FAC (Fluorescence Analysis)

Current Version: 8

Authors: Dean Brown, Perkin Elmer
Dave Frazer, Fluorescent Microsphere Resource Center
Comments/Corrections: frazer@u.washington.edu
Manual revised: January 22, 1996

Introduction

FAC (Fluorescence Analysis) is designed to read the fluorescence in multiple samples using a Perkin Elmer LS-50 or Perkin Elmer LS-50B luminescence spectrophotometer. FAC provides bookkeeping for multiple samples with a number of different fluorescent dyes in each sample. Measurements are written to screen and a text file, with optional output to a printer. The resultant text files are formatted such that they can be easily imported into spreadsheets on either DOS/Windows or Macintosh computers. The program works for the cuvette reader, the original 96-wellplate reader, and the configurable new wellplate reader. The program also calculates flow to each piece (ml/min) if a reference blood sample was obtained.

FAC is public domain software. Only the executable file is available for distribution. FAC will evolve to meet the needs of researchers measuring organ blood flow with fluorescent microspheres. Updated versions will be available through the FMRC.

Features

FAC is a menu driven program that runs under DOS. It runs separately from the Fluorescent Data Manager (FLDM™), available from Perkin Elmer. FAC is restricted to reading the fluorescent intensities of samples at up to 10 different excitation/emission wavelength pairs. FAC does not produce spectra or allow for synchronous scanning. However, FAC assists the user to setup a 'method' that, when stored, can be recalled and used without reentering the desired parameters during subsequent analyses. Storable parameters include excitation and emission slit widths, emission filter settings, the PMT voltage and the excitation/emission wavelength pairs. During analysis fluorescent values are written to both screen and text file, with an option to print a hard copy. When using cuvettes the user has the option of accepting or rejecting the value after each sample is read. Only accepted values are written to the text file and printer. There is a comment line that can be used for each sample as well. The output to the text file can be formatted for either DOS (tab delimited, carriage return/linefeed) or Macintosh (tab delimited, carriage return). The user has the option of calculating blood flow (volume/time) if a reference blood sample has been obtained and the fluorescence measured.

Installing FAC

FAC can be installed anywhere on your hard disk, as long as locations for the program and preference file are present as well as data files and method files locations. These subdirectories must exist prior to running FAC for the first time. An accompanying INSTALL.BAT file creates the needed subdirectories on the chosen drive. To use the INSTALL.BAT file, type **install** followed by the drive (e.g., **install c**) to which you wish to install FAC and the needed subdirectories.

The program comes configured initially as follows:

C:
I_____FA.....Program and preference file (C:\FA)
I_____METHODS.....Method files (C:\FA\METHODS)
I_____DATA.....Data files (C:\FA\DATA)

FAC Operation

The program is initiated by typing FAC while in the same directory as the FAC program. Once initiated, user input is menu driven. By convention, the choice enclosed by squared brackets is the default choice and can be chosen by simply pressing the return or enter key.

SETUP PROCEDURE

When FAC is run for the first time, you will be asked to initialize a preference file (FAC.PRF). On subsequent program initializations the program skips to the **Confirm Setup** section below. The preference file saves user supplied hardware and software configurations for future use. The following questions are asked during the setup procedure:

Computer Display	Explanation
Preferences File Not Found **Setup Required** **Fluorometer Hardware** **1. Perkin Elmer LS-50** **2. Perkin Elmer LS-50B** **Hardware Used (1-2):**	Choose which spectrophotometer you will be using.
Is there a printer available (Y/N):	Is there a printer attached to the control computer? If so, FAC can create a hard copy of the intensity data as they are read. FAC will prompt the user to turn the printer on if it is off.
Default output file format. Mac/IBM (M/I):	Select which file format should be selected on a default selection. The only difference between the two is the end of line character sequence used. Each operating system recognizes different sequences to indicate end of line.
You must now declare where program files are to be stored. The program preferences file must be in the same location as the program. If you ever move the program be certain and also move the file FAC.PRF. **There are two pathnames to declare:** **Data path - stores fluorescence counter result files** **Methods path - store methods data files** **These paths must exist. This program does not create them for you. The default paths are displayed. To accept them just press the ENTER or RETURN keys.** **Data file path [C:\FA\DATA]:**	Press return to accept displayed setting, otherwise enter a valid path name. If the path name that you enter does not exist you are prompted to reenter or optionally enter Q to exit the program so that you can create the needed subdirectory.
Methods file path [C:\FA\METHODS]:	Press return to accept displayed setting, otherwise enter a valid path name. If the path name that you enter does not exist you are prompted to reenter or optionally enter Q to exit the program so that you can create the needed subdirectory.

