Xi'an Jiaotong-Liverpool University
西交利物浦大学

General Operating Procedure for:

Cary Eclipse Fluorescence Spectrophotometer

Department of Health and Environmental Sciences

By Yili Cheng

Contents

1. Purpose

The purpose of this GOP is to regulate the operation, usage and maintenance of Cary Eclipse fluorescence spectrophotometer.

2. Scope

This GOP applies to all personnel using and managing Cary Eclipse fluorescence spectrophotometer.

3. Procedure

3.1 Switching on the machine

3.1.1 Turn on the instrument. The switch is at the front panel.
3.1.2 Turn on the computer; the password is “ENV”.
3.1.3 Wait till the instrument emit green light.
3.1.4 Click  at the bottom left corner of the screen to enter the desktop. Double click on the Cary icon 

on the desktop.
3.1.5 Choose the desired application.

3.2 Fluorescence Scan

3.2.1 Set up a method

Click on the scan icon  to open the scan application.

Select File/Open Method from the menu to load a previously defined method or select the Setup button to display the Setup dialog and specify the method parameters for a new method (fig.1).

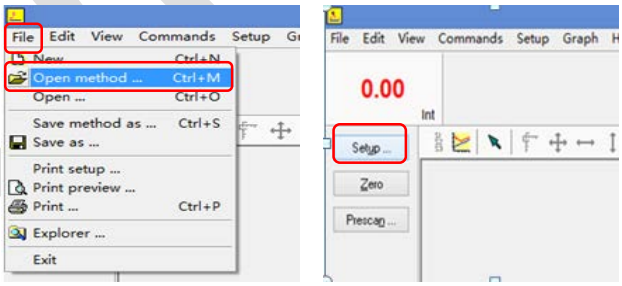

Fig.1 Setting up a method

3.2.1.1 Set the instrument parameters

Setup dialog box / Cary page (fig.2)

a. Set the data mode to fluorescence;

b. Set the Scan Setup mode to Excitation, Emission, or Synchronous;

- Excitation: fixed emission wavelength and scan excitation monochromator. Allow the user to determine the optimum excitation wavelength.
- Emission: fixed excitation wavelength and scan emission monochromator.
- Synchronous: scan both at the same time, usually with a fixed difference in wavelength. Good at identifying complex mixtures.

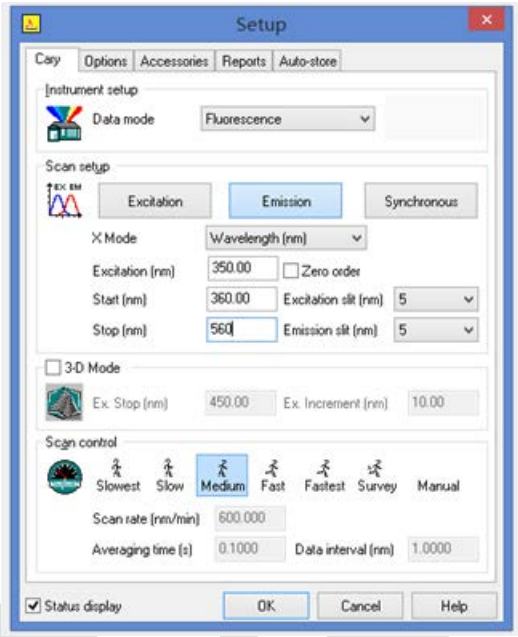

Fig.2 Scan / Cary page

c. Set the X Mode to Wavelength;

d. Enter an Excitation (nm) / emission (nm) value;

e. Enter an Excitation slit (nm) value of 5 and an Emission slit (nm) value of 5 to start. Slits determine the resolution of the spectrum. If a compound is highly fluorescent and has reasonable signal intensity the slits can be set quite narrow (i.e. 5).

f. Enter a Start (nm) value and Stop (nm) value. Typically the Stop (nm) value should be set to 150-200 nm greater than the Start (nm) value. Also enter the delta value for synchronous mode.

