Quick Reference How-To Guide for SLM Upgrades and the DM 45

Turning on the spectrofluorimeter

1. Make sure all electronic components are off, then turn on the lamp.
2. Turn the main power switch on.
3. Ensure that the control box is on.
4. Turn on the computer and open GlobalWorks program.
5. Cancel **Open File** window and click on **SLM** or **DM-45** button on tool bar.
6. The spectrofluorimeter will initialize and calibrate.

Changing modes of detection

- Several modes of detection can be chosen from in the **Operational Modes** tab under **Data Reduction Mode**. The modes available are dependent on the number of detectors and polarizers:

  One detector options are:

  L-Format Anisotropy: This option is available with at least two polarizers. Data is collected as fluorescence intensity for both vertical and horizontal emission polarizers and computed to give anisotropy.

  L-Format Polarization: This option is available with at least two polarizers. Data is collected as fluorescence intensity for both vertical and horizontal emission polarizers and computed to give polarization.

  Signal: Signal is reported directly with no mathematical manipulation of the data. The signal is reported in volts for a PMT and counts for a photon counter.

  Two detector options are:

  T-Format Anisotropy: This mode is available with two detectors, and three polarizers. Data is collected as a ratio of detector signals (one channel vertically polarized, the other horizontally). This data is converted automatically to anisotropy.

  T-Format Polarization: This mode is available with two detectors, and three polarizers. Data is collected as a ratio of detector signals (one channel vertically polarized, the other horizontally). This data is converted automatically to polarization.

  Ratio: In this mode, the emission signal is reported as a ratio of sample signal to reference signal. Fluctuations in lamp intensity are corrected for by this method.

  Single Beam: This mode reports the raw signal from the sample and reference channels.

Taking a fluorescence emission scan

1. Open the **Operational Modes** tab and set the **Data Collection Mode** to **Scan**.
2. Go to **Live Display** tab.
3. Move excitation and emission monochromators to the approximate intensity maximum by highlighting current wavelength with a mouse click and entering the desired wavelength (If maximum wavelengths are unknown, perform a scan with a small integration time to find the maximum).
4. Click on **Live Mode** button and adjust **PMT HV** value to give an acceptable signal (If you have photon counting you cannot change **PMT HV**).
5. Change emission scan range to desired range (click on the number to highlight).
6. Enter the desired number of increments to be collected and the integration time for each point.
7. Ensure that the excitation scan **From** and **To** wavelengths are identical and equal to the desired excitation wavelength.
8. Click on the **Collect Data** button to begin scan.

Taking a fluorescence excitation scan

1. Open the **Operational Modes** tab and set the **Data Collection Mode** to **Scan**.
2. Go to **Live Display** tab.
3. Move excitation and emission monochromators to the approximate intensity maximum by highlighting current wavelength with a mouse click and entering the desired wavelength (If maximum wavelengths are unknown, perform a scan with a small integration time to find the maximum).
4. Click on **Live Mode** button and adjust **PMT HV** value to give an acceptable signal (If you have photon counting you cannot change **PMT HV**).
5. Change excitation scan range to desired range (click on the number to highlight).
6. Enter the desired number of points to be taken and the integration time for each point.
7. Ensure that the emission scan **From** and **To** wavelengths are identical and equal to the desired excitation wavelength.
8. Click on the **Collect Data** button to begin scan.

Taking repeated scans

1. Under **Repeated Scans** tab, change **Number of Scans** to the desired number. Scans can be made automatically as a function of time, or manually. In the **Auto** mode, the time selected is the total time to complete all scans. In the **Manual** mode, scans are started by pressing the spacebar.
2. Select **Manual** or **Auto** as the **Scan Mode** on this page.
3. Ensure that **Time Units** are correct. These can be changed in the **Operational Modes** tab.

- All repeated scan data will be saved as a single, 3-D data set.
- Selecting **Average Scans** results in an output of a single, averaged scan.

