SOP for collecting Scanning Kinetics data on Cary UV-Vis
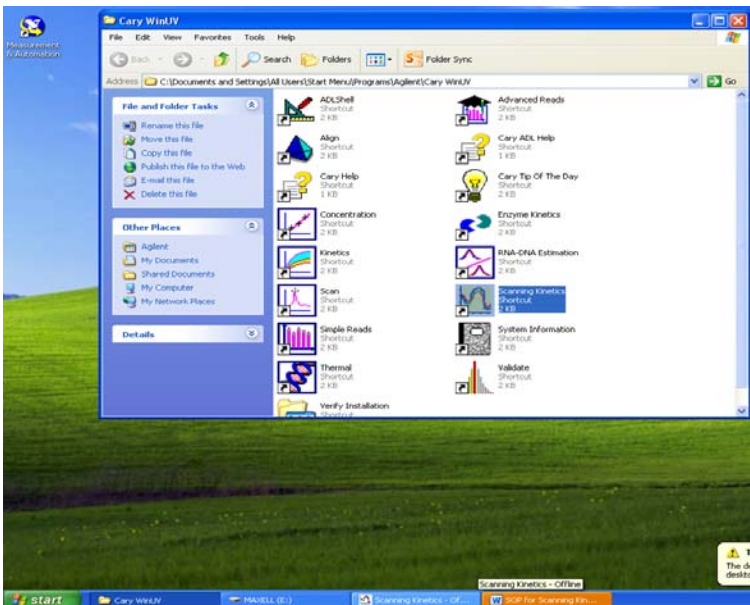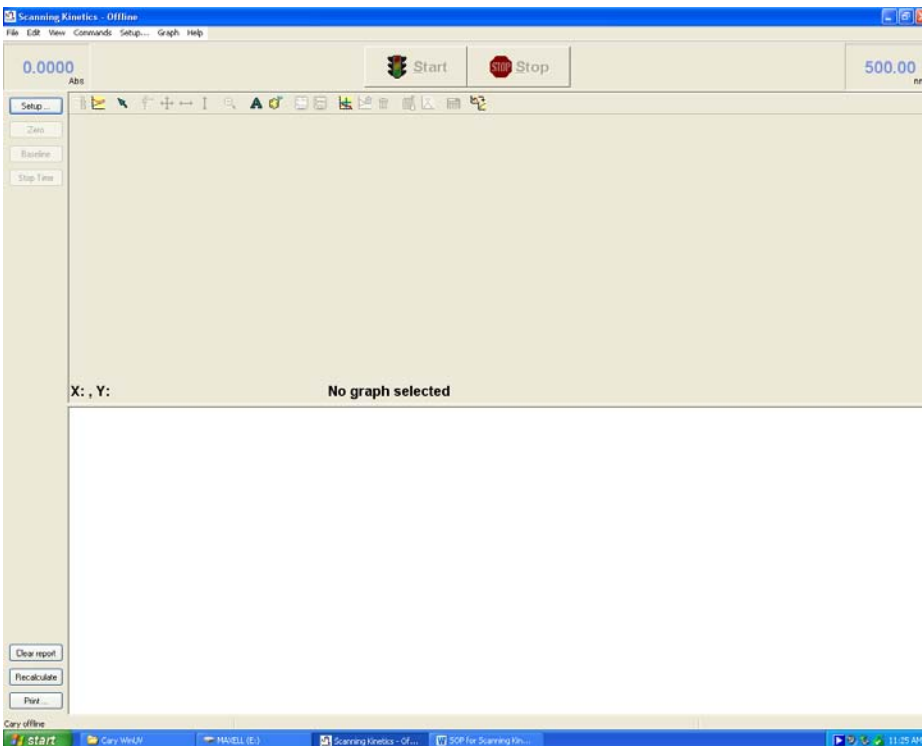
1. Turn on the instrument power switch and let the lamps warm up for about 15 minutes
2. If using temperature control, turn on the Cary temperature controller circulator.
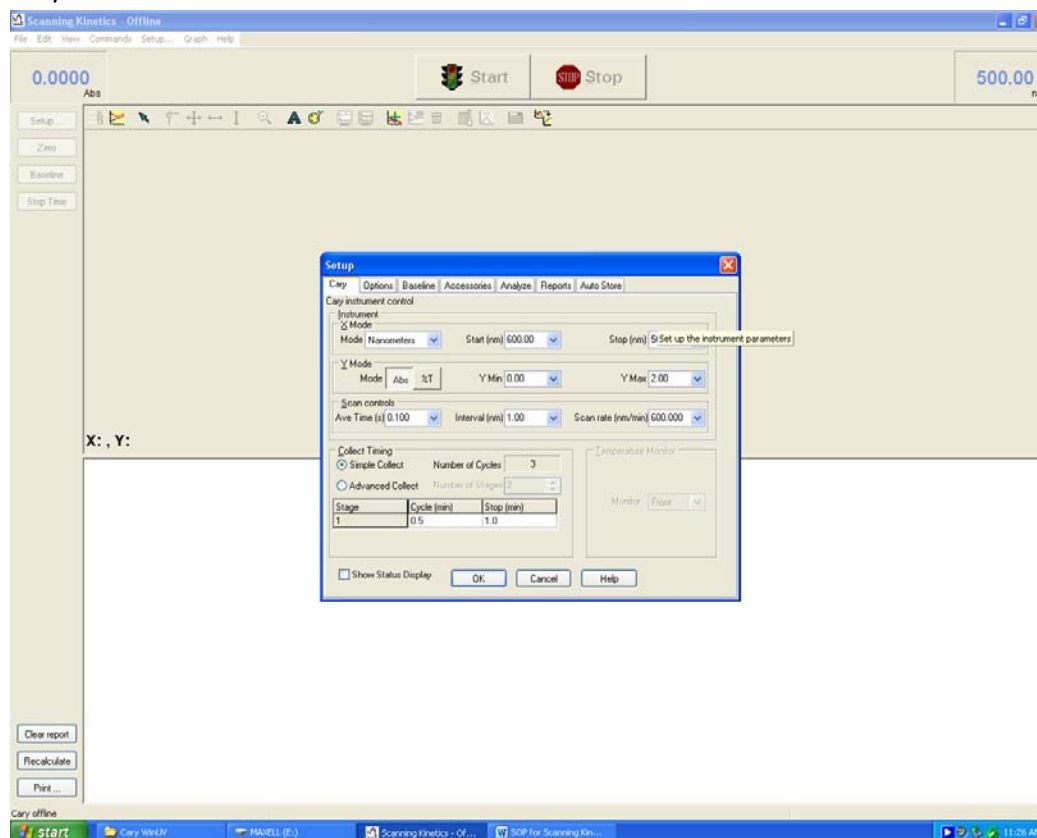3. Open the Cary WinUV program on the desktop and select the scanning kinetics icon.

