Instructions for setting up and running the demo of scanlsf least-squares spectral fitting program.

Ed Berry 2007

Download the programs and demo for windows from: http://sb20.lbl.gov/berry/scanlsf/
Install the demo by unzipping the file to a location on your hard drive or a floppy disk (The size is about 700kB). It unzips to a directory "scanlsf" with three subdirectories, `bin\`, `speclib\`, and `demo\`.
To uninstall simply delete the directory scanlsf and all its contents (Installing and running the program does not affect the registry or list of programs in start menu)

The demo involves fitting a set of spectra of purified cyt bc1 complex obtained after adding different amounts of different reducing agent to reach different stages of partial reduction of the cytochromes. If you are in a hurry, just doubleclick the file "`ademofit.bat`" in the `demo\` directory (part 3 below).

Note- The programs doing the actual work are old DOS programs, and they have to run in full-screen mode if run from windows. If you need to get to your windows desktop while a program is running, or if a program terminates and stays in full screen mode, you can minimize the dos window with <alt>-<tab> or convert it to a (frozen) normal window with <alt>-<enter>. This is also a quick way to make screen-shots of the display: hit alt-enter to restore to a normal window; alt-print-screen, and paste it into a new photoshop picture. Flatten image, convert to indexed mode, and save it as a gif. To make high quality postscript figures, see “1. continued” below.

1. Examine the standard basis spectra.
First examine the standard spectra used for fitting the experimental spectra. These are in the `scanlsf\speclib\bfbcallo.mat` file.

[The .mat extension is short for matrix, but you will never see the extension in a window because microsoft uses .mat for microsoft access table or something, and does not display the extension. However the distinctive icon is easy to recognize as a 2-D spectral dataset once you get used to it. Actually the extension is not important for our programs, you can call it what you like.]

Open the `scanlsf\speclib` folder in a window and make it small enough to open the `scanlsf\bin` folder in another window. This lets you drag the .mat file onto the `scanedit` program's icon to run `scanedit` and display the contents of the .mat file. (Later you can make a shortcut on your desktop to drag spectra onto).

Drag `bfbcallo.mat` onto scanedit.exe. A DOS window opens up, maximizes to full screen mode, and displays the spectra. The blue spectrum which is positive everywhere is the oxidized bc_1 complex. The sharper spectra that go negative and hence may get clipped in the default display are difference spectra of cytochromes

present in the bc1 complex (as well as cytochrome aa3 which is not present unless as a contaminant).

To get a better view, expand the scale and allow negative values. Below the display is a numerical menu, items are selected by entering the digit 1-8. Digit 9 always displays the next screen of menu, cycling back to the first after the last. Hitting the space bar returns immediately to the first menu. Hence commands are series of digits, like 94 (set vert display scale). Type 9 then 4 (no <enter>). Enter 1000 for full scale range (units are mAU). Then enter 0 to put zero at midscale. "6PA" redraws the spectra at the new scale. (That has to be uppercase PA, like some other things that are case sensitive, so its a good idea to set the caps lock on when you start the program.)
Now you have seen the standard spectra. "998" (quit) and go on to part 2, or if you want to play with the editor some more, continue at "1. continued" further below.

2. Examine the experimental spectra.
Navigate up one level from the `scanlsf\speclib\` directory and down into the demo directory. Most of the files here are a series of spectra named beef2-n where n is 11-38. Drag `beef2-11` onto `scanedit` in the bin directory. The program will recognize from the -11 that this is part of a series, and it will keep loading successive spectra until it fails or fills all 30 traces. In this case there are only 28 spectra, so all are loaded. If desired adjust full scale absorbance and center value as in (1) above. As you can see, the three peaks of the different cytochromes appear independently due to the different redox potentials. 998 (quit) and go on to part 3.

3. Least squares fitting spectra.
3a. Run a non-interactive pre-defined demo by clicking the `scanlsf\demo\ademofit.bat` icon. This sets some env variables and runs the script `scanlsf\bin\fitbbc.bat` which fits the indicated experimental spectra using the standard spectra in `speclib\bfbcallo.mat`.
Double click it:

The full-screen graphics comes on and displays the experimental spectra in rapid succession, with a list of numbers printed for each one. The actual fitting is taking place in this phase. The lists of numbers are the best-fit concentrations of each species. But this display and lists are pretty pointless as it goes by too fast to see.

When this is finished the second pass starts, in which each spectrum is loaded and plotted (blue), the best-fit linear combination of standard spectra is constructed from the coefficients calculated in pass 1 and plotted (green), and the difference is plotted (red). Ignore the prompts (cr to continue, x for no more wait, F to plot decomposition figure) since this is being run noninteractively from a script. When it finishes the results are saved in a .lft (leastsquares fit) file. This is then formatted and printed to a text file (.PRN) which can be opened in notepad or Word. Each row corresponds to one

spectrum (but they will be numbered 1-28 instead of 11-38). Each column gives the concentration of each spectral component. The standard spectral components from the .mat file are listed below the table for a reminder.