Collecting an L-format anisotropy fluorescence excitation scan

1. Ensure that an excitation and emission polarizers are installed.
2. Choose the desired PMT to use under the **Parameters** tab.
3. Under the **Operational Modes** tab set the **Data Collection Mode** to **Scan**.
4. Select **L-format Anisotropy** for the **Data Reduction Mode** under the **Operational Modes** tab.
5. Place sample in sample chamber, change the slits to an appropriate slit width, and open the appropriate shutters.
6. Choose the appropriate excitation and emission wavelengths.
7. Enter into **Live Mode** and adjust the **PMT HV** to give an appropriate signal (preferably between 2-8 volts). If a photon counter is used, signal should be less than 1,000,000 counts per second.
8. Move the polarizers to all possible positions in **Live Display** to ensure that PMTs are not saturated at any polarizer position. PMTs will give a signal of 10.000V when they are saturated.
9. Click on **G-factor** to collect a G-factor value. The G-factor is calculated as I_{HV}/I_{HH}.
10. If the G-factor is acceptable, click on **Accept G-factor**. **Collect Data** will replace the **G-factor** button, and the excitation polarizer will be moved to the vertical position, where it will remain for all anisotropy measurements.
11. Enter in the desired excitation scan range, the emission wavelength in both start and ending wavelengths, the desired number of points, and integration time.
12. Begin data collection by clicking **Collect Data**. The final data presented will be anisotropy as a function of excitation wavelength.

Collecting a T-format anisotropy fluorescence excitation scan

1. Ensure that excitation and emission polarizers are installed (at best, 2).
2. Choose the desired PMTs to use under the **Parameters** tab.
3. Ensure that the red PMT is on emission channel 1 and the blue is on emission 2. This will ensure that the red signal corresponds to the vertically polarized signal.
4. Under the **Operational Modes** tab set the **Data Collection Mode** to **Scan**.
5. Select **T-format Anisotropy** for the **Data Reduction Mode** under the **Operational Modes** tab.
6. Place sample in sample chamber, change the slits to an appropriate slit width, and open the appropriate shutters.
7. Choose the appropriate excitation and emission wavelengths.
8. Enter into **Live Mode** and adjust both **PMT HV** to give an appropriate signal (preferably between 2-8 volts). If photon counters are used, the signals should be less than 1,000,000 counts per second.
9. Move the excitation polarizers to both positions in **Live Display** to ensure that PMTs are not saturated at any polarizer position. PMTs will give a signal of 10.000V when they are saturated.
10. Click on **G-factor** to collect a G-factor value. The G-factor is calculated as I_{HV}/I_{HH}.
11. If the G-factor is acceptable, click on **Accept G-factor**. **Collect Data** will replace the **G-factor** button, and the excitation polarizer will be moved to the vertical position, where it will remain for all anisotropy measurements.
12. Enter in the desired excitation scan range, the emission wavelength in both start and ending wavelengths, the desired number of points, and integration time.
13. Begin data collection by clicking **Collect Data**. The emission polarizer will move to the horizontal position (to collect I_{VH}), and collect data as a function of excitation wavelength and presented as anisotropy.

Taking an assay (time drive)

1. Under the **Operational Modes** tab, set the **Collection Mode** to **Assay**.
2. Enter **Total Assay Time** in the **Live Display** tab.
3. Enter the desired excitation and emission wavelengths in the respective current wavelength displays.
4. Enter **Number of Points to Collect** and **Integration Time** per data point.
5. To subtract an offset from the data, click on the **Zero Instrument** button.
6. To begin the assay, click on the **Collect Data** button and press spacebar when prompted.