4. The Cary Scanning kinetics home screen will appear. When the blue bar at the top reads online you can continue.

5. To develop your method, click the setup button on the left side of the screen.
6. In the setup window, use various tabs to set your parameters:

a. Cary tab:

    i. Leave the modes as they are.

    ii. Set the wavelength range. Either you can collect scans over time at one specific wavelength or a range of wavelengths can be scanned during each data collection over time.

    iii. Adjust Scan controls as necessary. Usually the default values work fine. Adjusting the average time, scan interval, and scan rate may increase the quality of your data. It may be important in your experiment if your reaction is very fast and scans need to be taken as quickly as possible to scan only one wavelength, decrease your ave time and maximize your scan rate.

    iv. Under collect timing check the advanced collect box. This allows you to increase your number of cycles and adjust your parameters. Each cycle is a particular set of cycle (min) (this is how long before the instrument waits to take another scan) and stop (min) (this is the total duration the instrument will collect scans, which are set to collect at the time interval typed under cycle). For example: if a cycle is 3 and stop is 15, the instrument will scan every 3 minutes for a total of 15 minutes and you will have a total of 5 scans. A new stage begins when you change the cycle and stop parameters. For example, if you reaction is fast in the beginning and slow for the rest of the reaction, you may have the first stage with a cycle of 1 and stop of 5 to collect a scan every minute for the first 5 min of the reactions. The next stage may be a cycle of 5 and 60. This stage will

> collect a scan every 5 minutes for the next 55 minutes, so that the total reaction time at the end of the experiment will be 60 min.