This completes the setup section of the program. Please skip to the **Major Options** section below.

CONFIRM SETUP

After the initial setup run, the program initially displays the current setup. An option is presented to allow the preferences to be changed as hardware configuration or the user's needs vary. With each subsequent use of FAC, the current preference settings are displayed as follows:

Current Program Setup
Fluorometer is *LS-50* (LS-50B)
A printer *is* (is not) available
File formatting is for *Macintosh* (IBM)
Data file path is *C:\FA\DATA*
Methods file path is *C:\FA\METHODS*
Change Setup (Y/N) [N]:

If the setup is correct, accept the default by pressing the return or enter key. To change the setup, enter **Y;** you will be prompted with the questions as listed in section on **Setup Procedure**.

MAJOR OPTIONS

FAC supports two primary options. The first reads the sample fluorescence and the second retrieves previously acquired data and calculates flow per sample. The next menu allows the user to choose which FAC option is to be used. See section on **Flow Analysis** for instructions for that option.

Available Options
1 - Use Fluorometer
2 - Flow Analysis
3 - Exit Program
Enter Option (1-3) [1]:

Use Fluorometer

The first step in measuring fluorescence is selection of parameters required for measurement of each sample. FAC gives the user the option of recalling a previously defined method (parameter set) or creating a new one.

Parameter Selection
1. Recall a Method
2. Make a New Method
Selection (1-2) [1]:

Option 1 (Recall a Method)

Method listing (Y/N) [Y]:

If the user responds with 'Y', then all of the methods currently stored in the “methods” path sub-directory will be listed. Otherwise the user is immediately prompted for a user name.

Method Name (Maximum 8 characters - No Extensions)?

Once the user has entered a method name, please skip to the Transport device section below.

Option 2 (Make a New Method)

Computer Display	Explanation
Enter Excitation Slit (2.5-15)? **Enter Emission Slit (2.5-20)?**	First, the excitation and emission slit widths are entered.
Emission Filter (Y/N):	Second, the user is asked if an emission filter is to be used. If a filter is to be used, 5 choices are presented.
Select one of the Following **1. 290 nm cutoff filter** **2. 350 nm cutoff filter** **3. 390 nm cutoff filter** **4. 430 nm cutoff filter** **5. 530 nm cutoff filter** **Selection (1-5):**	If you want an emission filter then select which one you want.
Default or Selected PMT voltage (d/s):	The user can also specify if the default or a selected voltage is to be used for the photomultiplier tube (PMT).
PMT voltage (0-900):	If default PMT voltage (d) is chosen, the PMT voltage is determined by the spectrophotometer based on the excitation and emission slit widths. If the selected PMT voltage (s) is chosen, the user enters the voltage at the prompt shown to the left.
Enter Number of Analytical Wavelengths (Max 10):	The number of excitation/emission wavelength pairs is then entered with a maximum of 10.

The user is then prompted to enter each pair in **ascending** wavelength order. Unless the wavelengths are entered in ascending order the program will not function properly.

Excitation Wl 1 (200-800 nm):	**Emission Wl 1 (200-900 nm):**
Excitation Wl 2 (200-800 nm):	**Emission Wl 2 (200-900 nm):**
.	.
.	.
.	.
Excitation Wl n (200-800 nm):	**Emission Wl n (200-900 nm):**

Any Changes (Y/N):	After all wavelength information is entered, the operator is given the opportunity to make any changes to the wavelength entries before saving the new method file.

Enter the Number to be changed:	If yes, the operator is prompted to select the analytical wavelength pair of values to be changed. For instance, if an invalid number is entered in the second emission wavelength pair entered, you would enter 2. You are then reprompted to enter both the excitation and emission wavelengths for the second pair.
Enter Method Name (No Extension):	After the revised pair of numbers has been entered the user is asked to select any other changes. If no further changes are to be made the user is prompted to select a method name in which to store the parameters on disk. Any standard DOS name can be used up to 8 characters and without file extension.

Now the declaration of the method, or stored parameter set, is complete and available for subsequent uses by recalling an existing method, as shown above.

