

MacSpec II

A Macintosh Application for Spectrophotometer Control

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1 Introduction

This program allows a Macintosh computer to control a simple single-beam spectrophotometer via a serial port connection. The original version was written more than ten years ago by Jing Dong Liu, then a graduate student in the Biology Department at the University of Utah, for a biochemistry lab course. More recently, we have written an updated version, MacSpec II, using REALbasic, an outstanding software-development tool described below. In addition to its use in the teaching lab, we use the program extensively in our research.

1.1 System Requirements

MacSpec II will run on any Macintosh computer with a PowerPC processor. The computer must either have a serial port or a serial port adaptor. Both USB and PCI card adaptors are available.

Two separate versions of MacSpec are provided: one for the classic Mac OS and one for OS X (named MacSpec II Carbon). The two versions were built from the same source code, but, because the operating systems handle the serial port differently, they had to be compiled individually. We have only carried out minimal tests with the OS X version, but it seems to work well. Unlike the classic Mac version, it does not lock out other processes during a kinetics run.

1.2 Features

The major use of MacSpec is for kinetic experiments in which absorbance measurements are made at timed intervals from one or more cuvettes. The computer sends commands to control the spectrophotometer and records the data it receives back. If the spectrophotometer has a multi-cuvette holder that can be controlled via the serial port, the program can collect data from several cuvettes simultaneously. This is particularly useful for enzyme kinetics experiments.

Features of the latest version, MacSpec II 1.1, include:

1. Timed data collection
2. User specified total run time and time intervals (subject to the limitations of the spectrophotometer)
3. Automatic plotting of kinetic data.
4. Linear least squares fitting of data.
5. Printing data tables and graphs.
6. Export of data in tab-delimited text files.
7. Computer-control of basic spectrophotometer functions, including setting the wavelength, turning lamps on and off and moving the cell positioner.
8. Versions for the classic Mac OS and for OS X.

9. A demo mode so that you can play with the program and see how cool it is even if you don't have a spectrophotometer!

Eventually, we would like to add the capability for wavelength scanning.

1.3 Spectrophotometers

MacSpec is intended to be used with relatively inexpensive spectrophotometers that can be controlled via a serial port. There are several such instruments on the market, typically with prices in the range of \$5,000 - \$10,000. Using a computer with a simple spectrophotometer can be a cost-effective way of adding a great deal of functionality, provided that suitable software is available. Unfortunately, while many instruments can be controlled via a serial connection, each has its own command set which must be incorporated in the software. The current version of MacSpec can control four different spectrophotometer models. Sadly, three of them, the Spectronic 2001, Pharmacia Ultrospec III and Spectronic Genesys 8, have been discontinued by their manufacturers. The fourth is the Beckman DU 600 series and is currently in production. The program is written in a modular way so that it should be relatively easy to modify it for use with other instruments, so long as the instrument has a serial port and the command set is available. Usually, this information is provided in the manual for the spectrophotometer or can be obtained from the manufacturer. We would be happy to provide the source code for MacSpec and might even be able to help a little with the modifications.

1.4 About serial connections

The serial connection is one of the oldest bits of computer technology that is still with us, if for only a while longer. It was originally designed to connect computers or terminals to modems, but it has also been used to connect computers to each other, to printers and to a variety of peripheral devices including scientific instruments. The good thing about a serial connection is that it is relatively easy to write software to transmit data over it. The worst, probably, is the fact that it is one of the least standardized standards in the computer industry. Different connectors are used, and, even worse, different equipment manufacturers have different ideas about how the connector pins should be wired! Finding a cable with the right kind of connector at each end is no guarantee that it will work. The serial connection is also relatively slow by modern standards, but that is not at all a problem for this application.

To the extent that they follow any standard, most instrument manufacturers will specify that the serial port is an "RS-232" connection. None of the current Macintoshes have serial ports, but there are adaptors that allow serial devices to be connected via the USB port found on the newer machines. These, and the serial ports found on older Macs, are not actually RS-232 connections, but are RS-422 ports, and use the small circular DIN-8 plug. Fortunately, the two standards are similar enough that it is usually possible to get the Mac to talk to a serial device, though it may take some trial and error and some soldering.