- Emission: The start (nm) value should be set to the Excitation (nm) value plus the sum of the slits.
- Excitation: The stop (nm) value should be set to the Emission (nm) value minus the sum of the slits.
- Synchronous: check the example below for the determination of delta. (fig.3)
  Picture below is the excitation and emission spectrums of vitamin B2 and B6. Delta B2= 523-

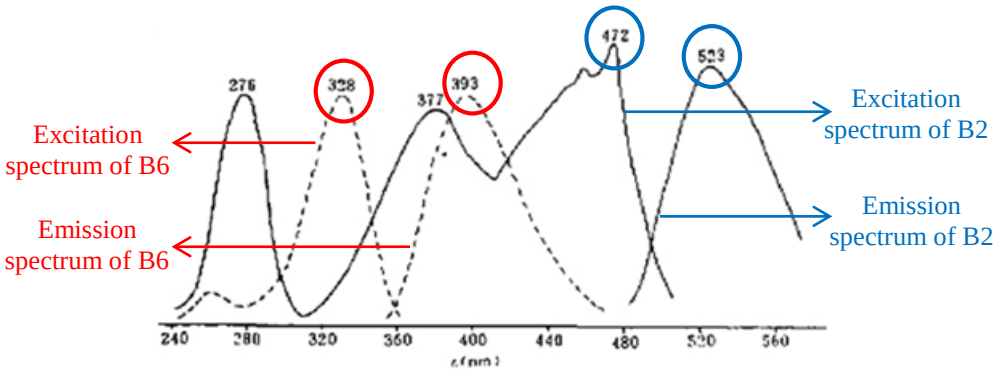

Fig. 3 Fluorescence spectrum of vitamin B2 and B6

472=51nm; delta B6 =393-328=65nm. Select a value between 51 and 65 as the delta for the synchronous mode.

g. Usually, do not need to select '3-D Mode'. Select it and define the Ex.stop and Ex. Increment if you really need.

h. In the 'Scan Control' group; select a scan speed button (i.e. Medium). Alternatively, you can select Manual and enter an Ave Time and Data Interval.

i. Select the Status Display check box so that you can view various instrument parameters during the scan to setup visual system monitoring.

3.2.1.2 Set up the scan options

Setup dialog box / Options page (fig.4)

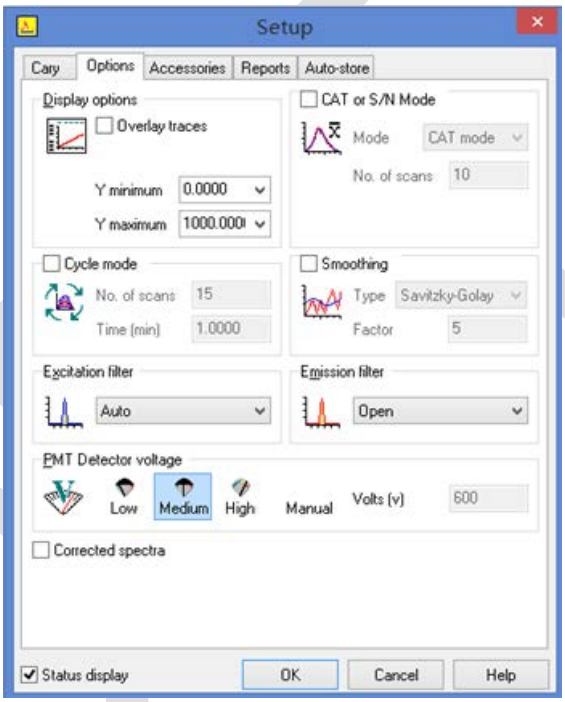

Fig.4 Scan / Options page

a. In the **Display Options** group, select the way in which you want the data displayed as it is collected. Select Overlay Data to superimpose the collected data of each sample in the Scan run in one graph box; if not selected the data will be displayed in individual graph boxes.

b. Enter the minimum and maximum Y values to be displayed on the graph during data collection.

c. If selected, clear the **CAT or S/N Mode**, **Cycle Mode** and **Smoothing** check boxes.

d. Set the Excitation filter to **Auto** and Emission filter to **Open**.

e. Set the **PMT Detector Voltage** to Medium. This setting can later be adjusted if results are over-range

3.2.1.3 Make sure that no accessories are selected on ***Setup dialog box / Accessories page.***

3.2.1.4 Once you are satisfied with your method setup select OK to confirm any changes you have made and close the Setup dialog. Save the method if you plan to use it regularly.

