Horiba DeltaFlex Modular Fluorescence Lifetime System (Date: 1/22/16)

Using the instrument:
Users must have completed the laser and instrument safety training with Prof. Takematsu before operating the instrument alone. [Your name must be on the list of authorized personnel]. If you have not yet received authorization, contact Prof. Takematsu or Celeste Morin Renaud.

Secure the area by closing the door to Druckenmiller 259 and replacing on the outside of the door the green laser off sign with the appropriate red or yellow laser use sign. Users must remove any jewelry, watches, badges, etc. that may scatter light. Locate the laser personal protective equipment: the laser goggle, nitrile gloves, and lab coat. Alert all persons in the room that you are going to be operating the instrument.

Write in the instrument log book: your name, date, start/finish time, objective, and any problems/comments that you observe. Please make sure to save all data files in your personal data folder: \\microwave\research\takematsulab\StudentAcess\Group_members\NAME. Note: it is highly suggested that you create separate subfolders with the date or page number of your lab notebook to organize your data.

The following are abbreviated protocols for the use of the DeltaFlex instrument. All persons should read over the protocol before proceeding. Manuals containing detailed information about the instrument, individual components, and data collection and fitting software are available in the lab.

Sections include:
- Turning on the instrument
- Lifetime measurements
- Setting up the temperature
- Shutting down the instrument

Turning on the instrument: [Locate the blue labels on the instrument, #1 – 4]. (Date: 1/22/16)

1. Turn on the main black unit power switch, located on the side of the plate connected to the main instrument. (Blue Label #1)

2. Turn on the three power switches located on the back of the three white subunits controlling the detector power supply (DPS 1), picosecond diode controller (DDC 1), and DeltaHub (DDH-HT high throughput TCSPC controller). You should feel cool air coming out of the back of each unit. Do not block the ventilation. Note: The detector and diode have an additional key control. Do NOT turn the keys on at this time. (Blue Label #2A, B, C)

3. Turn on the two power switches to the bath temperature control, one located on the white Quantum Northwest Temperature Control unit and the other located on the black Koolance Liquid cooling system. You should feel cool air coming out of the back of the control unit and see the fan and blue light turn on for the cooling system. Again, do not block the ventilation for the units. (Blue Label #3A, B).

   Note: The control for the stir bar is located on the front side of the Temperature control unit. Turn the dial to control the stirring speed. You CANNOT control the stirring speed through the DataStation program even though there is a Sample Stirrer option written into the program.

4. Turn on the computer and log into your account. (Blue Label #4).

5. If you need temperature control for your experiment, go to the instructions on "Setting up the Temperature." Otherwise, make sure to record the temperature of the cell holder (which should be close to the room temperature). This value appears on the front side of the Temperature control unit. This value may fluctuate slightly throughout the experiment if you do not turn on active temperature control.

6. Double-click on the

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Lifetime measurements: (Date: 1/22/16)

Before you proceed, you should know the following information about your sample:

- x Absorption spectrum: the absorbance at the excitation wavelength should be 0.1 or less.
- x Steady state emission spectrum: identify the emission or detection wavelength.
- x Estimate the lifetime of the system.

If you do not have this information, you are not yet ready to do a lifetime measurement. Proceed to shut down procedures.

If you are ready to proceed, remember to have your lab notebook with you to record the instrument settings and file names.

1. After you open the DataStation program, click and highlight the icon "Lifetime" and then click "New."

2. You will observe two sections on the left hand side of the screen: TCSPC Measurement and System Hardware.

3. Horiba provided us two control samples: (L): LUDOX_TMA colloidal silica, 34 wt% suspension in distilled or deionized water (420859_1L Sigma Aldrich) and (P): POPOP in MeOH prepared at absorbance 0.1 A units. The first sample is the standard for the scatter prompt. (In the manual, they recommend a 0.01% dilution of Ludox AS40 colloidal silica Sigma Aldrich 420840). The latter is the standard for Horiba time resolving instruments. The lifetime should be 1.32 ns ± 30 ps for a properly operating instrument (10,000 counts in the peak channel). Before you run your experimental sample, we will run these samples to make sure that the instrument is operational. The samples are available in two quartz cuvettes, labeled (L) and (P), respectively. Check that the solvent level has not decreased in (P). If needed, add methanol to the cuvette and check the UV/Vis spectrum. [If you

have to prepare a new sample (P), see instructions on "How to prepare sample (P)"]. Gently wipe the outside of the cuvettes with a Kimwipe to remove any dust or fingerprints.

