detectors are assigned as: the reference detector connected to channel Ref, the emission connected to channel CCD, and the absorption connected to channel Abs.
A-TEEM	A-TEEM™ stands for Absorbance-Transmittance Excitation Emission Matrix. A fluorescence EEM (excitation emission matrix) scans the wavelength range at which a solution or sample is excited and measures the emission spectrum at each scanned excitation, giving a 3D plot of excitation vs. emission vs. intensity. An A-TEEM™ is the method for which the absorbance/%transmittance spectra are also collected for the same sample and these are applied to the EEM to correct for inner-filter effects (IFE) to give a more accurate 3D contour of the fluorescence profile. Inner-filter effects are explained further in Section 1 of the Duetta manual (Introduction), but without IFE correction, spectra, including fluorescence EEMs may be inaccurate due to reabsorption from the sample and inadequate illumination of the sample due to high concentrations. Duetta is unique in that the absorbance and fluorescence EEM can be measured on the same instrument without moving the sample, and IFE corrections are automatically applied with EzSpec software.
Band pass	The wavelength range of light passing through the excitation and emission spectrometers. The wider the band pass, the higher the signal intensity. The band pass is selectable.

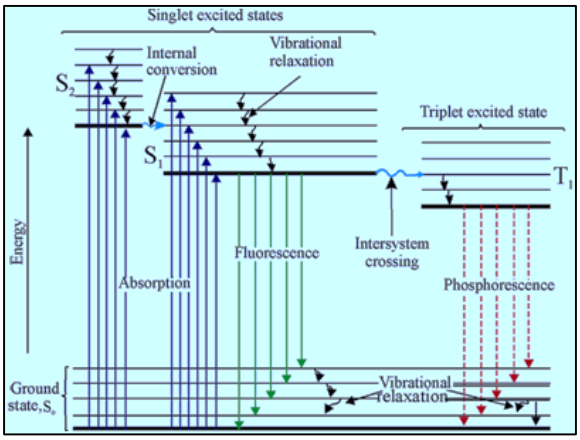
Term	Definition
Band pass filter	Optical element that selectively transmits a narrow range of optical wavelengths.
Bioluminescence	Emission of light originating from a chemical reaction in a living organism.
Blank subtraction	The removal of the spectral response of the solvent (and sample container) from the sample's spectral response. To accomplish this, an identical scan is run on the solvent just before running the actual sample. Proper use of a blank can remove solvent luminescence artifacts, scattering events, and any artifacts from the sample cuvette or container.
Blaze wavelength	The wavelength at which a grating is optimized for efficiency. Generally, the gratings are efficient to ⅔ before the blaze wavelength to twice the blaze wavelength. The excitation and emission gratings are blazed in the UV and visible respectively.
Chemiluminesence	The emission of light during a chemical reaction that does not produce significant quantities of heat.
Concentration determination	A function of the Capture Value Single Point type of scan that calculates the unknown concentration of a sample. The user runs known concentration samples to create a calibration curve, which can be used to calculate the unknown concentration.
Corrected emission scan	An emission scan that has been corrected for the wavelength response of the emission monochromator and the signal detector. To obtain a corrected emission scan, an emission spectrum is multiplied by the appropriate emission correction-factor file. A set of emission correction factors is supplied with the instrument and stored under the name Mcorrect.
Corrected excitation scan	An excitation scan corrected for the wavelength characteristics and age of the Xenon lamp, and the gratings in the excitation spectrometer. To obtain a first-order correction of the excitation scan, the emission detector signal is ratioed to the reference signal after the dark current and detector wavelength-response factors are applied for CCD_{corr} and Ref_{corr} (i.e., CCD_{corr} / Ref_{corr}). This provides correction for the lamp and emission and excitation-monochromator spectral responses. To obtain a completely correct scan, the excitation correction factors (Xcorrect) is included.
Correction factors	Compensate for the wavelength-dependent components of the system, like the Xenon lamp, gratings, and signal detector. Emission and excitation correction-factors are included with the software in a single file called

