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LightPilot™ by Wilson Analytical

Software

User's Manual



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Installing and Running the *LightPilot* Software

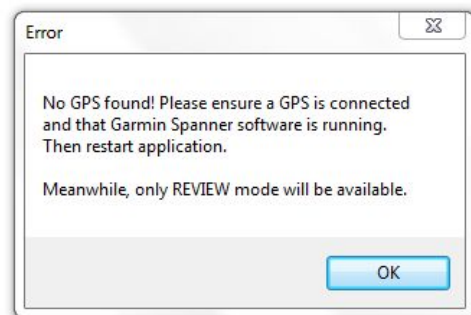
If you are using a netbook or other computer supplied by Wilson Analytical, *LightPilot* will have already been installed and configured. Once the netbook or computer is running, 'double-click' the blue icon to launch *LightPilot*.

If the *LightPilot* software has not already been installed on the computer, the program can be easily installed, either by inserting the *LightPilot* USB flash drive that was supplied, or by downloading the latest version of the software from the Wilson Analytical website, at www.wilsonanalytical.com.

To install the software from USB, simply insert the *LightPilot* USB into the appropriate drive in your computer, and double click on the 'install' icon. Then follow the instructions on the screen. Once completed go to the Wilson Analytical folder located in program files, then double click on Omni Driver, Java and redistrib in order to ensure they are downloaded.

In all cases you will be alerted when the installation is complete, at which point you can launch *LightPilot* by 'double-clicking' the blue icon on the computer desktop. If complications persist do not hesitate to contact us.

Once a successful download has been completed you may run the *LightPilot* Software with any Wilson Analytical instrumentation. If you choose not to use the WhereBox an error message may appear prompting you to connect the GPS device.



You can change it by simply opening the 'tools' menu on the toolbar, and selecting "Change Setup". This will open a dialog box prompting you to enter the current password (the default password supplied to you, to access *Control* mode). After you have done this, a new window will open up, called "Setup". Ensure the "Require GPS" box at the bottom of the "setup" pop up is deselected.

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Overview of the *LightPilot* Software

The *LightPilot* software was designed to make using the instrumentation straightforward and an enjoyable process, while still ensuring that the data is both accurate and reliable. With that being said the scientific jargon and complexity is kept behind the scenes, and the user is guided through the measurement process in a logical and straightforward sequence.

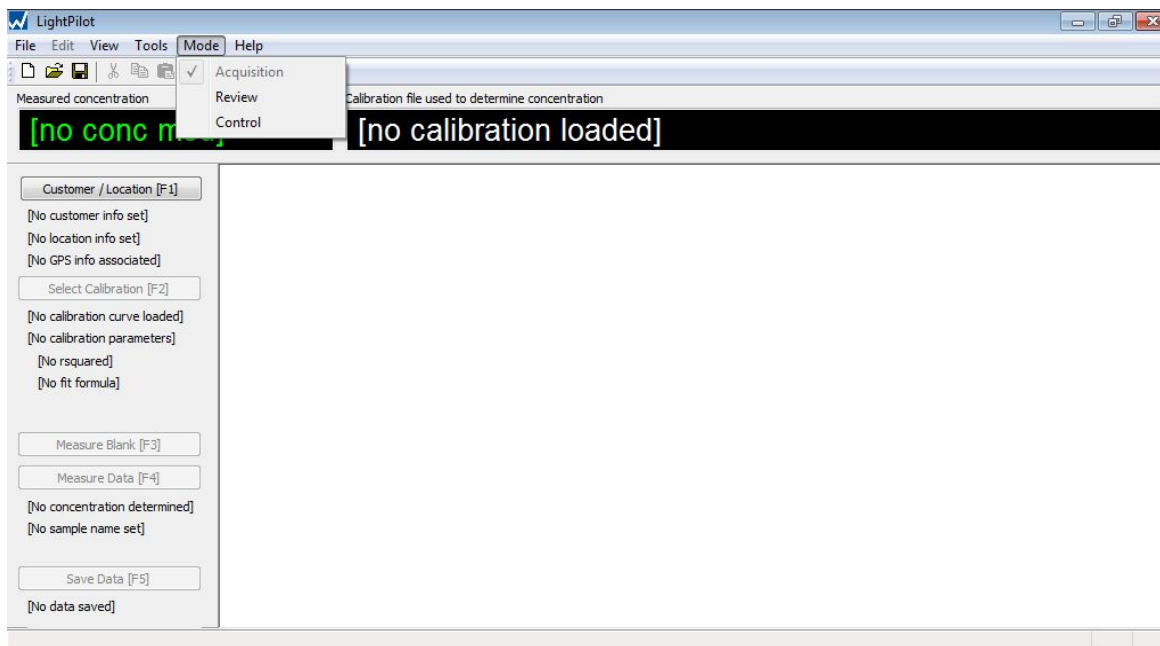
The first thing to know is that the *LightPilot* software has three modes of operation, each dedicated to a particular task. Each of the modes is discussed in detail in the remainder of this section. The three modes are:

Control mode, used to create or modify a calibration curve for the chemical or chemicals that will be monitored;

Acquisition mode, used to take measurements on the samples of interest; and

Review mode, used to display, review and print data files.

The user can switch between these modes using the 'mode' menu in the toolbar. Note that because the validity and the accuracy of its data hinges on the quality of the calibration curves created by the user, access to *Control* mode is password protected.



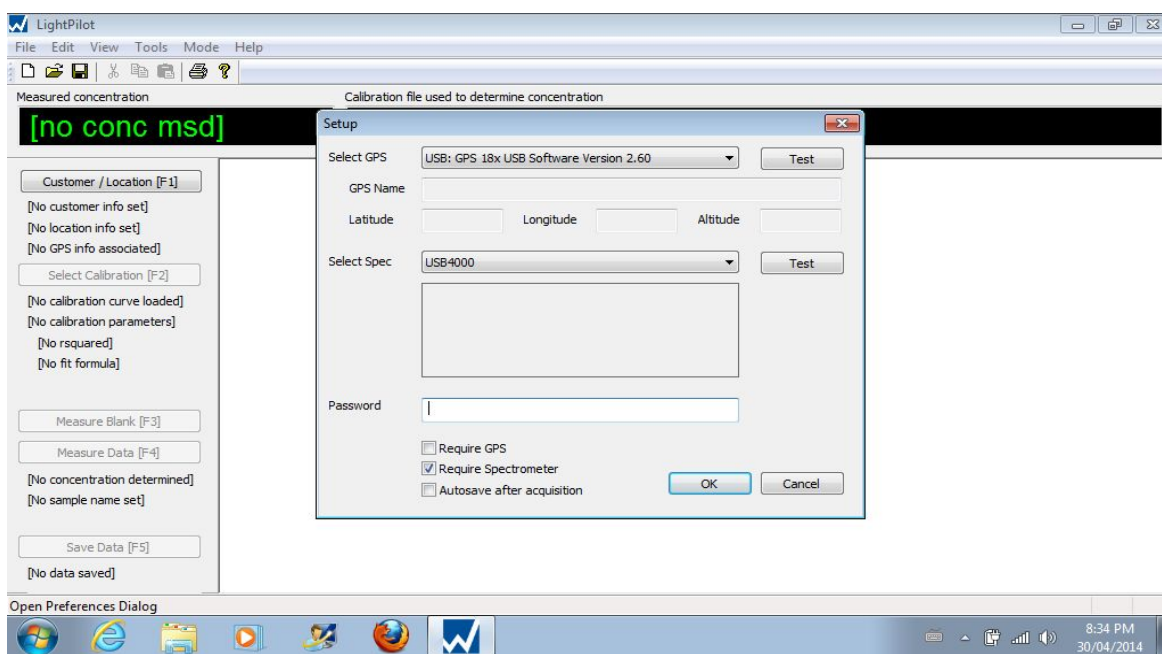
In each of the three *LightPilot* modes, the software has been designed to guide the user through the completion of the appropriate activity (calibration, acquire data, review data) in a logical sequence. For example, *Acquisition* mode will not allow a chemical sample to be measured until a calibration curve for that chemical has been selected. The software guides the user by having steps 'grayed out' until they are ready to be completed.

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Note that the *LightPilot* software has been designed so that it can be used without a mouse. Each of the selection buttons in each of the modes can be activated by pressing the appropriate function key on the keyboard, for example “Acquire Baseline [F1]”

LightPilot Setup

Though a default password to access *Control* mode has been supplied to you, you by no means have to keep this password. You can change it by simply opening the 'tools' menu on the toolbar, and selecting ‘Change Setup’. This will open a dialog box prompting you to enter the current password (the same one that is used to access *Control* mode). After you have done this, a new pop up will open appear, called "Setup".



From the "Setup" box, you can change your password simply by deleting the existing password from the "Password" box and entering your own, then clicking "OK" at the bottom. In the “Setup” box you can also test your connections with both the WhereBox (GPS receiver) and the spectrometer, to ensure that both are connected and working properly. In order to do a check click "Test" beside the appropriate device in the dialogue box.

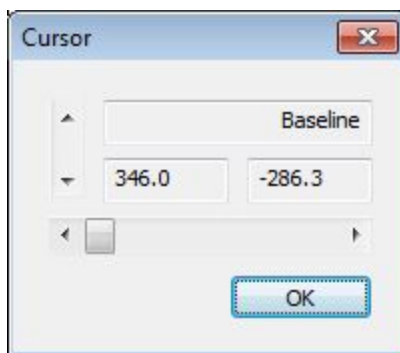
Note the ‘Require GPS’ box at the bottom of the “setup” box is deselected allowing the user to run samples without the WhereBox. If you choose not to use the WhereBox during testing please ensure the ‘Require GS’ box is deselected. Once you have saved these setting, they will remain the same even once you exit LightPilot.

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Though the default view on the software is to display the data, the raw spectrum and the baseline you do not have keep this setting. In order to change the data spectrum that is displayed simply open the 'view' menu on the toolbar, hover over 'Data Spectrum' and deselect the datapoints you do not want displayed. It is important to remember that the default setting will be displayed each time the program is closed and reopened, meaning you will have to change the view each time you open the software.

Cursor

In each of the three modes, it is possible to view the number of counts at varying wavelengths by opening the 'View' menu in the toolbar and selecting "Cursor". A dialogue box will open up, called "Cursor". From here, you are able to select a wavelength at which to get the counts. By default, it is set to the lowest wavelength at the far left of the graph.



There are two ways to adjust the wavelength: to change by large intervals, move the scroll bar side to side; to move by smaller intervals (0.2 nm of a wavelength), you can click on the left and right arrows to either side of the scroll bar. Note that the cursor line starts at the left of the spectrum (short wavelength end). Using this cursor, you can determine the height of any peaks that appear on the graph. The wavelength you are reading at any given time is represented on the graph as a black line.

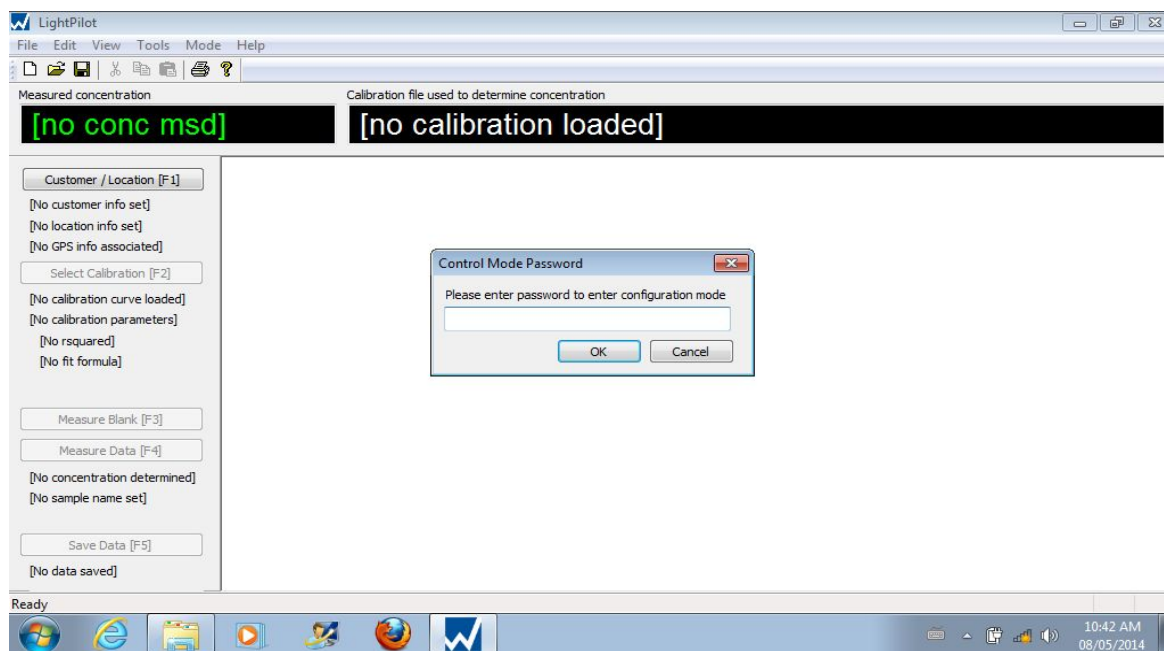
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‘Control’ Mode Introduction

LightPilot works by taking the measurement of the fluorescence intensity from an unknown sample and comparing it to the expected intensity from the same chemical, measured at a series of standard concentrations. The chart of ‘fluorescence intensity *versus* concentration’ for any given chemical is called its calibration curve.

Without a calibration curve, the data measured by the instruments is useless, and so it is the purpose of *Control* mode to guide the user through the creation and/or modification of calibration curves. The necessary steps are listed on the left hand side of the *Control* mode screen. In this section, we will discuss each of these steps in turn.

Choose “Control” from the Mode menu, and a password dialog box will open up. Type in the password to access the Control Mode.



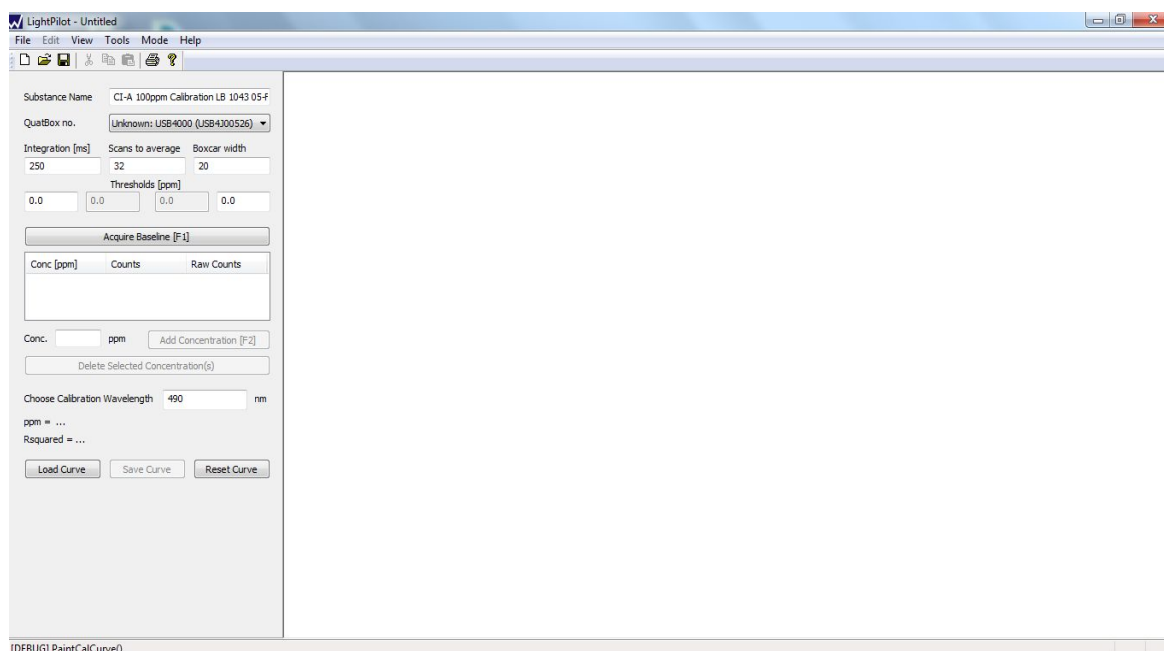
Let us assume that we want to create a calibration curve for a corrosion inhibitor chemical called C-100. Type in “C-100” (no need to type the quotes) in the ‘Substance Name’ text box. When the calibration file is saved, at the end of the process, it will be given the name “C-100.cal” (again, without the quotes). More information can be added to the name if multiple calibration curves will be needed for the same chemical. If you are re-doing a calibration curve for a chemical, we recommend copying and pasting the previous calibration name into the new curve and then changing the date. This ensures proper consistency and organization.

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Each instrument contains a spectrometer with a unique serial number. The *LightPilot* software keeps track of the spectrometers used to create calibration and data files, and you may occasionally see a warning alert that data you are using was created with a different unit.

This is perfectly normal and there is no reason for the user not to proceed with measurements using calibration curves created on other Wilson instruments. The light output from each instrument has been carefully calibrated to be equivalent, so that clients with more than one unit can work efficiently by sharing data and calibration curves as desired. However this is assuming the instruments are using the same wavelength for the light source and the same type of spectrometer. Various light sources are available for installation during the construction of the unit.

There is a drop down menu immediately below the 'Substance Name' text box, which allows the user to select the instrument that is currently detected by the *LightPilot* software, or devices that have been detected in the past. In most cases, only one serial number will appear. In the event that two or more serial numbers are displayed, the user should select the unit that will be used for their testing.



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Three Spectroscopy Parameters for the Calibration

Wilson Analytical's fluorescence instrumentation exposes a sample to a constant source of light, and records the intensity and wavelength of the light that is re-emitted by the sample. This 'emitted intensity' is recorded as a number of counts at each wavelength between 380nm and 1100nm, and is displayed by the *LightPilot* software as a spectrum on the computer screen. Different chemicals emit light at different characteristic wavelengths, and the normal procedure is to pick a wavelength of maximum intensity (i.e., a peak) as the wavelength for the calibration of that particular chemical.

The next step is to choose values for the three-spectroscopy parameters required to take a measurement. These are:

Parameter 1: Integration Time

Parameter 2: Scans to Average

Parameter 3: Boxcar Width

Now these parameters may not mean much to you, but they are important and will affect the intensity of the fluorescence signal and the quality of the data that you obtain. The parameters have the following effect:

Parameter 1: Integration Time

Think of integration time like the shutter speed on a digital camera. The longer the integration time, the longer the 'shutter' is open, and the more light (signal) reaches the detector. Just like in photography, you want your picture (your data) to have the right exposure. Too much exposure, and you will lose contrast and linearity. Too little, and you will lose sensitivity and be unable to measure low concentrations accurately. And, just like in photography, you sometime have to play around with the settings a little bit to get the exposure just right!

If we are planning to use C-100 at 500ppm, we might want a calibration curve that spans 50ppm (10x lower) to 2500ppm (5x higher). Now, remembering that one of the four secrets to accurate fluorescence measurements is keeping in the linear range, we know that the maximum concentration we can measure directly is probably 50ppm.

As the dilution factor in this case must be at least x50, we may as well pick x100, as this gives us a margin of safety, plus easier arithmetic. With a dilution factor of x100, the calibration for C-100 will require samples with concentrations ranging from 0.5ppm to 25ppm, for example 1ppm, 5ppm, 10ppm and 25ppm. If necessary, more concentrated samples at 50ppm and 100ppm could be prepared, to see at what point, and to what extent, the calibration curve becomes non-linear.

The basic principle in selecting the correct integration time is that a doubling of the time will double the amount of light reaching the detector, and hence double the number of counts recorded in the spectrum. The detector will show a maximum of 64,000 counts at any particular wavelength, so the integration time in this case must be such that at 25ppm, the maximum number of counts is less than

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64,000. At the low concentration end of the measurement, we would like to be able to see 0.5ppm reliably, and the minimum number of counts necessary to do this is around 10. In the middle of the range, where we hope to see most of our samples fall, we would like the 5ppm sample to have hundreds or maybe a couple of thousand counts at the chosen wavelength.

In most cases, there will be a range of integration times that are suitable to allow the user to 'see' the concentration range of interest quite comfortably. A good starting point for the integration time is 250ms (250 milliseconds). If there are insufficient counts at the low end of the concentration range, increase the integration time. Conversely, if there are tens of thousands of counts at the high end of the chosen range, decrease the integration time accordingly.

Note that if for any reason a single integration time value that covers the whole concentration range appropriately cannot be found, the user has two choices. The first is to select a value of the integration time that makes the low concentrations 'work', and simply further dilute samples that are off-scale. Or secondly, construct two calibration curves, one for higher concentrations, and one for lower.

It is important to note that once an integration time has been chosen, and a measurement taken, all further measurements for that calibration curve will use the same integration time. This feature is hard-wired into the *Control* mode so that the user cannot inadvertently take measurements with different parameters and try to construct a single curve. It only makes sense to take sample measurements with the instrumentation set up to the same configuration that was used to construct the calibration curve. This is one of the things that the *LightPilot* software looks after 'behind the scenes'.

Parameter 2: Scans to Average

This parameter is fairly self-explanatory. It is simply the number of fluorescence measurements that the instrument will obtain and average together before displaying on the screen. A big advantage of the fluorescence technique is the speed at which measurements can be taken; a few seconds at most. It is a good idea to take multiple data sets and average them together, as it takes so little time to do so, and the statistical accuracy of the data is thereby improved. A good default value for this parameter is 32, and the user can decide through experiment if there is a particular advantage in a greater or lesser value.

As with the integration time, once the 'scans to average' parameter has been set, and a measurement taken, all further measurements for that calibration curve will use the same value.

Parameter 3: Boxcar Width

The boxcar width is a technical parameter that has to do with smoothing of the data. A default value of 20 is acceptable for the majority of applications, and in most cases, the user will see little difference from adjusting its value. Boxcar width has been retained as a parameter for the more expert user, and for those requiring the most extreme precision in their work.

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As with the 'integration time' and 'scans to average' settings, once the 'boxcar width' parameter has been set, and a measurement taken, all further measurements for that calibration curve will use the same value.

Thresholds

Beneath the boxes for 'integration time', 'scans to average' and 'boxcar width' are four boxes labeled "Thresholds". Of these boxes, the middle two are 'greyed out', while the leftmost and rightmost ones are able to have data entered into them.

The two 'greyed out' boxes represent the range of the calibration curve, that is the lowest and highest ppm standard measured in making the curve, respectively. These boxes will fill in automatically as you make your calibration curve.

The leftmost white box allows you to enter the lowest ppm sample that falls outside the range of the curve you want to be reported. The rightmost white box, similarly, lets you enter an upper threshold, or the value that falls above the range of the curve that you want to be reported. Those values that fall into the areas between the threshold values and the range of the curve will be reported in orange, while those that fall outside the threshold values will be reported in red. Values that fall within the range of the calibration will be green.

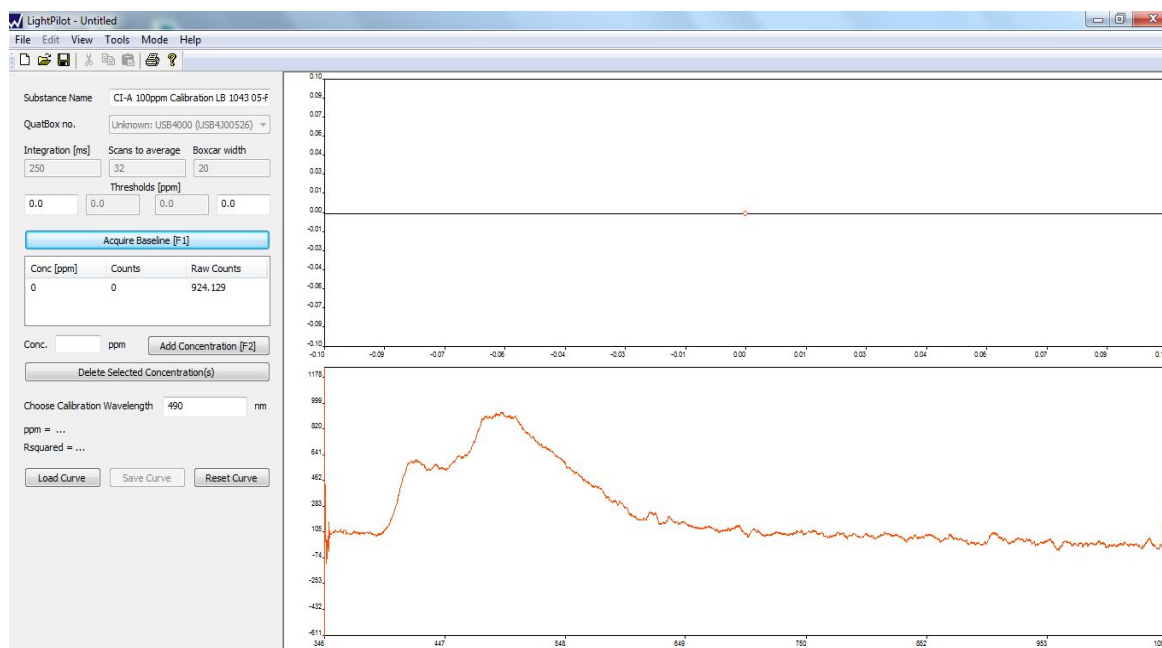
Note that it is not mandatory to set threshold values; if you leave them blank, they will be assumed to be the range of the curve, and thus any values falling outside the range of the curve will be reported in red.

Creating the Calibration Curve

The next step is to take a measurement with a 'blank' sample. This gives the instrument a baseline response (from the cuvette, solvent, etc.) that is subtracted from all subsequent sample measurements. The blank sample should be the same as the calibration standards in all respects other than the chemical itself. For example, if you decide to make the calibration standards for C-100 in a 95% water, 5% IPA solution that contains 100ppm of sodium chloride, then this solvent matrix should be used for the blank measurement.

It will only be possible to acquire a spectrum from the blank sample if the instrument is in operational mode, as indicated by the status LED's on the front of the instrument being green. Once the LED's indicated the instrument is ready, insert the blank sample into the cuvette holder and close the cover. The *Interlock* LED should now also be green. If it is red, the cuvette cover is not closed. Select *Acquire Baseline*. When the measurement is complete (*Power or data* LED on the front changes from blue to green), remove the blank sample. The baseline spectrum will be displayed, as well as the zero concentration counts at the calibration wavelength.

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Now that the three-spectroscopy parameters have been configured, and a baseline measurement has been taken, the actual calibration curve for C-100 can be created.

One way of developing a calibration curve is to create a 'dilution series' of the chemical of interest in distilled or deionized water. For example, a series of samples with 1ppm, 5ppm, 10ppm, 25ppm and 50ppm concentrations of chemical would form a very good calibration sequence. In practice however, chemicals are used in 'real world' water systems that may or may not contain salts, surfactants or other chemicals that could potentially interfere with the fluorescence measurement. If possible therefore, it is a good idea to use the same environment, or matrix, to prepare the calibration samples as will be encountered in the field doing the actual measurements.

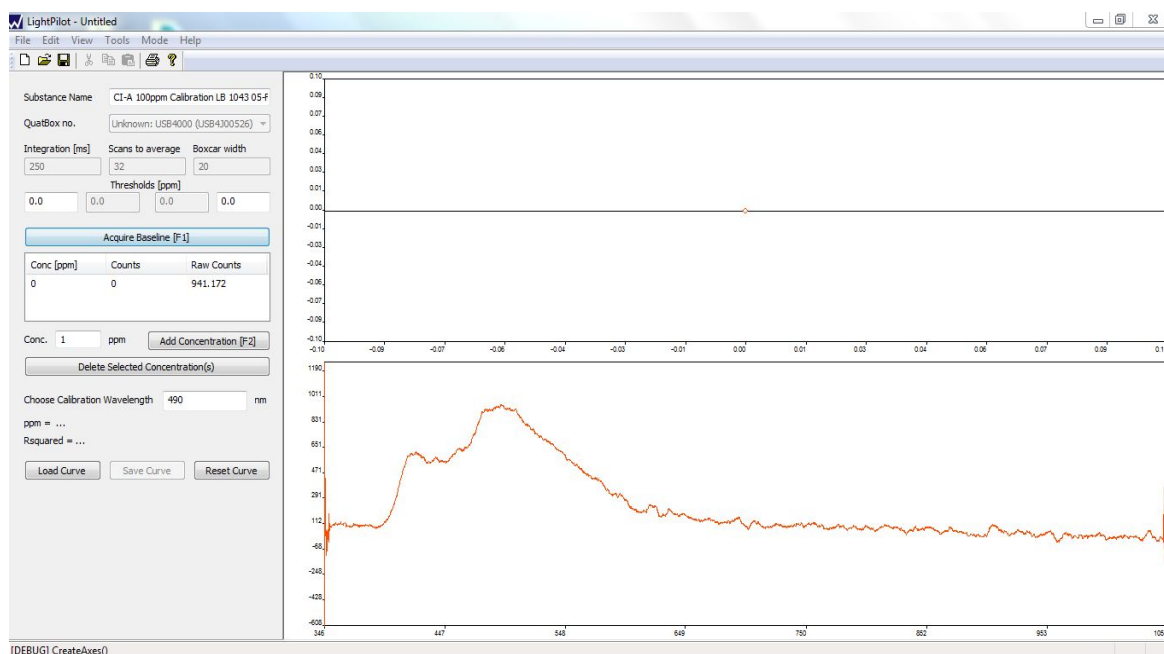
For example, if a corrosion inhibitor is being used at 500ppm in a system where the produced water has a salinity of 5% (50,000ppm), a useful calibration series could be formed by making an accurate 500ppm solution of inhibitor in the produced brine and diluting it by factors of x50, x100, x500, x1000 and x5000, to give solutions of 10ppm, 5ppm, 1ppm, 0.5ppm and 0.1ppm. Alternatively, solutions of 10ppm, 5ppm, 1ppm, 0.5ppm and 0.1ppm could all be prepared in a 100ppm brine (corresponding to the expected strength of the matrix; 500ppm in a 50,000ppm brine, diluted by x500).

Note that it is not necessary to prepare five or six samples to create a calibration curve. The *Control* mode will assume that the fluorescence intensity at zero concentration (0ppm) will be zero, i.e. all calibration curves go through the origin. Therefore, even a single sample, say 10ppm, will result in a straight-line calibration that could be used for subsequent measurement of unknowns. A two-point calibration is however ill-advised, because it will not confirm that you are operating in the linear range, and can easily result in large errors between the measured and actual chemical concentrations.