3.2.2 Sample measurement

a. Place the blank solution in the sample compartment of the fluorimeter. Make sure not to touch the side of the cuvette while doing so.

b. Click 

to zero the system. When the result is zeroed, the word 'Zeroed' will appear in the Y display box in the top left corner of the Scan Application window.

c. Preform a scan of the blank to check for any irregularities in the baseline. Select the Start button 

to commence a data collection. The Sample Name dialog is displayed. In the Sample Name dialog, enter the appropriate name and select OK. The scan will commence and the trace will appear in the Graphics area.

d. Insert sample into the holder. Select the Start button 

to start the test.

e. Once the run is finished, select the Save As command from the File menu. Enter the file name and select the type of file.

3.3 Simple reads

3.3.1 Perform a measurement at a single wavelength

a. Open the simple reads  application. Insert the blank into the sample compartment and zero the instrument by clicking the Zero button.

b. Click the Setup button. Select the ‘Data Mode’ you require. Enter the required excitation and emission wavelengths at which you want to perform the read. Click OK.

c. Insert the sample into the sample compartment. Wait while the instrument changes to the specified wavelength. Click the Read button to perform the read. The result will be displayed in the Report area.

3.3.2 Perform measurements at two wavelengths and subtract the result

a. Open the simple reads  application. Insert the blank into the sample compartment and zero the instrument by clicking the Zero button.

b. Click the Setup button. Select the ‘Data Mode’ you require.

c. Select User Collect. The 'Em. Wavelength' field will change to enable you to enter a simple Read command. You can also select a default command from the drop-down list. To do this, click the arrow to the right of this field (fig.5).

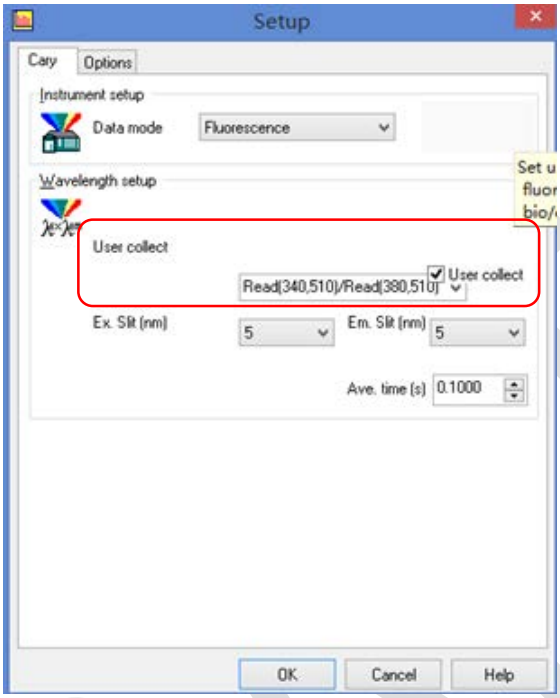

Fig.5 Simple reads / Cary page

d. Set the slit widths of the excitation and emission monochromators; enter the averaging time. Click OK.

e. Insert the sample into the sample compartment and click the Read button 

.

3.4 Concentration

3.4.1 Set up a method

3.4.1.1 Set up instrument parameters

a. Open the concentration  application. Click Setup to display the 'Setup' dialog box.

Setup dialog box / Cary page (fig.6)

b. Select the 'Data Mode' you require. Enter the required excitation and emission wavelengths at which you want to perform the read. Click OK.

c. Set the slit width of the excitation and emission monochromator.

d. In the 'Ave. (averaging) Time' field, enter the required value. A good starting point is 0.1 seconds.

e. Select the Status Display check box to display various information fields on your current reaction.

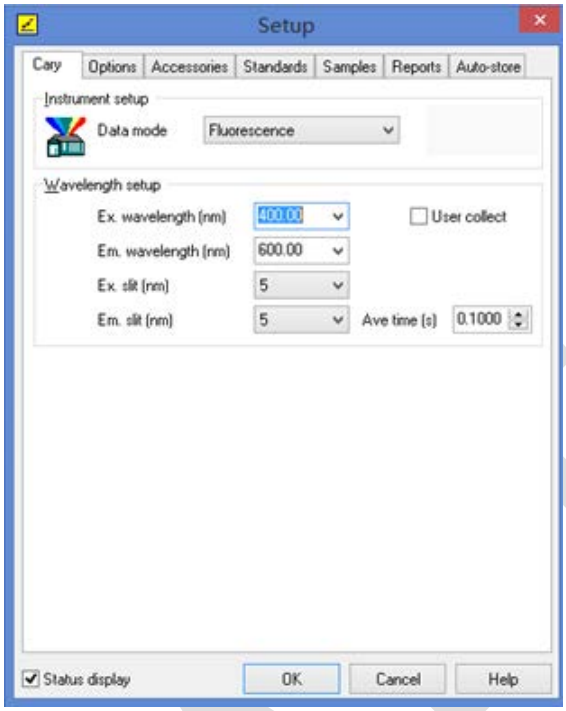

Fig.6 Concentrations / Cary page

3.4.1.2 Set up the filter and voltage options

Setup dialog box / Options page (fig.7)

a. Set the Excitation filter to Auto and Emission filter to Open.

b. Set the PMT voltage. A 'Low' setting is recommended for highly-emitting samples, while a 'High'

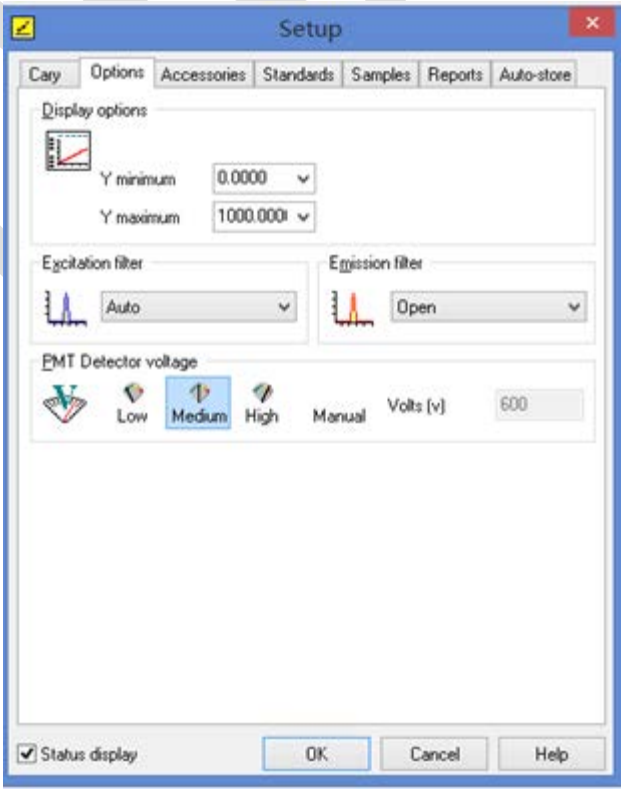

Fig.7 Concentrations / Option page

setting is recommended for low-emitting samples.

3.4.1.3 Make sure that no accessories are selected

Setup dialog box / Accessories page

3.4.1.4 Set up the calibration

Setup dialog box / Standards page (fig.8)

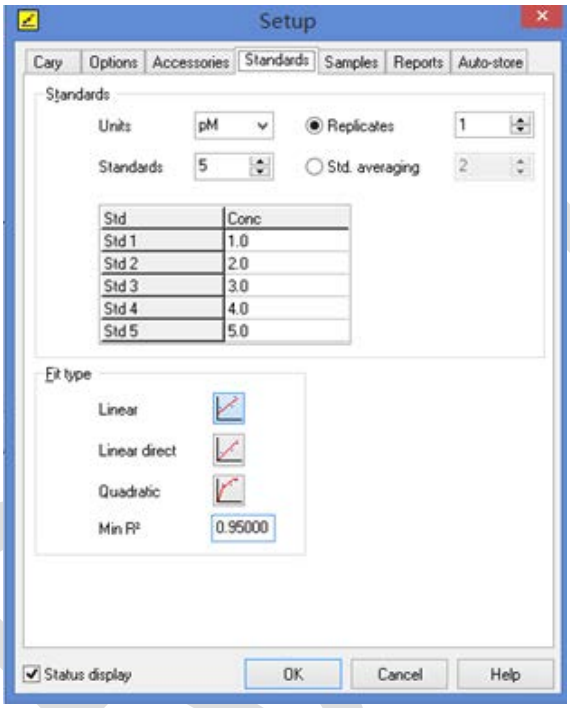

Fig.8 Concentrations / Standards page

a. Set the units and number of standards, In the 'Standards' table, enter the concentration of each standard in the 'Conc.' column.