4. Under System Hardware and Data Acquisition, select "Measurement Range." A small window will pop up. On the left hand side, you will be asked to choose a measurement range. It is recommended that you select a time range approximately 10 – 20 times that of your expected lifetime. This way, you will capture the whole decay (including the tail) and collect an appropriate number of data points in the decay. For the test sample (P), go ahead and use the default 200 ns time range. [Even though the expected lifetime for POPOP in MeOH is 1.32 ns, operating the excitation source at 100 ns or 220 ns versus 60 ns reduces the rep rate for the excitation source, prolonging its lifetime].

   On the right hand side of the window, you will be asked about the Trigger source and Phos. Trigger Source. We currently have DeltaDiodes DD-330 and DD-280 for our DeltaFlex system, so the TCSPC Trigger source should read "Diode." Mak.002 Mak.001 2 0 TD 0 Trs6 TD

photons are reaching your detector. You will need to either reduce your bandpass or close the shutter manually and insert a neutral density filter, cutoff filter, physical excitation/emission barrier, or etc.

Insert the cuvette with sample (P). Once

Decay. Make sure that the settings under System-Hardware have not changed, and then proceed as before: click on Decay and then Start.

Note: Ideally, the peak of the decay should be located to the left side of the screen. If it is not, you may want to change the timing or delay time of the excitation source to take advantage of the entire data

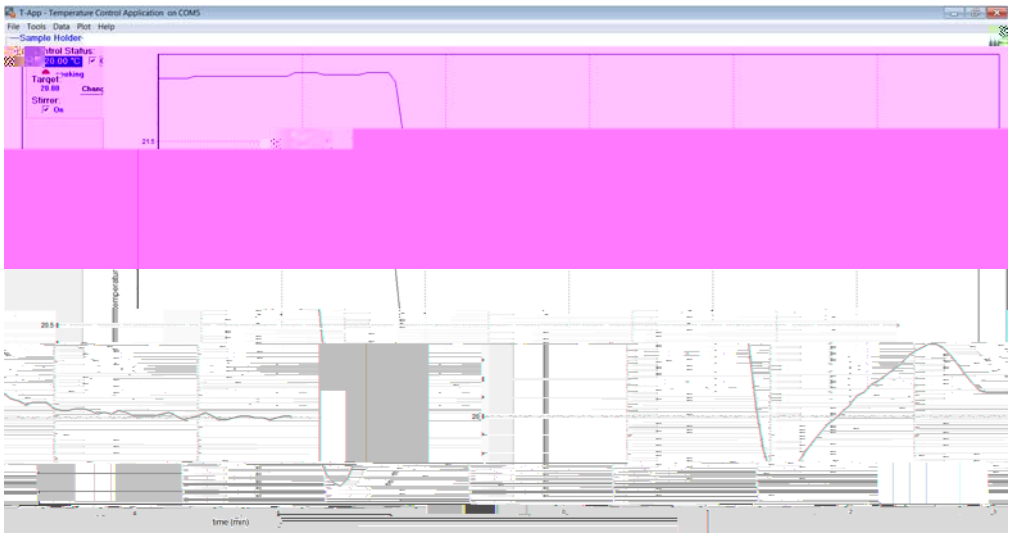
You can gradually increase the bandpass to your experimental condition as you add the neutral density filters on the excitation side].

Once you have found the optimum conditions, select Prompt under TCSPC measurement and click start on the toolbar. The prompt measurement will terminate once it has reached the peak preset (i.e. 10,000 counts). You should notice that the red “X” next to Prompt has been replaced with a green

Settingup the Temperature(Date:1/22/16)

1. Doubleclickon the TAppTemperatureControlicon on the desktop.

2. Onthe left handsideof the screen,clickon the “Change”button andenter the sampleholdertarget temperature.Thenclick the box below Control Statusto havethe temperature adjusted.You can follow the progressin temperature versustime on the graph plot. The final value of the holder should also be reflected on the front panel of the TemperatureControl Unit. Note: You can also makethese adjustmentsusingthe Toolmenu.



If you decideto work at temperaturesin which condensationor excessheat may becomean issue, contact Prof. Takematsuor Celesteas we may need to add a purge gasto the system.Alwaysbe awareof the freezingand boiling point of your solventor sampleof interest before you adjustthe temperature.

3. If you want to stir your sample,confirm that the stirrer box is checkedon the right handsideof the screen.Checkthat you haveaddeda stir bar to your cuvette.

4. Once you have reached the desired temperature, minimize the screen and proceed to your measurementsNote1: You can saveyour temperature log, by going to Data and Save(cell holder data). Note2: If you closethe screen,the programwill askyou whether you would like to maintain temperature control. If you want to do your measurementat that constanttemperature, click yes andexit the program.

Shutting down the instrument: