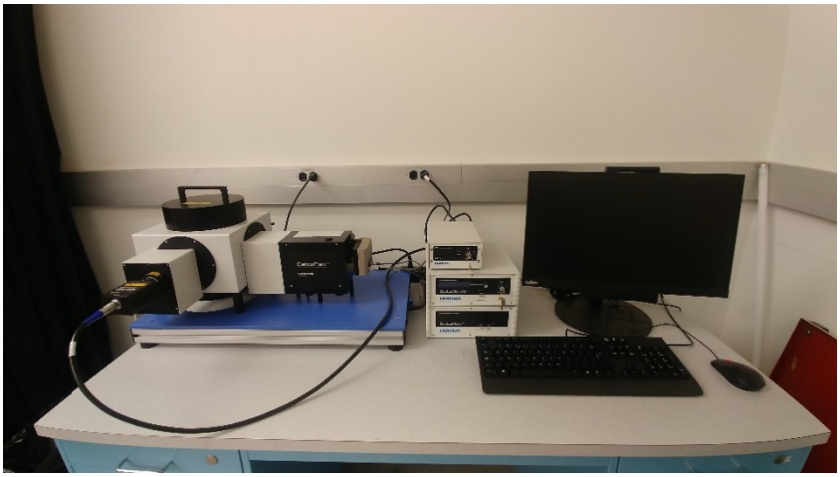
IOWA STATE UNIVERSITY
Chemical Instrumentation Facility

Horiba DeltaFlex Time-Correlated Single Photon Counting Fluorometer



Location: **1240 Hach Hall**
Contact: **Brett Boote, 1234 Hach Hall**

The Horiba DeltaFlex is a turn-key fluorometer in the standard 90° configuration, but with pulsed excitation sources and fast collection electronics and that allow for single-photon counting capabilities. It is capable of measuring the lifetimes of excited states from ~1 ns to over 1 s with high precision, as well as fluorescence anisotropy. Additionally, it is possible to use third party excitation sources (such as a visible wavelength pulsed laser) with suitable coupling mechanisms, however this must be discussed and approved by CIF staff, as well as be operated by personnel with laser training.

Getting Started

Double check the configuration of the instrument (correct excitation source installed) prior to powering up any instrument modules.

Log in to the *LockScreen* window using your unique password to unlock the computer. There are many power buttons as each component of this system has its own power supply/control module. For best system communication, the recommended powering order is as follows:

1. DPS (detector)
2. DeltaDiode (excitation source)

3. Base Unit (to the right of the instrument base)
4. DeltaHub (timing unit)

Once all components are powered, launch the **EZTime** software. This software package includes instrument control, real-time data acquisition, as well as automated (or manual) analysis of collected data. The recommended warm up time is 20 minutes for all components.

Loading Samples

Two Suprasil quartz cuvettes with Teflon stoppers are provided for standard (3.5 mL) samples in the top left drawer, one of which has a colloidal solution for collecting the Instrument Response Function (IRF). The other will be stored with methanol to preserve the interior cuvette surfaces from dry residues. For routine samples, use the second standard cuvette. Half-full cuvettes will have sufficient sample volume for analysis.

In general, the samples should be dilute in order to provide accurate lifetimes.

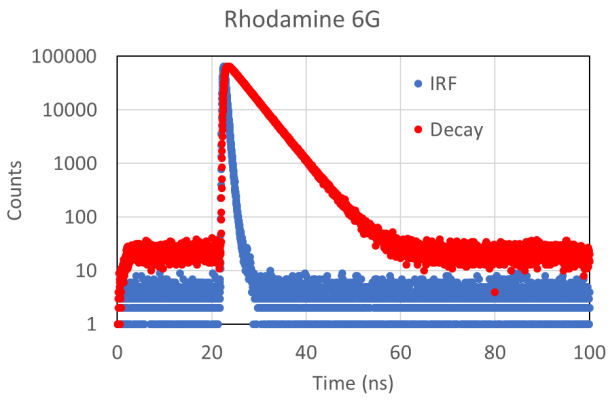
For limited-volume samples there is also a microcell (3x3 mm) and adapter for the cuvette holder.

Setting Instrument Parameters

There are currently two sources available, at ~280 and ~350 nm. Select the source based on the absorbance of the sample for the most efficient signal generation. Install the source onto the DeltaFlex platform using the provided 2 mm allen wrench and attach the cable from the DeltaDiode controller prior to powering the controller on.

To begin collecting data, turn the interlock keys for both the DPS detector module and DeltaDiode driver module. These turn on emission from the excitation source and apply high voltage to the PMT, allowing for data collection. In the **EZTime** software, begin at the *Instrument* tab at the top right of the view. This gives options for the monochromator position and timing electronics, which update in real time as changes are applied. For an optimal decay curve, the monochromator should be set to the maximum emission wavelength of the sample (in order to determine this for a sample without a literature value, jump to the *Steady State* section below).