b. Select the type of curve fitting required for your calibration under 'Fit Type'.

c. Enter the required R2 value or correlation coefficient in the 'Min R2' field. The closer the number is to 1.000 the better the fit. Typically, 0.95 is used.

3.4.1.5 Set up your samples

Setup dialog box | Samples page (fig.9)

Enter the number of samples that you are going to use and the name of each sample. You can enter up to 20 characters for each name.

If you would like the samples to have the same name with a different numeric extension, enter the name in the first sample position and then click the 'Increment' button.

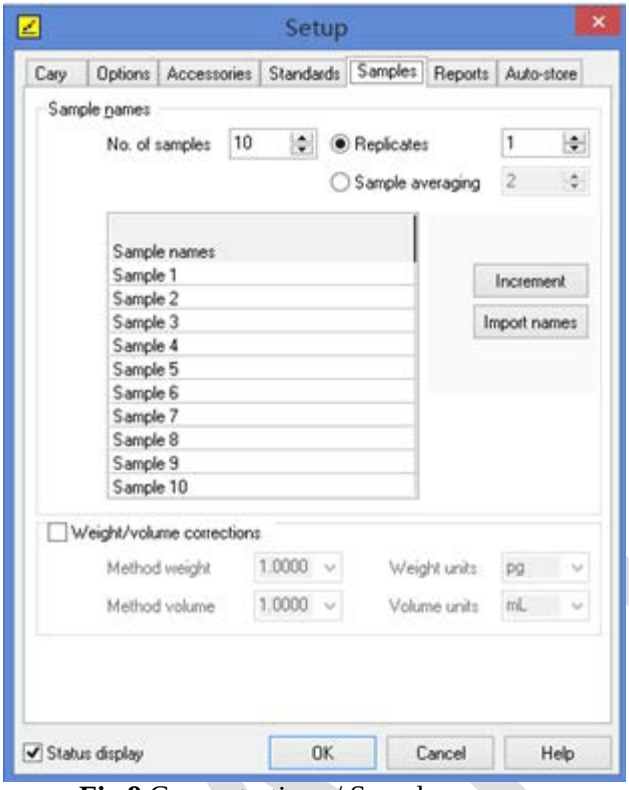

Fig.9 Concentrations / Samples page

3.4.1.6 Click OK to finish the method setup.

3.4.2 Perform the calibration and measure the samples

a. Insert the blank into the sample compartment and zero the instrument.

b. Click the Start button 

. Then select the standards and samples to be used in the analysis. Click OK to close The 'Standard/Sample Selection' dialog box.

c. The 'Cell Loading Guide' dialog box will be displayed. Load your standards as indicated; then click OK to start reading the standards. If additional 'Cell Loading Guide' dialog boxes are displayed, continue to load your standards as indicated and click 'OK' to continue loading the standards. The Cary Eclipse will measure the standards and calculate the calibration curve.

d. A 'Cell Loading Guide' will be displayed for the samples. Load your samples as indicated and click OK. The Cary Eclipse will then measure the samples and calculate their concentration.

e. Save your data.

3.5 Cleaning

Any spills in the sample compartment should be immediately wiped up.

The exterior surfaces of the Cary Eclipse spectrophotometer should be kept clean. All cleaning should be done with a soft cloth. If necessary, this cloth can be dampened with water or a mild detergent. Do not use organic solvents or abrasive cleaning agents.

3.6 Switching off the instrument

Exit the Cary Eclipse application;

Turn off the instrument and computer.

Document information:

Document No:	GOP-Cary Eclipse-01	Version No:	0.1 (draft)
Drafted/ modified by:	Yili Cheng	Effective date:	
Comments:			