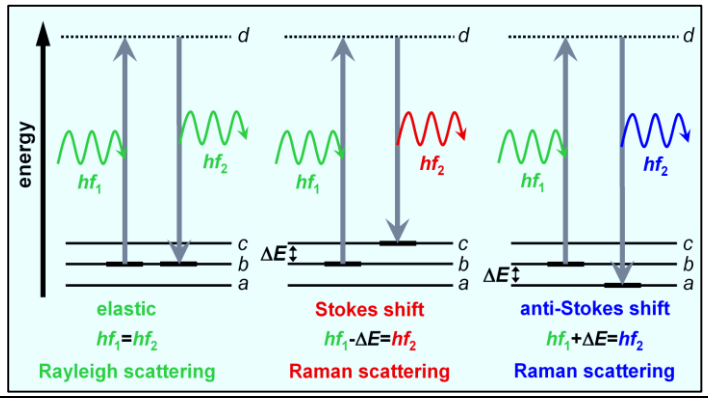
Term	Definition
	corrections.ezspec_data which travels with the instrument. These corrections are applied automatically in the EzSpec™ software.
Cut-on filter	Optical component that passes light of a higher wavelength.
Cut-off filter	Optical component that passes light of a lower wavelength.
Dark offset	The software correction used to subtract dark counts (or dark signal) on a detector from a spectral acquisition. This feature is implemented automatically in EzSpec™ software.
Data file	A file used to store spectral or timebased data, analysis data, or other recorded data.
Dispersion	The range of wavelengths of light across the field of view of the entrance and exit apertures. Dispersion depends on the focal length of the monochromator, the groove density of the optics, and the *f*-number (speed) of the monochromator. Dispersion is usually expressed in nanometers of spectral coverage per millimeters of slit width (nm/mm).
Emission Spectrum	A scan showing the spectral distribution of light emitted by the sample. During an emission scan, the excitation monochromator remains at a fixed wavelength while the emission detector scans a selected region.
Energy transfer	The transfer of the excited energy from a donor to an acceptor. The transfer occurs without the appearance of a photon and is primarily a result of dipole-dipole interactions between the donor and acceptor.
Excitation/emission matrix (EEM)	A three-dimensional plot showing the total luminescence from a sample across all useful wavelengths. See: **Total Luminescence Spectroscopy**
Excitation monochromator	Located between the xenon lamp and the sample compartment, this monochromator is used to scan the excitation spectrum of a sample. The excitation monochromator on the Duetta™ is a single grating monochromator with slit apertures at the entrance and exit and an excitation shutter located directly after the excitation exit slit aperture to protect the sample from photobleaching between measurements.
Excitation Spectrum	A scan showing the spectral distribution of light absorbed by the fluorescent components of the sample. To acquire an excitation scan, the excitation monochromator scans a selected spectral region while the emission detection remains at a fixed wavelength region.

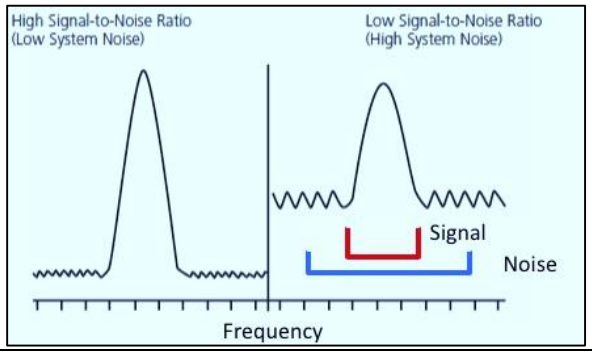
Term	Definition
Excited state (S_1)	The energy level to which an electron in the ground level of a molecule is raised after the absorption of a photon of a particular wavelength.
Extrinsic fluorescence	Inherent fluorescence of probes used to study non-fluorescent molecules.
Filter	An optical element used for the selection of certain wavelengths of light. Types of filters include high-pass, low-pass, band pass, and neutral density.
Fluorescence	The emission of light during the transition of electrons from the lowest excited singlet state to the ground state after being exposed to light. Fluorescence typically occurs within ~10^{-9} seconds. 
Fluorescence lifetime (τ)	The average length of time that a molecule remains in the excited state before returning to the ground state.
Fluorophore (Fluorescent probe)	A molecule or compound that has a known fluorescence response. Normally, fluorophores are used to provide information on concentration, size, shape, and binding, in a particular medium. They are also stable over a wide pH and temperature range, and they are resistant to photobleaching
Front-face detection	A mode of detection in which fluorescence is collected off the front surface of the sample. Front-face detection usually is selected for samples such as powders, thin films, pellets, cells on a cover-slip, and solids.
Grating	Optical element in the monochromator, consisting of finely scribed grooves that disperse polychromatic light into its components.
Ground state (S_0)	The lowest energy level in a molecule.
High-pass filter	Optical component that passes light of a higher wavelength.