More information about serial port connections is available at the Goldenberg lab web page: <http://www.biology.utah.edu/goldenberg/>.

1.5 USB Connections

As noted above, none of the current Macs have serial ports, so you will have to use a USB-serial adaptor to use MacSpec with one of these machines. (On the other hand, controlling a spectrophotometer is an ideal application for an older Mac that is being pushed aside for one of the new shiny machines.) There are several adaptors on the market, each with its own driver software. Our limited experimentation indicates that some work better than others, but we have had pretty good luck with the Keyspan Twin-Serial Adaptor.

At some point, instrument manufacturers may stop incorporating serial ports and start using USB for connecting to computers. While this will certainly represent technological progress, it's not clear whether or not it will actually make things better for Macintosh users. Each USB device requires its own driver, which is a relatively deep part of the operating system, so it may be much more difficult for users to write their own software to control these devices. Support from the manufacturers may be limited to PCs.

1.6 About REALbasic and PlotCanvas

The current version of MacSpec was written using REALbasic, a computer language and development environment that is quite similar to, and somewhat compatible with, VisualBasic, a widely used Microsoft product. Like VisualBasic, REALbasic uses a graphical interface that makes it relatively easy to build an application with its own sophisticated interface. The language is object-oriented and bears almost no resemblance to the original Basic language. Programs written in REALbasic are compiled to create stand-alone applications and can be compiled for either the Classic Mac operating system or for Mac OS X, making REALbasic one of the easiest ways to create native OS X applications. The “professional” version of REALbasic can even compile applications for Windows. So, it should be relatively easy to port MacSpec to Windows, though we haven't tried it.

To add the ability to plot kinetic data, we used and adapted a REALbasic “class” for graphing written by Rob Frohne of Walla Walla College. With Rob's permission, we are making our version of PlotCanvas available for other REALbasic programmers. Our version can be found on the Goldenberg lab web page, <http://www.biology.utah.edu/goldenberg/>, and Rob's original version is available at http://www.wwc.edu/~frohro/REALbasic/rEALbasic_cartesian_plot_c.htm.

2 Connecting the Hardware and Setting up the Software

2.1 Hardware Connection

To use MacSpec, the Macintosh and spectrophotometer must be connected via an appropriate serial cable. The three spectrophotometers currently supported each require a different connection. Although special cables could be wired for each, it is probably easiest to use the standard “hardware handshaking” cable intended for a modem and adaptors as described below. Those who want the gory details of how these connections work are referred to the separate documentation on serial port connections.

Most Macintoshes with serial ports have two ports: one labeled “modem” and the other “printer”. If possible, use the modem port, since the printer port is not intended for two-way communication at higher rates.

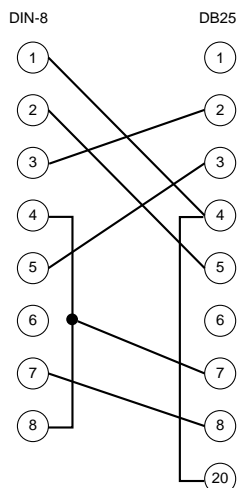
If you are using a newer Macintosh without serial ports, you will need to use either a USB-Serial adaptor or an accessory board with a serial port. We have only tried a couple of USB adaptors and, of these, the Keyspan Twin-Serial adaptor worked best. You will also need to install the appropriate driver software. This software should allow you to identify one of the serial ports on the adaptor as the “modem” port. It is probably best to use this port for the connection to the spectrophotometer.

2.1.1 Disclaimer

We do not have any professional credentials in computer hardware or serial interfacing. We cannot accept any responsibility for damage to your computer, your spectrophotometer or your sanity.

2.1.2 Pharmacia-LKB Ultrospec III

This spectrophotometer is relatively easy to connect; all that is necessary is the hardware handshaking cable. This cable has a male DIN-8 connector at one end and a male DB25 connector at the other, with the pins of the connectors wired as shown below:



The DIN-8 connector is a small round plug with 8 pins and connects to the Macintosh (or a USB-Serial adaptor), and the DB25 is a wide flat connector with 25 pins. Only 9 of the pins of the DB25 connector are actually used, and some cables include only these pins.

Be aware that not all cables with a DIN-8 connector at one end and a DB25 connector at the other are actually wired this way! Some cables, in particular, are intended to connect a Macintosh to a printer, rather than a modem, and are wired differently. These cables will not work for this application.

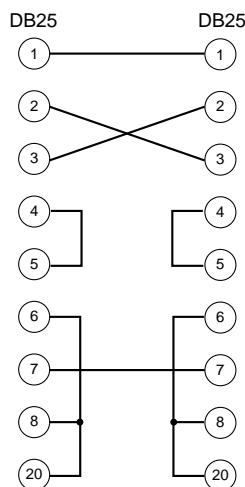
2.1.3 Beckman DU 600 series

These spectrophotometers should be connected using the same hardware handshaking cable as described above for the Ultrospec III.

2.1.4 Spectronic 2001

To connect this spectrophotometer, you will need the same cable as described above and two additional components:

1. A “null modem” adaptor. This is a small box with a male DB25 connector on one side and a female DB25 connector on the other. The pins of the two connectors are wired together as follows:



2. A male-to-female DB25 “gender changer”.

Connect the DB25 end of the cable to the female end of the adaptor, attach the gender changer to the male end of the adaptor and then attach the assembly to the male DB25 connector of the spectrophotometer.

2.1.5 Spectronic Genesys 8

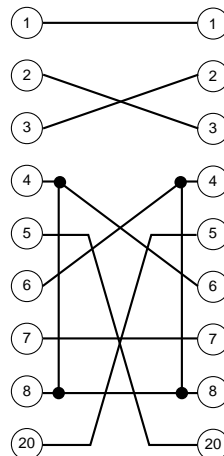
Connecting this spectrophotometer is somewhat more complicated and involves a bit of soldering. This instrument uses the smaller DB9 connector rather than the DB25 for its serial port. A relatively simple method for making the connections uses the following components:

1. Standard Macintosh hardware handshaking cable as described above.
2. A Serial Jumper Box with DB25 connectors (Part no. 276-1403 from RadioShack). This is simply a small box with two DB25 connectors (one male and one female) and provisions to wire the pins together in any combination. The connections are made by soldering jumper wires to a printed circuit board.
3. A cable with a male DB25 connector on one end and a female DB9 on the other (to fit the spectrophotometer).
4. A male-to-female “gender changer” that allows the jumper box to be connected to both cables.

The DB9-to-DB25 cable is relatively well standardized and readily available. But, make sure that it is wired as follows:

DB9 Pin	DB25 Pin
1	8
2	3
3	2
4	20
5	7
6	6
7	4
8	5
9	22

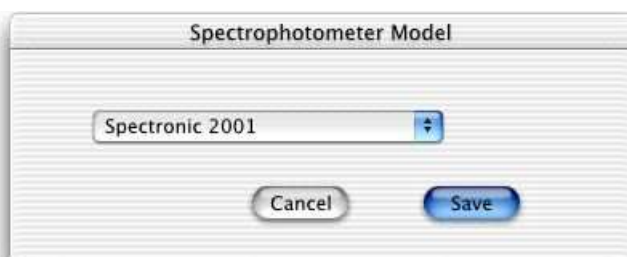
The connectors of the jumper box should be wired as shown below:



After wiring the box, attach its female connector to the DB25 end of either of the cables and then use the gender changer to connect it to the other cable. Connect the DB9 connector to the spectrophotometer and the DIN-8 plug to the Macintosh.

