

DeltaFlex™

Modular Fluorescence Lifetime System

System guide

HORIBA
Scientific

info.sci@horiba.com

www.horiba.com/scientific

USA: +1 732 494 8660
UK: +44 (0)20 8204 8142
China: +86 (0)10 8567 9966

France: +33 (0)1 64 54 13 00
Italy: +39 2 5760 3050
Brazil: +55 (0)11 5545 1514

Germany: +49 (0)89 4623 17-0
Japan: +81 (0)3 6206 4717
Other: +33 (0)1 64 54 13 00



Table of contents

1. Safety precautions	5
1.1 Safety symbols	5
1.2 DeltaFlex safety notice	6
1.3 DeltaHub safety notice	6
1.4 NanoLED safety notice	7
1.5 DeltaDiode safety notice	7
1.6 SpectraLED safety notice	8
1.7 PPD safety notice	8
2. Introduction	11
3. Unpacking and installation	15
3.1 Mounting	16
3.1.1 DeltaDiode / NanoLED / SpectraLED pulsed light source.....	17
3.1.2 PPD picosecond photon detection module.....	17
3.2 Cables and connections	17
3.2.1 Timing electronics power inlet and power switch.....	17
3.2.2 Your computer (USB).....	18
3.2.3.1 Fluorescence (DeltaDiode / NanoLED) light source controller.....	18
3.2.3.2 Phosphorescence (SpectraLED) light source.....	18
3.2.4 PPD picosecond photon detection module.....	18
3.2.5 Baseplate (SDB).....	18
3.2.6 Simplified schematic connection schemes (viewed from the rear).....	19
3.3 Software installation (not necessary if a computer has been supplied)	20
3.3.1 Launching EzTime.....	20
4. Operation	21
4.1 Measurement modes	21
4.2 General considerations	22
4.3 Performing time-resolved measurements using EzTime	25
4.3.1 Data storage concept.....	26
4.3.2 Measurement set up – Instrument page.....	27
4.3.3 Data collection – Data page.....	29
4.3.4 Data analysis – Data page.....	32
4.3.4.1 How do I know if I have a good data fit?.....	33
4.3.4.2 Interpretation of and exporting fitted data.....	34

4.4 Histogram streaming measurements	36
4.4.1 Analysis for streamed histogram data.....	37
4.4.2 Experimental considerations for streamed histogram measurements	38
4.5 Photon streaming measurements	39
4.6 Automated scripts	43
4.6.1 Time-resolved anisotropy measurements.....	43
4.6.1.1 Anisotropy data analysis	45
4.6.1.2 Anisotropy measurement considerations	45
4.6.2 Time-resolved emission spectra (TRES) measurements.....	46
4.6.2.1 TRES data analysis.....	47
4.6.3 Turret scan measurements	48
4.7 Steady state measurements	49
4.7 Steady state measurements	49
4.8 Advanced functions / settings	50
The advanced setting form has several tabs enabling the Start and stop inputs to be configured for choice of excitation source, which stop channel is active etc.	50
4.9 Other light sources	51
4.10 Initial system check	51
5. Maintenance and troubleshooting	53
5.1 General	53
5.1.1 If no Stop (and / or Hit) ratemeter signal seen when expected	54
5.2 Accessing Log files	55
6. Specifications	57
Appendix A. Luminescence and techniques to measure it	59
Appendix B. DeltaPro Sample chamber	63
Appendix C. FluoroHub-A+ timing electronics	65
Appendix D. Detector Options	68
Appendix E. Temperature Control Options	69
Appendix F. Sample Holder Options	72
Appendix G. Accessories – Mounting polarisers	73
Appendix H. Computer settings (USB / power)	74
Notes	77

1. Safety precautions

1.1 Safety symbols

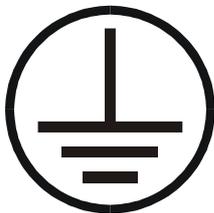
Terms and symbols described in this section are used throughout the HORIBA Scientific product line. These symbols may appear in this user guide only, or they may be marked on the outside of the product or other equipment that is intended for use with the product.



The product will be marked with this Exclamation Symbol when it is necessary to refer to this user guide in order to prevent personal injury or equipment damage. This symbol also appears in this user guide to indicate areas where personal injury or equipment damage is possible.



The Electrical Shock Symbol indicates a hazard arising from dangerous voltage. Any mishandling could result in irreparable damage to the equipment, and personal injury or death.



The Grounding Symbol indicates that connection to an external ground conductor is required for protection against electric shock in case of a fault. Failure to provide correct grounding or mishandling could result in irreparable damage to the equipment, and personal injury or death.



The Laser symbol indicates the presence of radiation emitted from visible or invisible laser light sources. This symbol may also be used to indicate the presence of invisible ultraviolet or infrared light. Do not look directly at the beam. Failure to follow laser safety rules or mishandling could result in eye injury leading to permanent loss of sight or vision impairment.

WARNING

This calls attention to a procedure, practice, or condition that could possibly cause bodily injury or death.

CAUTION

This calls attention to a procedure, practice, or condition that could possibly cause damage to equipment or permanent loss of data.

1.2 DeltaFlex safety notice



WARNING

On opening the DeltaFlex sample chamber, care should be taken as the light source can be operative. Practice care when inserting objects into the chamber because of the possibility of reflected or scattered excitation light. Also see safety notices relating to light sources.



CAUTION

The chamber gas purge inlet should only be used with inert gases, such as dry air or nitrogen.

When connected to a recirculating water bath ensure that the connections are properly made to avoid leakage. It should be noted that the DeltaFlex should only be used in the temperature range -40 C to 70 C.

The use of caustic substances should also be avoided.

1.3 DeltaHub safety notice



WARNING

This product must be electrically earthed during operation. The IEC mains power inlet includes a protective conductor terminal that must be connected to earth.

Live and Neutral fusing is implemented on this product. Both fuses are located adjacent to the IEC mains power inlet. Fuse ratings are listed in Specifications.



If this product is used in a manner not specified by the manufacturer then any safety protection provided by the product may be impaired.

This product has no user serviceable parts. Please contact HORIBA Scientific if this product requires servicing.

This product should be operated only by personnel who have read this user guide.

Confirm that the product's input voltage, as indicated next to the IEC mains power inlet, is appropriate for your local mains voltage supply before using for the first time. Different fuse ratings are used for 110V 60Hz and 240V 50Hz. If you think that the voltage indicated is not appropriate for your local mains voltage supply then do not attempt to use the product and contact HORIBA Scientific for advice. Correct fuse ratings are listed in Specifications.

Do not use the product if any cables connected to it are damaged or appear worn; disconnect from the mains power supply immediately and contact HORIBA Scientific for advice.



CAUTION

Airflow inside the module depends on the top cover being in place. Adequate airflow must be ensured to avoid damage to certain components inside the module. Do not obstruct the any fan on the rear panel and do not operate the module without the top cover in place.

Always ensure that the product is switched off while connecting or disconnecting cables attached to it.

1.4 NanoLED safety notice



WARNING

Some NanoLED excitation sources generate laser light or ultraviolet / near infrared radiation. **Do not look directly at the beam** and always observe appropriate safety procedures. Always refer to the information provided on the label attached to the NanoLED. If in doubt please contact your organisation's laser safety officer.

1.5 DeltaDiode safety notice



WARNING

Some DeltaDiode excitation sources generate laser light or ultraviolet / near infrared radiation. **Do not look directly at the beam** and always observe appropriate safety procedures. Always refer to the information provided on the label attached to the DeltaDiode. If in doubt please contact your organisation's laser safety officer.

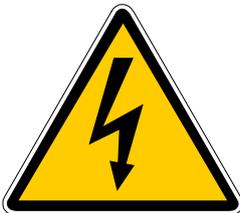
1.6 SpectraLED safety notice



CAUTION

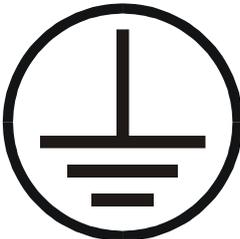
Some SpectraLED excitation sources generate ultraviolet or near infrared radiation. **Do not look directly at the beam** and always observe appropriate safety procedures. Always refer to the information provided on the label attached to the SpectraLED. If in doubt please contact your organisation's radiation safety officer.

1.7 PPD safety notice



WARNINGS

Lethal high voltages are generated inside this product. Do not attempt to operate before reading this user guide. Improper operation could lead to personal injury or death, or damage to the product and any equipment connected to it.



This product must be electrically earthed during operation. The power connector includes a protective conductor terminal that must be connected to an external power supply with an earth terminal.

If this product is used in a manner not specified by the manufacturer then any safety protection provided by the product may be impaired.

This product has no user serviceable parts. Please contact HORIBA Scientific if this product requires servicing. Do not attempt to open the casing. Opening the casing is dangerous and will invalidate your warranty. Continued technical support is conditional on the anti-tamper seal not being broken. Should the photomultiplier tube inside the product become loose or show signs of damage then stop using the product immediately and contact HORIBA Scientific for advice.

CAUTION



This product is designed solely for the detection of low light levels in photon counting mode. Do not use this product for any other purpose. The single-photon sensitive photomultiplier tube used inside this product is operated at high gain and is intended for low light level detection only - it can be permanently damaged if exposed to ambient light levels while the power is on. The product should be stored in darkness when not in use and should be

protected from strong sources of illumination (e.g. direct laser illumination) at all times.

Always ensure that the product is switched off while connecting or disconnecting cables attached to it.

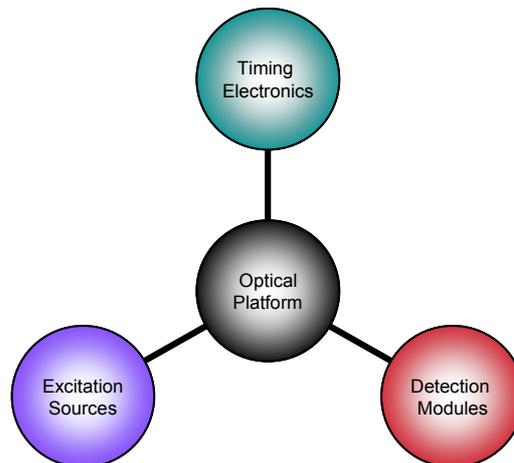
If in doubt, please contact your HORIBA Scientific representative for advice.

2. Introduction

Thank you for choosing this HORIBA Scientific product.

The DeltaFlex is a compact, highly modular, time-correlated single photon counting (TCSPC) based lifetime system. It is capable of measuring luminescence lifetimes from 5ps to 2sec, depending on the choice of light source and detection module. It offers picosecond time resolution over a wavelength range from the UV to the near infrared (NIR). The modular nature of the design means that it can be easily extended and upgraded using the comprehensive range of products available from HORIBA Scientific. This makes DeltaFlex an easy to use, versatile system in the field of lifetime determination, with the high sensitivity associated with the TCSPC technique.

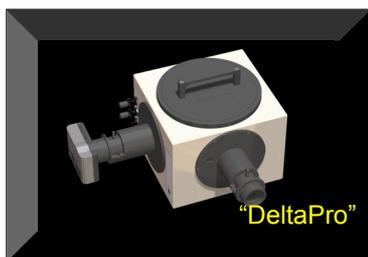
The system consists of the following components, shown schematically below.



The components parts communicate by a purpose built communication system called “F-Link” and are controlled using EzTime software. This software also controls data acquisition and analysis. The F-Link communication bus enables upgrades to be effortlessly incorporated into the system and recognised by the EzTime software. As EzTime can communicate with the component parts of the system via F-Link, it can also be used for diagnostic purposes in the case of a system fault.

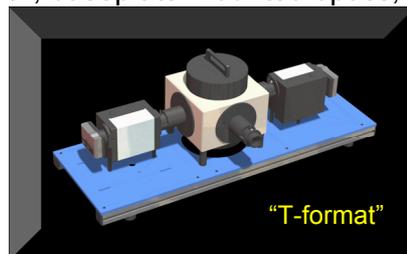
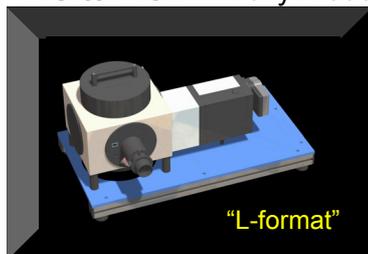
Because of the highly modular nature of the DeltaFlex system many different Detection Modules, Excitation Sources and even Timing Electronics can be added to the Optical Platform. The Optical Platform itself can also be configured in several ways and on the following page some of the common options are illustrated.

Optical Platform



DeltaPro – Filter based, independent sample chamber

DeltaFlex – Fully modular, baseplate mounted optics, eg



Timing Electronics



General purpose, high speed Lifetimes 25ps to 1s



High time resolution Lifetimes 5ps to 2s

Detection Modules



PPD
230nm-920nm
TTS <180ps



HPPD
220nm-890nm
TTS from <50ps



MCP
185nm-850nm
TTS 25ps



NIR (R)
300-1700nm
TTS 1.5ns



NIR (H)
950nm-1700nm
TTS 400ps

Excitation Sources



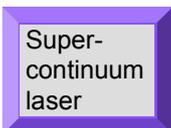
Lasers & LEDs
Rep rate to 100MHz
Coverage 250-1310nm



LEDs for exciting Phosphorescence
Coverage 265-1275nm



Lasers & LEDs
Rep rate to 1MHz
Coverage 260-1310nm



It should be noted that future upgrade to your system is possible and if required please consult your HORIBA representative concerning possible options. Because of the modular nature of the system it can be possible to have other options apart from those shown above. Also more than one excitation source / detector may be required to cover the whole wavelength range indicated above.

The previous illustration showed the DeltaPro sample chamber, this is a filter based entry level system coupled with DeltaHub timing electronics. It has similar capabilities to DeltaFlex systems, except that the emission is selected using a filter rather than a monochromator. The DeltaPro sample is designed for simplicity and is not suitable for some temperature control options, such as cryostats. In terms of measurement capability, the lack of a monochromator means that TRES measurements cannot be performed and that the DeltaPro is only recommended for use with a PPD detection module. All other detection modules are best used with a shutter to avoid exposure to ambient light, which is incorporated within the TDM monochromator as standard. For further information concerning the DeltaPro consult **Appendix B**.

The light source controller and detector can be connected via USB and it is recommended that the USB link to the computer is made to either the DeltaHub or FluoroHub-A+, with USB connections to the other components “daisy-chained” from them. This enables the EzTime measurement control software application to “manage” the devices. Decay data can be both measured and analysed using EzTime.

Please refer to the relevant user manual for more information concerning software usage. An in-depth description of the hardware items can also be found in their respective manuals and the users are advised to familiarise themselves with them. This user manual is primarily concerned with the installation and providing an overview to operating the DeltaFlex system.

This user guide assumes that the reader has some existing knowledge of fluorescence techniques, however **Appendix A** will provide a reminder of some of the basics involved.

3. Unpacking and installation

The DeltaFlex fluorescence lifetime system is a sensitive instrument and should be handled with care at all times. Inspect the packaging for signs of rough handling and report any damage to HORIBA Scientific immediately. Do not use the DeltaFlex if you suspect any component may have been damaged during shipping. Because of the modular nature of the system, just a brief overview is given of the installation process.

The shipment will comprise of items similar to those below.

- Optical system comprising of a baseplate with sample chamber (containing a sample holder with stirrer and temperature sensor) and TDM monochromator attached to it (unless a DeltaFlex-00). There is a system distribution board (SDB) attached to the bottom of the baseplate to provide an electronic communication interface. In the case of a DeltaPro sample chamber no baseplate is provided.
- Timing module (DeltaHub or FluoroHub-A+)
- Specified light source(s) (DeltaDiode / NanoLED / SpectraLED) with appropriate controller
 - Note, SpectraLED sources are controlled directly from the Timing electronics
- Detection module (plus hex key for attachment)
 - Optional power supply (DPS-1), which is required for longer wavelength PPD versions (-850 & -900)
 - Optional base unit required for HPPD
 - Optional PHV and CFD-2G required for MCP
- Cables for connecting
 - Light source drive cable (head to controller)
 - Detector power and Stop cables
 - Light source sync to Start
 - Optical system (SDB) to Timing electronics (Spectrometer F-Link)
 - USB cable (long) for connecting the Timing electronics to your host PC
 - USB cable (short) for connecting Light source controller to Timing electronics
- DeltaFlex User Guide, with software, drivers and instructions on a USB stick
- Relevant manuals
- Accessories specified at time of order

Carefully unpack each component carefully removing any protective packaging material and identify it according to the list above.

3.1 Mounting

To prepare the **DeltaFlex fluorescence** lifetime system for use, place the modules on a flat surface next to your PC. An example is illustrated below (Figure 1), including optional components (*). Avoid positioning the system in a location where,

- it is difficult to access the power switches
- ventilation to the fans is restricted
- there is a risk of spillage of water or other liquids

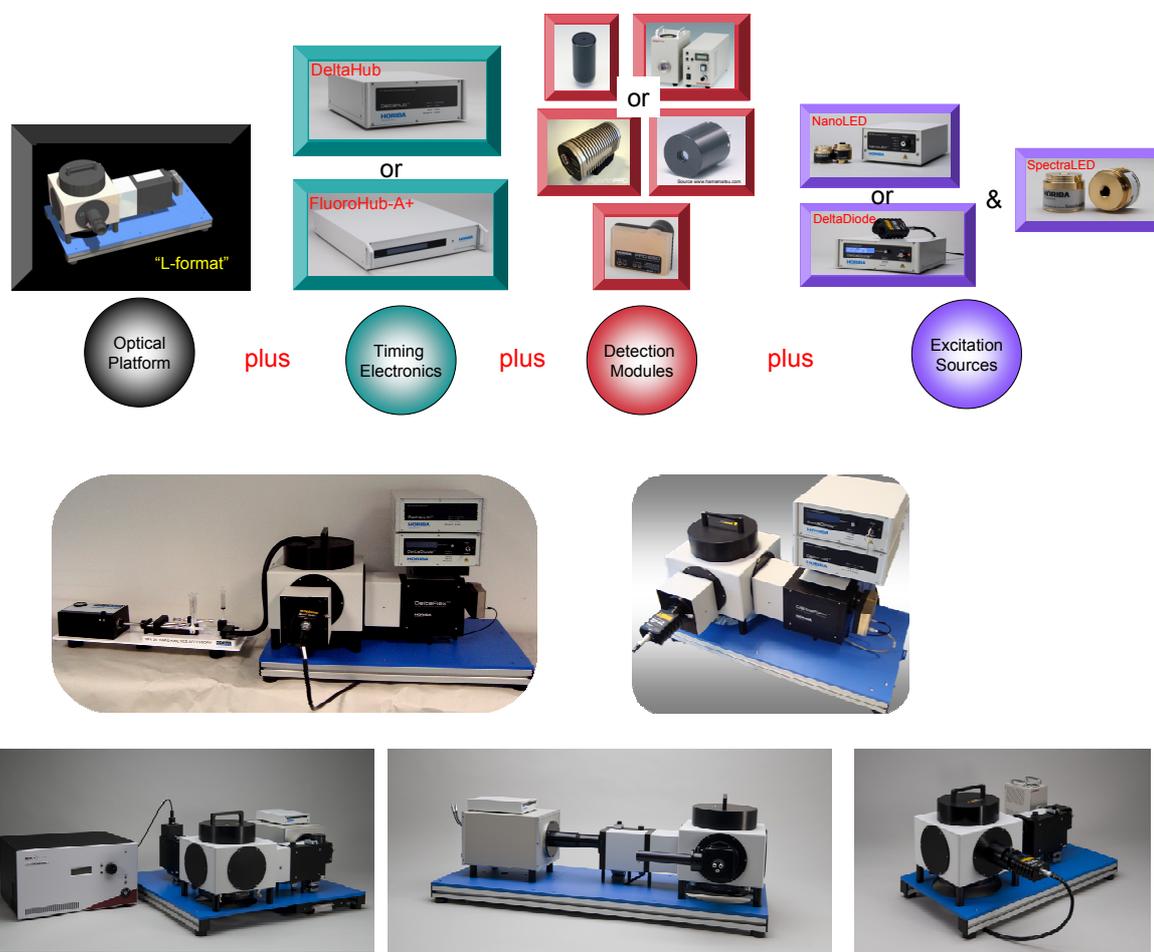


Figure 1. Schematic (top) of component system parts and (below) illustrations of possible configurations for the DeltaFlex, including optional shelf for organising the electronics modules.

