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

Windows VB Version
Ed Berry 2007-9

Installing the program

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). (Linux: tar –tzvf scanlsf.tgz) 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). For linux, cd to the scanlsf/demo directory and "./ademofb.csh". This runs the demonstration. To understand what is going on, read the description below.

There are several versions of the suite depending on your platform and tastes. The most completely functional and highly tested is the original set compiled with MS QuickBasic. These are MS-DOS programs, but they work quite well in Windows (at least up to XP), and some drag-and-drop functionality has been added. However they must run in full-screen mode, and the mouse is not functional. Furthermore since they use the Microsoft proprietary runtime libraries, they are not open-source and cannot be licensed under the Gnu GPL license.
Ports are in progress to FreeBasic (www.freebasic.net), allowing to run in a normal window under Windows or Linux and be licensed entirely under the Gnu GPL license (www.opensource.org/licenses/gpl-2.0.php). Although not extensively tested, the spectral fitting routine is now completely functional. It is also being implemented in MS Visual Basic, allowing it to run in Windows and utilize GUIs and dropdown menus in the user interface. Thus this tutorial is provided in four versions to accommodate the slightly different user interfaces:

Windows:
- DemoQB.pdf – Quick-Basic compiled programs
- DemoWFBC.pdf – free-basic compiled programs
- **DemoVB.pdf – Visual Basic program (all in one)**

Linux:
- DemoLFBC.pdf – free-basic compiled for Linux

VisualBasic program

Note- The functionality of the several programs of the original DOS suite are being combined in one single executable under VB. Not all the functions have been added at this time, but most of the basic functions as well as spectrum deconvolution have been, and most of these are accessible through Windows-style GUIs as well as DOS-style menus (which are being kept for now to ease the transition- an option will be available to make these disappear)

Getting familiar with the program and running the demo

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 may be invisible in windows . . . (See the FAQ)

Open the `scanlsf\speclib` folder in a window and make it small enough to open the `scanlsf\bin` folder in another window beside it. This window contains the programs used. Drag the bfbcallo.mat file from the speclib folder onto the `scaneditvb` 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, to view them in scanedit).

When you drag `bfbcallo.mat` onto scaneditvb.exe a window opens up 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 except as a contaminant). The assumption is that any spectrum of the bc1 complex in any redox state can be fit by a linear combination of these spectra. If the complex is fully reduced, only the first spectrum will be required. If any cytochromes are partially oxidized, an appropriate amount of each difference spectrum will be subtracted from the reduced spectrum to fit the experimental one.

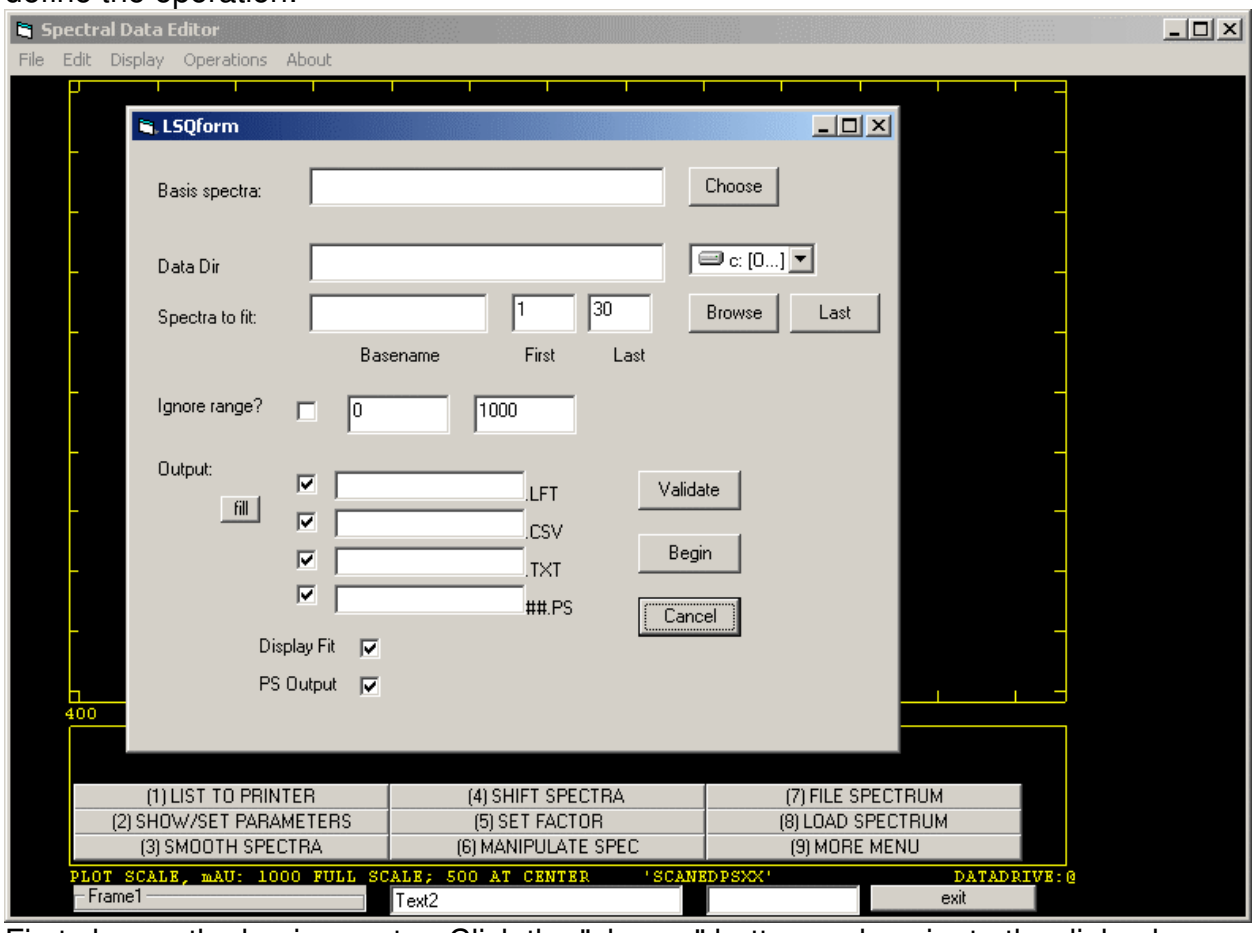
To get a better view, expand the scale and allow negative values. Changing scale will be in the "display" drop-down menu, but for now is only available through the old numeric menus.