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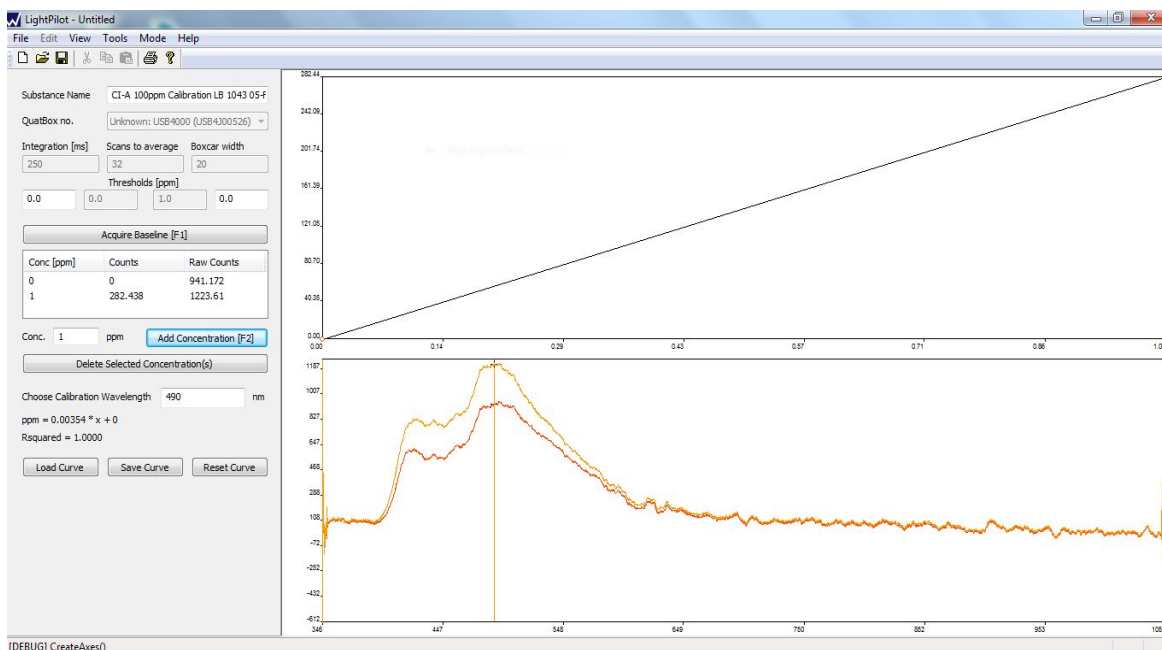
It is advisable to use at least three chemical dilutions to create a calibration, and the concentrations chosen should cover at least an order of magnitude in concentration range. In other words, 1ppm, 10ppm and 50ppm will create a much better calibration curve than 1ppm, 2ppm and 5ppm.

Let us say that we have prepared six calibration samples for C-100, at 1ppm, 5ppm, 10ppm, 20ppm, 50ppm, and 100ppm. Place a cuvette containing the 1ppm solution into the sample holder and close the sample cover. Type '1' in the white text box that appears between the symbols 'Conc.' and 'ppm', and then select the 'Add Concentration' button. The fluorescence spectrum from the 1ppm sample will be displayed on the lower right hand side of the *Control* screen.



LightPilot will now construct a data table that shows the concentrations run so far, the units, counts and raw counts. This data is the actual calibration curve, and displayed graphically on the upper right of the screen. Do not worry if it appears to be non-sensical at this stage, because you have not yet chosen a calibration wavelength (see below).

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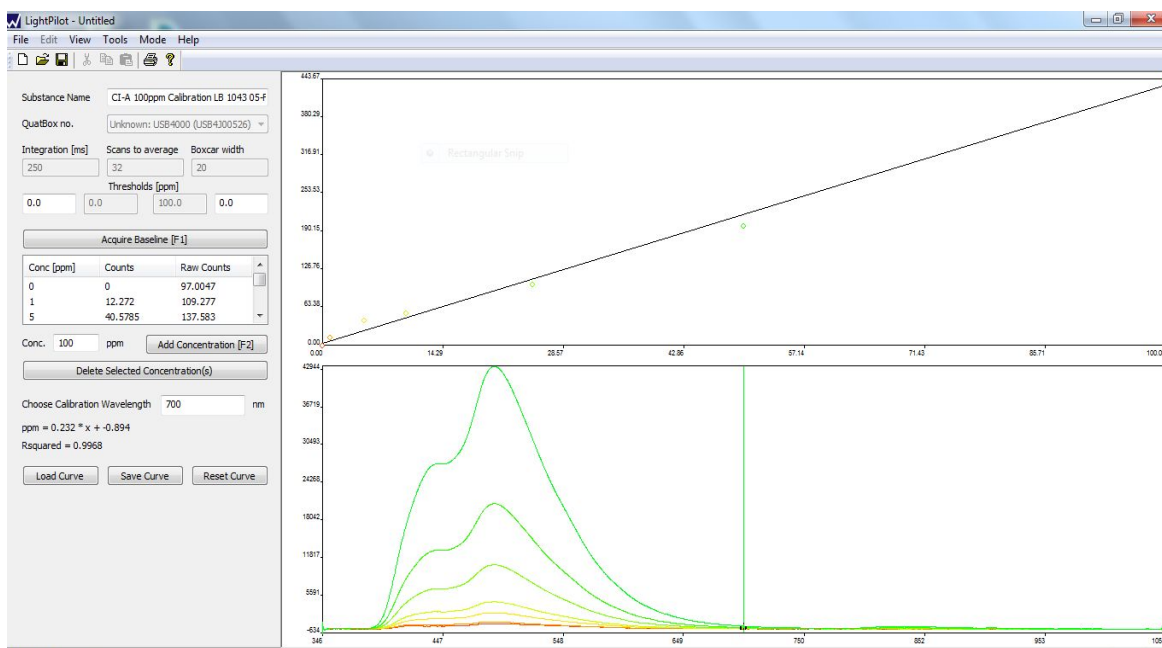


Proceed with the creation of the calibration curve by adding the 5ppm, 10ppm, 20ppm 50ppm, and 100ppm concentrations in the same way as you did for the 1ppm sample. In each case, place the cuvette with the new concentration in the cuvette holder, close the cover, type the new concentration in the text box on the left hand side of the screen (between the symbols 'Conc.' and 'ppm'), and select 'Add Concentration'.

If at any time you make a mistake, or simply wish to remove one or more of the data sets, simply highlight that measurement or measurements and select 'Delete Selected Concentration(s)'. You can delete sample if something was typed incorrectly or if you would like to redo a data point.

When you have measured all six calibration samples, you should see six spectra displayed at the bottom right hand side of the screen, each with the same shape (peaks in the same place) but with different intensities. To complete the creation of the calibration curve, you must now select a calibration wavelength.