b. Options tab
   i. Parameters were left as is
c. Baseline tab
   i. Parameters were left as is. Under correction, none was selected. Therefore there was no baseline correction in the set up. The baseline will probably start at around 0.1-0.5 abs instead of zero depending on your particular UV-Vis cell. In our experiments, a quartz cell with a septum for an air tight seal was used. If your cell contains a precipitate this will cause the baseline to be much higher than 0, around 1 abs, and this indicates your data contains a large amount of error.
d. Accessories tab
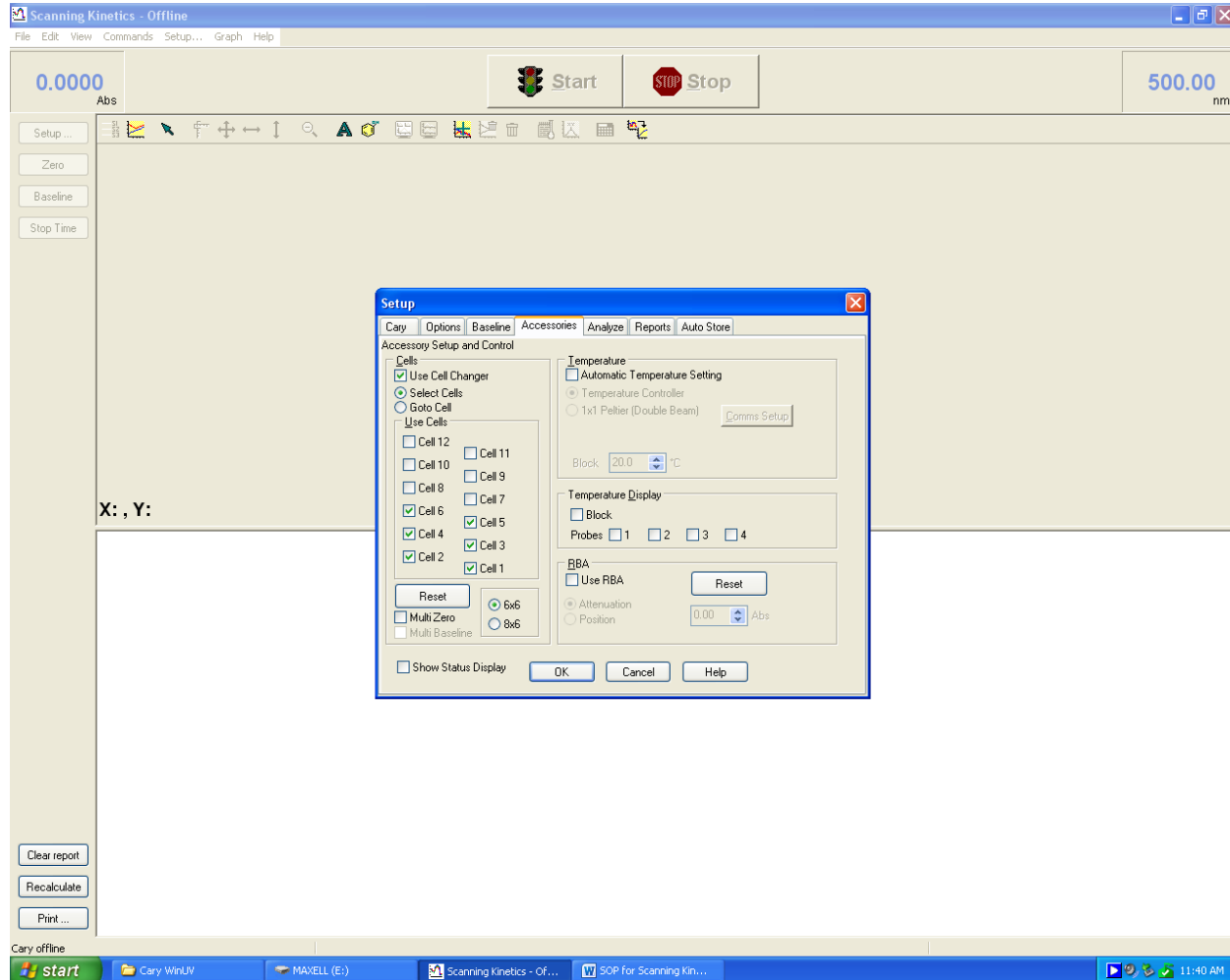
   i. Use cell changer was selected, even if only one UV-Vis cell was placed in the instrument. Check the cell(s) you wish to use. Only by having the cell changer checked are you able to check automatic temperature setting on the right.

When this box is checked you are then able to enter your desired temperature where it says Block. Nothing was checked under Temperature display or RBA.

    e.   The analyze, reports, and auto store tabs were left as is.

7. Once the parameters are set. Click Ok in the set up window.

8. The start button at the top of the screen should be lit up and allow you to click on it. Once you've clicked on it, a new window will appear that resets the cell changer so the proper cell is lined up with the beam. A countdown to collection will be displayed. This time can be used to inject substrates, clean the uv-vis cell, etc. You can bypass this count down and simply tell the instrument to take the first scan whenever you are ready.

9. During data collection, traces of the scans will be displayed. Each new scan will be overlayed on the previous scans. A plot of Abs versus Time can be created at any time during collection by clicking on the create kinetics continuum button.
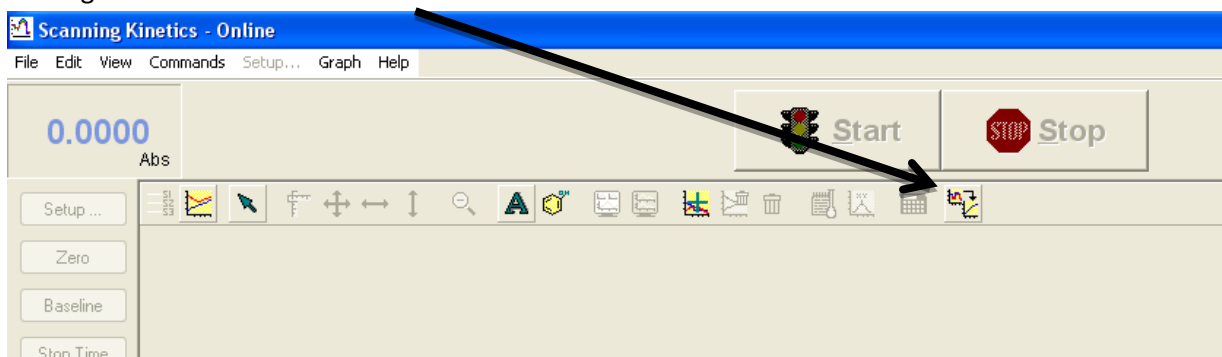
10. The data collection can also be stopped at any time by clicking the Stop button at the top of the screen next to the start button.