

Olis-computerized HP 8452 Quick Reference Guide

1. Initializing the instrument

- i. Be sure that the instrument has been turned on before proceeding to initialize the instrument. To begin using the instrument use the Startup menu, which opens automatically upon starting the software, and move to the 'Data Collection' tab. Click on the instrument name and click on 'Open'. Note that occasionally the software is used to control two instruments or two hardware settings which are different enough to be considered as two instruments by the software.
- ii. When the instrument has finished calibrating, it will return to the last used settings. Once this has finished the instrument will enter 'Idle Mode' meaning that it is waiting for a command.

2. Entering and exiting Debug Mode

- i. The software is equipped with a Debug Mode in which the software can be run with no instrument present. In this mode, a green button labeled Debug Mode will appear in the upper right corner of the Live Display screen.
- ii. To exit debug mode, ensure that the instrument is powered on and click on the green debug mode button. The instrument will calibrate as it does during startup.
- iii. To enter debug mode from the normal operating mode, press the ctrl+alt+shift+d keys simultaneously. A confirmation message will appear. Answering 'OK' will place the instrument in Debug mode.

3. Collecting a wavelength scan

- i. Ensure that the Data Collection Mode in the Operational Modes page is set to 'Scan.'
- ii. Collecting a reference scan
 - a. Insert an appropriate reference sample into the cuvette holder. This is typically a cuvette containing solvent or buffer, but no sample.
 - b. Click on the Collect Ref button on the Live Display
 - c. Click OK when asked to insert the reference. The reference will be collected and applied to subsequent data until the integration time is changed or the Apply Reference checkbox is unchecked.
- iii. Collecting an absorbance scan
 - a. On the Live Display page, enter the wavelength range, Number of Increments, and Integration time (expressed as Reads per Datum in some instruments). Note that the software may automatically adjust the ending wavelength value if the entered Number of Increments is too large for given wavelength range.
 - b. Collect a reference by clicking on the 'Collect Ref' button.
 - c. Place the sample in the cuvette holder.
 - d. Click Collect Data to obtain the scan.

4. Collecting multiple scans at less than one scan per second

- i. Choose an appropriate integration time. This is the collection time for the entire spectrum.
- ii. Collect a reference by clicking on the 'Collect Ref' button.
- iii. Place the sample in the cuvette holder.

- iv. On the Repeated Scans page, choose the desired number of scans to collect. Choose Collect Scans as a function of 'Time.'
- v. Choose the total time for data collection. The number of scans will be spaced evenly over that time. Pressing the spacebar during the incubation period between scans will automatically start the next scan and recalculate the times for the remaining scans. The shutter will close between each scan.
- vi. Click Collect Data to begin obtaining scans.

5. Collecting multiple scans at faster than one scan per second

- i. Choose an appropriate integration time. This is the collection time for the entire spectrum.
- ii. Collect a reference by clicking on the 'Collect Ref' button.
- iii. Place the sample in the cuvette holder.
- iv. On the Repeated Scans page, choose the desired number of scans to collect. Choose Collect Scans as a function of 'Time.'
- v. Click on the Rapid Scanning checkbox.
- vi. Choose the total time for data collection. The data will not be displayed until data collection has finished.
- vii. Click Collect Data to begin obtaining scans.

6. Posting Data to GlobalWorks

- i. At the end of data collection a message will appear asking if you would like to Post the Data to GlobalWorks.. Choosing 'Yes' will result in the current data being transferred to the Experiment Window. Data processing procedures are described in the GlobalWorks manual. Choosing 'No' will post nothing to GlobalWorks. The current data will be overwritten the next time data are collected (including Live Mode).
- ii. At any time, except during data collection, the current data may be transferred to GlobalWorks, by clicking on the 'Post these Data' button located in the upper right corner of any screen.

