

IMSERC User Manual for Fluorometer

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INTRODUCTION

Use of this instrument is allowed only by qualified users after receiving training by a staff member. Do not run this instrument without approval from IMSERC staff. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees and/or removal of all IMSERC privileges. This set of instructions is meant to serve as a guide for 'routine' data collection on the instrument. For custom experiments that are not covered in this user manual, contact a staff member. For the full list of modes, capabilities, and potential custom experiments that could be run on this instrument, please either contact a staff member or check the corresponding capabilities section at <http://imserc.northwestern.edu/pcm-instruments.html>. Please read this user manual and acquaint yourself with the instrument.

A hard copy of this user manual can be found near the instrument. An electronic version of this user manual is linked to the desktop of the instrument computer and also available under the corresponding instrument section at <http://imserc.northwestern.edu/pcm-instruments.html> by pressing on the 'User manual' button. If while using the system, something happens that you do not understand, please **stop**, and **get help**. In any event, be completely prepared to justify your actions. The cost of even minor repairs could be considerable.

SAFETY

All users of IMSERC must review the general safety policies at <http://imserc.northwestern.edu/about-policies.html>. To become an independent user of this instrument, you must have the following safety training and certificates under your LUMEN profile:

- Laboratory Safety
- Personal Protective Equipment

You need the above certificates to be able to reserve time for this instrument on NUcore. Online classes and certification are offered at <https://learn.northwestern.edu>. Upon completion of the certificate, it will take an overnight to filter through the different systems and get into the files that NUcore uses. Additionally, familiarize yourself with the location of standard safety stations like eye wash and shower stations found next to room DB033A and outside the DB area in the hallway, respectively. Gloves should be removed when using the computer.

Please collect all waste such as samples, gloves, kimwipes, etc. and dispose of them properly in your lab. There are no containers for hazardous chemical waste in DB022.

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DATA MANAGEMENT

Your personal data folder is created during training. Please save data under your personal folder, which must be located under your supervisor's group folder, otherwise you might not be able to access your data remotely. See a staff member if you do not have a personal folder on this instrument yet. For users that prefer to name their data folders using dates, use the order of YYYY-MM-DD or YYYYMMDD in the name, so that folders can be sorted chronologically by the operating system if needed.

Data from this instrument are copied on your group folder on 'imsercdata.northwestern.edu' under 'others/Fluorometer' once every hour. Please follow instructions at <http://imserc.northwestern.edu/about-general-faq.html#data> for details about data access.

SOFTWARE

Data acquisition and real time plotting are performed with the 'FluorEssence' software that uses Origin for creating graphs. Software is installed on the instrument computer. For offline analysis after your instrument reservation is complete, please ensure that you have exported your data as TXT files for using 3rd party data processing software for smoothing and background subtraction. You have the option to use the instrument computer for analysis, but you must reserve instrument time through NUcore.

DEFAULT INSTRUMENT STATUS

The default measurement mode using the Fluorometer spectrometer is **room temperature measurements of bulk liquid or solid samples**. Please notify the appropriate staff member well in advance if you would like to run an experiment in a different mode. For the full list of modes and capabilities, please check at <http://imserc.northwestern.edu/pcm-instruments.html#fluorometer>. Additionally, put a note on your NUcore reservation indicating the preferred mode of your measurement.

The default working condition of the Fluorometer is as follows:

1. The computer screen is turned off by default. You must start your reservation through NUcore to be able to turn on the computer screen. If screen is already on, start your reservation through NUcore
2. The default 'Fluorometer' user account should be logged in. In case the computer is restarted, the password for the 'Fluorometer' account is _____ (see hardcopy by the instrument)

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3. Acquisition software (FluorEssence) should not be running. Close the acquisition software when you are done with the measurement
4. Power on the spectrometer should not be on. Spectrometer must be turned off at the end of the measurements
5. There should be no error messages on either the front panel of the instrument or the acquisition software. Please check the [‘Troubleshooting’](#) session for a potential solution before reporting the error

If there is an error or problem with the instrument that is not covered under the [‘Troubleshooting’](#) section, please report the issue by following at least one of the steps below:

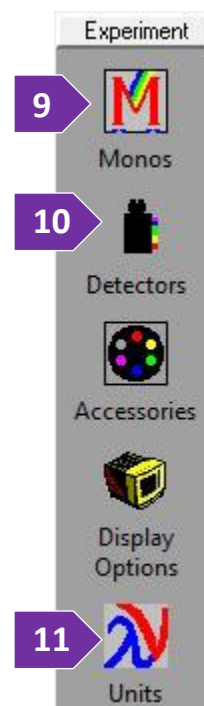
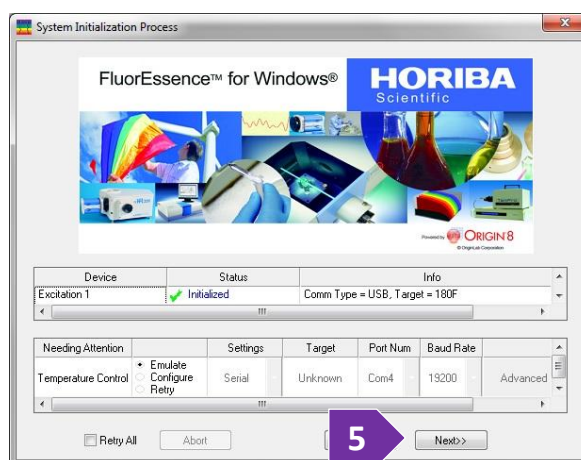
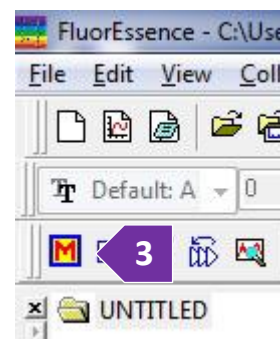
1. If you have already started your reservation using NUcore, please end your reservation and select the error reporting option with a brief description about the issue. Place the ‘Stop’ sign near the instrument computer to notify users immediately after you. ‘Stop’ signs are located on the shelf above the computers in BG51
2. If you have not started your reservation using NUcore, please report problems with the instrument at <http://imserc.northwestern.edu/contact-issue.html> and place the ‘Stop’ sign near the instrument computer
3. Contact a staff member for instructions

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COLLECTING SPECTRA

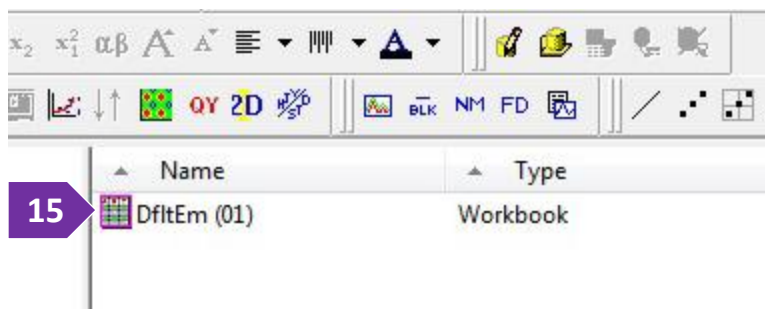
The following procedure should be followed by the users who want to use the default instrument configuration.

1. Turn on the spectrometer by flipping the power switch to the 'On' position. Power switch is located at the right side of the spectrometer next to the power cord. It takes about 20 minutes for the system to properly warm up, which is important for quantitative measurements
2. Double press on the 'FluorEssence' icon on the Desktop to launch the acquisition software
3. Press on the 'Experiment Menu' icon at the top left corner of the window to bring up the main experiment menu and initialize the instrument (figure 3)
4. A pop-up window entitled 'System Initialization Process' will appear and initialization should be completed within a few seconds with no errors
5. Press on 'Next' button to start the software (figure 5)
6. From the 'Main Experiment' menu, press on 'Spectra'
7. An 'Experiment Type' window will appear. Click 'Emission' to collect emission data, alternatively you can click 'Excitation' to collect absorption data. It is highly recommended to have some absorption data of your sample, so that you have a good starting point regarding emission and exaction scan ranges
8. In the 'Fluorescence Division – Experiment Setup' window, under the 'Experiment' panel, optionally you can name the experiment conditions and where the file should be saved (to your group folder)
9. Select the 'Monos' icon on the top left of the 'Experiment Setup' window (figure 9). This is where you can modify the slit width, scan range, and wavelength settings for the excitation and emission monochromators. Enter the excitation wavelength under 'Excitation 1' and under 'Emission 1' Next, select the "MONOS" icon on the top left. This is where you can modify the slit width, scan range, and wavelength settings for the excitation and emission monochromators. Enter the excitation wavelength under "Excitation 1" and under "Emission 1" choose the wavelength range