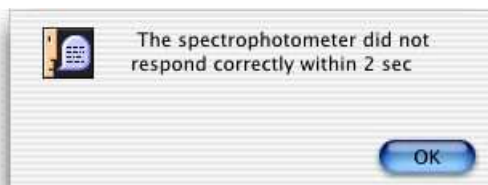
2.2 Software settings

MacSpec stores information about the spectrophotometer model being used and the serial port settings in a preference file (MacSpec prefs), located in either the Preferences Folder in the System Folder (System 9 and older) or the individual user's preferences directory (OS X). If the multiple users feature of OS 9 is turned on, the preference file is stored in the individual users' preferences folders. The first time the program is started, it will ask you to identify the spectrophotometer you are using:

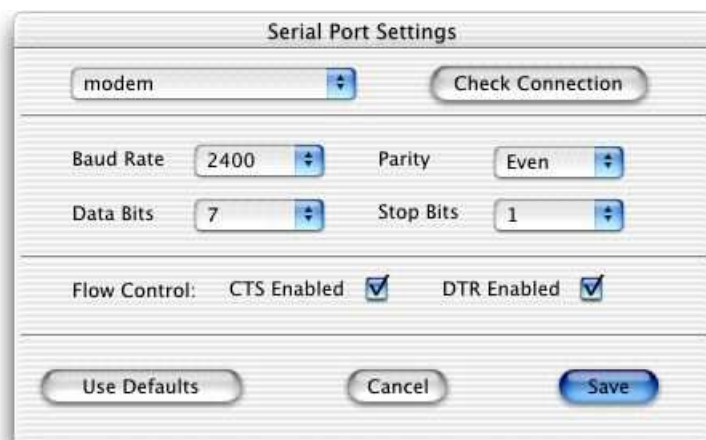


Simply choose the appropriate spectrophotometer name from the drop-down menu and click the **Save** button.

You will likely then see an error message:



Click **OK**. At this point, make sure that the spectrophotometer is on and connected, and then choose the item **Serial Port Settings** from the **Edit** menu. This will bring up a dialog box from which the various parameters for the serial port can be set.



From this dialog, you can select the serial port to use for the connection (preferably the modem port) and set all of the standard serial port parameters. For all of the spectrophotometers currently supported, both of the flow control (hardware handshaking) options should be turned on. For the other settings, each of the three spectrophotometers has its own set of default settings, and clicking the **Use Defaults** button will set the parameters for the currently selected spectrophotometer type. To varying degrees, these parameters can be adjusted on the different spectrophotometers, so you should make sure that the spectrophotometer settings match those set in the dialog box. The settings that can be adjusted by the user are as follows:

- Beckman DU 600 series - The two hardware handshaking options in MacSpec, CTS and RTS, should be turned off, and the XON/XOFF options on the spectrophotometer should be turned off. The other communications settings can be changed using the spectrophotometer's interface. You can either set these to match the defaults from MacSpec or change the settings in the program to match the spectrophotometer's settings.
- Spectronic 2001 - Nearly all of the settings can be changed using DIP switches on the serial interface board. You can either set these switches to match the defaults from MacSpec or change the settings in the program to match the hardware settings.
- Spectronic Genesys 8 - There are no adjustable settings. Just use the defaults for this spectrophotometer.
- Pharmacia-LKB Ultrospec III - The baud rate can be set from the keypad on the spectrophotometer. Make sure that this setting matches the one set in the program.

After setting the various parameters, you can test the connection by clicking the **Check Connection** button. You should see a dialog box that reports the time delay between when the test was initiated and when the computer received an appropriate return signal. If the spectrophotometer does not respond correctly, recheck all of the connections and settings.

After the correct settings have been established, click the **Save** button. The settings will now be stored in the preferences file, and you shouldn't need to change them again unless you change something on the spectrophotometer.

3 Controlling the Spectrophotometer from the Computer

The **Spec** menu contains several commands for controlling the spectrophotometer:



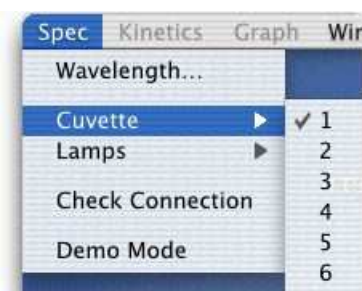
These functions are detailed below.