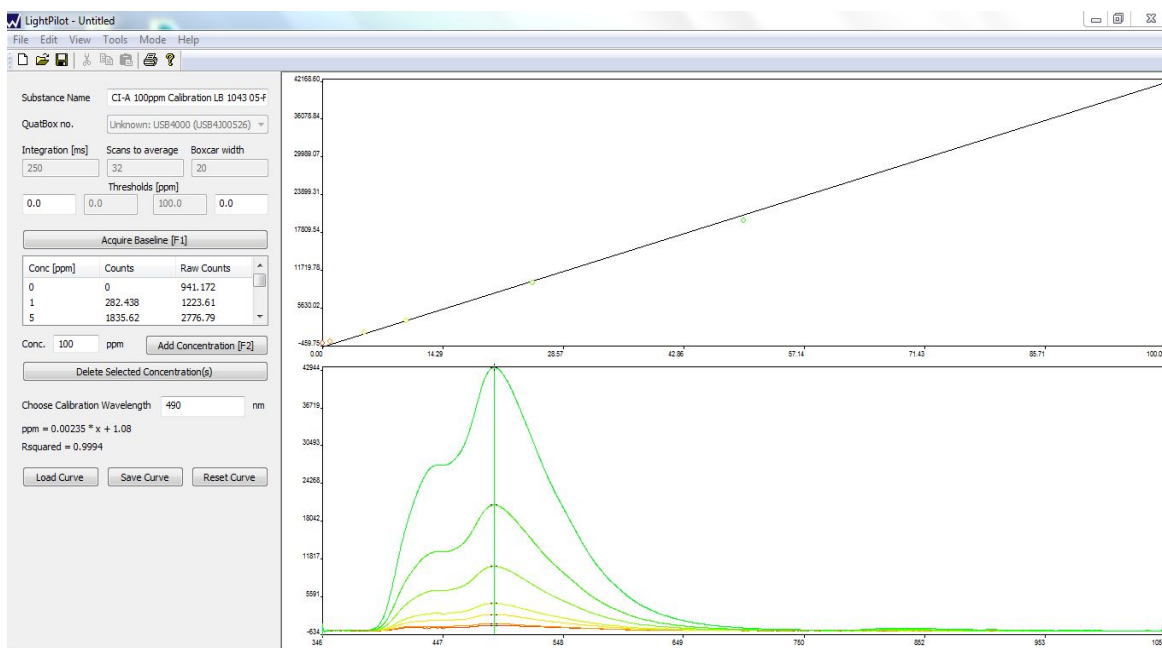
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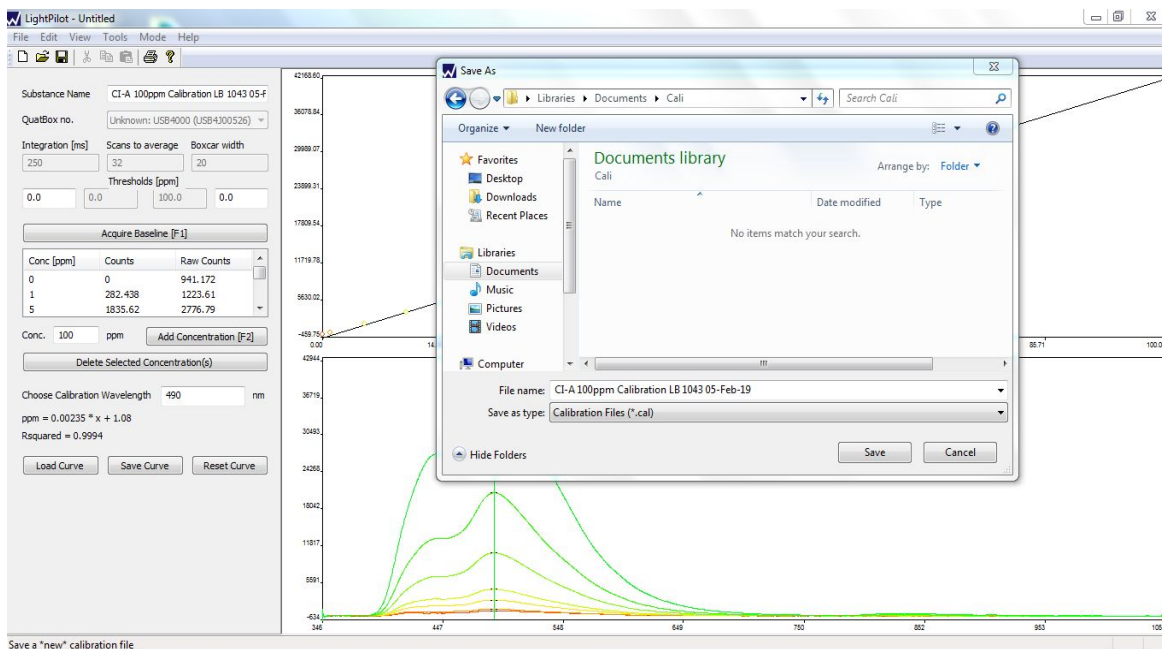
In the spectrum display box on the bottom right hand side of the screen, there is a single vertical line. This line moves to the wavelength that you type into the text box to the right of the words 'Choose Calibration Wavelength' on the lower left hand side of the screen. So, for example, if you see that your fluorescence spectra have peaks around 480nm, you should type '480' into the 'Choose Calibration Wavelength' box. The vertical line will move to 480nm in the spectrum display box, and you should now see a calibration curve on the upper right hand side of the screen that is close to linear. If the vertical line is not at the peak maximum, simply adjust (move) the line by typing a different number into the wavelength box. For example, if the line is too far to the right, try typing '475' in the 'Choose Calibration Wavelength' box and the line will move 5nm to the left.

At this point, the user has complete control over the calibration and can add or subtract samples with different concentrations, or choose a different wavelength for the calibration, until calibration curve at the top right hand side of the screen is as linear as possible (Rsquared value of 0.98 or greater is ideal). The equation of the calibration curve and the Rsquared value are calculated and displayed immediately underneath the 'Choose Calibration Wavelength' box, as well as on the calibration curve itself.

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The final step is to save the calibration curve by simply selecting 'Save Calibration Curve' at the bottom left hand side of the screen. Once you have done this, the C-100 calibration curve has been successfully created and can be recalled and used in *Acquisition* mode.