3b. Run the fitbbc noninteracitve script from the command line.
Edit fitbbc.bat and change the first two lines to set the filename and directory of the .mat file containing standard spectra, save with a new name. Omit the extension .mat and the trailing slash on the directory:

```
set std=BFBCALLO
SET PTH=..\speclib
```

have the scanlsf\bin directory in your path. If you start your dos shell using the dos icon in the bin directory, it runs the autoexec.bat in the same directory to set your path. (Be aware it assumes it is being run from the bin directory and actually puts the current directory in the path, so if you source this from another directory you need to edit autoexec.bat to give the absolute path of bin directory).
For now just doubleclick the DOS SHELL icon in bin. In the shell window that opens type CD and PATH to verify you are in the bin directory and it is in your path.
Now cd ..\demo
and run the script (arguments are basename of the experimental spectra, first, and last numbers to process, separated by spaces or commas):
fitbbc beef2 11 38

From here on everything goes as with the auto demo. but here you could have fit different spectra, or by copying fitbbc.bat to fitcplab.bat and editing it to use chlorophyll standard spectra you could be fitting to different standards. In practice this is probably the most convenient way to run the program for routine analysis

3c. Run scanlsf.exe from the command line.
This program is superficially like scanedit.exe but doesn't use dropped files.
cd to the directory with the data and invoke the program (bin is in your path):
scanlsf
select menu option 1 (fit spectra),
M (starting from matrix not LS inverse; I should take this out)
..\speclib\bfbcallo.mat<enter> (path and name of standard spectra)
<enter> (unless you want to ignore part of the spectral range because offscale or something)
<enter> to use the default name and location for lsinv matrix (temp.lsi in current dir)
beef2,11,38 (same param as for script but now must be separated by commas)
<enter> for default- obsolete option
T.LF' (or any valid filename, for an unimportant temporary file)

Now it takes off and does pass 1, plotting the experimental spectra and calculating coefficients. At the end it asks if you want to calculate residuals. Always answer "Y".
Now it wants name for .lft file of results. say beef2.lft
(you might be fitting with the experimental spectra in this directory with several different choices for fitting spectra, so name result for fitting spectra)

Now it takes off on pass 2, plotting spectrum, best fit, and residual. At the end of each it waits for input before going on, to give you time to examine the fit. If you enter "X" (which is what the script does), it waits 2 seconds between plots. If you don't enter X you can enter F on any one to make the "decomposition figure" showing the required amount of each standard spectrum and their sum compared to the experimental spectrum.

1. continued (more stuff in scanedit)-
Make a new spectrum which is the sum of the oxidized bc1 and the three reduced-minus-oxidized difference spectra, which should be equal to the spectrum of the fully reduced complex. Simple arithmetic operations are under menu item 6 (manipulate spectra).

When you hit 6 it will tell you the number of the next empty trace. Remember this so you can put the new spectrum in it. You will also see a list of options. Select 1 (add or subtract two spectra). Then you get a syntax hint: "n1=n2+n3, n1=n2-n3" n1 means the number of the trace to put it in (which can be one of the original traces if you want), n2 and n3 are the traces being added or subtracted, and +/- tells which. Type "8=1+2" and hit enter. Before plotting the result, it asks for a comment for the new spectrum. Say "bc1 with c1 reduced" if you want, or just hit enter to leave the comment blank. So the whole process was:
61<enter>8=1+2<enter>bc1 with c1 reduced<enter>.
now add the other two difference spectra one at a time:
61<enter>9=8+3<enter>bc1 with c1 and bH reduced<enter>.
61<enter>10=9+4<enter>bc1 fully reduced<enter>.

Lets save the last one for future use:
hit 8 (save spectrum), it asks you which trace, 10<enter>
it shows you the comment for 10 and asks for a filename,
Filename can be any 11 alphanumeric characters; if longer than 8 then the others will go in the extension. Don't put a dot; dash is OK. Say bovbc1red.

Now lets make a postscript figure from the standard spectra. This is 92 (plot on paper). First question, which traces? You can enter a range (separated by dash) or single trace. Say 1-4 to get the oxidized and three difference spectra from the original file, and <enter>. That's "1-4<enter>"
now add in trace 10, the fully reduced: "10<enter>"
hit <enter> one more time to indicate you're through.
On the next question enter 1 to cycle through the colors starting with color 1 (blue). Then hit enter 4 or 5 times until you see a lot of activity as it writes the traces to the file (another version of this routine lets you preview the figure onscreen, but that's not in here yet). Hit <enter> one more time at the question about the arrow, and it closes the file and tries to copy the file to LPT1:. This will probably crash if you don't have printing set up ("net use lpt1: \\printhost\printer"), but by now it has already created the plot in "temp.ps" which you can send to a color printer or open in Illustrator or ghostscript.

Contents:

BIN directory:

`scanedit.exe` - spectrum viewer, editor, simple arithmetic operations on one or two spectra. (Not used in the demo, but for viewing the sample and standard spectra)

`scanlsq.exe` - many functions, including the Sternberg-et-al. least squares fitting algorithm.

`fitbbc.bat` - script for fitting spectra, now set up to use the standard spectra of the bovine cytochrome bc1 complex. Edit for other standards as described above.

"DOS SHELL" - shortcut to start a dos shell with the current (bin) directory in your path, from running the programs from command line.

`autoexec.bat` - startup file invoked by DOS shortcut, edit to tailor your shell

SPECLIB directory:

`bfbcallo.mat` - standard basis spectra for fitting bc1 complex in different redox states

DEMO directory:

`ademofit.bat` - script to fit experimental spectra using bfbcallo.mat

`beef2-11` etc. experimental spectra to be fit

Reference:

Sternberg, J., Stillo, H. & Schwendeman, R. (1960). Spectrophotometric Analysis of Multicomponent Systems Using the Least Squares Method in Matrix Form. Analytical Chemistry 32, 84-90.

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