Wavelength... This command brings up the following dialog box:

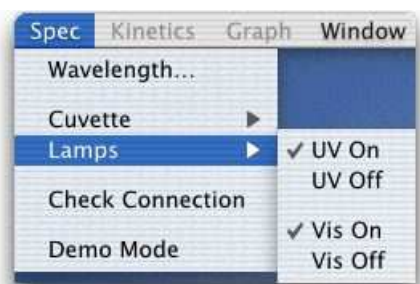


When the dialog box first appears, it should contain the wavelength that is currently set on the spectrophotometer. To change the wavelength, enter the new value and click **OK**. If the chosen wavelength does not lie within the range of 190 to 950 nm, an error message will appear.

Cuvette This menu item has a submenu that allows the user to set the position of the cuvette holder, if the spectrophotometer has a multi-position holder.

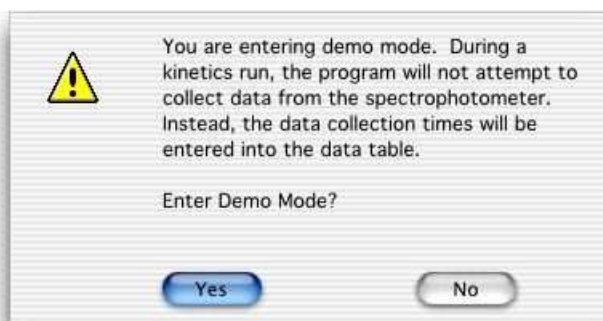


Lamps This menu has a submenu that allows the user to turn on or off the UV or visible lamps. Not all of these functions are implemented on some of the spectrophotometers.



Check Connection This command sends a signal to the spectrophotometer and waits for a return message to confirm that the connection is working. A dialog should appear reporting the time required for a response or an error message indicating that no response was obtained after 2 sec. If the test fails, check to be sure that the spectrophotometer is on, the connection to the computer is correct and the serial port settings are correct.

Demo Mode This command allows the program to be run in a demonstration mode, in which a kinetics run can be simulated without a connection to a spectrophotometer. In this mode, the data collection times are entered in the data table, rather than absorbance values. The following dialog box appears to confirm the command:



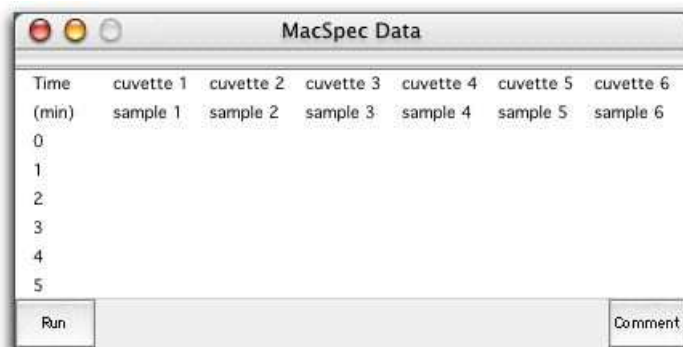
When a kinetics run is started in demo mode, another dialog box will appear to ask the user whether or not to proceed with the run in demo mode.

4 Setting up and Running a Kinetics Experiment

The major function of MacSpec is to control kinetic experiments in which absorbance measurements are made at regular time intervals from one or more cuvettes. As the run progresses, the data are entered in a table, and after the run is completed the absorbance values are automatically plotted as a function of time.

4.1 The Data Window

When the program is first started, a kinetics data window will open automatically:



This first window will be setup for a kinetic run that uses the maximum number of cuvettes available with the selected spectrophotometer and to collect a total of 6 six data points. These parameters can be changed as described below.

In the data window, each column has two headings. The top heading indicates the cuvette number and is fixed. The second heading has default entries "sample 1", sample 2", etc. These entries can be changed to more meaningful labels. Simply click on the current entry to make it active for editing.

Clicking on the "Comment" button will open a dialog box in which information about the experiment can be entered:



The comment field will automatically contain information about the program and the date. The contents of this field will be saved along with the absorbance data when a file is generated with the **Save** or **Save As** command.