Below the display is a 3x3 block of buttons labeled with the digits 1-9 and their function. 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. Clicking a button has the same function as typing its number. 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; or select "plot all" from the display drop-down menu. (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) (or ctrl-Q or exit button or file:exit) 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 `scaneditVB` 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.
As far as I can tell the VB program is not scriptable- but gui's are being made to perform the more complex operations that would benefit from scripting. Double click scaneditVB.exe in the bin directory to start the program. Under the "operations" dropdown menu select LS Fit. A form appears with places to enter the parameters to define the operation:

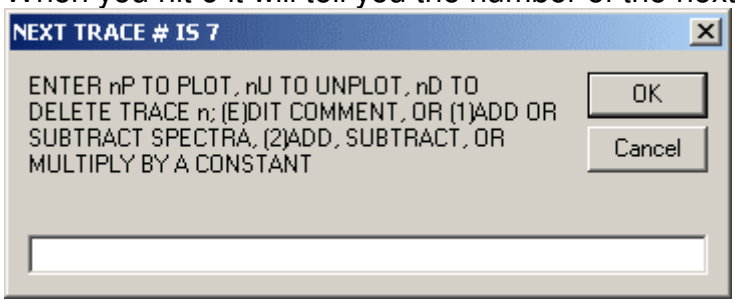


First choose the basis spectra. Click the "choose" button and navigate the dialog box that appears to the scanlsf\speclib directory (later you can set an env variable to make this dialog always start in your speclib directory). Select BFBCALLO.MAT. Next define the data to be analyzed. click the "Browse" button and browse to scanlsf\demo. Select the file beef2-11. The dialog disappears and the next three boxes of the dialog are filled

in with that file's location. You still need to fill "last". Either type "38" or click the "last" button and select beef2-38. Skip the part about "ignore range" in this case (leave it unchecked). Under output, click the "fill" button to generate a name for the output files based on the filename of the standard basis spectra. Uncheck the box by .lft and .txt if you don't want to generate those old reports. Leave "Display fit" and "PS Output" checked (this last seems redundant). Now click "begin" (The "validate" button doesn't do anything, but if the procedure fails it returns you to this form, ideally after displaying an error message telling you what went wrong). Fitting proceeds as in the DOS version, first displaying the experimental spectra while calculating the coefficients; then displaying the fit of each (blue experimental spectrum, green best fit, red residuals). If all completes successfully, you are returned to scanedit form with the screen cleared ready for further operations. Minimize it or click the exit button. Find the .csv file, double click it to load it in Excel. There will be one .ps file for each sp[ectrum fit, showing the same view seen during fitting but in vector graphics. Use acrobat, ghostscript, or ps2pdfmulti to convert into single pdf document.

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>.
("Lin.Comb." under the "operations" menu has a more convenient way of doing this)

Lets save the last one for future use:
hit 7 (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:

`scaneditVB.exe` - spectrum viewer, editor, simple arithmetic operations on one or two spectra, linear combinations of multiple spectra, least-squares fitting as in scanls, fourier transform and inverse.

SPECLIB directory:

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

DEMO directory:

`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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