7. Collecting and averaging multiple scans

- i. On the Live Display page set the instrument parameters as desired for a single scan.
- ii. On the Repeated Scans page ensure that 'Repeated scans as a function of... ' is set to None
- iii. Set the Number of Scans to the desired value on the Repeated Scans page.
- iv. Click on the Average Scans checkbox. This will result in one final two-dimensional scan which is the average of the scans. If this checkbox is not checked, the entire group of scans will be retained. These may be averaged later in GlobalWorks
- v. Choose the Scan Option which determines whether scans will occur automatically or user prompted.
- vi. On the Live Display page, click on 'Collect Data' to begin data collection.

8. Collecting Scans as a function of time

- i. On the Live Display page set the instrument parameters as desired for a single scan.
- ii. On the Repeated Scans page ensure that the 'Repeated scans as a function of ... ' is set to Time.
- iii. Set the Number of Scans to the desired value on the Repeated Scans page.
- iv. Set the Total Time for Scans to the desired total time.

- v. Choose the Scan Option to Auto or Manual which determines whether scans will occur automatically or user prompted.
- vi. On the Live Display page, click on 'Collect Data' to begin data collection. If the Scan Option is set to Auto the scans will be automatically spaced out over the given time. At any time the incubation time between scans can be circumvented by pressing the spacebar.

9. Changing the axis scaling

- i. On the X and Y Axes page enter the desired minimum and maximum values for the Y-Axis. Uncheck the Autoscale checkbox to use the entered values. If autoscale is desired, check the autoscale checkbox.
- ii. The X-axis value can be changed by entering new values in the X-Axis entry box.
- iii. The scaling changes in SpectralWorks do not affect the dataset once it has been posted to GlobalWorks. Scaling in GlobalWorks is achieved by right-clicking on the data and choosing Scaling from the pop-up menu.

10. Changing the background color

- i. On the X and Y Axes page, click on the 'Change Background Color' button.
- ii. Use the color palette to choose the desired background color. Click 'OK.'

11. Changing the line colors

- i. Choose the line number of the line that you wish to change.
- ii. On the X and Y Axes page, click on the 'Change Line Color' button.
- iii. Use the color palette to choose the desired background color. Click 'OK.'

12. Creating, saving, and opening protocols

- i. Protocols allow the saving of instrument settings so that a given procedure can be saved as a file. To save a protocol, set the instrument to the desired settings.
- ii. Exit Live Mode if necessary.
- iii. Choose the Save Protocol from the File menu to save a protocol.
- iv. Enter a file name for the protocol and click OK
- v. To open a protocol, choose 'Open Protocol' from the File menu. Note that all protocols are located in the Program files\OLIS Globalworks\Protocols.
- vi. Answer 'OK' to the protocol confirmation message. This indicates that current parameter settings will be replaced with ones from the protocol.

13. Attaching a protocol to a dataset

- i. Collect a dataset using the instrument settings that are intended to be saved in the protocol.
- ii. Click on 'Save Dataset with Protocol' from the File menu.
- iii. Enter a name for the protocol. Protocols will be stored in the Protocols folder of the OLIS GlobalWorks folder and will have a '.olp' file extension.
- iv. Choose a name for the dataset. The dataset location is not limited as the protocol is.
- v. When the file is opened, a message box will appear indicating a protocol is associated with it. Choose 'Yes' to load the protocol or 'No' just to open the dataset.

14. Using an automation script

- i. Using the procedure described in 'Creating, Saving, and Using Protocols,' create a protocol for each data collection procedure. For example, for a collection of a scan, set up the scan as you were about to collect it. Save this as a protocol. Repeat this for each scan or assay (or set of repeated scans or assays).
- ii. Using the Windows program 'NotePad' or a word processing program which does not automatically format the data, write a protocol script according to the following format: Enter the name of the protocol (in brackets) to be used followed by the name of the data file to be saved. Enter a line in the script, using the same format, for each procedure to be automated. If desired, enter a pause, written in brackets. This will require a user input in order for the instrument to proceed with the measurement. After this script has been written, save it using the word processing software.
- iii. Initialize the Experiment script by double clicking on the experimental script file.
- iv. After initialization has been completed, a message will appear indicating this
- v. The instrument will collect data as directed by the experiment script. If a pause is encountered, a message will appear indicating this:
- vi. Datasets will be saved as described in the Experiment script