Transport device

Reading from cuvette, wellplate or liquid handler (C/W/L) [C]:	How will samples be transported to the fluorometer for analysis? The two primary options are via cuvettes or wellplate reader. The liquid handler is in the experimental and not ready for use at this time
Wellplate	If you choose the wellplate option, the following questions are asked
New or Old Wellplate reader (N/O)[N]:	Select either the original or new configurable Perkin Elmer wellplate reader.
Enter Wellplate Geometry Name (No Extension):	If using the new wellplate, you are prompted to enter the name of the file that describes the geometry of the wellplate. This is not necessary for the original reader as the geometry of the plate is fixed.
Are a blank and standard in wells 1 & 2 (Yes/No)[Y]:	The program is set to read a blank (solvent only) and a standard in wells 1 & 2. This allows comparison between wellplates. If you are using this method enter Y. If study samples are in wells 1 & 2, enter N.

Stop after each well or each plate (W/P)[P]:	This gives the operator the option of accepting or rejecting fluorescent intensities after each sample is read or waiting until all of the samples are read. If the operator chooses to stop after each plate, the minimum and maximum values for each excitation/emission wavelength pair is displayed at the end of the plate reading.
Enter maximum acceptable value:	If you choose to stop after each plate you are prompted to enter a number above which you wish to receive notifications. Each time a fluorescent intensity exceeds this value, a beep is sounded to alert you to the large intensity. After beeping, the program continues to measure the fluorescence in the remaining wells

.

Output Options

Output results to printer as collected (Y/N) [Y]:	The next step is to determine the output preferences. If the initial setup indicated the presence of a printer, the user is asked if measurements should be printed and stored on a disk.
Format result file for Macintosh or IBM (M/I) [M]:	The user is asked how the data file should be formatted; either a Macintosh format or a DOS format can be selected.

Miscellaneous Information Gathered

This information is gathered to further identify the samples/study on the disk file and printouts.

Amount of solvent used per sample (ml):	Used to keep track of differences which might be caused by variation in the amount of solvent used.
Scan speed (-1500 to -10, 10-1500) [480 nm/min]:	Sets the speed for scans performed during the operation of the program.
Read value time (0.1-100) [1.0]:	This sets the amount time (in seconds) that a read operation will average data. The longer the period, the more stable the results.
Analyst:	Who read the fluorescence measurements?

Sample Identification:	Identification (text) for the samples currently being measured.
Enter starting sample number:	This is used to identify each sample. Any number can be entered. If the samples being run are a continuation of a previous data set, the true sample number of the first sample to be read should be used. If you are using a blank and standard in wells 1 & 2, the sample in well 3 corresponds with this number.
Starting Well Number (1-96):	If a wellplate reader is being used, the operator is asked to enter which well to start the fluorescence reading. All readings are performed on sequential wells, starting at this number well. This is the number of the first well to be read whether it contains a blank or a real study sample.
Enter Total Number of Samples to be Read:	This declares how many wells will be read. If the starting well number plus the total number of samples to be read exceeds 96, the user is prompted to put in the next wellplate and readings start at well #1 on that plate.
File Name Already Exists **Select one of the Following** **1. Rename File** **2. Overwrite File** **3. Append File** **Selection (1-3):**	If the filename already exists, the user is presented with appropriate options. The last option is used when all of the samples from a given experiment cannot be run in a single session. This allows large numbers of samples to be compiled in a single text file. If the appropriate starting sample number was chosen, the sample numbers in the large text file will be continuous.

Reading Fluorescence

So far the computer has neither sent nor received any signals from the fluorometer. At this point the computer communicates with the spectrophotometer to set it up as the user has specified during the setup and initialization procedures. During this period the computer displays the following:

Setting-up Instrument ...Please Wait

The screen is then initialized with columns for the sample number and each excitation/emission wavelength pair. An example is shown below:

	490	**530**	**565**
Sample	**506**	**552**	**598**

Instructions are displayed at the bottom of the screen to prompt the user. The first prompt directs the user to ready a new device for sampling (either wellplate or cuvette).

Place New Cuvette into Sample Holder! Continue or Quit (C/Q):

or

Place New Plate into the Well Reader! Continue or Quit (C/Q):

The user should then enter either a 'C' to start reading the sample(s) or a 'Q' to quit FAC.

The program handles sample fluorescence measurements differently, depending on whether the samples are presented in cuvettes or the new or old wellplate. In both the old wellplate and the cuvette modes each wavelength pair is "read" for a well before moving on. Output is immediately displayed on screen and printed as the wells are read. In this instance the screen output is as shown below. The fluorescence at each excitation/emission wavelength pair will be read and displayed on the screen:

	490	**530**	**565**
Sample	**506**	**552**	**598**
1	**32**	**456**	**231**
2	**23**	**498**	**174**

At the completion of reading all wavelengths for a well the values are displayed on the printer if requested. At the completion of each cuvette measurement cycle the following prompt is displayed.