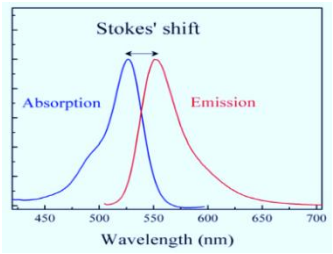
Term	Definition
Increment	The spacing between adjacent measurement points in an acquisition. Typically, increments take the form of wavelength (nm) or time (seconds or milliseconds).
Inner-filter effect	This occurs when components in a concentrated sample reduce the signal intensity and create artifacts in the spectra. To avoid this phenomena, it is recommended to use concentrations of <0.05 OD in a 1 cm path length cell. EzSpec™ software can automatically correct these deviations.
Integration time	The amount of time that each data point is collected from the detector(s), specified in seconds. Longer integration times can help improve the signal-to-noise ratio for a measurement, whereas shorter integration times prevent saturation of the fluorescence detector. Choose integration times to optimize the signal-to-noise ratio.
Internal conversion (or Non-Radiative Transition)	Electronic transitions within an excited molecule that do not result in emission. This conversion usually involves changes in vibrational levels.
Intersystem crossing	The electronic transition from the excited singlet state to the excited triplet state before returning to the ground state. This transition involves a change of spin that is quantum-mechanically forbidden, giving a much longer timescale than fluorescence. This transition causes phosphorescence on the timescale of microseconds to seconds. See the Jablonski (energy) diagram below.
Intrinsic fluorescence	The natural fluorescent properties of molecules.

Term	Definition
Jabłonski (energy) diagram	A diagram that illustrates possible paths for fluorescence, phosphorescence, and non-radiative transfers and the various vibrational sub-levels available around each energy level. 
Linearity	Signal response. When the detector response is linear, doubling the light intensity means doubling the detected signal. See **Spectral Calibration** for more detail
Low-pass filter	Optical component that passes light of a lower wavelength.
Luminescence	The emission of light from an excited state to a lower energy state. See: **Bioluminescence**, **Chemiluminesence**, and **Fluorescence**.
Mercury lamp	Light source that emits discrete, narrow lines as opposed to a continuum. A mercury lamp can be used to check the monochromator's calibration.
Method file	A file that saves all system defaults and all accessory settings for a particular experimental setup. Use method files to archive scan settings for acquisitions that are performed routinely
Mirror-image rule	When the emission profile appears to be the mirror image of the absorption spectrum.
Molar extinction coefficient (ε)	The absorptivity of a particular substance, in M^{-1} cm^{-1}.
Monochromator	The component in a spectrofluorometer that is scanned to provide the excitation spectra. Monochromators are chosen for stray-light rejection, resolution, and throughput.
Neutral-density filter	An optical element that absorbs a significant fraction of the incident light. These filters usually are characterized by their optical density, on a

Term	Definition
	logarithmic scale. For example, a filter with OD = 1 transmits 10% of the incident light. Ideally, these filters absorb all wavelengths equally. See also **Absorbance**.
Optical density	A synonym of *absorbance*. See **Absorbance**.
Optical-density effects (Inner-filter effect)	High optical densities can distort the emission spectra as well as the apparent intensities. See **Inner-filter effect** for more detail.
Parallel Factor Analysis (PARAFAC)	A multi-way canonical decomposition-analysis method. – Delete this topic?
Phosphorescence	The emission of light or other electromagnetic radiation during the transition of electrons from the triplet state to the ground state. Phosphorescence is generally red-shifted relative to fluorescence and occurs within ~10^{-6} to ~1 second. To enhance phosphorescence, samples often are frozen at liquid-nitrogen temperature (77 K). 
Photobleaching	The reduction in fluorescence from a photosensitive sample due to overexposure to excitation light. To prevent this, keep the sample out of the room light. The Duetta™ scans rapidly and from low energy to high energy to minimize photobleaching
Principle Component Analysis (PCA)	Uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of uncorrelated variables called “Principle Components.” – Delete this topic?
Quantum yield (Fluorescence quantum yield)	The percent quantum yield can be reduced by factors such as scattering, quenching, internal conversion, and non-radiate effects among other specialized processes $$\text{Quantum Yield} = \frac{\#\ \text{photons emitted}}{\#\ \text{photons absorbed}}$$