To start the run, either click the “Run” button in the data window or select the **Run** command in the **Kinetics** menu. As the run progresses, the data table will be automatically filled with the absorbance values from the spectrophotometer. Under OS 9 or earlier, the computer will be locked during the run, but under OS X, the program will continue to execute the run even if the user leaves the application to do other work on the computer. The run can be aborted by pressing the command and period keys.

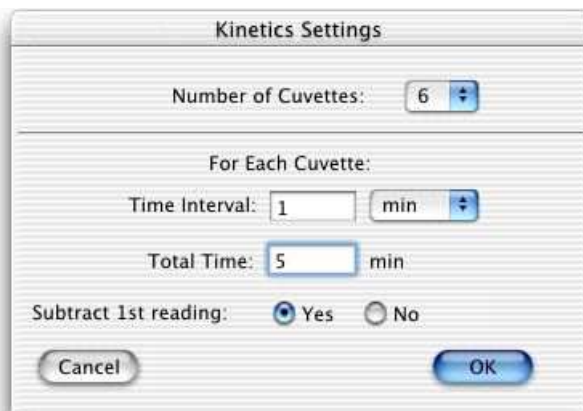
When a Beckman DU 600 series spectrophotometer is used, the wavelength dialog box will appear before the kinetics run begins. This is because of a quirk in the command set for these spectrophotometers that requires that the wavelength be specified for each absorbance reading. Enter the correct wavelength and click the “Set” button.

After the run, the “Run” button will be replaced with the “Graph” button. Clicking this button will redraw the graph for the data window and bring the graph to the front of the screen.

To set up another kinetics run, select the **New** command in the **File** menu. The parameters from the last executed run will be used as the defaults for the new data window.

4.2 The Kinetics Settings Dialog Window

The parameters for a kinetics run can be changed by selecting the **Settings...** item in the **Kinetics** menu, which opens a dialog box:



The following settings can be changed:

- The number of cuvettes from which absorbance measurements should be recorded, ranging from 1 to the maximum number for the spectrophotometer being used.
- The time interval between readings for each cuvette. This can be specified in either minutes or seconds. There is a minimum interval that depends on the spectrophotometer model and the number of cuvettes being used. If your choice is less than this minimum, a dialog box will appear when you click **OK**. The dialog will indicate the minimum interval for the currently set spectrophotometer and number of cuvettes.

If only one cuvette is being used, intervals as short as 5 sec can be used. When more than one cuvette is used, extra time has to be allowed to move the cuvette holder and take measurements for the other cuvettes.

When the program is in demo mode, the interval can be as short as 1 sec.

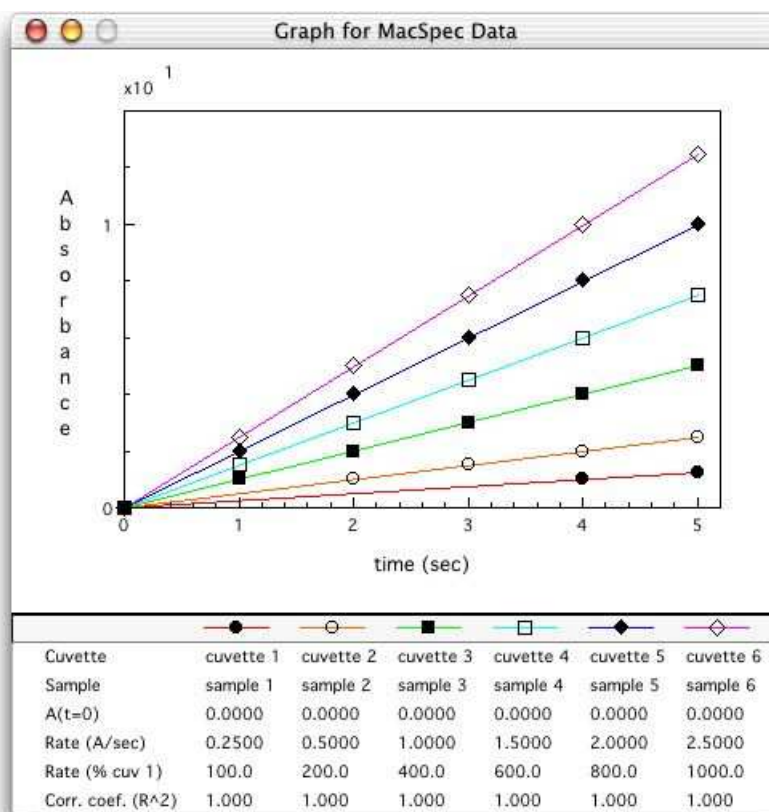
- The total time over which data are recorded for each cuvette, in the same units as specified for the interval between measurements.
- Subtract 1st reading. For many kinetics experiments, only the rate of change in absorbance is really important, and it may be convenient to express all of the measurements as a difference from the absorbance at time zero. When the **Yes** button is selected, the first data point for each cuvette will be set to 0, and all subsequent points will be recorded as a difference from the initial value. If the **No** button is selected, the absorbance values will be recorded directly.

