INITIALIZING THE INSTRUMENT:

- Turn on the computer and turn on the instrument power switch located on its right front corner. Start the software by double-clicking on the **SCAN** icon on the desktop.
- Once the instrument is initialized, the screen should have the **Start** and **Stop** buttons at the top center. If **Connect** appears in place of **Start**, press **Connect** in order bring the instrument online.

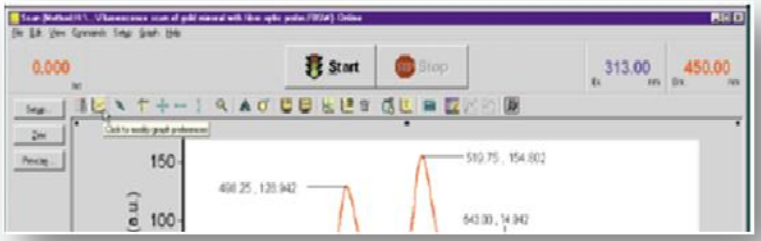

Figure 1. Scan Toolbar.

SETTING UP THE EXPERIMENT:

- Press the **Setup** button, a dialog box with method parameters will appear.
- On the **Cary** tab, Select the **setup** button to displace the setup dialog and specify the method parameters.
- Set **Data Mode** to **Fluorescence**. Set the **scan setup** mode to **Emission** or **Excitation** based on shich spectrum is desired. Set the **X Mode** to **Wavelength (nm)**. Enter the **Excitation (nm)** value, which can be determined by quickly running the sample on the UV-Vis and establishing the wavelength of maximum absorbance.
  Enter an **Excitation slit (nm)** value of **5** and an **Emission slit (nm)** value of **5,** these can be adjusted to maximize the signal.
  Enter a **Start (nm)** value (perhaps the excitation value previously entered added to sum of the slits) and enter a **Stop (nm)** value (about 150-200 nm higher the start value.
  Clear the **3-D mode** check box. Select the **Scan Control** to **Medium**.
- On the **Options** tab, Select the **overlay traces** check box to overlay the results of the traces on one graph. Then clear **CAT** or **S/N Mode, Cyclo Mode** and **Smoothing** check boxes. Set the **Excitation filter** to **Auto**. Set the **Emission filter** to **Open**. Set the **PMT Detector Voltage** to **Medium**.

- On the **Reports** tab, enter **Operator Name**. To automatically label peaks on the spectrum, select **Maximum Peak** or **All Peaks** options. The peak threshold limit and labeling options are altered by pressing the **Peak Information** button.
- On the **Auto Storage** tab, select whether to save the scans before or after a run. Selecting after the run (not the default choice) will avoid saving bad data.

Figure 2. Set up Dialog Box.

DATA COLLECTION:

- In order to zero the instrument, place the blank solution in the sample compartment #4. Press the **zero** button to zero the system. When the result is zeroed, the word '**Zeroed**' will appear in the Y display box in the top left corner of the scan application window. DO NOT discard the blank, you must zero the system before each reading.
- Replace the blank with the sample to be analyzed in compartment #4 and press the **Start** button. You will be asked for a sample name then the spectrum will start running and the trace will appear in the graphics area. **Autoscale** the spectrum if it goes offscale. Once the run is completed, a save file box will be presented to allow naming the file. Any peak information will be printed in the report box (the bottom half underneath the plot).
- Text can be added to the plot by pressing the **A** button. When running multiple scans in one session, pressing the traces button (the farthest left button in the toolbar) to select individual data sets can be selected and made visible in the current plot. Please note that all data plots (including any baselines) are still accessible through the **Graph** menu, and all will be printed out when printing the data. In order to omit any data set, right click on the graph with that data and select **Remove Graph**.

- In order to manually mark a certain point on a spectrum, right click at that point and select **Print x-y Point**. The coordinates will be printed in the report window. Text can be added or changed in the report window by selecting **Edit Report** on the **Edit** menu and manipulating the text as needed. Double clicking on the report window will allow to expand it to full size.
- To save the session as-is, including the report window contents and alterations made to the scans, select **Save Data As**… from the **File** menu and enter the name for the *batch file*. In order to export some of the data for use in other programs, click on the graph of that data, select **Save Data As**…from the **File** menu, and select **Spreadsheet ascii (*.CSV)** from the **Save Files As** box.