Term	Definition
Quenching	Reduction in the fluorescence intensity of a sample due to chemical or environmental influences. Quenching may be static, dynamic, or collisional in nature.
Raman scattering	Scattering caused by vibrational and rotational transitions of molecular atomic bonds. Raman bands generally appear red-shifted relative to the incident electromagnetic radiation. The primary characteristic of Raman scatter is that the difference in energy between the Raman peak and the incident radiation is constant in energy units (cm^{-1}) for a given molecular bond's vibrational mode.
Rayleigh scattering	LIGHT SCATTERING BY PARTICLES IN A MEDIUM WITHOUT CHANGE IN WAVELENGTH. THIS TYPE OF SCATTERING is inversely proportional to the fourth power of wavelength, so that shorter wavelength violet and blue light will SCATTER more than the longer wavelengths (yellow and especially red light)
Reference detector	The detector used to monitor the output of the Xenon lamp.
Resolution	The ability to separate two closely spaced peaks. Resolution can be improved by decreasing the band pass and the increment (step size).
Right-angle detection	Collection of fluorescence at 90° to the incident radiation. Right-angle detection typically is selected for dilute and clear solutions.
Sample changer (automated)	An automated accessory that automatically positions up to four cuvettes held in the sample compartment. This accessory is used to run up to four samples at one time for a small assay, or to run blanks with the samples simultaneously. Automated sample changers are thermostatted and possess magnetic stirrers.
Saturation	The effect of having too much signal incident on a particular detector. Saturated detectors give erroneous results, and do not show any response for small changes in signal.
Scatter	A combination of Raman, Rayleigh, and Rayleigh-Tyndall scattering, which can distort fluorescence spectra with respect to intensities and wavelengths. 
Signal channel	See: **Acquisition modes**.

Term	Definition
Signal-to-noise ratio (*S/N*)	The measurement of the signal observed divided by the noise component seen in that signal. 
Single Point	The Duetta™'s EzSpec™ experiment type Capture Value is designed for performing single-point fluorescence measurements at discrete wavelength pairs.
Singlet state	A molecular electronic state such that all electron spins are paired. That is, the spin of the excited electron is still paired with the ground state electron (a pair of electrons in the same energy level must have opposite spins, per the Pauli exclusion principle).
Spectral calibration	The accuracy of a monochromator with respect to its wavelength alignment. Monochromators are traditionally calibrated using line-spectra sources, such as mercury lamps.
Spectral correction	The removal of the wavelength sensitivity of detectors, optics, sources, and backgrounds from the spectrum taken on a sample. In the Duetta™, the EzSpec™ software applies all corrections automatically
Spectral response	The measure of a detector's response to radiation at a specified wavelength. The spectral response of a detector is often expressed graphically in a plot of responsivity versus wavelength
Spectrofluorometer	An analytical instrument used to measure the fluorescence properties of a molecule or substance. The device consists of at least two monochromators, a source, a sample compartment and detection electronics. However, newer spectrofluorometers provide may provide automated options such as polarization, temperature, titer plates, and pressure among others.
Stokes shift	It is the difference (in wavelength or frequency units) between positions of the band maxima of the absorption and emission spectra (fluorescence and Raman being two examples) of the same electronic transition. It is named after Irish physicist George G. Stokes.

Term	Definition
	
Technical spectrum	A spectrum acquired on research instrumentation with instrumental bias remaining in the measurement. This spectrum must undergo proper spectral correction in order to match the theoretical spectrum. HORIBA's specrofluorometers offer several correction methods that include spectral correction, dark offset, and blank subtraction among others
Temperature scan	This scan is used to monitor a sample's response to temperature. Such scans require a Peltier sample holder, which must be compatible with Duetta™'s EzSpec™ software. The circulating water bath is not controlled by the software, other than the stirring on the SampleSnap-WJ.
Throughput	The amount of light that passes through the spectrofluorometer for a particular measurement. Like band pass, throughput has an inverse relationship with resolution. When the throughput is increased the resolution decreases.
Time-based scan	Scan type in which the sample signal is monitored as a function of time while both the excitation and the emission spectrometers remain at fixed wavelengths. Time-based data are used to monitor enzyme kinetics, dual-wavelength measurements, and determine reaction-rate constants.
Total luminescence spectroscopy (TLS)	This technique is devoted to monitoring changes to the entire excitation/emission matrix of luminescence on a sample. This discipline is best applied to fast kinetics measurements of samples during reactions, temperature curves, or changes in other parameters
Transmission	Light that passes through a sample without being absorbed, scattered, or reflected. Transmission is usually measured as a percentage of the incident light at a certain wavelength.
Triplet state (T_1)	The spin-paired ground or excited state formed from the excited singlet state, in which electrons are unpaired. The triplet state gives rise to phosphorescence.
Tyndall scattering	The Tyndall effect, also known as Willis–Tyndall scattering, is light scattering by particles in a colloid or else particles in a very fine suspension. It is named after the 19th-century physicist John Tyndall.