After making any changes, click on the **OK** button to save the changes or the **Cancel** button to close the dialog box without saving the changes. The frontmost data window will be updated to reflect any changes.

5 Graphs

5.1 The Graph Window

At the end of a kinetics run, the absorbance data will be automatically plotted as a function of time:



The axes are automatically scaled, with the labels in scientific notation. The power of ten (if it is other than 0) is indicated at the top of the absorbance axis or the far right of the time axis.

The data for each cuvette are also automatically analyzed by least-squares linear regression, and the table at the bottom of the graph includes the following information:

- The intercept of the least-squares line on the absorbance axis, representing the estimated absorbance at time zero.
- The slope of the least-squares line, representing the estimated rate of absorbance per time unit (min or sec).
- The rate expressed as a percentage of that for cuvette 1.
- The correlation coefficient, R^2 . This is a statistical parameter that reflects how well the experimental data are fit by the line. A value of 1 indicates a perfect fit, and a value of 0 indicates that there is no correlation between the absorbance values and time. More precisely, R^2 indicates the fraction of the variation in the independent variable that is accounted for by the fit linear function.

5.2 The Graph Menu

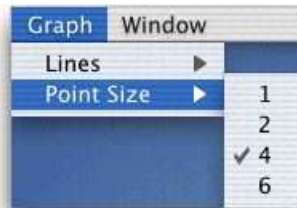
The appearance of the graph can be customized a bit using the **Graph** menu items:

The **Lines** submenu:



offers three three choices: The least-squares line, line segments drawn from one time point to the next or no lines.

The **Point Size** submenu menu



offers four choices for the size of the symbols used to plot the data.

For more elaborate graphs or data analysis, the data can be saved in a text file and transferred to a graphing or spreadsheet program, as described in the following section.

The graph window cannot be saved, but it can always be regenerated by clicking on the “Graph” button in the data window.

6 The File Menu

The **File** menu contains the basic commands to open, save or print files:



Only a few comments are necessary about these commands:

- The **New** command creates a new data window for a kinetics run.
- The **Open** command opens a previously saved data file. Graphs cannot be saved or opened, but they can be regenerated by clicking on the “Graph” button of the data window.
- The **Close** command closes the frontmost data or graph window.
- The **Save** and **Save As...** command is only active when the frontmost window is a data window. The data in the window are saved as a simple tab delimited text file. This file can either be reopened in MacSpec or opened in a graphing or spreadsheet program, or a word processor. The text in the comment box is saved at the top of the file, with each line prefixed with the “#” character.
- The **Print** command is used to print the contents of either data or graph windows. When the data window is printed, the output includes the data table, the contents of the comment box and the linear regression results from the graph window. When a graph window is printed, the output includes the graph, linear regression statistics and the comments.
- The **Page Setup** command is not currently active.

7 Editing Data

After a kinetics run is completed the **Lock Data** item in the **Edit** menu will become activated:



Selecting this item will remove the checkmark and allow the user to edit the contents of the data window. Only the absorbance values can be changed. The major use of this feature is to delete an aberrant data point, such as might arise from a failure of the cuvette positioner. The inclusion of this feature is not meant as an endorsement of data manipulation!

8 Contact Information

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