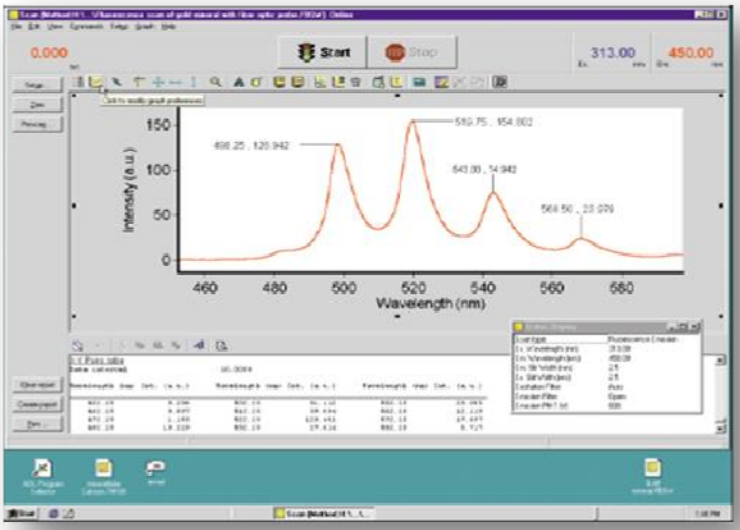

Figure 3. Peak Labels.

- Once the plot has been drawn, you may use the cursor mode to switch between **Free mode** and **Track Mode** in order to monitor the X and Y values by dragging the intersecting lines along the graph to the point of interest.

SIMPLE READS:

- This feature allows for sample analysis at one wavelength. Access the **Simple Reads** icon from the **Cary Eclipse** folder and click on **Setup.**
- Set up the experiment in the same manner as in the **Scan** mode.
- Proceeds to zero the instrument and press on **Read** to begin collecting data.

CONCENTRATION:

- This feature allows for sample analysis at one wavelength. Access the **Simple Reads** icon from the **Cary Eclipse** folder and click on **Setup.**
- Set up the experiment in the same manner as in the **Scan** mode.

- In the **Standards** tab enter the desired **Unit Selection.** Enter the number standard samples to be analyzed. In **Replicates** enter one less than the standard sample number. Clear the **Std. Averaging** option and enter the various concentrations in the appropriate boxes.
- In the **Fit Type** tab highlight the type of fit appropriate for the data and adjust the fit by altering the default of 0.9500 R^2 in **Min R^2**.
- In the Samples tab enter the **Number of Samples** and **Replicate**s and label samples.
- In order to run the standards and samples, zero the instrument and select the samples to be used for calibration from the **Standard/Sample** selection. Available samples can be Selected for Analysis by using the arrows.
- Click and foow the on screen direction in order to **Present Standard** to be analyzed. Once all standards are analyzed a calibration curve with be automatically displayed.

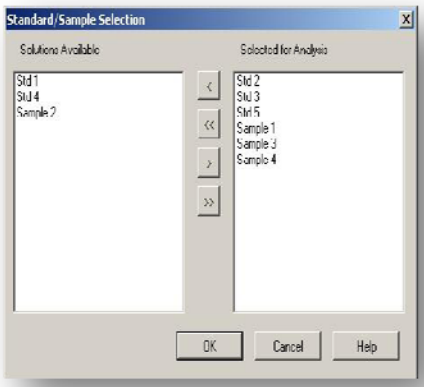

Figure 4. Sample Selection.

- Sample analysis is analogous to standards.

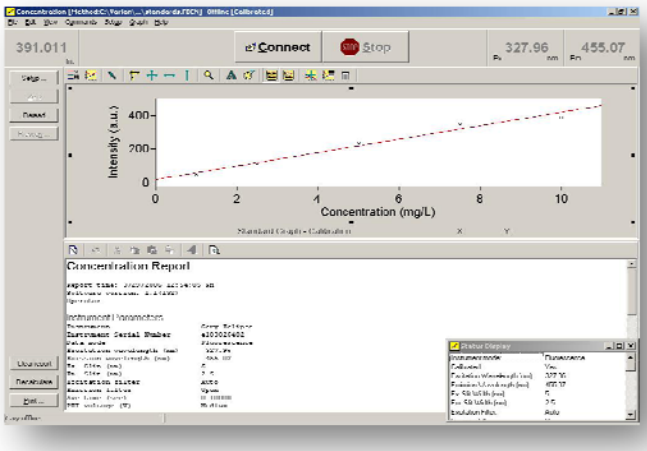

Figure 5. Sample Concentration Results.