3.1.1 DeltaDiode / NanoLED / SpectraLED pulsed light source

These sources connect directly to the excitation port and are fastened using a hex key provided.

3.1.2 PPD picosecond photon detection module

First remove the protective PVC cap and attach the module by slotting it onto the emission port and then tightening the three grub screws, using the hex key provided, to hold it in position.

3.2 Cables and connections

All initial cable connections must be made before switching the power on or installing the software. The connections are described in more detail in the respective individual manuals, but the basic connections are as follows.



CAUTION

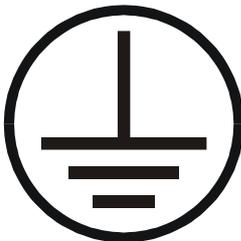
Always ensure that the Timing electronics, computer and any associated modules are switched off while connecting or disconnecting cables. The Timing electronics power switch is located at the rear of the module.

3.2.1 Timing electronics power inlet and power switch

The Power inlet connects the Timing electronics to your mains electricity supply using the cable provided. The power inlet type is a filtered IEC 3-pin socket with fused live and neutral.

The module is switched on and off using the switch next to the inlet.

The mains cable must be connected to a power outlet with an earth connection. For fuse rating and type see Specifications elsewhere in this document. Colour coding of the supplied IEC cable is –



Brown: Live

Blue: Neutral

Yellow/Green: Earth or ground

WARNING: This equipment must be earthed

3.2.2 Your computer (USB)

The Timing electronics connects to the USB port on your PC using the cable provided. Note that the Timing electronics will not function correctly if connected to a USB 1.1 port. The PC requirement is for Windows 7 (32 or 64 bit) or Windows 10, English language version.

3.2.3.1 Fluorescence (DeltaDiode / NanoLED) light source controller

The sources connect to the **head** socket on their respective controller using the supplied drive cable. The **sync** output (NIM) can be connected directly to the **Start-A** input (Lemo 00 to Lemo 00 cable) of the timing electronics (Stop input on the FluoroHub-A+ for reverse mode). The TTL sync output and sync input connections are not used with this system configuration.

3.2.3.2 Phosphorescence (SpectraLED) light source

The SpectraLED sources connect to the **SpectraLED socket** on the Timing electronics using a drive cable. The start signal connection is made internally and overrides the connection to the Start-A input. This means that when using a SpectraLED there is no need to remove any cable attached to Start-A.

3.2.4 PPD picosecond photon detection module

The PPD's power is connected via a 5 pin Lemo connector from the socket labelled **Aux power** on the back of the Timing electronics to the 5 pin **Power** socket on the side of the PPD module. If the PPD features a cooler for the photocathode, then it is powered by a separate DPS-1 power supply module.

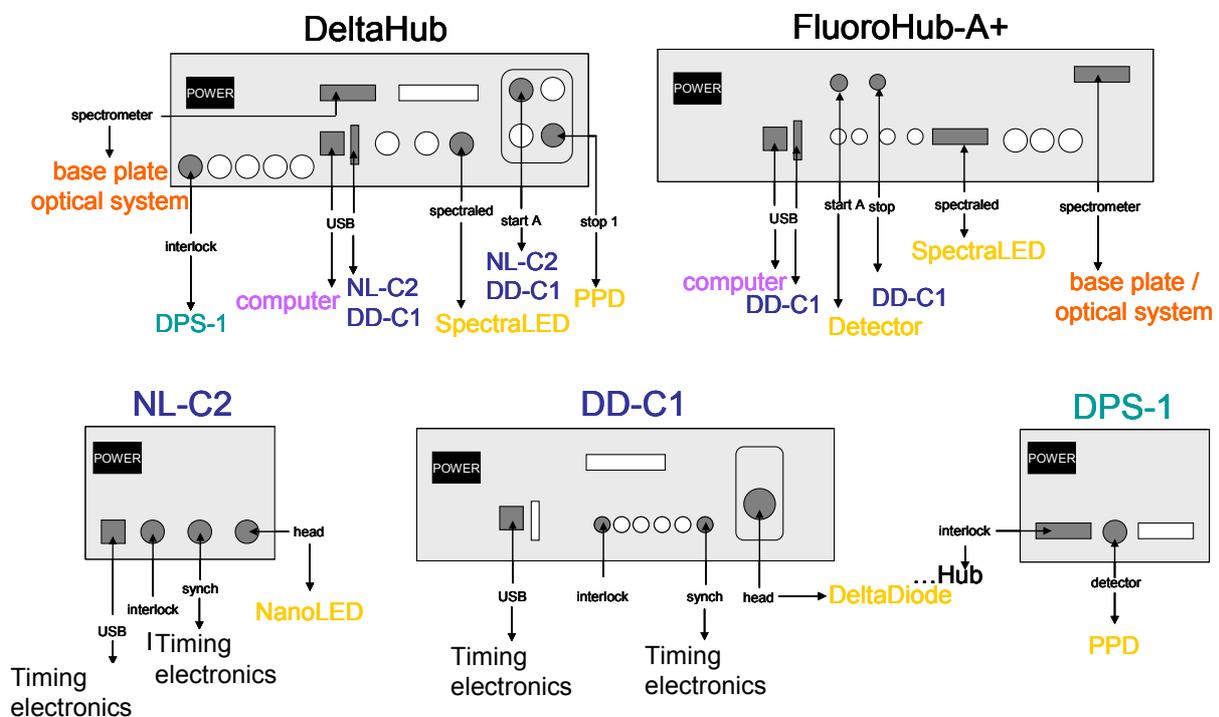
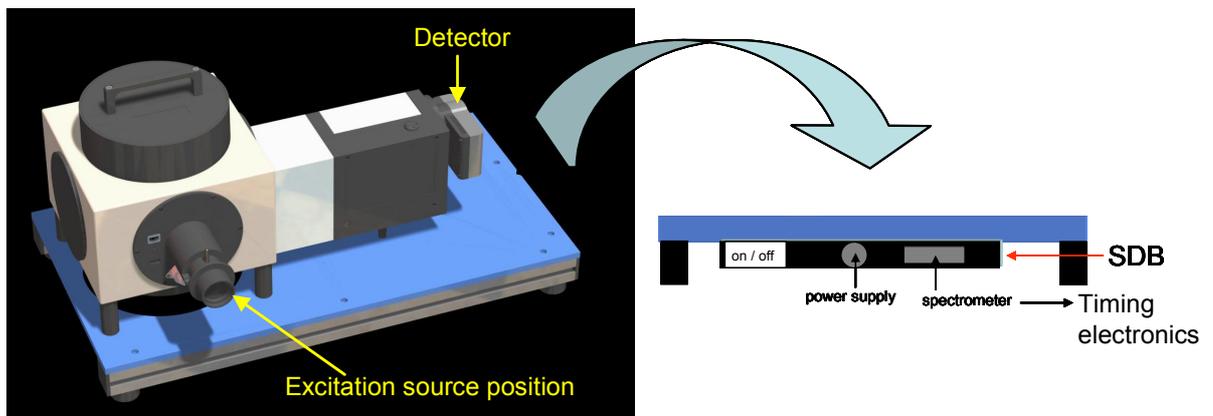
The signal cable (Lemo 00 to Lemo 00) connects from the **NIM out** on the PPD to the **Stop** socket on the back of the DeltaHub (**Stop 1** for single channel use).

3.2.5 Baseplate (SDB)

The baseplate SDB (system distribution board) connects from the **Spectrometer (F-Link)** to the **Spectrometer Interface** on the rear of the Timing electronics via a ribbon cable. An AC-DC power supply provided also connects to the SDB.

3.2.6 Simplified schematic connection schemes (viewed from the rear)

It should be noted, that it is possible to have both (DeltaDiode or NanoLED) and SpectraLED sources connected electrically simultaneously (although only one can be operated at a given time) and the required source then selected in EzTime. The timing electronics can either be a DeltaHub (for a majority of applications) or a FluoroHub-A+. The FluoroHub-A+ is designed for use where short-lived fluorescence is to be measured making use of short transit time spread detectors, such a microchannel plate PMTs. MCP-PMTs couple via a CFD-2G discriminator amplifier to the Start-A input with the excitation source connected to the Stop in order for the equipment to operate in “reverse mode” – see Appendix C.



3.3 Software installation (not necessary if a computer has been supplied)

The DeltaFlex with EzTime acquisition and analysis software is designed for use with **Windows 7** (32 or 64 bit) and **Windows 10**, operating systems (**English language** version only). During the installation process your equipment does not need to be connected. The installation files may be supplied on DVD or USB Stick. The process should be intuitive, but to summarise, on your computer:

- Locate the folder containing your new software
- Click on the *set up* icon
- Follow instructions

If you have purchased a computer with your HORIBA system then the software will have been pre-installed.

Note that EzTime requires a specific version of the Microsoft .Net framework to operate. If your PC does not already have this framework installed then you will be prompted to install this during the installation process. You should use administrator permission to install and activate EzTime. Also, some adjustment maybe required so that the computer does go into “sleep” mode and turn off communication to the system, see **Appendix H** concerning USB power management settings.

At the end of the installation there will be an option to launch the program.

3.3.1 Launching EzTime

Once the software is installed, EzTime can be launched by clicking on the desktop icon . The first time it is launched you will be prompted to enter a registration key. The key is obtained from HORIBA Scientific by providing the system ID. Note the key is specific to the computer. EzTime may be run in “Trial mode” for 30 days without the key, so you can proceed immediately but please be sure to note the system ID and request the key to avoid any later interruption to your work.

When launching, EzTime will attempt to connect to the DeltaFlex instrument. If the instrument is not connected (via USB) or it is powered off, then EzTime will start in the Offline mode. In Offline mode the software may be used to analyse data files that have already been measured, and the Instrument page will not be available. So unless you only wish to perform data analysis in Offline mode, please ensure the instrument is switched on and ready to communicate. If the instrument is not detected, you will be reminded to do so.

NOTE – If upgrading an existing system running DataStation with EzTime, the DeltaHub firmware needs to be V2.6 or above for the program to function. If this is not present then the program will not run and you will be advised to update the DeltaHub firmware. This is included in the installation package and it is vital that the update process can be performed without interruption to avoid firmware corruption. Just follow instructions and as the update can take up to 30 minutes. Please ensure, if using a laptop, that it is fully charged and connected to a power supply. Consult the update guide for details.

4. Operation

The main interface for performing measurements is the EzTime software, which controls the DeltaFlex through the DeltaHub. The DeltaHub is software driven and does not feature any front panel controls; locations of the signal connections for lifetime measurements with DeltaDiode, NanoLED and SpectraLED sources can be seen in section 3.2.6. Users are advised to familiarise themselves with the individual manuals for these products. This section will only deal with the basics of operating the equipment with the view of obtaining and analysing basic time-resolved measurements of a liquid sample in a standard 10mm pathlength fluorescence cell. However, similar measurement principles apply to other types of samples.

4.1 Measurement modes

The principal modes of measurement that can be utilised are given below

- **Lifetime** – for measurements of decay times from 25ps to 1s*, shortest data acquisition time of 1 ms
- **Steady state** – uncorrected emission spectrum using your chosen pulsed excitation source ^[a]
- **Anisotropy** – fluorescence and phosphorescence timescales ^[b]
- **TRES (time-resolved emission spectra)** – collect decays at specified wavelength increments (fixed dwell time or peak count preset) on both fluorescence and phosphorescence timescales ^{[a] [b]}
- **Histogram streaming** – measures 1 to 26,000 sequential decays, from 1 ms to 1 min per decay. Only available for one stop channel at a time ^[c]
- **Photon streaming** – measures individual photon arrival events in terms of macrotime (time from start of experiment) and microtime (time in relation to optical pulse). These photon events can be converted to histograms and up to 26,000 histograms can be analysed ^[c]

* Depending on excitation source

^[a] Not available for DeltaPro systems as requires monochromator

^[b] Script based measurement, also configurable using an automated script feature



^[c] Configured and run from the Preview tab on the Instrument page. Photon streaming requires appropriate hardware to be present and streaming modes not available for FluoroHub-A+ based systems.

4.2 General considerations

After checking that the appropriate hardware is connected, turn on the individual components and computer. For critical measurements it is recommended to leave the hardware for several minutes to stabilise.

Prior to commencing a measurement it is helpful to know a few details about the **sample**, such as;

- Excitation wavelength (λ_{exc}) and emission wavelength (λ_{em}) – obtained from steady state absorption and emission measurements
- An approximate idea of the decay time – either from a previous measurement or literature

The former will determine the selection of excitation wavelength and hence the choice of excitation source, along with the filter to select the emission. It should be noted that to avoid problems of self absorption it is advisable to prepare a sample with an optical density < 0.1 at and above the excitation wavelength. As the emission will always be at longer wavelengths (**see Appendix A**), if using an emission filter it should be selected to at least remove any scatter at the excitation wavelength. An idea of the expected decay time will aid in choosing between a DeltaDiode / NanoLED for a fluorescence measurement (25ps to 10 μ s) and a phosphorescence measurement (1 μ s to 1sec) using a SpectraLED. Note that there is some overlap in the time range that the excitation sources can access. Once the appropriate light source for excitation and the emission wavelength have been selected, the procedure for making a lifetime determination is based on the following steps;

- Use of the EzTime software to optimise and control the experimental conditions
- Collect and analyse the data to determine the lifetime
- Save, display or export the results

When setting up for a time-resolved measurement there are some considerations to keep in mind

(a) The first step is to choose the **Time Range** (in Instrument page). It is recommended to select a time range approximately 10 to 20 times that of the expected lifetime, so that all of the decay can be seen on the screen. The timescale should be selected as to provide;

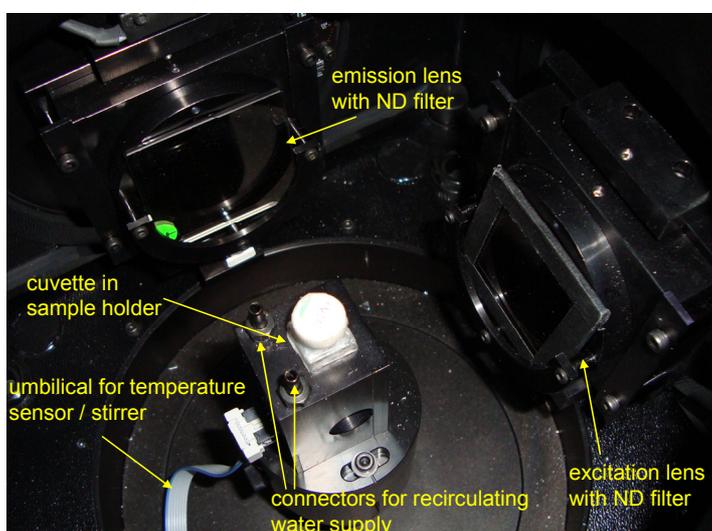
- the required time resolution
- reasonable data acquisition time
- an appropriate number of data points in the decay

(b) Check that the chosen **excitation source** is attached. The type of light source should match that selected in the Advanced Settings, Start Inputs tab. Note that it is possible to exchange light source heads with the DeltaFlex powered on, although it is recommended to put the key switch on the DD-C1 or NL-C2 controllers to standby first.

(c) The wavelength can be changed by selecting the Optics dialogue on the Instrument page, which will activate a drop down form. Wavelength can be changed either by using the arrows or by typing in the number, followed by Apply or OK. The bandpass and shutter can also be accessed here. Typically the shutter is in “Auto”, where its state will depend on the interlock signal provided by the microswitches sensing the presence of the sample chamber lid (no lid = shutter in closed position).

Note – The microswitches in the sample chamber also activate a shutter (when the lid is removed) on the excitation optics. This means the path of the excitation source is automatically blocked when the sample chamber lid is removed.

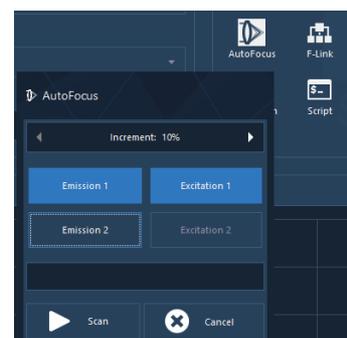
(d) Place the **neutral density filters** (always use more to avoid excessive light at the detector and then reduce to increase intensity). Then place the **sample** into its holder (different types, available as accessories are given in **Appendix B**).



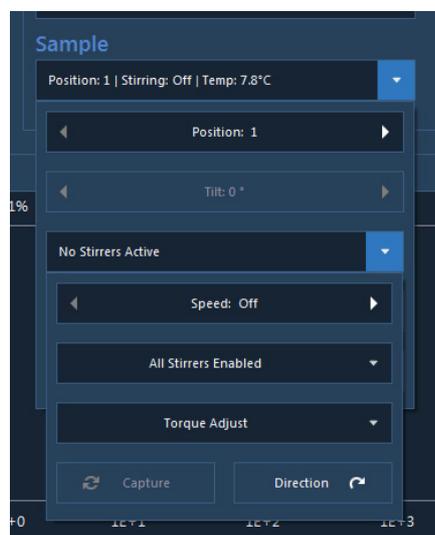
(e) Close the lid; this activates the shutter situated within the monochromator. With the lid open the shutter (unless overridden) is automatically closed to protect the detector.

(f) If motorised polarisers are to be used and fitted see **Appendix C**. For example they can be employed for magic angle conditions; vertical (0) on excitation and at the magic angle (54.7°) on emission, which is advisable if using laser excitation sources with certain samples to remove depolarisation effects. These can be set in the Optics form (Instrument page); use Apply to set the angle. If using manual polarisers in graduated mounts, these should be adjusted accordingly.

(g) The signal strength (count rate) can be further optimised by changing the position of the lenses to adjust the focus. This can either be done through the Optics form or by using the **Autofocus** feature. This is located in the **Tools** section of the Instrument page. The appropriate optical path should be selected and the “scan” chosen which will initiate an optimisation of the selected lenses to optimise the signal strength (count rate).



(h) If the sample requires **stirring** make sure that an appropriate stirrer bar is present in the cuvette. The stirring speed and direction can be controlled by selecting the dialogue under Sample.



Note that if the stirring bar becomes trapped, the “**capture**” button can be used, which momentarily stops the stirrer and then ramps up the speed to aid coupling between the “stirring” magnetic field and the bar. If a Sample Turret is installed it can also be controlled from this dialogue.

The temperature of the sample holder is also monitored and is displayed here.

The sample holder has two connections which allow it to be coupled to a recirculating water bath. Connections for tubing are present inside and on the outside of the sample chamber. An inlet is also present to connect oxygen free nitrogen to flush the sample chamber. This is recommended when using temperatures below 10°C to avoid condensation forming on the sample.

Note that the specified temperature range is -40 °C to +70°C.

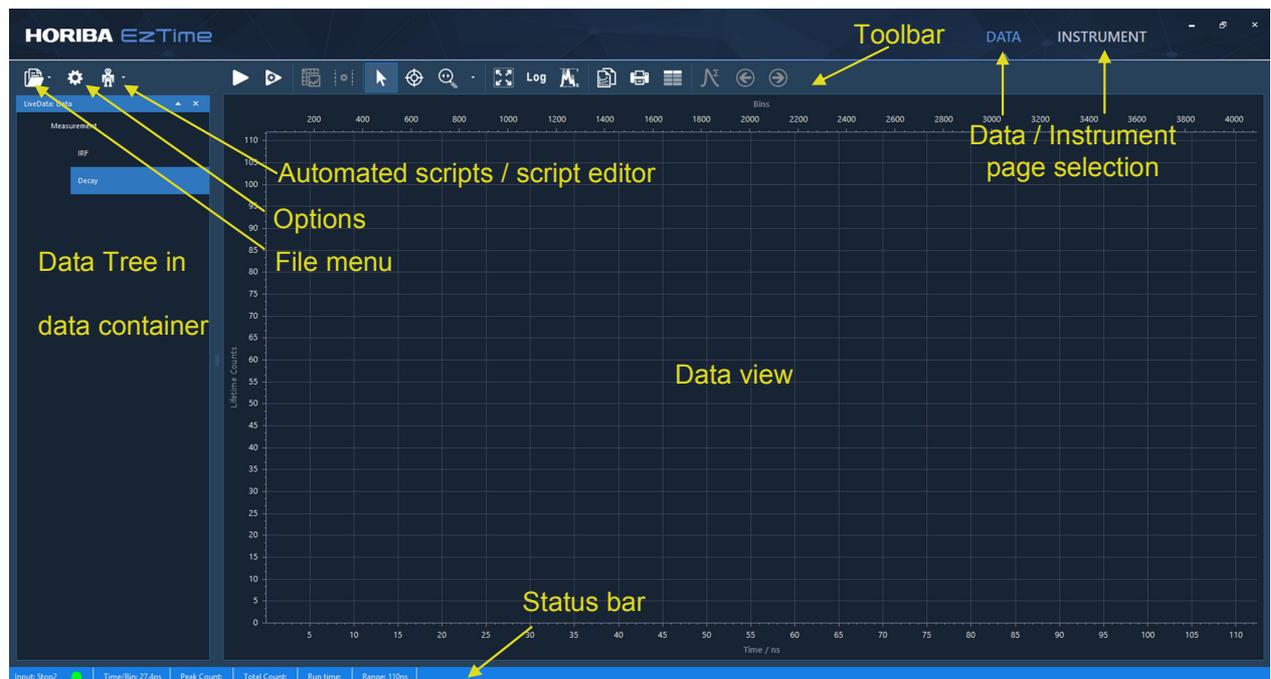
4.3 Performing time-resolved measurements using EzTime

After checking that the appropriate hardware is connected and the optical system is turned on at the baseplate, turn on the computer and electronics (ie. DeltaHub, light source and detector power as appropriate). Make sure that they have initialised (eg the power indicator on the DeltaHub becomes constant) prior to launching the software. For critical measurements it is recommended to leave the hardware for **30 minutes** to stabilise.

Launch the EzTime software by clicking on the icon on the desktop. This software should automatically find and initialise any accessories present. If it does not load check the connections and retry. Leave the DeltaFlex until the power indicator on the DeltaHub becomes constant before launching this application.



Once EzTime has loaded, the **Data page** appears. This is the page for acquiring (both time-resolved and steady state) and analysing data. All data are stored in “containers” which can be saved and opened in EzTime.



The tool bar functions are summarised below



4.3.1 Data storage concept

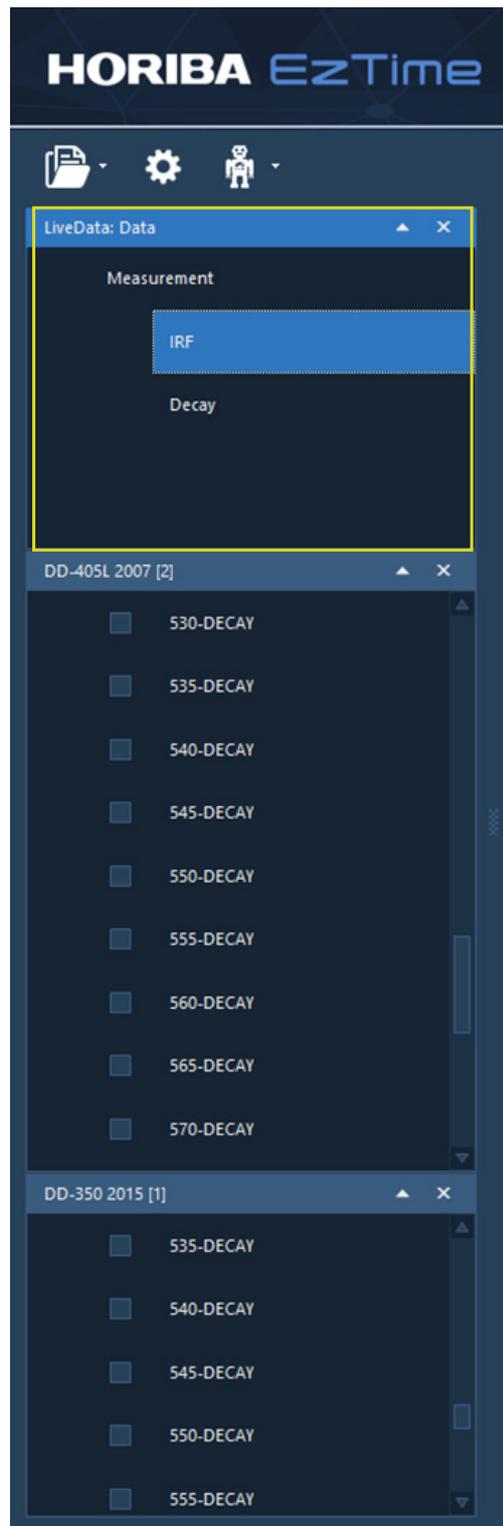
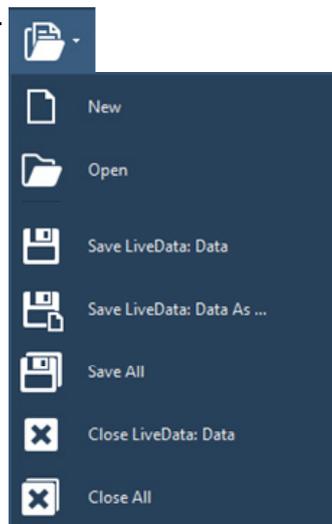
Data is stored in “containers”

- The primary “LiveData” container (always positioned uppermost left) is the only container that can be used to collect new data. Any other visible containers are “locked” - no new data can be collected into them.
- Analysis is allowed in both “live” & “locked” containers.

Each container can have many nodes; i.e. decays / IRFs / spectral data. Additional nodes can be added by tap / right click on Measurement, which brings up a radial menu shown below.

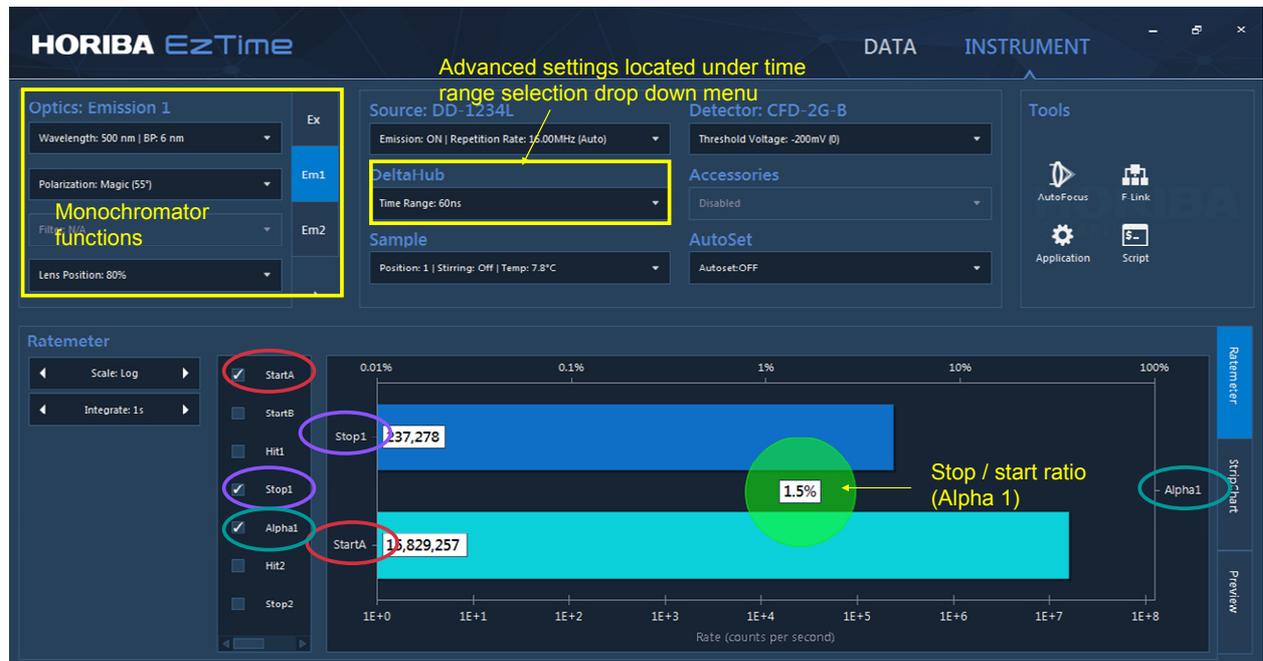


New containers can be made from the file menu (below) and existing containers can be opened and closed (saved) using this menu.

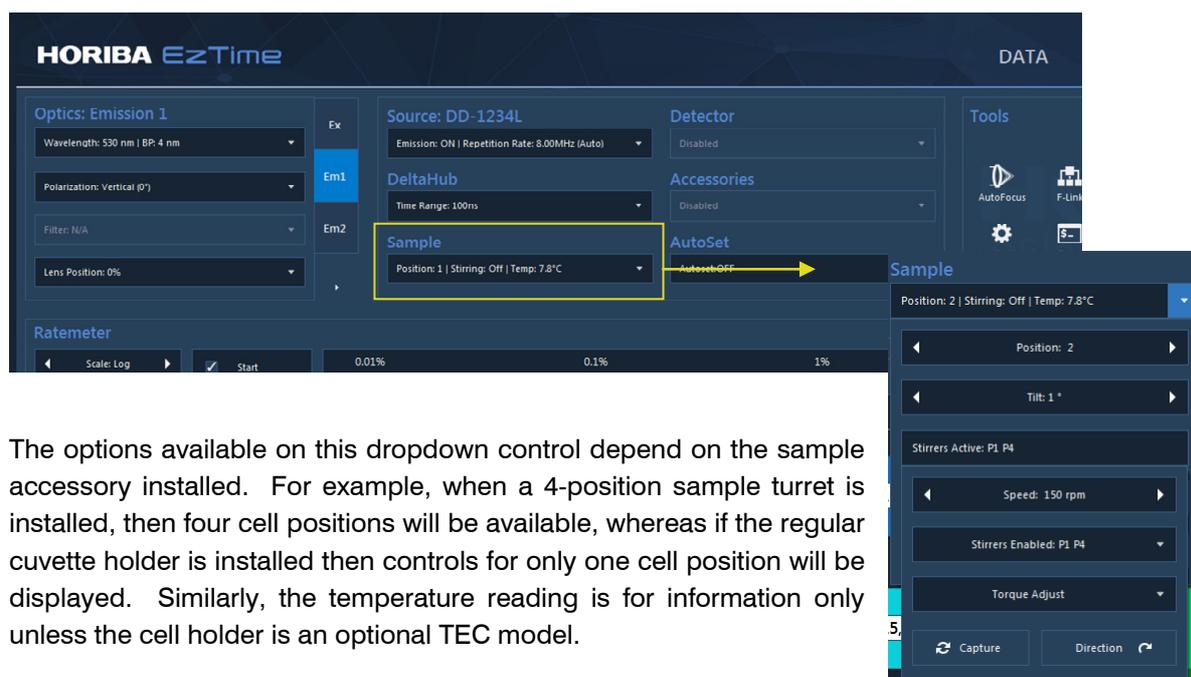


4.3.2 Measurement set up – Instrument page

The conditions, such as time range, optical parameters and count rate can be set up in the **Instrument page**. This is selected by clicking (tapping) on Instrument on the top on the right of the EzTime form. It is recommended that these parameters are set up and optimised prior to performing a measurement.



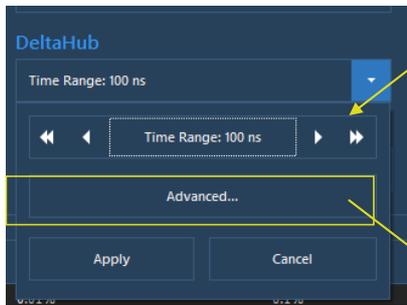
(a) Sample control: position, temperature, stirring.



The options available on this dropdown control depend on the sample accessory installed. For example, when a 4-position sample turret is installed, then four cell positions will be available, whereas if the regular cuvette holder is installed then controls for only one cell position will be displayed. Similarly, the temperature reading is for information only unless the cell holder is an optional TEC model.

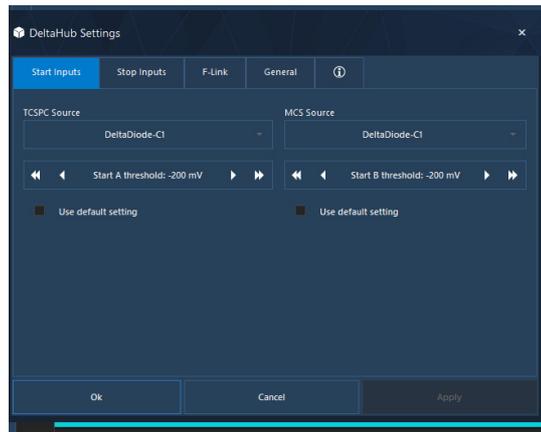
(b) Time Range control

This dropdown control selects the time window to be measured. Changing the Time Range also selects the allocated excitation source (chosen in Advanced Settings) and sets the optimal operating parameters for that source to suit the newly selected Time Range e.g. Laser diode repetition rate.



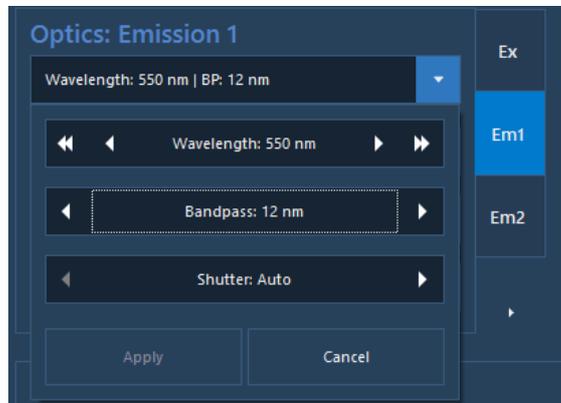
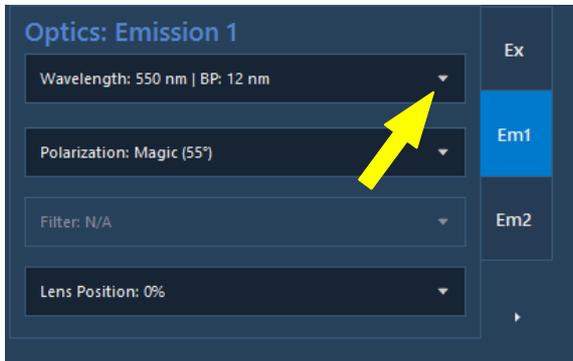
Time range selection

Advanced settings can be used to change sources for TCSPC and Phos plus Stop channel inputs etc.



Note: For changes to be registered the “Apply” button must be clicked

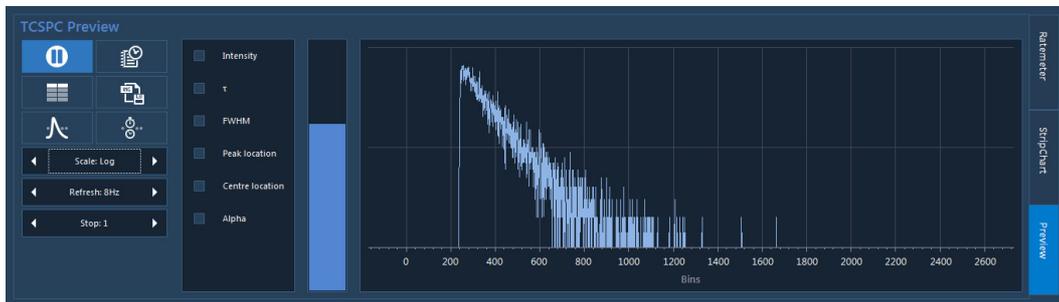
(c) The monochromator emission wavelength, bandpass and any (optional) motorised polariser position can be selected in the optical control forms.



(d) The count rate can be seen using the **Ratemeter** tab. The alpha indicator which relates to the stop to start ratio will change colour (red from green) if the recommended 2% ratio is exceeded.



As well as using the ratemeters, the preview mode allows optimisation of measurement conditions.



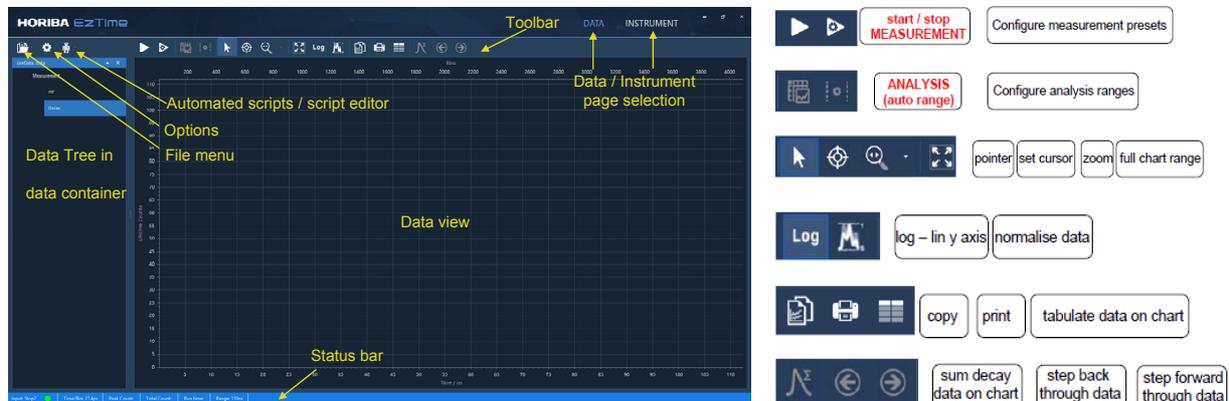
Running the preview mode (view continually refreshes) enables the IRF / decay shape to be seen and temporal position to be altered.

Different rates and widths can also be displayed to help set up (more complicated) systems.

In MCS ranges the preview acts as a similar way to a measurement (rather than continuously refreshing) to set the conditions for the measurement.

4.3.3 Data collection – Data page

Once experimental conditions are set go to the Data page to collect and analyse your decays.

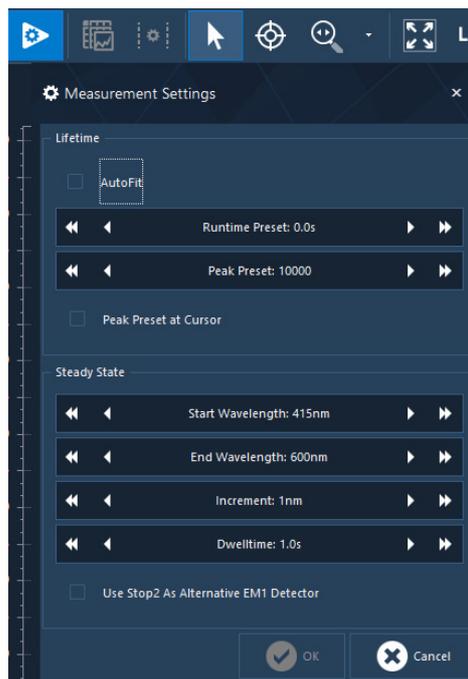


(a) Configuring peak or run time presets

Selecting  to the right of the start/stop measurement button opens the Measurement Settings form. Here the presets for new lifetime and steady measurements may be entered.