Collecting stopped-flow fluorescence data

1. Ensure that the stopped flow apparatus is on the instrument and the gas pressure is between 75 and 90 psi.
2. Attach two luer-lock disposable syringes to the ports above the flow valves and fill them with your desired reagents. We recommend that you degas buffers as well to reduce the probability of bubbles entering the flow system.
3. Move fill valves to the “Fill” position and carefully draw back stopped flow syringes to fill.
4. Ensure that correct PMTs are active in **Parameters** tab.
5. Set the **Data Collection Mode** to **Stopped-Flow** in the **Operational Modes** tab.
6. Choose the desired **Data Reduction Mode** in the **Operational Modes** tab.
7. Under the **Live Display** tab, adjust he current excitation and emission wavelengths, slit width, and open the appropriate shutters.
8. Enter **Live Mode** by pressing the **Live Mode** button.
9. Adjust the **PMT HV** to give a good fluorescence signal. Note that a fluorescing sample must be in the flow cell at this time.
10. Choose the appropriate **Data Collection Time**, **RC Time Constant**, and whether or not pre-trigger data will be shown.
11. If a baseline offset is desired, click on **Zero Baseline**. This will subtract the current intensity from all subsequent measurements.
12. Ensure all valves are in the flow position and syringes are in contact with the plunger block. The green “Ready” light on the electronics box should be on. If not, a red indicator light will be on, indicating a valve is out of position.
13. Press **Collect Data** to begin data collection. If the valves are not in their proper positions, the program will not collect data and will indicate which valves are improper. If the instrument is ready, pressing the spacebar will initiate the flow and data collection.

Fitting 2-D data set

1. Click on dataset to be fit.
2. If you desire to fit only a portion of this data, select **Create Data Subset** in the **Tools** menu. When prompted, enter the desired range. Click on new dataset to select it.
3. Select **2-D Fits** under the **Fits** menu and select the desired model to fit the data. *If you would like a data fitting model added to the software, please contact Olis.*

Fitting a 3-D data set

- There is a tutorial under the **Help** menu which describes SVD data processing and fitting.

Smoothing a 3D dataset using SVD

1. Click on the desired dataset in the **Experiment** window.
2. Click on **SVD** to generate the SVD eigenvectors.
3. Choose **Reconstruct 3D from SVD Data**.

Naming a dataset

1. Double click on the **Name** property in the **Properties** window.
2. Enter a name for the dataset.
3. Press enter to assign the name. *This name will remain with the dataset and is distinct from the file name.*

Saving a dataset

1. Click on the desired dataset in the **Experiment** window.
2. Add any comments, and change the dataset name if desired.
3. Choose **Save Dataset** or **Save dataset as...** under the **File** menu. *Choose an appropriate directory and file name.*

Saving an experiment

1. Click on the desired experiment in the **Experiment** window.
2. Choose **Save Experiment** under the **File** menu.
3. The program will prompt for file names for each data set in the experiment. *When the experiment is reopened all the accompanying datasets will be opened.*

Changing the axis scale on a data set

1. Select desired data set
2. Right-click on graph
3. Select scale and enter desired values.

Viewing more than one set of data

1. Open all desired sets of data.
2. Select a dataset to be viewed (move between data sets in the **Experiments** window on the right).
3. Select **Copy Slice** under **Edit** menu.
4. Select second data set to view.
5. Select **Paste Slice** under **Edit** menu

- To hide a slice from view (and from the printer), select it and select **Hide Slice** under the **View** menu.
- To switch between hidden slices and viewed slices, select **Swap Hidden/Unhidden Slices** under **View** menu.

Exporting a 2-D data set

1. Select a data set to be exported.
2. Right click on the chart and select **Save as Ascii**.
3. Enter the filename when prompted.

- Alternatively, data can be exported directly into Excel by selecting **Export to Excel** under the right-click menu.

Smoothing a scan

1. In the **Experiment** window, select a dataset by clicking on it.
2. Right click on the desired dataset and choose **Select** from the pop up menu.
3. Right click on the dataset again and choose the **Smooth** option under **Data Processing** in the pop up menu.
4. Choose the degree of smoothing (3-25 points per average).
5. A new smoothed dataset will be generated in the **Experiment** window. The name will by default be "[original data file name]-smoothed."