Term	Definition
	
Vibrational states	Sublevels within an electronic energy level resulting from various types of atomic motion in a molecule. Within an energy level, transition between these states does not involve a large change in energy, and it is considered a non-radiative transition. In larger electronic transitions such as fluorescence, a molecule drops from the lowest vibrational level of the excited state to the highest vibrational level of the ground state. This emission is termed the Stokes shift between the S_1 and ground states.
Xenon lamp	Lamp that produces a continuum of light from the ultraviolet to the near-infrared for sample excitation.

16 Bibliography

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Valeur, B. (2002). *Molecular Fluorescence: Principles and Applications.* New York: Wiley-VCH. .

17 Compliance Information

17.1 CE Declaration of Conformity

Manufacturer:	HORIBA Canada Incorporated
Address:	347 Consortium Court London, ON N6E 2S8 Canada
Authorized Rep:	HORIBA FRANCE SAS
Address:	16-18 rue du Canal 91165 Longjumeau Cedex France
Product Name:	Duetta, Duetta-Bio, LampSnap, SampleSnap
Model Numbers:	5700007000 – Duetta 5700004300 - Duetta-Bio 5700012885 - SampleSnap-1Pelt 5700012859 - SampleSnap-4Pelt 5700012880 - SampleSnap-PLQY 5700012886 - SampleSnap-LN 5700012882 - SampleSnap - FF 5700012883 - SampleSnap-SS 5700012884 - SampleSnap-PWD 5700012881 - SampleSnap-WJ 5700006701 - SampleSnap 5500357868 – LampSnap

Conforms to the following Standards:

Safety:	EN 61010-1: 2010 (3rd Edition)
EMC:	EN 61326-1: 2013 (Emissions & Immunity)
RoHS:	EN 50581:2012

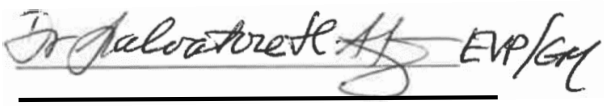
17.1.1 Supplementary Information

The product herewith complies with the requirements of the Low Voltage Directive

2014/35/EU and the EMC Directive 2014/30/EU.

The CE marking has been affixed on the device according to Articles 16 and 17 of the EMC Directive 2014/30/EU.

The technical file and documentation are on file with HORIBA Canada Incorporated.



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17.2 UKCA Declaration of Conformity

Manufacturer:	HORIBA Canada, Inc.
Address:	347 Consortium Court, London Ontario N6E 2S8 Canada
Authorized Rep: **Address:**	HORIBA UK Limited Kyoto Cl, Moulton Park Moulton Park Industrial Estate, Northampton NN3 6FL, United Kingdom
Product Name:	Duetta, Duetta-Bio, LampSnap, SampleSnap

Part Numbers:		
	5700007000 - Duetta	5700012882 - SampleSnap - FF
	5700004300 - Duetta-Bio	5700012883 - SampleSnap-SS
	5700012885 - SampleSnap-1Pelt	5700012884 - SampleSnap-PWD
	5700012859 - SampleSnap-4Pelt	5700012881 - SampleSnap-WJ
	5700012880 - SampleSnap-PLQY	5700006701 - SampleSnap
	5700012886 - SampleSnap-LN	5500357868 – LampSnap

The above listed product(s) Conform to the following Statutory Requirements and Designated Standards:

UK SI 2016 No. 1101 and amendments:	Electrical Equipment (Safety) Regulations EN 61010-1: 2010 (3rd Edition)
UK SI 2016 No. 1091 and amendments:	Electromagnetic Compatibility Regulations EN 61326-1 (Emissions & Immunity)
UK SI 2012 No. 3032 and amendments:	The Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment Regulations 2012 EN 63000:2018

17.2.1 Supplementary Information

This declaration is issued under the sole responsibility of the Manufacturer.

23rd September 2022
Date of issue

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General Manager and Executive Vice President
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Matt Bryanton
Head of Quality and Business Process
HORIBA UK Limited
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Moulton Park Industrial Estate,
Northampton NN3 6FL, United Kingdom

17.3 Applicable CE and UKCA Compliance Tests and Standards

Test	Standards
Emissions, Radiated/Conducted	EN 55011: 2009 + A1:2010, FCC Part 15:2017, ICES-003 Issue 6
Radiated Immunity	EN 61000-4-3: 2006 + A1:2008 + A2:2010
Conducted Immunity	EN 61000-4-6: 2009
Electrical Fast Transients	EN 61000-4-4: 2012
Electrostatic Discharge	EN 61000-4-2: 2009
Voltage Interruptions	EN 61000-4-11: 2004
Surge Immunity	EN 61000-4-5: 2006
Harmonics	EN 61000-3-2: 2014
Flicker	EN 61000-3-3: 2013
Safety	EN 61010-1: 2010 (Third Edition)

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