Enter A [accept], R [reject], S [standard] (A/R/S) [A]:

If the user selects 'A' (accept) the values are written to the text file and the printer if the printer option was selected. By selecting R (reject) the user can rerun the same sample after altering the sample (e.g., diluting the sample if the fluorescence was out of an acceptable range). It is recommended to read standards [S] periodically throughout a session to verify that the spectrophotometer remains stable throughout the session. When the user selects [S] the readings are written to a .CTL file which is stored in the same location as the .FA data file. The number of the current sample is used for the standards reading in the .CTL to enable the user to locate when the standard was read, but the sample number is not increased.

As each color completes, the screen is erased and the next color's values appear on the screen.

Once a cuvette sample is accepted, the user is asked if a comment is to be added to this sample.

Comment [None]:

This is helpful for noting where a sample is from, how much it was diluted, or any information about the sample. The comment is written to the text file only. Asterisks are printed on the hard copy if a comment has been entered.The user is then reprompted to place a new sample in the cuvette holder.

When using the new wellplate, a single wavelength is selected in the LS-50 or LS-50B and all wells are read for that color before monocromators are reset to the next color. This saves wear and tear on the monocromators and dramatically lowers the read time for the entire plate. As the measurements are made they are displayed on the screen in the order shown. The program is setup for using a 96-wellplate. We can provide other configurations if requested.

1	**9**	**17**	**25**	**33**	**41**	**49**	**57**	**65**	**73**	**81**	**89**
2	**10**	**18**	**26**	**34**	**42**	**50**	**58**	**66**	**74**	**82**	**90**
3	**11**	**19**	**27**	**35**	**43**	**51**	**59**	**67**	**75**	**83**	**91**
4	**12**	**20**	**28**	**36**	**44**	**52**	**60**	**68**	**76**	**84**	**92**
5	**13**	**21**	**29**	**37**	**45**	**53**	**61**	**69**	**77**	**85**	**93**
6	**14**	**22**	**30**	**38**	**46**	**54**	**62**	**70**	**78**	**86**	**94**
7	**15**	**23**	**31**	**39**	**47**	**55**	**63**	**71**	**79**	**87**	**95**
8	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**

When all the samples have been read, the user is asked if s/he wants to run another set of samples from the same experiment, begin reading the samples of a different experiment, or quit to DOS.

1. Run another set of samples from the same experiment
2. Start a New Experiment
3. Flow Analysis
4. Exit Program
Select One of the Above (1-4):

FLOW ANALYSIS

If, at the beginning of FAC, the user chooses the option to analyze flow, the following questions are displayed:

Pathname of data files:
Name of data file (no extensions):

FAC reads the requested data file into memory and displays the header and number of samples in the retrieved file.

Number of Wavelengths 4
Analyst Dave
Excitation Slit 4
Emission Slit 4
Cutoff Filter 2
PMT Voltage 820
Sample Identification 1
Date/Time 12-21-1992 15:33:33
ex 430 490 530 600
ex 467 506 552 635 Comment
Number of samples in file: 9
Is this the file (Y/N):

If this is the correct file, the user is then asked to enter the fluorescent intensity of the reference blood sample at each of the excitation/emission wavelength pairs.

Pair 1 430/467:
Pair 2 490/506:
Pair 3 530/552:
Pair 4 600/635:

The user is then prompted to enter the flow rate of the reference blood withdrawal sample.

Flow rate of withdrawal sample (ml/min):

The flow to each sample during each injected color is calculated and written to a text file specified by the user.

Name of flow file (No extensions):

The extension, '.FR', is appended to each flow file.

FAC History

FAC7E - Add support for standards.
FAC7B - Add read time and scan speed support

Support for FAC

In an attempt to limit the number of different versions of this program and to coordinate its evolution for all users, the Fluorescent Microsphere Resource Center will support FAC. Dean Brown of Perkin Elmer will also support the program, but requests should be made through the Fluorescent Microsphere Resource Center by one of the following routes:

Internet:	glenny@pele.pulmcc.washington.edu
Phone:	(206) 543-7063
FAX:	(206) 685-8673
Mail:	Robb Glenny University of Washington Div. of Pulmonary & Critical Care Medicine Box 356522 Seattle, WA 98195-6522 U.S.A.

The FMRC is keenly interested in making FAC useful to researchers using fluorescent microspheres. Any suggestions to enhance the program or this documentation will be gladly considered.