Doing math on a dataset

1. In the **Experiment** window, select a dataset by clicking on it.
2. Right click on the dataset and choose **Select**.
3. Repeat this procedure for any datasets to be included in the mathematical operation.
4. Right click on the dataset again and choose the desired mathematical operation under the **Data Processing** menu. *These options are also available under the **Tools** menu.*
5. The new mathematically manipulated dataset will be generated in the **Experiment** window.

Printing a data set as a report

1. Select chart by clicking on dataset.
2. Select **Print Preview** under **File** menu and choose **Color** or **Black and White**.
3. Click on **Print**.

Pasting a dataset into Microsoft Word

1. Select chart by clicking on dataset.
2. Select **Send Chart to Clipboard** under **Chart** tab.
3. Open Microsoft Word document.
4. Choose **Paste Special** under **Edit** menu.
5. Double click on graph to edit it using Microsoft Draw.

Turning off the spectrofluorimeter

1. Exit the GlobalWorks software by selecting **Exit** under the **File** menu.
2. Exit Windows and turn off main power switch.
3. Turn off power to lamp.

Deleting a slice from a dataset

1. Left click on a dataset to highlight it.
2. Choose **Edit Dataset** under the **Edit** menu.
3. Click on **Edit Axis Data** of the axis of the slice to remove.
4. Left click axis points or drag mouse to select multiple points.
5. Right click and select **Remove Axis Points** under **Axis Options**.
6. Click on **Save Axis Data**.
7. Click **Post Data to GlobalWorks**.

Changing the axis titles on a dataset

1. Left click on a dataset to highlight it.
2. Choose **Edit Dataset** under the **Edit** menu.
3. Change axis title and units. *Axis values can be changed by clicking **Edit Axis Data**, changing axis values, and clicking **Save Axis Data**.*
4. Click **Post Data to GlobalWorks**.

Collecting repeated scans as a function of a titrator script

1. In the Repeated Scans tab, set **Repeat Scans as a function of** to **Titrator Script**.
2. Follow instructions for calibration.
3. Load solution into titrator using the **Titrator Control Panel** to move syringes.
4. To edit a script file, click on **Edit Script**.
5. Select appropriate data collection parameters in the **Live Display** and **Operational Modes** tabs.
6. Click on **Collect Data** to begin Scans.

Taking repeated scans as a function of a temperature script

1. In the **Repeated Scans** tab, select the desired temperature script by entering or browsing to the correct file.
2. To edit a script file, click on **Edit Script** and change the number of scans, temperatures and integration times.
3. Check that the temperature controller is set to **On** in the **Temperature Control** tab.
4. In the Repeated Scans tab, set **Repeat Scans as a function of** to **Temperature Script**. *The **Number of Scans** value should change to be equal to the number of scans in the temperature script.*
5. Select appropriate data collection parameters in the **Live Display** and **Operational Modes** tabs.
6. Click on Collect Data to begin Scans.

Building a 3-D dataset

1. Collect individual 2-D traces to be included in 3-D dataset.
2. Click on dataset.
3. Choose **Edit Dataset** under **Edit** menu.
4. Change Y axis title and units to new axis.
5. Repeat for each trace to be included. *Cut and paste may be used.*
6. Select all datasets to be included by right clicking each in the **Experiment** window and choosing **Select**.
7. Right click on a dataset in the **Experiment** window, choose **Build 3-D from 2-D** under **Data Processes**.
8. Select all datasets to be included by right clicking each in the **Experiment** window and choosing **Select**.
9. Click on the new dataset, choose **Edit Dataset** under the **Edit** menu.
10. Choose **Edit Axis Dat**a, enter new values and click **Save Axis Data**.
11. Click **Post Data to GlobalWorks**.