Once the emission wavelength is set, the amount of light reaching the detector can be varied by changing the bandpass filter (from 1 nm to 32 nm). The graph at the lower part of the screen monitors counts from the excitation source and detector, and standard practices specify that a ratio (alpha value) of less than 2% is optimal. Naturally, the higher the alpha value the faster decay curves can be collected. If a ratio below 2% cannot be obtained, dilute the sample with more solvent or install an ND filter in the excitation path.



The next optimization is to set the time window for data collection, selectable in the *EM1* settings. The collection window can be varied between 40 ns and 11 s, which changes the duration of each data channel. To view a real time display of the data, select the *Preview* tab at the lower right and press the play button to start. Best practices are to use about half of the collection time for the decay trace, as shown above. This way, there are plenty of data points to fit the long-lived part of the state to the baseline. Once parameters are optimized, select the *Data* tab to collect and analyze time-resolved data.

Time- Resolved Data Collection

In **EZTime**, data is stored in containers. The default starting state for the software is one IRF and one decay trace in the data container, but more can be added by hovering over the *Measurement* icon and clicking the notebook icon and selecting the appropriate data type. Additionally, measurements can be renamed by clicking their own notebook icon and selecting *Edit*.

Before collecting any decays and for any new experimental conditions, it is best practice to collect a new IRF as the delay will change at each collection window setting, and the IRF is dependent on the width of the time channels as well. To collect an IRF, tune the detector to the wavelength of the excitation source in use and load the cuvette containing the scattering solution. *Ensure the ratio of excitation to emission counts is less than 2% by adjusting the bandpass as previously noted.* To begin data collection, press the button. The data collection will proceed until the counts threshold (65000) is reached or for the specified run time (300 seconds), whichever occurs first. These values can be modified in the configuration button, next to in the main window if desired.

With a suitable IRF collected, return to the *Instrument* tab to change the monochromator to the emission wavelength of the sample, and load the sample-containing cuvette in the system. Adjust the bandpass as necessary to maintain a 2% or less alpha value, then return to the *Data*

tab to collect the Decay. As before, counts will steadily increase in time channels, building up the decay curve over time.

Once all required data is collected, turn both interlock keys to the vertical (defeated) position. Then save the data container to a folder with the date in *D:\\Data\yourusername*, and for raw data press the spreadsheet button to generate a spreadsheet of the displayed data, which can be saved as an Excel file or as text.

Data Analysis

The **EZTime** software package comes with two modes of data analysis: autofitting (during collection) and manual (post) fitting. As always, by exporting the raw data third party software may be used as well.

Autofit

1. If autofitting is desired, check the *Autofit* box in the configuration settings menu. This will begin a fitting routine using 1, 2, and 3 exponentials directly after the data collection is done, using automatic fitting start and stop times. Press the disk icon below the fits which should be saved.
    - *In general, a good fit has a chi-squared value of less than 1.2, where adding an additional exponential term doesn't reduce the chi-squared value significantly.*
2. Click *Okay* to exit the fitting routine. Then the fits will appear nested below the decay they are associated with, and can be saved and converted to text as needed.

Manual Fitting

1. Decays can be manually fit as well, simply select the decay and associated IRF so they are displayed on the plot and press the *Analyze* button. If multiple decays are plotted they will be batch fit.
2. The number of exponentials used must be specified, so if several fits are to be compared they must be run successively.
3. Additionally, the *Configure* button allows for fine-tuning parameters for the fitting, such as setting the time limits to fit over for both the IRF and decay.
4. Once the fits are complete, they can be saved and exported to tabulated values.

Steady State Measurements

Through the incorporation of a monochromator, this instrument is also capable of steady state (counts vs. wavelength) measurements. To define parameters for the scan, press the configuration button where the start and stop wavelength and dwell time can be set. *Note: this monochromator scans slowly as it is not intended primarily for scanning measurements, please use the Perkin Elmer LS 55 for routine steady state data collection*. If decay curves and steady state data are displayed concurrently in the data view, the steady state signal is at the

right y-axis, with wavelength on the top x-axis. All data operations for steady state data are the same as for decays.

Sample Cleanup

When finished with measurements, place all generated waste in the appropriate container (based on solvent) in the back lab of 1238 Hach. In the event the sample or solvent should be in a special waste container, please retain and dispose of that waste in your research lab where there is likely a waste disposal strategy in place.

Thoroughly rinse the sample cuvette with DI water (if applicable), methanol, and then refill and cap with methanol for storage.

Instrument Shut Down

First close the EZTime software, then power down each component of the instrument. For reference, there are 4 power switches to toggle. Then, log off the *LockScreen* program using the lower right *Logoff* button. Return all loose components, tools, and cuvettes to the top left drawer of the instrument table.

Maintenance:

No user maintenance is necessary for this optical system. Contact CIF Staff if anything seems disconnected or out of the ordinary.

System alignment:

Any alignment adjustments will be made by CIF staff.