If Autofit is checked then the decay data will automatically be fitted to 1,2 & 3 exp models after the decay measurement stops. Suitable data fitting ranges will be automatically chosen. (Tip: if reconvolution with the IRF is required then the IRF needs to be measured before the decay, otherwise a “tail fit” will be performed.)

Note if both Presets are non-zero then the measurement will terminate when the first preset is reached. (Tip: 0=preset disabled). There is also a maximum limit of 65,000 counts in the peak channel when using a DeltaHub.



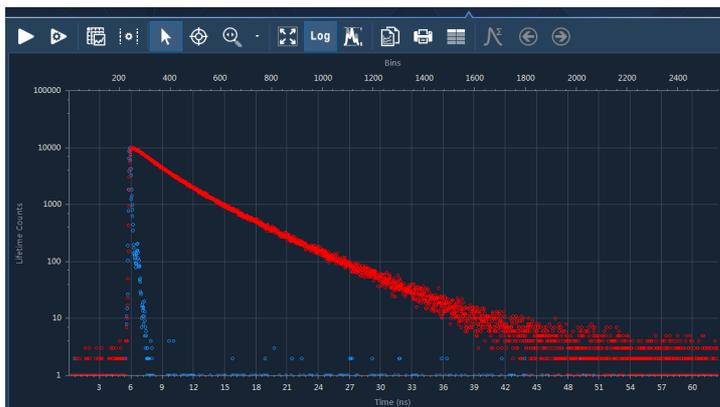
(b) Collecting data

Select IRF or Decay in the tree to measure an IRF / decay and then  to collect data.

IRF is blue

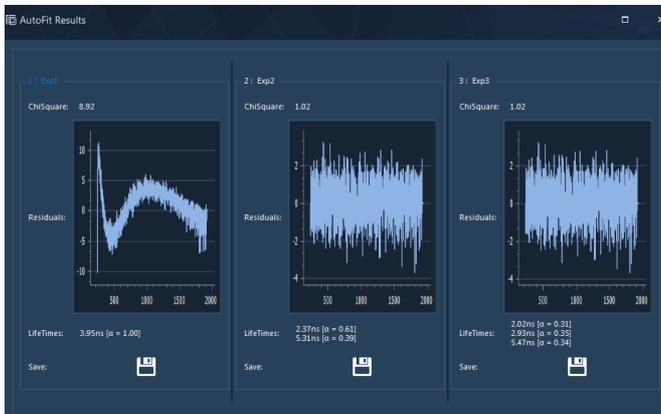
Decay is red

unhighlighted data - grey



If auto fit has been selected, then at the end of the decay data collection the fits using 1, 2 and 3 exponentials will be displayed.

Fits need to be saved and will then appear under the respective decay in the measurement tree. Otherwise the fit will be discarded. If further analysis is needed then this can be performed “manually” – see 4.3.4.



(c) Saving data and making new measurements

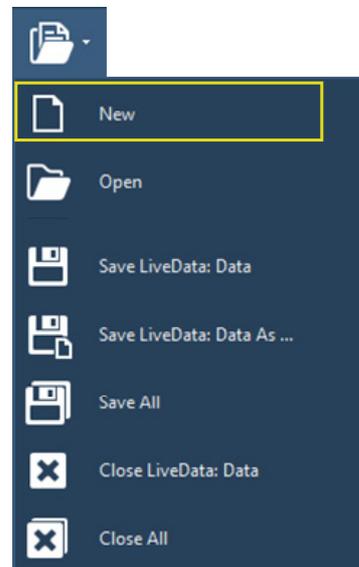
New measurement nodes can be created by:

(i) Adding individual nodes (IRF or decay) - right click or tap measurement to open radial menu...



(ii) Adding measurement pairs i.e. a measurement node with IRF and decay sub-nodes

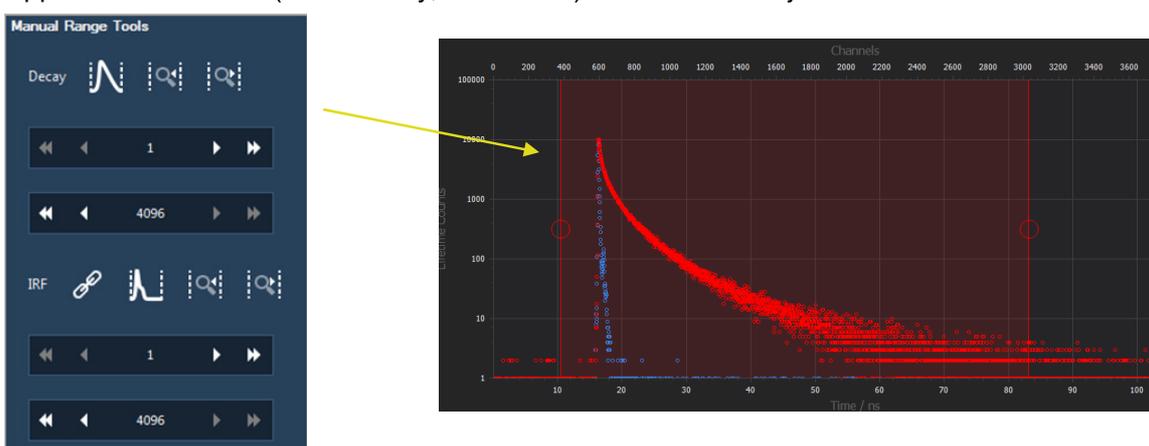
(iii) Selecting *New* from File menu (left) will open a new measurement container with its own tree view. This will lock the previously created container.



(c) Using selected data from the Data Tree – manual fit limits

As before select the data that you wish to analyse so that it is present in the Data View.

Select the configure icon  to the right of the Analyse button and put in the ranges for decay and prompt (if different). The form below will appear “docked” but can be made to float as shown. Cursors will appear on the screen (red = decay, blue = IRF) which can be adjusted



Data can then be analysed and viewed as before (see 4.3.4 (a))

Highlighted data containers can then be saved using the file menu. On exiting the program you will be reminded to save any unsaved data.

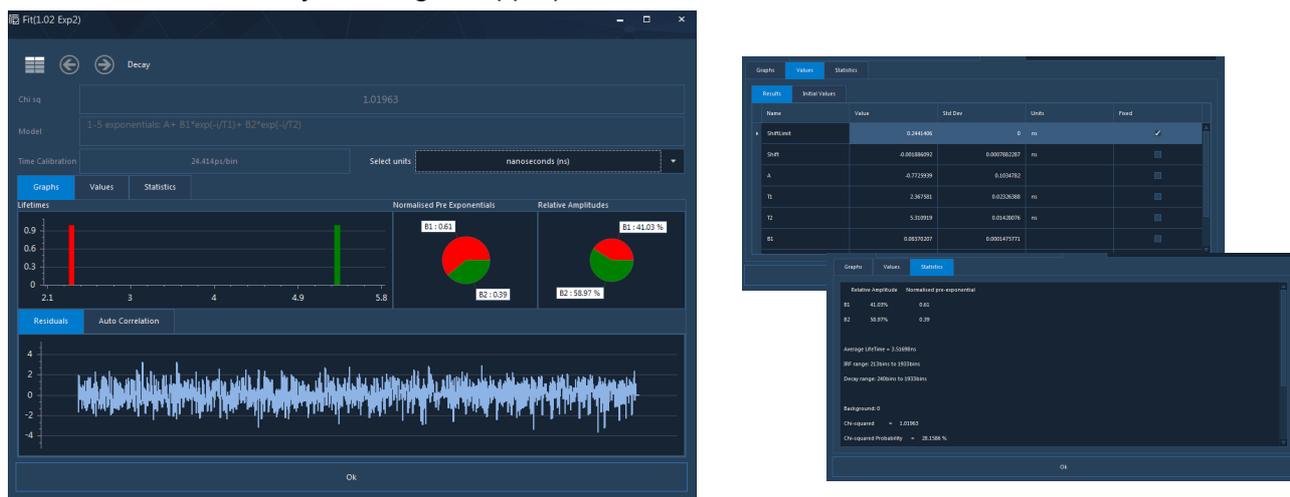
4.3.4.1 How do I know if I have a good data fit?

The usual criteria to assess whether a fit is satisfactory are to use the reduced chi-squared value and to see if there are any trends in the weighed residuals. Also the fitting of an additional exponential decay component should not produce any significant improvement. The returned values should also be sensible. Generally, when using reconvolution, it is only possible to measure a lifetime that is approximately 10% of the instrumental full width at half maximum. The following should be considered in assessing the fit data, along with any prior knowledge of the system studied.

- Chi-squared value below 1.2, not meaningfully improved by adding an extra decay component.
- Randomly distributed weighted residuals.
- Do the lifetime(s) correspond to previous reports (measurements or literature)?
- Lifetime values of 10^{-11} seconds (0.01 ns) or one data channel should be treated with caution and may relate to scattered excitation light reaching the detector.
- Large (e.g. more than ~ 3 channels) or negative shifts may indicate a problem.
- Excessive errors (s. dev) on the data.
- Negative pre-exponential components (B values) are not necessarily a sign of a bad fit, they can indicate the presence of an excited state process and are referred to as “rise times”.

4.3.4.2 Interpretation of and exporting fitted data

Once the data has been analysed the results can be accessed either by selecting the “view results” option just after the fit has completed or by selecting the fit node in the tree and using a right click or tap to bring up a radial menu with “view results”. If the “view results” option is chosen, then a graphical representation, such as that shown below can be seen. The initial view is graphical but values and statistics can be found by selecting the appropriate tab.



In the graphical view the lifetimes obtained are given along with their contribution to the overall fluorescence emission. Also in the statistics tab a value for the average lifetime can also be found. These are obtained in the following manner. If we represent the fluorescence decay as follows,

$$I(t) = \sum_i^n \alpha_i \exp(-t / \tau_i)$$

Then the normalised pre-exponentials, which are indicative of the relative “concentrations” of the fluorescing species.

$$\alpha_i = \frac{\alpha_i}{\sum_{i=1}^n \alpha_i}$$

Another way to represent the relative contribution of different fluorescing species is to use the relative amplitude or fractional. This is the relative “concentration” weighted by the lifetime and can be more easily related to the steady state emission.

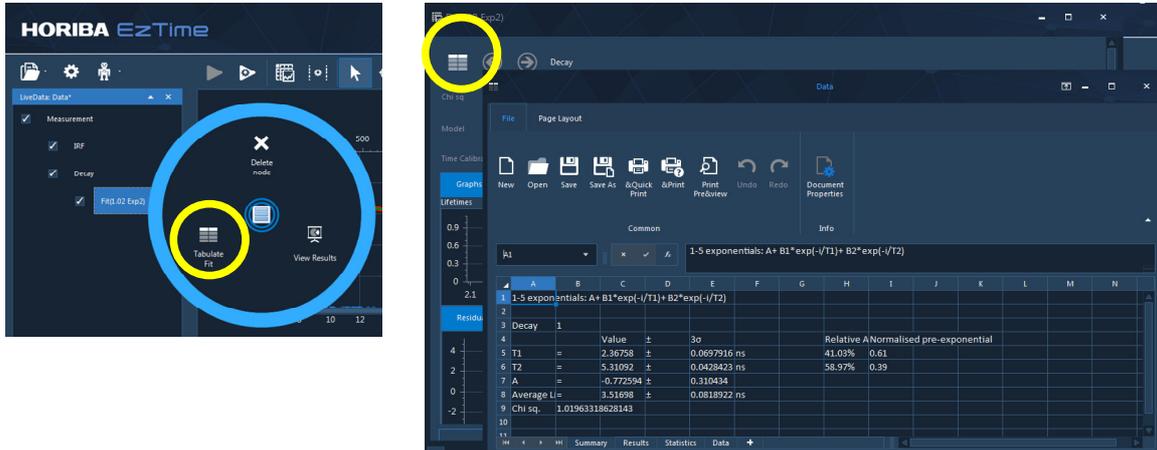
$$f = \frac{\alpha_i \tau_i}{\sum \alpha_i \tau_i}$$

The average lifetime is provided as $\tau_{ave} = \sum_{i=1}^n \alpha_i \tau_i$, however, if for example, Stern-Volmer quenching is

an application then the user may prefer to calculate the average in the manner shown below (not given in EzTime). Please consult the literature for the correct method for your application.

$$\tau_{ave} = \frac{\sum_{i=1}^n \alpha_i \tau_i^2}{\sum_{i=1}^n \alpha_i \tau_i}$$

The resultant graphics may be copied directly by using a right click / tap on the selected chart to bring up a radial menu. It is also possible to export data in a spread sheet (work book) format. This can be done by selecting the tabulate function, either from the radial menu when accessing the fit results or when viewing the results, see below.



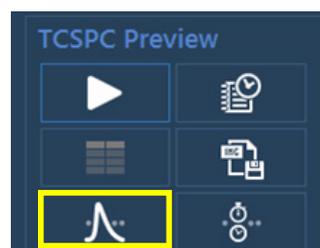
The work book, which can be saved outside of EzTime and opens as a MS Excel workbook, contains a summary of the fit results, as well as the “raw” data for the decay, IRF (if applicable) and the fitted function. This enables the decay data to be incorporated into third party software if required, ie for custom analysis and data presentation.

4.4 Histogram streaming measurements (Appropriate hardware required)

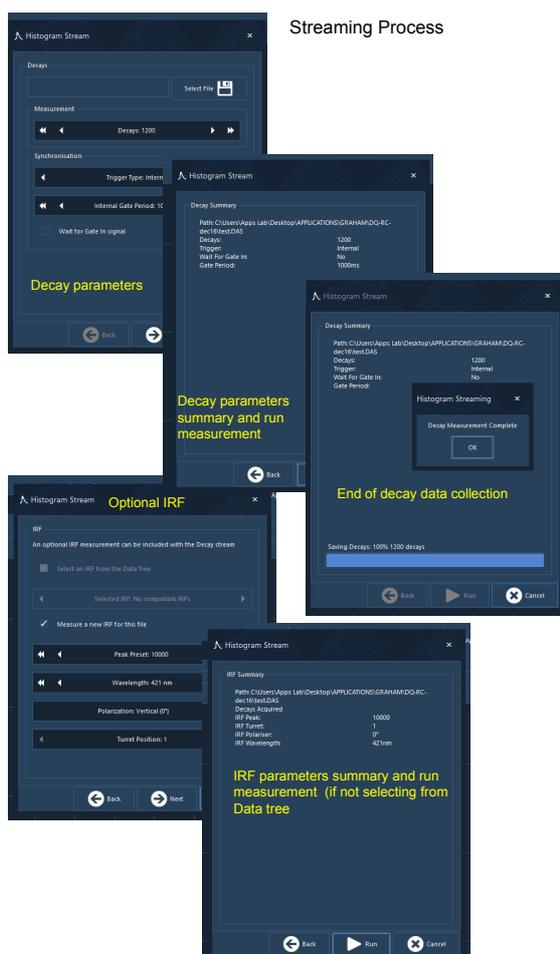
This mode, selected from the **Preview tab** on the **Instrument page**, permits the acquisition and analysis of up to 26,000 decays measured sequentially, without any delay between individual decays. The time per measurement can range from 1 ms to 60 seconds and the measurement can be triggered directly using EzTime or using an external (TTL) trigger connected to the Timing electronics. There is the option to save an instrumental response (IRF) along with the decay data. This can be acquired at the end of the measurement or selected from a previously measured IRF in the Data Tree. It should be noted that this measurement type is unavailable on the phosphorescence timescale.

The measurement can be performed in the following manner;

Histograms of decay data can be streamed by clicking on (tapping) the button on the Preview tab, right.



This then brings up the following forms enabling the measurement conditions to be set.



The number of decays and the time per decay (internal clock period if this is to be controlled via EzTime) should be adjusted to suit the sample being measured. This will depend on the timescale of the process occurring and also the strength of the fluorescence emission, i.e. low count rates may require longer times to obtain sufficient data.

After measuring the decays it is possible to either measure an IRF or pick one present in the Data Tree. The appropriate box should be checked to associate it with the file.

The triggering of the measurement can be made in the following ways;

Internal - commences by pressing Start on the form and uses internal clock period (expressed in ms).

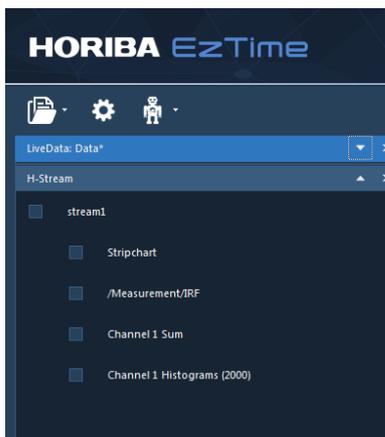
If the **Wait for Gate In signal** is checked then the Start needs to be pressed, but acquisition will only occur after an external trigger signal has been received (connects to Gate In on the rear of the DeltaHub). This makes use of the **internal** clock to provide the data collection period for each sequential histogram.

External - Start needs to be pressed, but the acquisition only commences when an external trigger is received, and uses the clock period of the external source.

When the data has been collected the corresponding data file will be written and only after **Done** has been selected should any further measurements or opening of the file be attempted. The length of time for writing the file will depend on the number of decays it contains and when the maximum number of 26,000 is used this can take several seconds and mean that EzTime may not respond.

4.4.1 Analysis for streamed histogram data

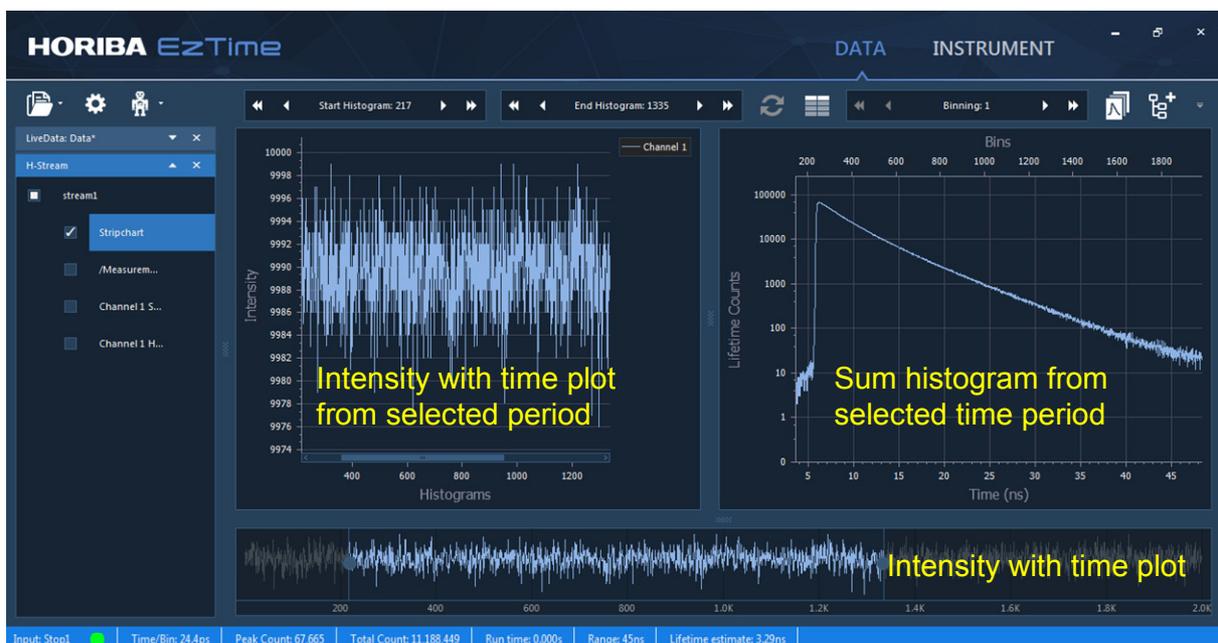
The file relating to the histogram stream data appears automatically after the measurement as a data container. It will typically have the nodes shown below for a measurement containing an IRF.