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10. Select the 'Detector' icon on the top left of the 'Experiment Setup' window (figure 11) and select the appropriate detector(s) for your measurements. For most experiments, the S1 detector (signal detector) should be enabled. The R1 (reference detector) is not necessary unless you need to compensate for any light source fluctuations
11. Optionally, select the 'Units' icon on the top left of the 'Experiment Setup' window (figure 11) and select the appropriate units (Time, Wavelength, Slit Width, or Temperature) for your experiment
12. Insert your sample
13. Click the 'Run' icon on the bottom right of the 'Experiment Setup' window. The 'Intermediate Display' window will pop-up which provides a real-time output of the spectral data
14. The 'Intermediate Display' window will ask you to enter a project name, enter a clear and consistent project name. **During the real time output of the data, ensure that the maximum intensity does not exceed 2×10^6 CPS.** It is important to decrease the slit width in the event the maximum intensity is reached to prevent saturation of the detector. Repeat the scan until a suitable slit width has been determined. The experiment can be easily modified by selecting the 'Previous Experiment Setup' icon which is next to the 'Experiment Menu' icon (figure 3). For quantitative measurements, ensure that the same slit width is used for each experiment
15. Every time you are running a measurement, a new 'Workbook' is created. Right press on the default name of the workbook ('DfltEm' in figure 15) and rename the workbook, so that you do not lose track of the name and conditions of each collection. All workbooks are saved under a single file which is the project name file you provided in step #14. It is highly recommended to 'Save As' the project again, so that you verify the path of the file which should be pointing under your group folder
16. The lambda max of emission can be determined from this data by pressing on the 'Data' icon and spotting the maximum intensity and its corresponding wavelength
17. Pressing on the 'Auto Run Previous Experiment' icon will allow you to perform additional runs under the same experimental parameters
18. The same process can be used to determine the optimal excitation wavelength
19. A scattering peak from the detector can be observed at twice the excitation wavelength. This peak can be removed during the data treatment, or you can use filters



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20. At high concentration and emission band can be observed at twice the first (major) emission of the sample.
Please make sure you are diluting your sample as low as possible to avoid such behavior or the emergence of emission bands from exciplex species
21. At the end of your measurements:
 - a. Save all files and experiments
 - b. Remove your cuvettes and clean them thoroughly
 - c. Close the 'FluorEssence' Program
 - d. Turn off FluroMax-4

EXPORTING DATA

It is highly recommended to export your raw data to an ASCII format file for offline processing unless you have access to the Origin plotting software. To export the spectra into an ASCII format that can be imported by 3rd party software which creates graphs:

1. Select the 'Data' tab on each spreadsheet that contains a measurement
2. Under the 'File' menu, select 'Export'
3. Provide the path for the exported file and ensure that you have selected TXT as a type

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PUBLICATION

A. EXPERIMENTAL SECTION

Modify the text below according to the setup and conditions you used during the measurement:

“Emission/excitation spectra were collected at room temperature using a Horiba Fluoromax-4 spectrometer. Sample was placed in a **plastic cuvette | quartz cuvette | quartz capillary | solid holder** and data were collected by using an exposure of **XX** seconds and averaging **YY** spectra.”

B. ACKNOWLEDGEMENT

“This work made use of the IMSERC (RRID:SCR_017874) Physical Characterization facility at Northwestern University, which has received support from the Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-2025633), and Northwestern University.”

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TROUBLESHOOTING

A. THE COMPUTER SCREEN WILL NOT TURN ON

Begin your reservation in NUcore to initiate access to the instrument

B. COMPUTER REQUIRES LOGIN AND A PASSWORD

The default 'Fluorometer' user account should be logged in. In case the computer is restarted, the password for the 'Fluorometer' account is (see hardcopy by the instrument). See '[Default instrument status](#)' section for more details.

C. WHY DOES MY SPECTRA SLOW UNEXPECTED PEAKS?

Raman peaks from the solvent can show up in emission spectra. If you are unsure if a peak is attributed to a Raman signal, run a blank measurement of the solvent. Alternatively, varying the excitation wavelength should cause the signal to shift in the same direction. In the case of a solid sample, a band pass filter can prove helpful for removing peaks that result from scattering. Be aware of the emergence of the scattering peaks at $2 \times \lambda_{exc}$.

D. WHY IS MY EMISSION REALLY LOW?

First, check that your sample is positioned properly where the beam will hit the sample. Second, lower the concentration – the low emission could be a result of aggregation. Third, play around with the slit width – you may need to have a wider slit width to see optimal signal.

E. WHY IS MY SPECTRA DISTORTED?

Check that you are not saturating the detector. This can occur at peak intensities greater than 2×10^6 CPS. At high intensities the detector cannot count all the photons and may damage the detector, this will also distort the spectra.

F. THERE IS AN ERROR/PROBLEM WITH THE INSTRUMENT THAT IS NOT ADDRESSED UNDER THE TROUBLESHOOTING SECTION

If there is an error or problem with the instrument which is not addressed under the troubleshooting section, please report the issue by following at least one of the steps below:

1. If you have already started your reservation using NUcore, please end your reservation and select the error reporting option with a brief description about the issue

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2. If you have not started your reservation using NUcore, please report problems with the instrument at <http://imserc.northwestern.edu/contact-issue.html> and place the 'Stop' sign near the instrument computer. 'Stop' signs are located on the shelf above the computers in BG51 and online at the link above. Email or talk to a staff member
3. Email or talk to a staff member

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REVISIONS

v1.01 2025/09/12	<ul style="list-style-type: none">• Release of original version of the user manual
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