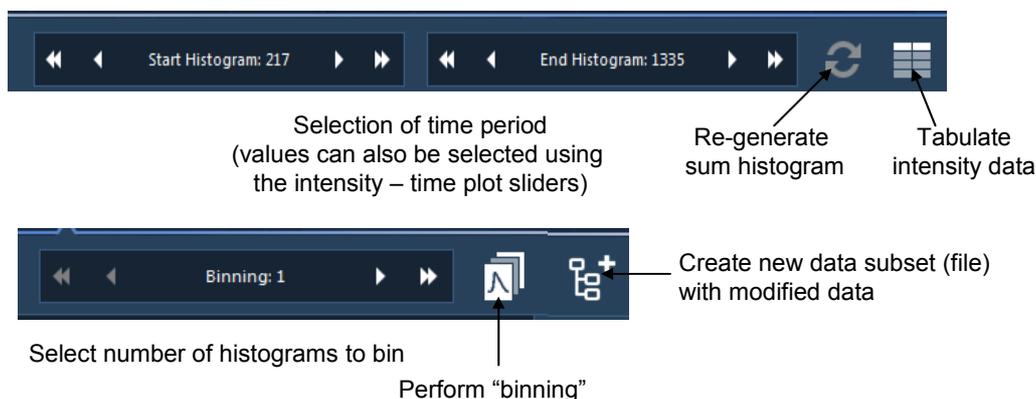
Amongst the nodes are those for the (optional) IRF and the sum of all the histograms collected (channel 1 sum in the example). Selecting these will show them in the usual manner.

All the streamed histograms are in one node (channel 1 histograms (2000) in the example). 2000 represents the number of individual histograms). Selecting this node will only show one of the streamed histograms at a time. They can be stepped through manually or automatically if required. They can be displayed at the same time as the IRF and sum histogram.

The other, stripchart, node changes the Data view to that shown below and cannot be displayed at the same time as the other nodes.



The node, as well as giving an indication of changes with time, allows for data manipulation of the histogram stream. Two “sliders” either side of the intensity - time plot allow truncation of the time range. It is also possible to “bin” subsequent histograms to obtain more counts for analysis. A new subset of the modified data can then be made and can be saved. Note, the original data file is not amended. The function of selected icons present on the “strip chart” node are indicated below.



If a data subset has been created, it will contain similar nodes to that of the original data. Fitting of the whole dataset is performed by selecting the histogram node (and IRF if required). The data can be fitted either using a global model (sum of exponentials) or a batch model. Using the preview button a representative individual decay or the sum of all the decays can be fitted and when satisfied with the fit, that decay model and starting estimates are then applied to all the decay data. The Global analysis will iterate across the dataset to provide common lifetimes for all the decay curves. The batch analysis will apply the same exponential fit to all the decays and fit each individually. A third option is to sum all the decays in the dataset (using the preview button in the menu bar) and fit this total decay curve. When a satisfactory fit is obtained the recovered lifetimes are then applied and should be fixed as common to the individual decay curves and the pre-exponential factors iterated for each.

It possible to view a summary of the recovered fit parameters, by first choosing the desired fit from the tree and then the **Tabulate** option. This outputs a spread sheet, which may later be copied into a third party program.

4.4.2 Experimental considerations for streamed histogram measurements

The careful planning of this type of measurement is important in order to obtain good quality data. The data quality ultimately depends on the number of photons collected. If there is not sufficient data collected then the analysis may be compromised.

More photons can be collected by

- increasing the excitation repetition rate (certain DeltaDiodes can be used up to 100 MHz)
 - lifetime dependent – need to avoid re-excitation of sample before it has decayed

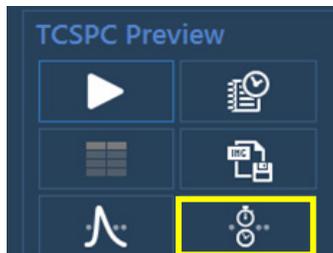
- increasing data collection period
 - dependent on timescale of the process to be observed
- exceeding the usual start to stop ratio (>2% can be tolerated if just observing relative changes)
 - but within reason, <10% and depends on accuracy of data required

Therefore to get the most out of a histogram streaming measurement both the quantum yield, lifetime and the timescale of the process need to be considered and it can be necessary to perform an initial measurement to optimise the conditions.

However if it is not possible to further optimise the experimental conditions, more photons can be analysed by binning sequential histograms, although this will be at the expense of time resolution.

4.5 Photon streaming measurements (Appropriate hardware required)

This mode requires the appropriate hardware to be present on the DeltaFlex. If it is not, the Photon Streaming function (see below), found on the **Preview tab**, will advise if selected.



Photon streaming, also known as “time-tagging” or “FIFO mode” records individual photon events rather than sorting them out into histograms. Each photon is “time stamped” in terms of its arrival time after the start of a measurement (the macrotime) and its time in relation to the optical excitation pulse (the microtime). Because of this the resultant file sizes can be quite large (many Mb to Gb). Using this mode it is possible to simultaneously collect data from two (or more) detectors and offers good time resolution, based on the macrotime clock, to follow kinetics. The data obtained can also be made into histograms and can simply be manipulated and analysed in the same way as histogram streamed data, see **section 4.4.1**.

To perform a measurement in Photon Streaming mode, first select the icon (shown above) on the Preview tab on the Instrument page. The forms given for performing the measurement follow overleaf. Note that it is also possible to associate an IRF with the streamed data. The IRF can be measured prior to the Photon streamed data or it is possible to combine with an IRF (in a different container) at the analysis stage. In both cases the measurement time range must be the same. The collection channel(s) that will be that selected under the Stop Inputs tab.

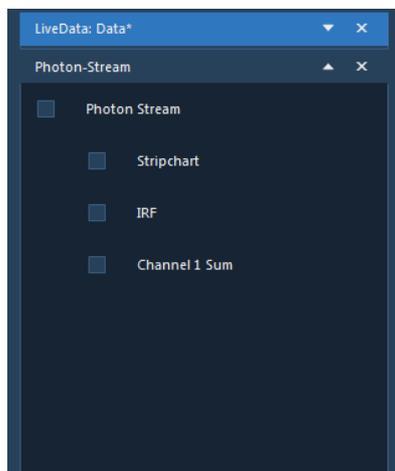


Selecting “Run” starts the measurement process and while the data is being acquired a progress indicator showing both the intensity and the summed photon events will be displayed.

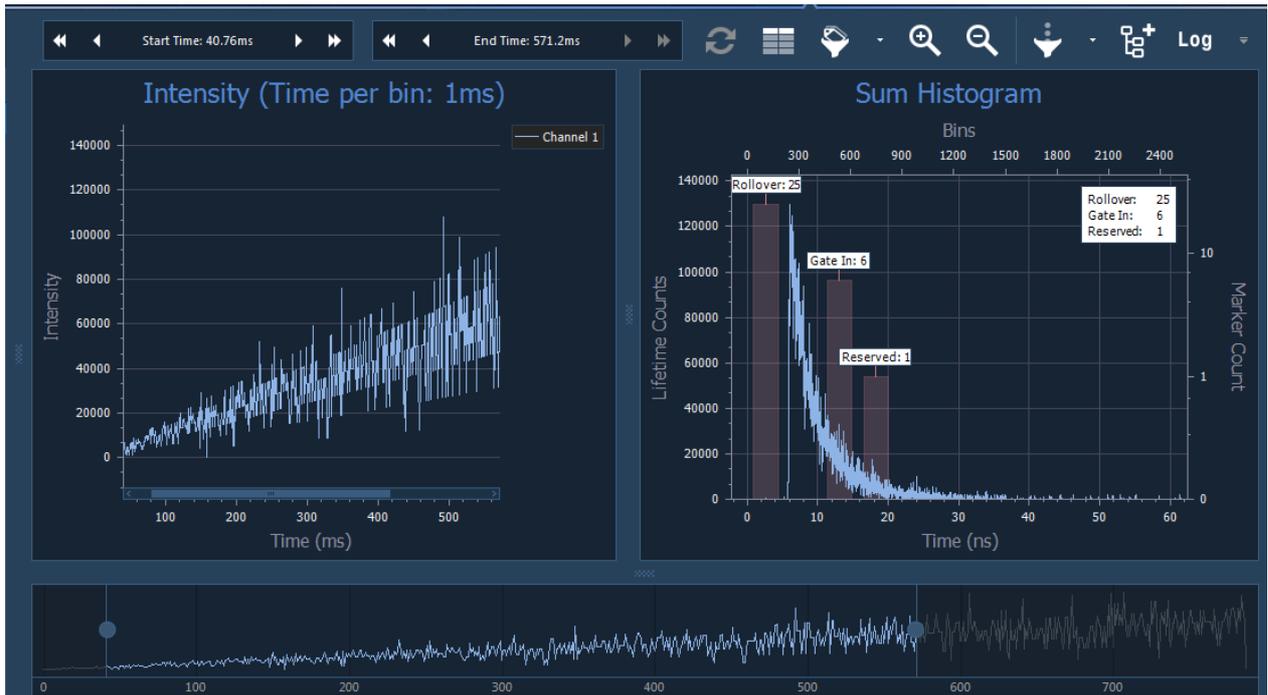
Once the measurement reaches a preset (time or file size) a message stating that the measurement is complete appears. Next, when continuing, is an option to associate a previously measured IRF with the measurement.

The measurement will now appear on the Data Page.

The file container will contain (for a single channel measurement) the nodes as shown below.



As with the Histogram streaming measurement there is a node relating to a histogram comprising of the sum of all the photon events and an optional IRF. There is again a Strip Chart node with a similar format, but in this case rather than consisting of histograms it contains individual photon events. An example of the Strip Chart view follows.



It is from this chart that data can be manipulated and histograms generated, that can be either analysed in a global or batch manner. The bottom intensity – time chart has “sliders” that can be used to select a certain time period, which is then displayed in the upper left panel. A histogram of these photon events can also be generated and is shown in the upper right panel. A summary of the icon functions associated with this view is given below.



Selection of time period
(values can also be selected using the intensity – time plot sliders)

Re-generate data

Tabulate intensity data



Marker display selection

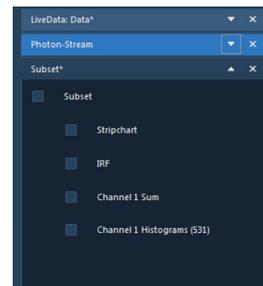
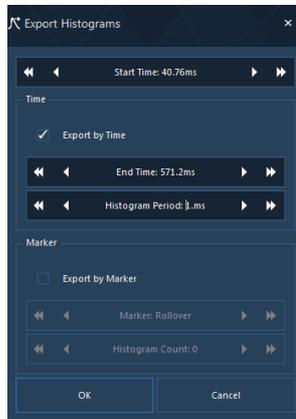


Channel display selection



Bin resolution in intensity chart

 Create histogram subset (file)



The upper left panel showing the selected time period is scaled so that it contains a maximum of 10,000 points in the chart. This determines the display (not data) resolution and adjacent bins summed to restrict the number of points plotted. If a higher time resolution is required, for example to tabulate and output, then a shorter time range needs to be selected to reduce the number of points in the chart. This can be achieved using the sliders in the bottom chart and the plus magnifier. If this is selected, it then plots 10,000 points again, but now with a higher time resolution. This process can be repeated if needed and if the whole time range is required again the minus magnifier should be selected. This brings the chart back to the full range (lowest resolution).

Once the data (time) range over which histograms are required has been selected, they can be generated by selecting the  icon. This will bring up a further dialogue in which histograms can be generated either based on time or by event marker. The data produced appears as another container, which then requires saving. This means that the original data is not amended. At this point the new container has a new node pertaining to histograms as well as the original nodes. However, it should be noted that if a shorter time compared to the original was used to generate this data then the new container will only relate to that time range. Histograms can now be analysed in a similar manner to those acquired in the Histogram streaming mode (section 4.4.1) and it should be kept in mind that a maximum of 26,000 histograms can be analysed at any one time using the EzTime software.

Note that by default the data is stored within the EzTime file format, but in the case of the native photon stream data there is the possibility to store in HDF5 format. This can be selected either at the beginning of the measurement, in which case an IRF cannot be associated, or the EzTime data exported after the measurement in complete.

4.6 Automated scripts

An easy to use script language is included with EzTime allowing measurements to be made in a predetermined manner. However for common measurements such as anisotropy and time-resolved emission spectra (TRES) there is an automated script feature that guides the user through these simple measurements automatically generating a script to control the experiment. It is not even necessary to view the script to run the measurement, although the script can be saved and edited to further optimise the measurement conditions. Before running an automated script ensure that the appropriate accessories are present (ie monochromator for TRES, polarisers for anisotropy and turret for turret scan).



During a script measurement the Data Tree and Instrument page functions will not be accessible. Full details of the scripting language and usage are in the appropriate user guide.

4.6.1 Time-resolved anisotropy measurements

Anisotropy measurements can be performed on both the fluorescence and phosphorescence timescales, making use of DeltaDiode, NanoLED or SpectraLED excitation sources. It can be run using motorised polarisers or manually, in which case the user will be prompted by on screen messages to move the polarisers to the required position. Prior to any measurement of anisotropy polarisers should be mounted in both the excitation and emission arms (see **Appendix G**).

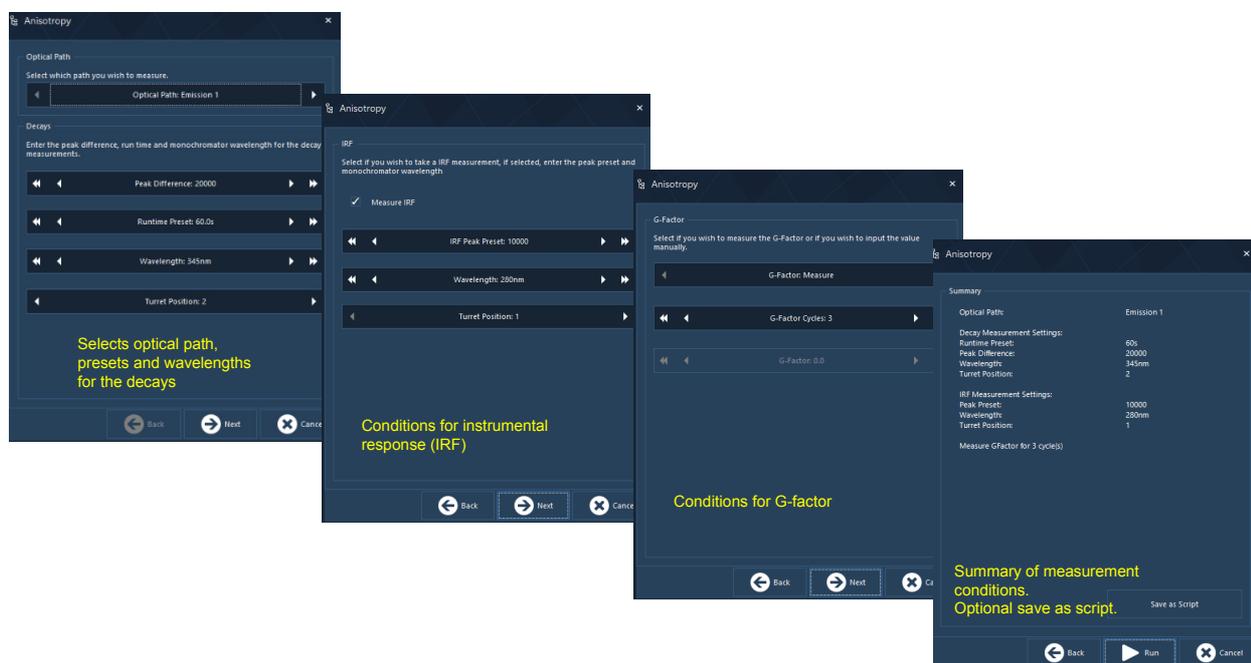
The measurement can be selected from the new measurement menu and can involve the acquisition of:

- The anisotropy decays
- The G-factor (to correct for the detection arm bias for each polarisation)
- The IRF (instrumental response function)

The first two measurements are made using the sample, while the last uses a scattering solution. The measurement conditions should be ascertained as for a standard time-resolved measurement, keeping in mind that the maximum intensity will be when both polarisers are vertically orientated. Also it should be noted that the maximum peak count in any individual measurement, when using a DeltaHub, is limited to ~65000 counts, so dwell times should be adjusted to make sure that this value is not overcome. It is, however, possible to have accumulative peak counts in excess of this value.

The measurement can be commenced by selecting the Anisotropy node in the automated script function.

Selecting Anisotropy brings up the following dialogue, enabling the presets and optical conditions to be set up for the anisotropy measurement.



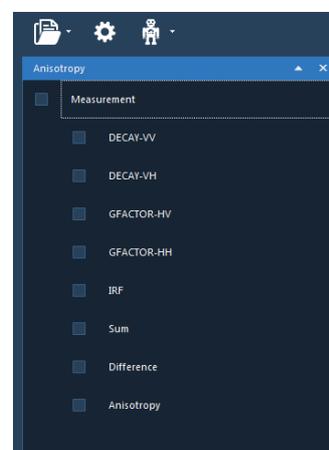
Anisotropy decay form allows the peak difference between VV and VH to be selected and the time per polarisation to be defined. The number of cycles will be determined by the time it takes to arrive at the chosen difference. The difference between the two polarisations will help determine the data quality and 10,000 counts is a reasonable estimate. However, this value will be sample dependent and with low emission samples it may be necessary to reduce the number of counts in the difference.

The **IRF** form allows the peak preset to be chosen and wavelength selected.

The **G-factor** form permits the dwell time and the number of cycles to be chosen or if know a number to be entered for the G-factor.

Note - The dwell times and number of collection cycles are very sample dependent

When the measurement is complete as well as the measured data the sum, difference and anisotropy files will have been generated automatically in the Data tree, eg right.



4.6.1.1 Anisotropy data analysis

It is recommended that the appropriate manual is consulted for full details of the significance of the data and its analysis as only a brief summary is given here.

The data obtained from an anisotropy measurement consists of the following;

- Sum – addition of the anisotropy decays, corrected using the G-factor. This contains data relating to the lifetime of the sample
- Difference – file containing rotational information allowing reconvolution of the anisotropy data with the IRF
- Anisotropy – data calculated directly from the decays without reconvolution of the IRF

In order to perform a fit on the difference data the sum needs to first be analysed and the result saved.

4.6.1.2 Anisotropy measurement considerations

Prior to the measurement it is advisable to check the measurement conditions to obtain sensible count rates, note that the maximum rate will be when the polarisers are in the VV position.

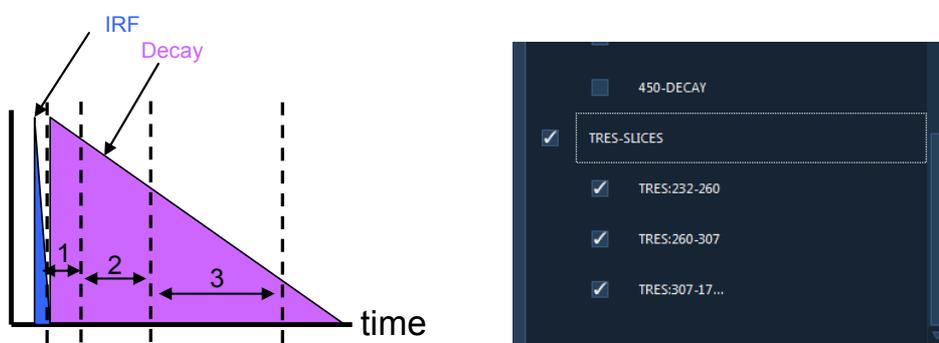
If short rotational correlation times are to be measured then fitting to the difference data (using reconvolution) is recommended as this should correct for instrumental factors.

4.6.2 Time-resolved emission spectra (TRES) measurements

The measurement can be commenced by selecting the required node in the automated script function.



It is recommended to limit the measurement to 100 wavelength dependent decays, which are acquired at fixed wavelength increments. They can be collected for a fixed period of time or up to a predetermined peak count (or with both presets chosen to whichever occurs first). The data acquired is automatically “Sliced” within EzTime to produce three “Time slices” using the approximate temporal positions indicated below. The actual time bins using in the slicing are indicated in the Data Tree, as shown in the example below.



Selecting the TRES measurement in the automated script function will guide the user through the measurement set up process, which will allow the measurement to be run with an option to save the resulting script. This can then be run again in the future or opened to edit. This allows further optimisation of the experimental conditions to be made and the measurement customized to the users requirements.

The forms generated by the automated script function consists of the following

The image displays three sequential screenshots of the TRES software interface, illustrating the configuration process for a measurement.

First Screenshot: Shows the 'Optical Path' selection (Emission1) and 'Decays' settings (Start Wavelength: 415nm, End Wavelength: 600nm, Increment: 5nm, Dwelltime: 5.0s, Polarization: Magic (55°), Turret Position: 2). Below the screenshot is the text: "Selects optical path, presets and wavelengths for the decays".

Second Screenshot: Shows the 'IRF' (Instrumental Response Function) settings, including 'Measure IRF' (checked), 'Peak Preset: 10000', 'Wavelength: 409nm', 'Polarization: Vertical (0°)', and 'Turret Position: 1'. Below the screenshot is the text: "Conditions for instrumental response (IRF)".

Third Screenshot: Shows the 'Summary' of measurement conditions, including 'Optical Path: Emission1', 'Decay Measurements Settings' (Start Wavelength: 415nm, End Wavelength: 600nm, Increment: 5nm, Dwelltime: 5.0s, Turret Position: 2, Polariser Angle: 55°), and 'IRF Measurement Settings' (Peak Preset: 10000, Wavelength: 409nm, Turret Position: 1, Polariser Angle: 0°). Below the screenshot is the text: "Summary of measurement conditions. Optional save as script."

4.6.2.1 TRES data analysis

On completion of the measurement 3 TRES slices will have been created automatically. If further slicing of the data is required, then the decays should be displayed in the Data view and the tabulate data on chart button on the tool bar used to put the decays into a spreadsheet. This can then be taken to a preferred spreadsheet program and additional analysis performed.

The TRES files allow for the use of the global analysis module in EzTime. This means that the sum of up to 5 linked common lifetimes can be used to describe the data set. This form of analysis also allows for automatic determination of the decay associated spectra. The user is recommended to see the appropriate manual and application notes (in the Fluorescence Lifetime section of the HORIBA Scientific website).

4.6.3 Turret scan measurements

To be accessible this automated script requires the sample turret to be present. This feature allows the programmed acquisition from (up to a maximum of) each of the 4 sample positions. By running over several cycles it is also possible to add (append) data into that already acquired for a particular sample position. Each position can also be defined as either decay data or an IRF acquisition. An example for this dialogue is given below making use of three turret positions; two for decay data and one for the IRF. Prior to running the measurement an option to save the conditions as a script is also given.

Data obtained using this feature can be analysed individually (see 4.3.4).

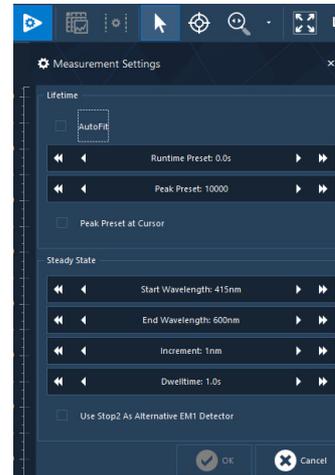
Define the selection of optical path, number of cycles and turret positions to be included

For each of the selected positions, the presets and wavelengths can be set. Choose if IRF or decay.

Summary of measurement conditions. Optional save as script.

4.7 Steady state measurements

Steady state measurements made using the pulsed excitation source can be simply performed by adding an additional node from the radial menu obtained in the Data Tree. This adds a steady state node to that of the IRF and decay. Presets for the wavelength and dwell time parameters are found along with those for the Lifetime measure.

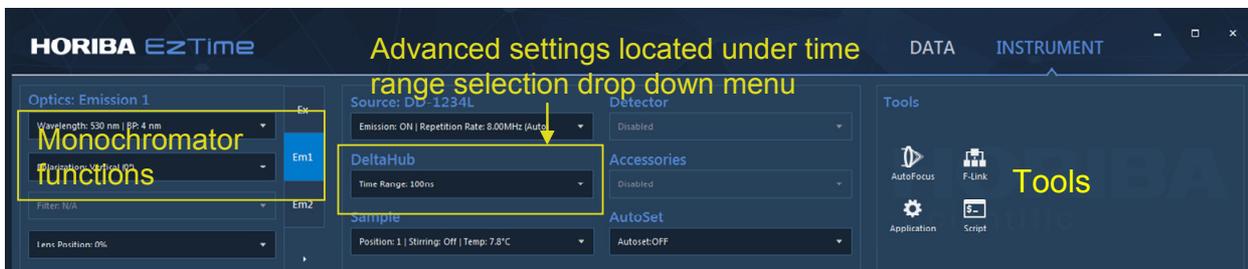


Selecting the steady state node and selecting the Start Measurement will enable collection of the spectral data.

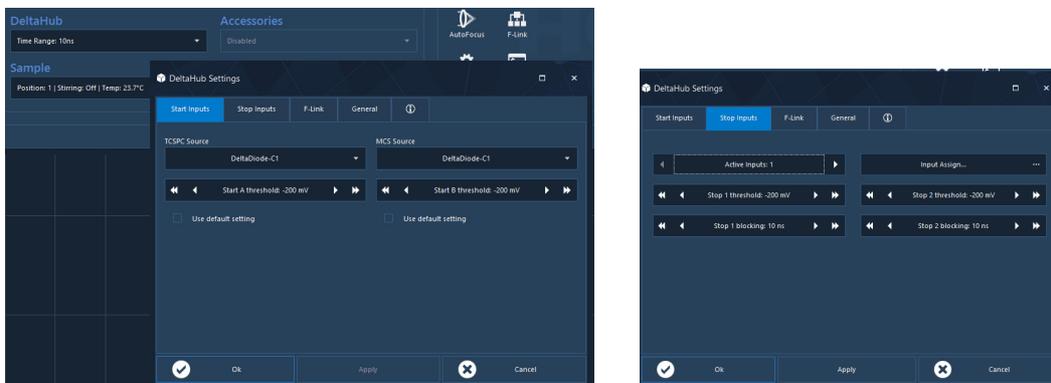
Note – the spectra obtained will be uncorrected for instrumental response and it is possible to display both spectral and temporal data at the same time..

4.8 Advanced functions / settings

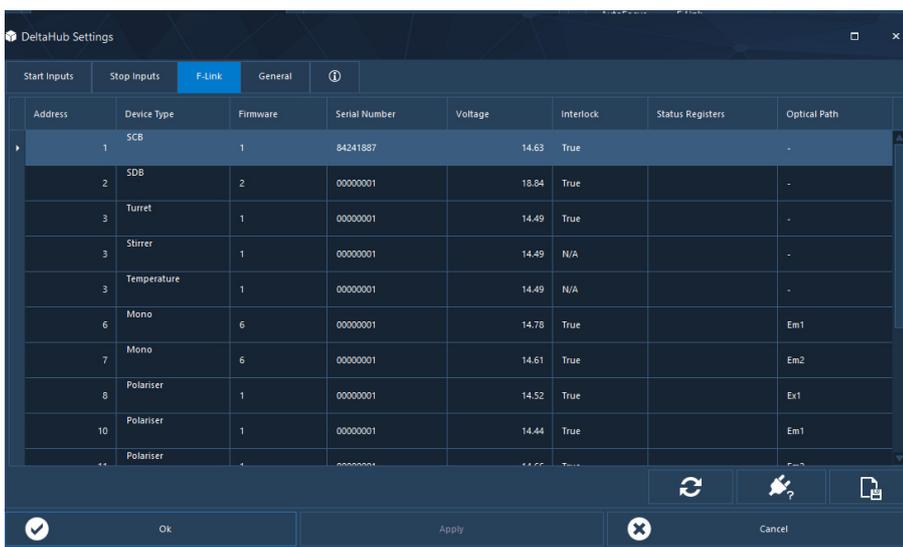
Also selectable from the Instrument page are some features that are not common usage and the user is advised to consult the **DeltaHub guide** for full details. However, a few of these will be described here.



The advanced setting form has several tabs enabling the Start and stop inputs to be configured for choice of excitation source, which stop channel is active etc.



It also has a F-Link tab. F-Link relates to the electronic communication system between the individual parts of the system and shows which elements are present. This is an important section as it shows what components are present in the DeltaFlex system and can provide useful diagnostic information. An example of this form is shown below. The population of this form will depend on the system configuration. This form can also be directly accessed via the  icon in Tools



Note

In the advanced settings forms it is possible to override the interlock on the auxiliary power sockets on the back of the DeltaHub. HOWEVER this should only be done with extreme care, if they are attached to a detector, as the microswitches on the sample chamber are disabled. I.e. the detector voltage will not be cut if the sample chamber lid is lifted.

The blocking width, used to reduce the effect of detector after pulsing, is normally set to a minimum to enable high repetition rate performance

4.9 Other light sources

As well as excitation sources from the DeltaDiode, NanoLED and SpectraLED ranges it is possible to make use of other light sources with this system. These options can be selected from the advanced settings dialogue.

4.10 Initial system check

- After the instrument has been set up we recommend that the DeltaFlex is tested using a standard sample of a known lifetime on an appropriate time range and taken to 10000 counts in the peak channel. We would recommend POPOP in methanol with excitation at 375nm and emission at 425nm, which should return a single exponential lifetime of 1.32 ± 0.1 ns at room temperature.

If a good fit is not achieved, verify the experimental conditions, also that the equipment has been left to stabilise for close to 30 minutes prior to measurement.

If continued problems persist contact HORIBA Scientific support staff.

5. Maintenance and troubleshooting

5.1 General

The DeltaFlex component parts (Timing electronics, sources, detection module) contain no user serviceable parts. Please contact your local HORIBA Scientific representative if you require technical support.

Note – the LED indicators on both the optical system (SDB) and components, principally the DeltaHub and excitation source controller are indicative of the state of the equipment and can be used to assist in trouble shooting any problem, along with the F-Link form (see section 4.7). Any correspondence relating to problems encountered with this equipment, where possible should include

1. An EzTime log file (see section 5.2)
2. Copy of the F-Link form (see section 4.8)
3. Information concerning which LED indicators are illuminated
4. Example data (EzTime file)
5. A brief description of the experiment and measurement conditions

Under normal circumstances the LEDs should show the following behaviour

SDB

Power – illuminated when connected to a power supply and turned on

Interlock – illuminates when the sample chamber lid (for example) is removed causing the shutter to close in the monochromator

F-Link – illuminates when a F-Link command is initiated, ie when a motor command is sent to a F-Link node (device such as focussing optics, polariser or monochromator).

DeltaHub

Consult the DeltaHub manual for full details, however these are summarised briefly below

Indicator (top to bottom)	Function
Start A	Illuminates when a signal is detected at the Start A input. The input rate is measured and displayed by the host software.
Start B/Trigger	Illuminates when a signal is detected at the Start B input or when the Trig out connector is in use.
Stop A	Illuminates when a signal is detected at the Stop A input. The input rate is measured and displayed by the host software.
Stop B	Illuminates when a signal is detected at the Stop B input. The input rate is measured and displayed by the host software.
System	Illuminates when a F-link connection exists. Blinks when there is communication traffic on the F-link bus.
Status	Illuminates (or blinks) to indicate a fault condition
Host	Illuminates when a connection to the host PC software exists
Power	Illuminates when the DeltaHub is powered from the mains supply. This indicator will blink for a few seconds when module is switched on while the microprocessor is initialising. Once initialised, the indicator will cease blinking. Wait until the indicator stops blinking before launching DataStation or other host software.

5.1.1 If no Stop (and / or Hit) ratemeter signal seen when expected

If the sample chamber lid has been removed, then the detector module has been disabled, so no signal is expected to be seen.

If a Deltadiode / NanoLED source has been selected and the controller turned on, then the start ratemeter will display the repetition rate. However the start ratemeter will only display a value for a SpectraLED source if DataStation is acquiring.

If no signal is seen on stop ratemeter – (a) check sample chamber lid on correctly

(b) check connections to detector firmly made and if DPS turned on

If only a “detector noise” signal seen – (a) check DeltaDiode / NanoLED turned on

(b) check appropriate filters in sample chamber, ie not too many ND filters and correct emission wavelength.

If in doubt, contact your HORIBA Scientific representative for assistance.

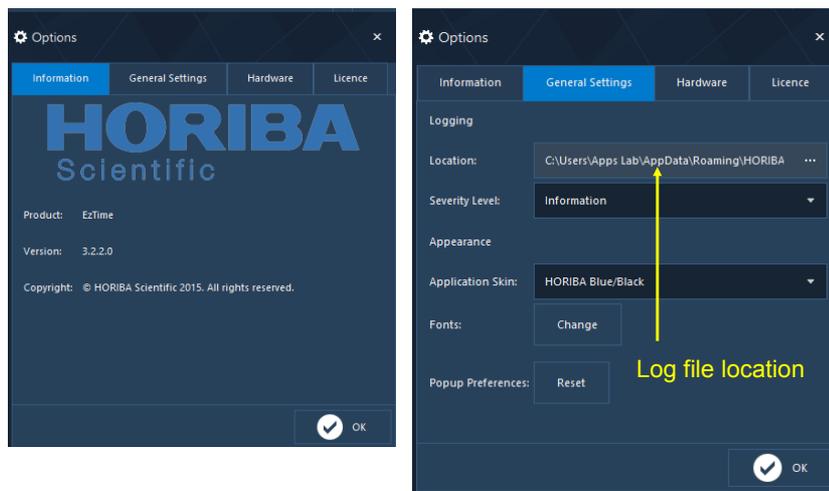
5.2 Accessing Log files

EzTime creates a log file that contains details about the particular session of the program. If anything goes wrong with the application then these log files can be a great help to HORIBA Scientific support staff in tracing the problem.

The log file can be accessed through the options dialogue on the Data page or Application (in Tools) on the Instrument page.



Selecting this brings up the following form and under the “General Settings” tab is the location of the log file. Clicking on this location should bring up the log file.



By providing this “history” to us will greatly assist in the trouble shooting process.

6. Specifications

A typical **DeltaFlex** fluorescence lifetime system consists of a sample chamber, DeltaHub, PPD detection module and NanoLED / DeltaDiode (and / or SpectraLED) source(s) – see component user guides for full specifications. The dimensions (footprint) and weight below are based on a DeltaFlex with DeltaDiode excitation.

Overall dimensions (footprint)	750 x 550 (WxD)
Overall weight	32 kg
Power consumption**	140 VA
Operating environment	<p>Operating temperature 5 °C to 40 °C</p> <p>Maximum relative humidity 80% non-condensing for temperatures up to 31 °C decreasing linearly to 50% relative humidity non-condensing at 40 °C</p> <p>Indoor use only</p>
Compliance	<p>This product complies with:</p> <p>EN 61326-1: 2006 (Emissions & Immunity)</p> <p>EN61000-3-2:2000 (Mains harmonics)</p> <p>EN61000-3-3:1995 (Voltage fluctuation)</p> <p>EN61010-1:2001 (Safety requirements for electrical equipment for measurement, control, and laboratory use Part1: General requirements)Supplementary Information:</p> <p>The CE marking has been affixed on the device according to Article 8 of the EMC Directive 2004/108/EC.</p> <p>The certificate is on file with HORIBA Instruments Inc. and documentation is on file with the CE test house, York EMC Services Ltd, Donibristle, UK.</p> 

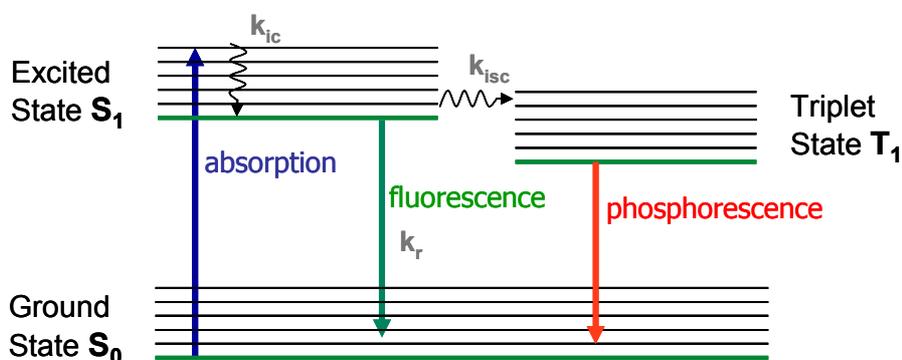
* No cables attached.

**typical for DeltaFlex-01-DD with PPD-850 (incl DPS).

Appendix A. Luminescence and techniques to measure it

Background

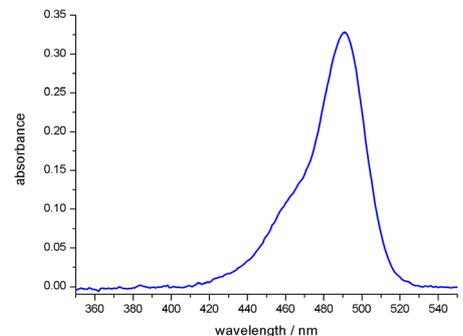
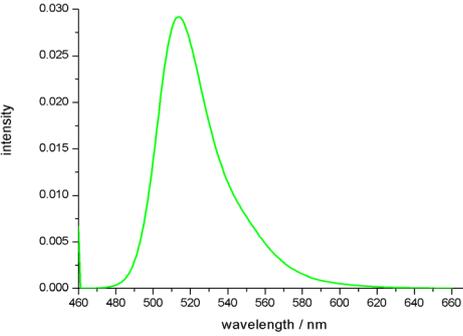
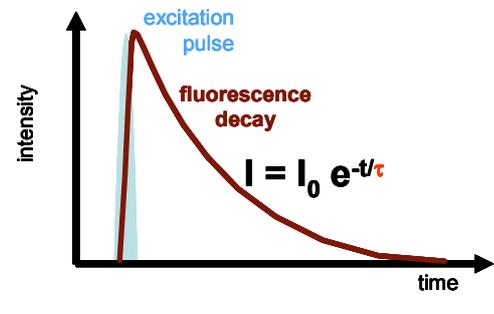
The term luminescence originates from the latin (*lumen* for light) and was first used as *luminesenz* by Wiedemann in 1888 for “*all those phenomena of light which are not solely conditioned by the rise in temperature*” [1]. It is now generally used to encompass the processes of *fluorescence* and *phosphorescence*, with these two phenomena simplistically distinguished by timescale. *Fluorescence* is associated with the absorption and re-emission of light on the pico- to nanosecond range, while the timescale for *phosphorescence* is normally associated with the microsecond to second range. The spectral range for these phenomena spans the ultra violet to near infra red regions. Both processes relate to the absorption of energy in the form of light, which causes a change in electron distribution, moving the molecule to an excited electronic state, which then relaxes back to the ground state via emission of light. In *fluorescence* this occurs between singlet energy levels, but with phosphorescence intersystem crossing occurs and the electronic triplet state becomes populated; emission to the ground state then occurs. As molecules tend to dissipate energy in the excited state this leads to the energy of the re-emitted light to be less than that of the excitation light, i.e. *fluorescence* and *phosphorescence* occur at longer wavelengths compared to the excitation. This is demonstrated in the simplified energy level diagram given below (known as a Jablonski diagram). Where k_{ic} , k_{isc} and k_r are the internal conversion, intersystem crossing and radiative rate constants respectively.

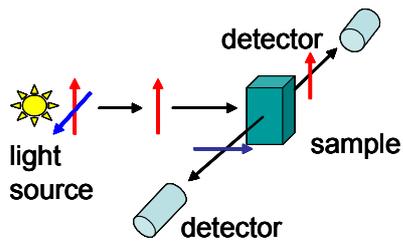
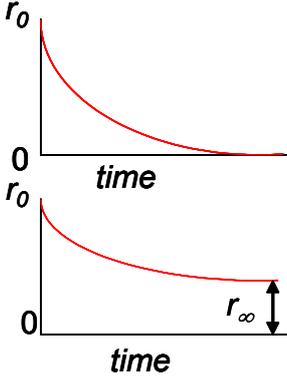
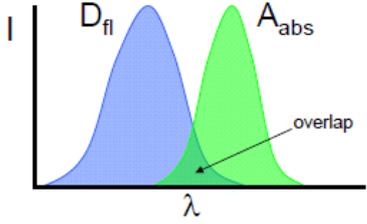


Many substances fluoresce (ranging from biological tissues to inorganic crystals) and this can be put to good use, since a molecule's fluorescent properties are highly dependent on its local environment. In some respects a fluorescent molecule can be considered a nanoscale reporter and a well characterised probe molecule can help elucidate information concerning local pH, viscosity, dielectric constant and molecular interactions. There are numerous books relating to this field and some worthy of note are those by Lakowicz[2] and Valeur[3].

Measurement techniques

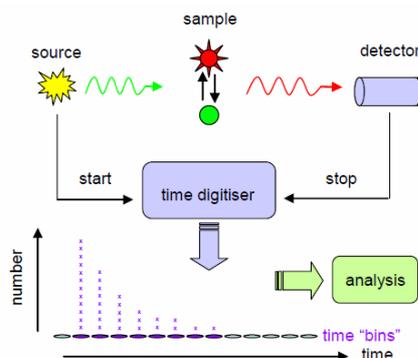
These can be roughly divided into steady state or time-resolved measurements. The former can be used to provide information concerning the wavelength, quantum yield, Stokes' shift and molecular interactions. Time-resolved measurements also give a measure of how long a molecule spends in the excited state and have the advantage that (within certain limits) they are independent of concentration, as each molecule has its characteristic decay time, independent of how many are present. Whereas, simplistically, doubling their number would give double the fluorescence intensity. Further to this making use of fluorescence anisotropy and resonance energy transfer allow local viscosities and (nanoscale) distances to be determined. A simplified schematic summary is shown below and it is recommended that users consult the literature to gain a fuller knowledge.

	measurement	information
absorption		<p>Using Beer-Lambert law the number of molecules can be obtained</p> $I = I_0 10^{-\epsilon [c] d}$ <p>absorbance = $\epsilon [c] d$</p> <p>..... ϵ extinction coefficient $[c]$ concentration d pathlength</p>
emission		<p>Quantum yield can be determined</p> $\phi = \frac{\text{number of photons emitted}}{\text{number of photons absorbed}}$ <p>Stokes' shift</p> $\lambda_{\text{max em}} - \lambda_{\text{max abs}}$
Time-resolved		<p>Fluorescence lifetime determination</p> $I(t) = \sum_{i=1}^n \alpha_i \exp(-t/\tau_i)$ <p>..... τ_i fluorescence lifetime(s) α_i pre-exponential(s)</p>

fluorescence technique		information
steady state anisotropy	 <p>polarisation measurement</p>	<p>molecular “freedom” or energy depolarisation. $r_{\max} = 0.4$ (or -0.2 if perpendicular dipoles), $r_{\min} = 0$</p> $r = \frac{(I_{ } - I_{\perp})}{(I_{ } + 2I_{\perp})}$ <p>.....</p> <p>r - anisotropy, I - intensity (with either parallel or perpendicular polarisers)</p>
time-resolved anisotropy	 <p>$r(t) = r_{\infty} + (r_0 - r_{\infty}) \exp(-t/\tau_R)$</p>	$\tau_R = V\eta / kT$ $2\left(\frac{r_{\infty}}{r_0}\right)^{1/2} = \cos^2 \theta + \cos \theta$ <p>.....</p> <p>r_{∞}-limiting anisotropy r_0-initial anisotropy τ_R-rotational correlation time V-effective volume, η-viscosity θ-semicone angle for hindered rotation</p>
resonance energy transfer	 <p>Need Donor and Acceptor spectral overlap – effective range between 10 to 100 Å</p>	<p>Critical distance R_0 (50% efficiency of energy transfer)</p> $R_0 = 9.78 \times 10^3 \left(n^{-4} \phi_{\kappa}^2 \frac{\int I(\nu) \epsilon(\nu) d\nu}{\nu^4} \right)^{1/6}$ <p>Rate has distance dependence allowing RET to be used as a nanoscale “ruler”</p> $k_{ET} = \frac{1}{\tau_d} \left(\frac{R_0}{r} \right)^6$

Fluorescence measured using time-correlated single-photon counting

This is the method used to measure fluorescence lifetimes on the **DeltaFlex** systems and has become the method of choice for those working with short lifetimes, weak fluorophores and high repetition rate excitation sources (>10kHz). Put simply, this technique measures the time between when a photon excites a fluorophore and emits fluorescence. A schematic of the method is shown below.



Experimental lifetime determination considerations

Several factors need to be considered before commencing a measurement. It is best if the sample itself is dilute. In practice this means less than approx. 5×10^{-5} M, although it is also recommended to have an optical density (OD) of 0.1 or less at the excitation and longer wavelengths. The purpose of keeping the OD low is to avoid problems associated with self absorption (e.g. apparent red shift in the emission spectrum and reduction in quantum yield), while using low concentrations also minimises molecular interactions that can cause concentration quenching.

Problems can also arise if the sample is scattering, this can be manifest in the recovery of apparent short lived decay times. When using cut-off filters it is advisable to check that there is sufficient wavelength separation between the excitation wavelength and the filter (remember filters will allow some light through at wavelengths shorter than their quoted value, which is usually the 50% transmission wavelength – more noticeable with scattering samples).

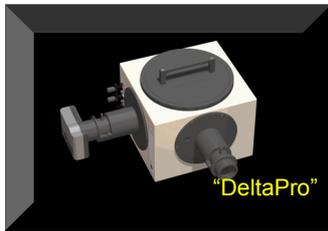
The sample geometry should also be taken into account when performing a measurement. Liquid samples in standard 10mm pathlength fluorescence cells are typically measured in the usual 90° configuration. More opaque samples require “front face” measurements. In order to minimise the influence of scattered light the sample holder is often tilted 60° to the vertical, as the horizontal position of the sample holder requires rotating so that the sample is 45° to both the excitation and emission arms of the sample chamber.

[1]. B. Valeur in *New Trends in Fluorescence Spectroscopy: Applications to Chemical and Life Sciences*. B. Valeur and J.-C. Brochon (eds), Springer Verlag 2001.

[2] J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer and the “Topics in Fluorescence Spectroscopy” series of books (J.R. Lakowicz (ed)), Springer.

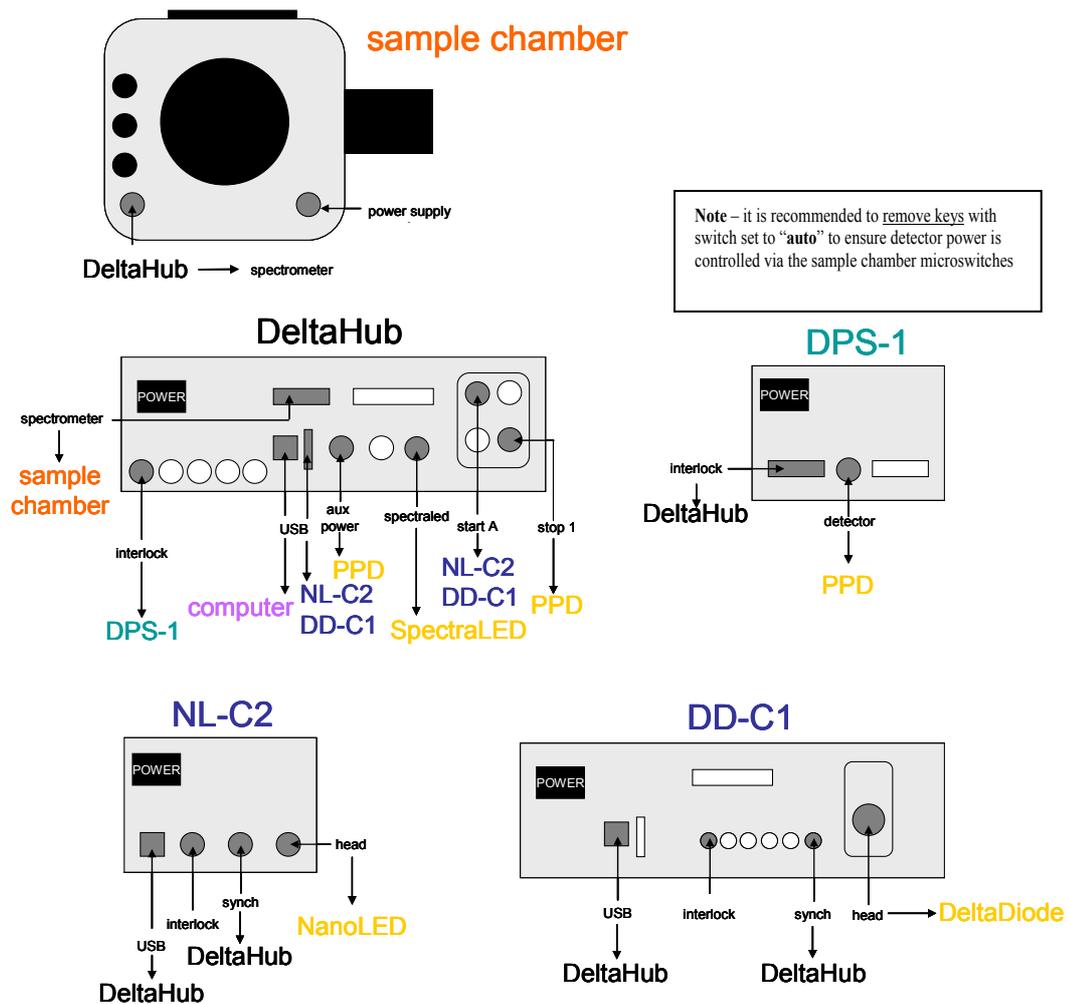
[3] B. Valeur, *Molecular Fluorescence: Principles and Applications*., Valeur, B., Wiley-VCH, New York, Chapters 1-3, pages 3-71 (2002).

Appendix B. DeltaPro Sample chamber



The DeltaPro is a filter based sample chamber with motorised focusing optics and a shutter on the excitation channel. Although it makes use of the same electronics as the DeltaFlex, because it does not comprise of a monochromator, with built in shutter, on the emission channel it is only recommended for use with PPD detectors. The microswitches in the sample chamber drop the voltage on the PPD detector when the sample chamber lid is removed to prevent the detector becoming exposed to ambient light. Also the DeltaPro sample chamber cannot be used with advanced temperature control options and is only suitable for use with a recirculating water bath in this aspect.

Since the DeltaPro does not have a baseplate, a connection is needed between the sample chamber and the timing electronics for communication purposes (ie for the interlock). Connections are shown schematically below.



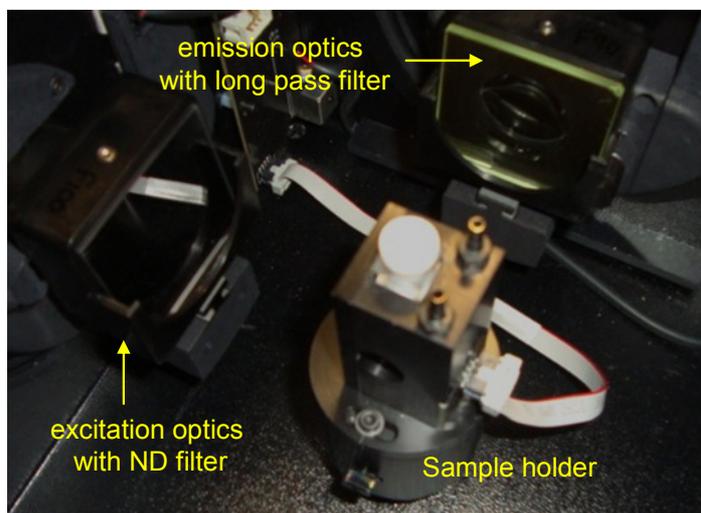
If a PPD-650 is used as the detector, then this can be powered directly from the Aux Power socket on the Timing electronics. If a PPD-850 or PPD-900 is used then a DPS-1 power supply is required and the Interlock cable connected.

Recommended configurations for a DeltaPro, with either DeltaDiode or NanoLED excitation sources are given below.



When operating with EzTime it will be noticeable that functionality involving a monochromator (eg. steady state, TRES) will be absent. Other functionality will depend on the configuration of the system.

Since the DeltaPro is filter based (the filter holders accept 50mm square filters, see below) it is recommended when performing a measurement to use more ND filters at the start, then to reduce to obtain the suitable count rate. The decay measurement should be performed using an appropriate wavelength selection filter on emission, which should be removed for measuring the IRF.



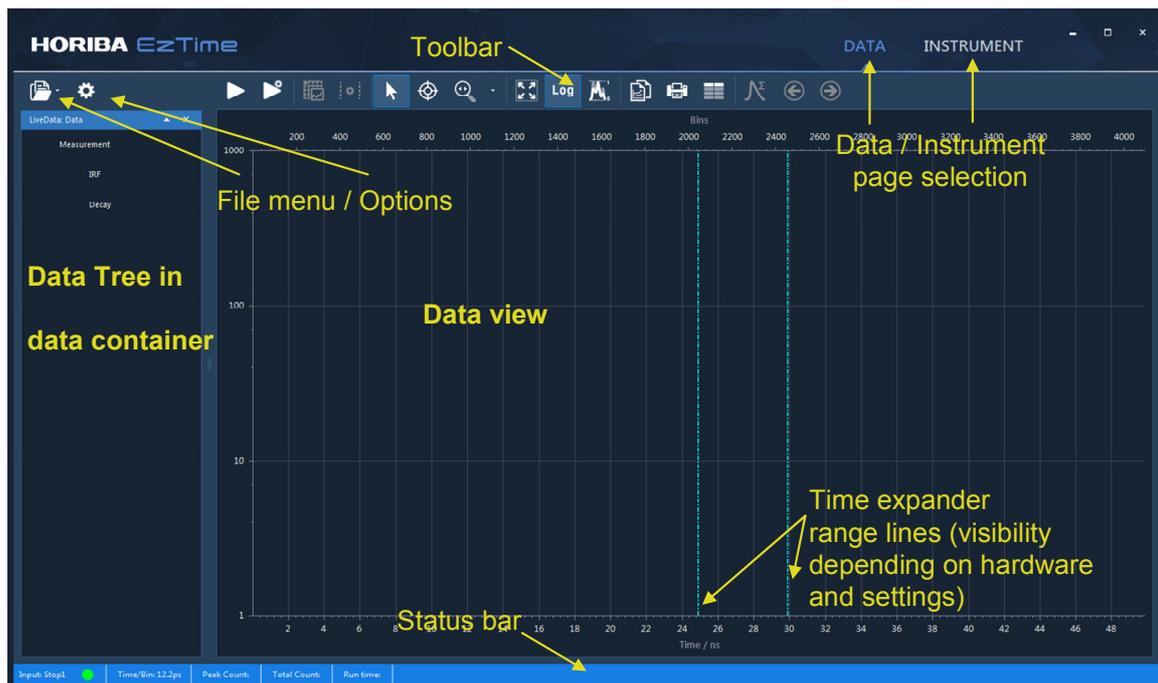
Apart from the sample chamber and associated optics the DeltaPro has the same functionality with EzTime as the DeltaFlex system.

Appendix C. FluoroHub-A+ timing electronics



The DeltaFlex is a modular system and can be configured with different excitation sources and detectors. Although the DeltaHub based system can satisfy a vast majority of measurement needs there are specific applications that require the measurement of very short fluorescence lifetimes, with a HPPD or microchannel plate detector for example. For these measurements a very high time resolution and low timing jitter electronics are recommended. The FluoroHub-A+ meets these requirements (time bins from 0.3ps and jitter < 10ps FWHM). It can be used in place of the DeltaHub on the DeltaFlex system. The EzTime software will function in the same manner as with the DeltaHub, although there are some different features present on both the Data and Instrument pages.

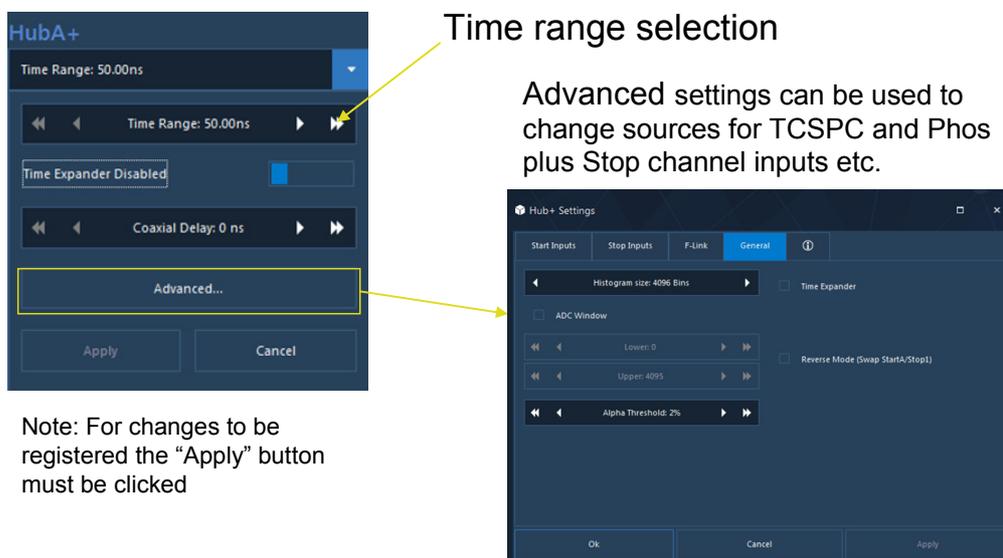
Data page



The noticeable difference is the appearance of two lines in the Data view. If the Time expander feature to get very narrow time bin widths is to be used the data should be positioned (using delays) within the two lines.

Instrument page

On this page to accommodate the functionality of the FluoroHub-A+ the advanced settings dialogue has some additional features.



Reverse mode / time range / co-axial delay

When using the DeltaHub, because of its very short deadtime, it is always run in “forward mode” (light source synchronisation to Start, detector to Stop input). However the FluoroHub-A+ with its time to amplitude converter it is recommended to run in reverse mode on the short timescales to optimise data collection efficiency. I.e. light source synchronisation to Stop, detector to Start input. It is important to inform EzTime of reverse mode operation and this is simply done by checking the Reverse Mode box in the “General” tab. For longer TCSPC time ranges (>200ns) and (always for) phosphorescence time ranges forward mode can be used.

Depending on the time range a delay will be required to position the data within the histogram time window. For short (50ns and 100ns) time ranges in reverse mode this is achievable using the co-axial delay within the FluoroHub-A+. When running reverse mode on the 200ns time range an additional delay to that incorporated within the FluoroHub-A+ may be required. Please note that, although co-axial cable delays exhibit good timing properties, when long delays are required by their nature they can attenuate the timing signal. Therefore cable delays over 200ns are not recommended. For longer time ranges (microsecond time ranges in forward mode) positioning of the data within the histogram time window may require the use of a third party delay generator.

Note – Delay should be added to the stop channel for both forward and reverse modes of operation.

Histogram size / ADC window

In the “General” tab there is the ability to select the histogram size (number of time bins) and for maximum resolution 16k is recommended. In the histogram because of the nature of TAC-based electronics it is advisable to remove time bins at both the beginning and end of the histogram to obtain the best quality data. This is factory optimised and applied by checking the ADC window box. It is recommended that the ADC window values (upper and lower levels) are always applied.

Time Expander

In order to get the highest resolution possible it is recommended to use the maximum number of time bins in the histogram and to enable the Time Expander feature. This can be done either under the FluoroHub-A+ dropdown or in the General tab. The data to be collected needs first to fall within the Time Expander range lines and this can be adjusted using delays – such as the coaxial delay under the FluoroHub-A+ dropdown dialogue.

Since the FluoroHub-A+ is optimised for use for high resolution timing experiments it is not designed for high throughput applications, such as photon and histogram streaming measurements. Therefore these features will not be accessible within the EzTime software.

Appendix D. Detector Options

The modular nature of the DeltaFlex means that it can be configured with different TCSPC detectors to cover different wavelength and temporal ranges. Please note that if a DeltaPro sample chamber is used then only the PPD is recommended. A selection of possible detectors is given below, but in addition it is also possible to use certain SPAD based detectors.

				
PPD 230nm-920nm TTS <180ps	HPPD 220nm-890nm TTS from <50ps	MCP 185nm-850nm TTS 25ps	NIR (R) 300-1700nm TTS 1.5ns	NIR (H) 950nm-1700nm TTS 400ps

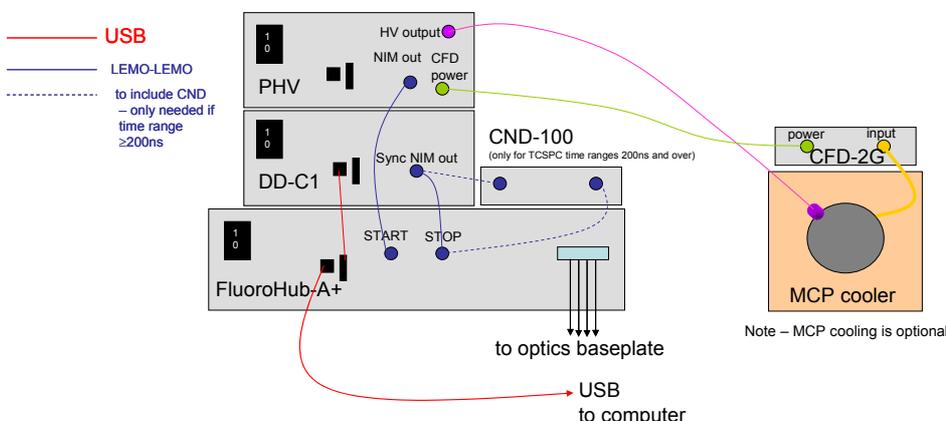
Each detector package (provided by HORIBA Scientific) should provide a NIM pulse to connect to the timing electronics as follows.

The **PPD** provides the NIM signal directly from the back of the module.

When using the **NIR detectors**, the NIM signal is provided from the CFD-2G amplifier / discriminator recommended for use with either the R or H NIR detector package.

The **HPPD** base unit provides the NIM output for the timing electronics.

The **MCP** package, includes a PHV high voltage supply and a CFD-2G discriminator / amplifier. There is close coupling between the detector and the components for optimum timing properties and to minimise any rf interference and the NIM signal can be obtained from the PHV unit. When considering the inclusion of a MCP in a DeltaFlex system it is recommended that for optimum performance it is used in conjunction with FluoroHub-A+ timing electronics. A schematic of the principal electrical connections are shown below for an example of a DeltaFlex with DeltaDiode controller and FluoroHub-A+ in reverse mode, viewed from rear.



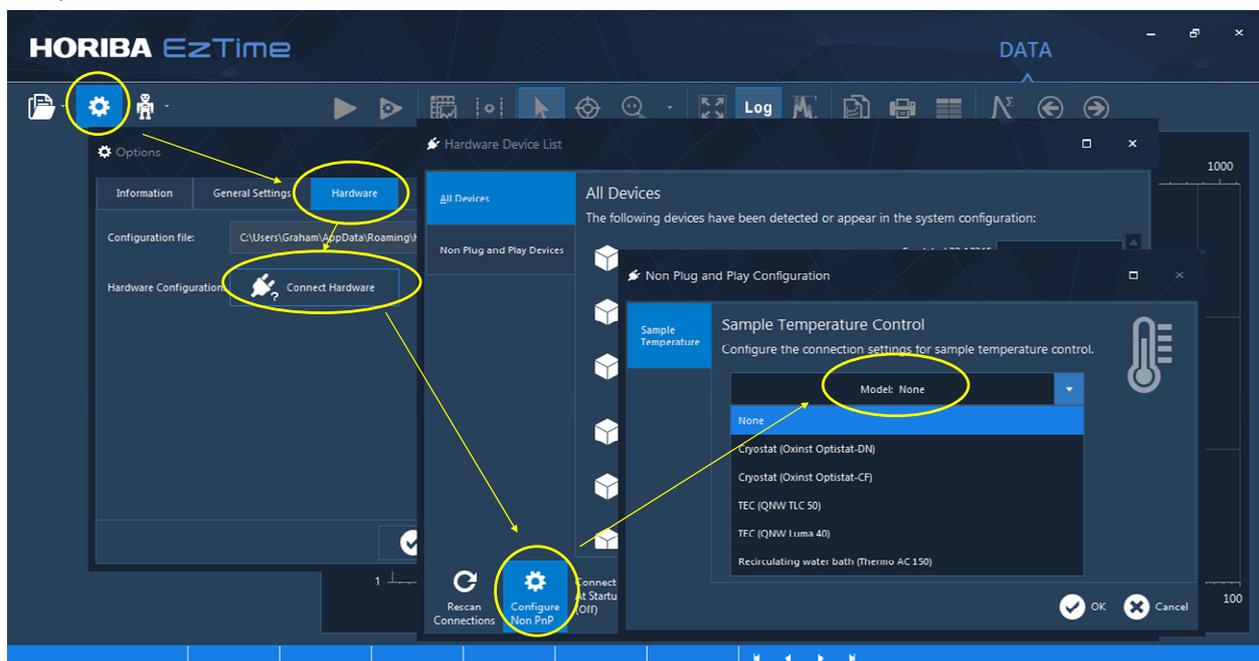
Appendix E. Temperature Control Options

There is a choice of temperature control options for the DeltaFlex system and the principal ones are given below. Note only the water bath is compatible with the DeltaPro sample chamber.

- Water bath (-25°C to 70°C)
- Peltier (-25°C to 105°C)
- LN2 cryostat (OxInst DN or Janis VPF-100)
- LH cryostat (OxInst CF)

The appropriate adaptors and connectors are required to physically mount the temperature control devices into the sample chamber. Here only the software control aspect will be addressed and the manuals pertaining to the chosen device should be consulted.

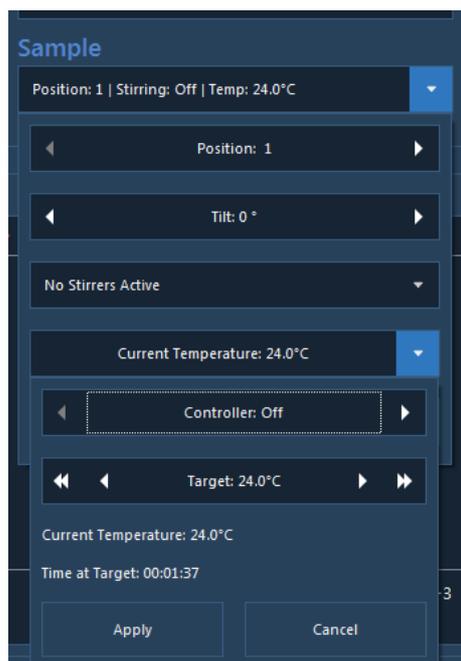
The EzTime software has the ability to control certain water baths, Peltiers and cryostats. However, since these are third party devices and not on the F-Link communication bus, they need to be configured within the EzTime software as a non “plug ‘n’ play” device. This can be selected from using the options icon, see below.



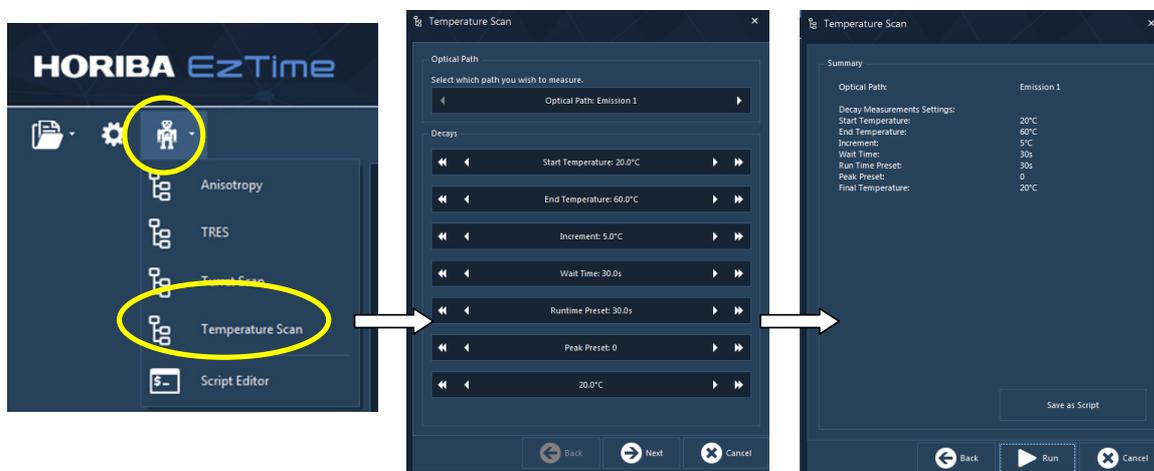
A drop down menu or use of arrows enables a supported temperature control device to be selected. On the first occasion that a temperature control device has been selected, the “Detect Port” button should be selected to initiate communication. The connection can be confirmed by the “Test connection” button. The status of these devices can be seen in the hardware dialogue (non plug and play).

In order to set the temperature control device, go to the Instrument page and use the Sample drop down to locate the controller and temperature settings (see right). This enables the setting of the temperature in the software of the supported device.

Note, that if a recirculating water bath is installed and used in conjunction with a standard sample holder (with stirrer and temperature sensor) or 4-position sample turret, then two temperatures will be displayed (see below). One relates to the temperature on the control device (TC) and the other at the sample holder (TH). It is only possible for EzTime to set the temperature on the water bath and thus it is recommended that a “calibration” is performed noting the set temperature on the water bath and that at the sample holder.



Once a temperature control device has selected it is now possible to perform a “Temperature scan” measurement, controlling measurement conditions and temperature. This can either be scripted or selected under the autoscript icon on the Data page – see below for an example of the dialogue.



When using temperature control devices, it is advised that they are turned on before starting the EzTime software and turned off after the software has been closed. If a device is not used, it can be disconnected using the hardware dialogue, so that the EzTime software does not report it as not connected. In the case of cryostat controllers, it maybe necessary to restart the software if the turn on order is not followed.

Appendix F. Sample Holder Options

There is a choice of sample holders available as accessories for the DeltaFlex fluorescence lifetime system, which enable a variety of different sample types to be measured

There is a choice of sample holders available as accessories for the DeltaFlex fluorescence lifetime system, which enable a variety of different sample types to be measured.



The first sample holder in the picture is a standard 90 degree sample holder (code DP-SC-11) with integrated stirrer and temperature sensor. The second is designed to be used with solid samples only (code DP-SC-10). The third holder in the picture is a front face sample holder, which can be used with solution based samples in standard cuvettes, as well as with solid samples (code DP-SC-09).

All three allow lateral movement (in one direction) to help optimise the fluorescence signal and the solid only sample holder also has a height adjustment. The solid sample holders are angled to reduce the influence of scattered excitation light, which is problematic when using solid or scattering samples. The holders capable of usage with cuvettes can be connected to a recirculating water bath, which allows for the control of sample temperature.

A **4-position Sample Turret** is also available, with sample positions controlled via the EzTime software. This Turret can be controlled using scripting and has connections for a recirculating water bath for temperature control. It also features individual stirrers a temperature sensor.



Appendix G. Accessories – Mounting polarisers

The polarisers can either be manually controlled or in motorised mounts. They are supplied in barrel mounts of diameter 30mm and length 25mm as shown in the figure below. Note that the polarising element is located closer to one end of the mount than the other. The polarising element is aligned within the mount so that the vertical plane of polarisation coincides with the v-groove along the length of the barrel. This v-groove facilitates automatic alignment within the holder.



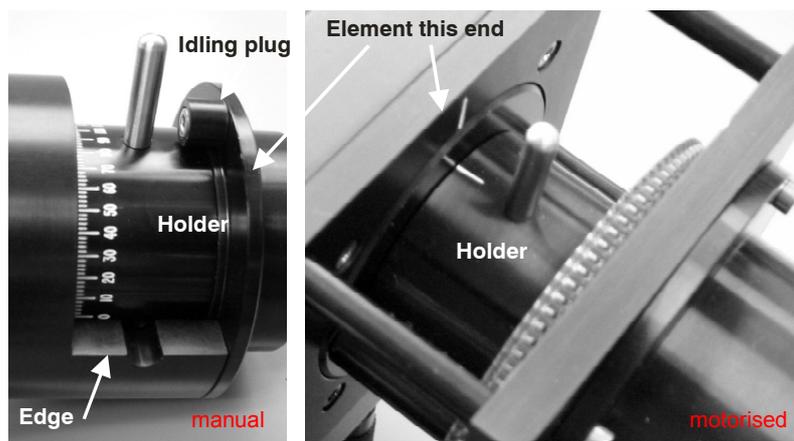
The polarisers



Polariser holders

The polariser mounts must be fitted in to the polariser holders before the anisotropy measurement can commence. These feature v-tipped setscrews that mate with the v-groove in the mount, and the mount will automatically align within the holder when the setscrew is tightened. The polariser holders are graduated to indicate the plane of polarization. Manual holders feature graduations. Motorised holders feature a single graduation to indicate the vertical position. The polarisers should be inserted into the holders so that the polarizing element is located at the end nearest the graduations.

Once the polarisers are secured in their holders, they can be fitted into their seats in the optical system as shown below. The holders are designed to be a tight fit to exclude ambient light, and it may be necessary to rock the holder from side to side to ease it gently into its seat. The polarising element should be located so that it is at the furthest point from the sample in order to maximise transmission through the element. Replace the idling plugs to keep the holders secure when they rotate.



The polarisers may be removed from the holders when not required by reversing the procedure described above. The polarisers should be stored in a cool dry place and protected from dust, liquid and scratches.

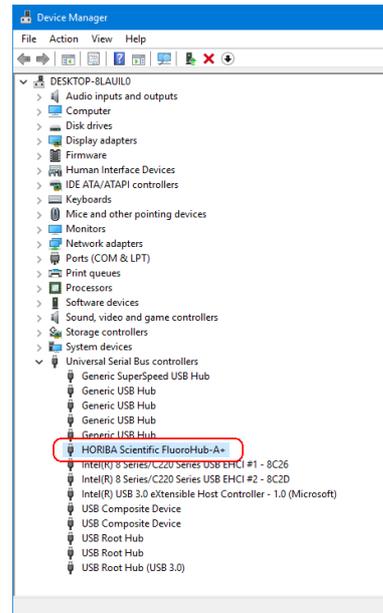
Appendix H. Computer settings (USB / power)

If a computer has been supplied with the system, then it should have been tested and its settings optimised for use with EzTime and the DeltaFlex system. If another (non HORIBA Scientific) computer is being used then first check that it fulfils the system requirements (eg Windows 7 or 10, English language version). For optimal performance it may be necessary to adjust some of the computer power and communication (USB) settings to avoid them “sleeping” and hence an interruption in communication between the computer and the DeltaFlex system. Also, note that, the USB from the computer should be connected to the Timing electronics, and any subsequent “daisy chained” connection made from this device.

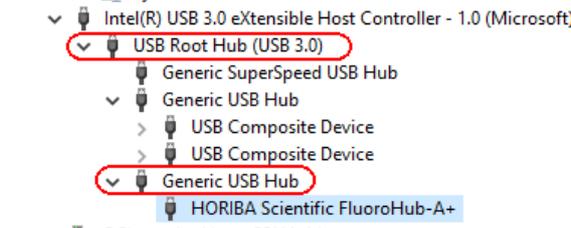
The power management settings on the computer that can be adjusted to avoid communication being interrupted are slightly different for the FluoroHub-A+ and DeltaHub and the following is an example using a Windows 10 operating system

FluoroHub-A+

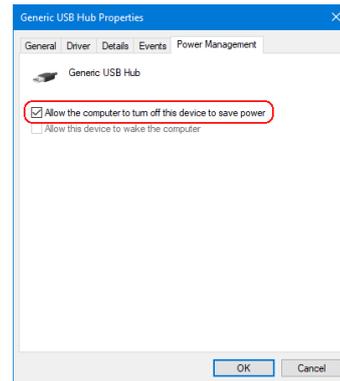
1. Connect the HubA+ and turn the power on.
2. Open Device Manager and expand the section “Universal Serial Bus Controllers”
3. Select the device “HORIBA Scientific FluoroHub-A+” (right)
4. In the View menu select “Devices by connection”.



5. This view shows a tree with all the USB connections that the hub is attached to. Locate the hub in the tree if it is no longer selected. The hub will be connected to a “Generic USB Hub” which may be connected to a “Root_Hub”.

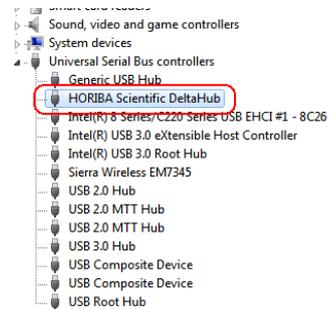
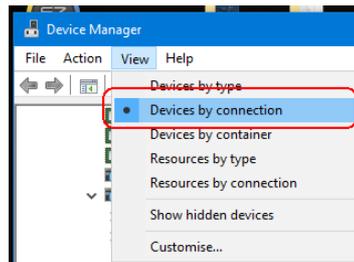


6. Select the “Generic USB Hub”
7. Right-Click and choose Properties
8. Go to the Power Management tab and **uncheck** the option “Allow the computer to turn off this device to save power”
9. Repeat these steps for the “Root Hub” if required.

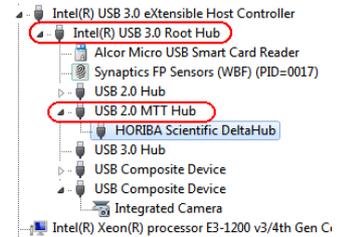


DeltaHub

1. Connect the DeltaHub and turn the power on.
2. Open Device Manager and expand the section “Universal Serial Bus Controllers”
3. Select the device “HORIBA Scientific DeltaHub”
4. In the View menu select “Devices by connection”.



5. This view shows a tree with all the USB connections that the hub is attached to. Locate the hub in the tree if it is no longer selected. The hub will be connected to a “USB 2.0 MTT Hub” which may be connected to a “Root Hub”.



6. Select the “USB 2.0 MTT Hub”
7. Right-Click and choose Properties
8. Go to the Power Management tab and uncheck the option “Allow the computer to turn off this device to save power”



9. Repeat these steps for the “Root Hub” if required.

Notes