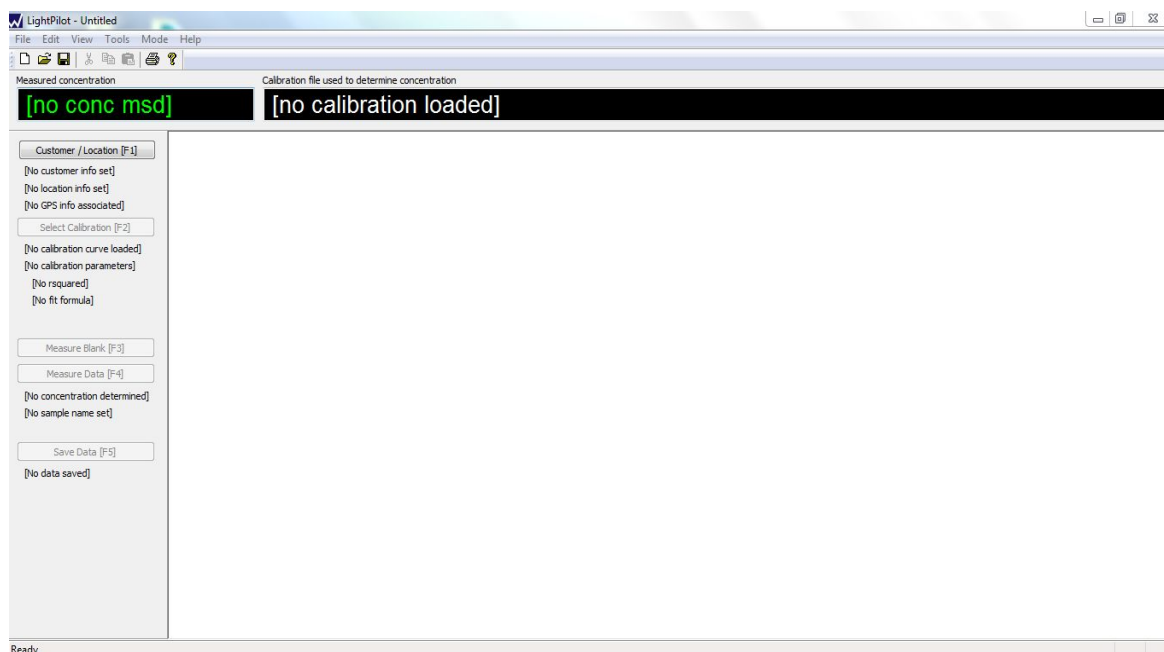


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‘Acquisition’ Mode Introduction

Once a calibration curve has been successfully created, Wilson’s instrumentation can be used to determine chemical concentrations in unknown samples. From the toolbar, select the ‘Mode’ menu, and then ‘Acquisition’. No password is required, and the *Acquisition* screen will be displayed.

Acquisition mode breaks down the measurement of each sample into five sequential steps, and the user is taken through each of the five in turn. The *LightPilot* software makes it impossible to take the steps out of sequence, although it is possible to go back and change previous steps. Options that are not yet available for a particular sample are ‘grayed out’. For example, once step one has been completed, step two becomes available. Once that has been completed, step three is available, and so on.



The five steps are:

- Entering information about the sample (e.g. name and location)
- Selecting a calibration curve
- Measuring a ‘blank’ sample
- Measuring the unknown sample
- Saving the data file.

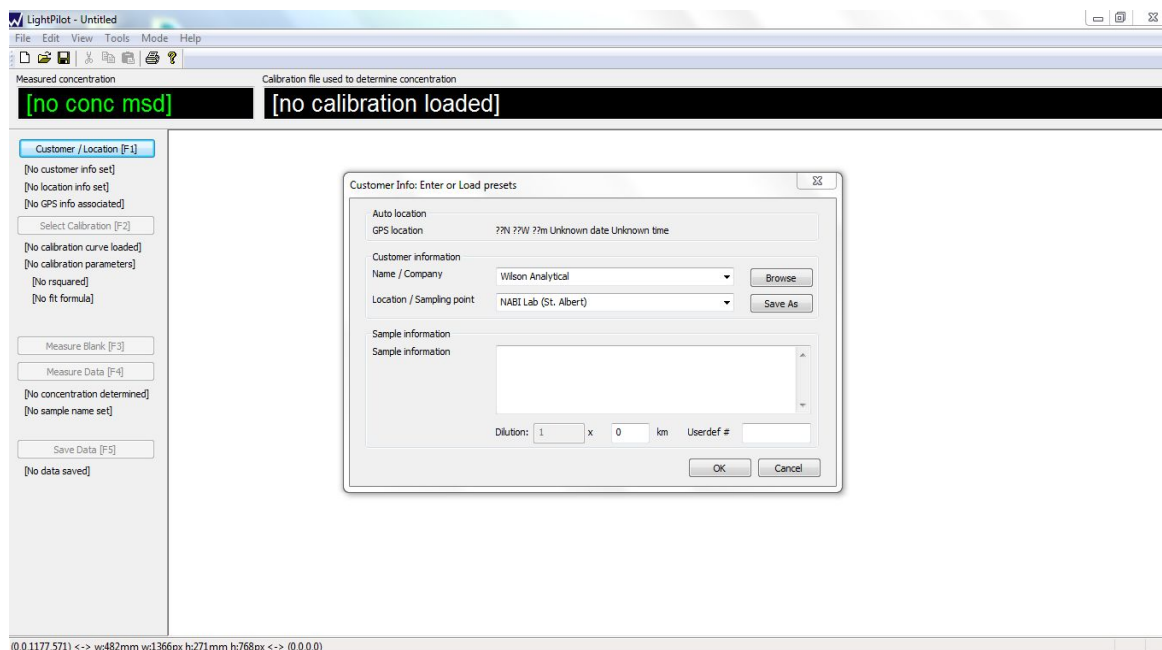
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Entering Information about the sample

If you have the optional WhereBox unit attached to the QuatBox™, the GPS coordinates will be displayed at the top of the screen (assuming that you are located somewhere where the GPS signal can be detected). GPS locators, plus a date and time stamp will also be automatically written to the data file that is being created. You will be prompted to enter information on the sample customer and location by the following dialogue box each time you take a sample.

Note that beside the 'Name/Company' and 'Location/Sampling point' boxes, there are arrows. You can click these arrows to open a dropdown menu of previously entered sample locations and company names. Selecting one of these will save you the need to enter this information again if you are sampling at the same location multiple times.

After filling in the 'Name/Company' and 'Location/Sampling point' boxes, you can click the 'Save As' button to the right of them to save this information to the GPS coordinates. In future visits, if all the information remains the same, you can simply click 'Browse' (located above the 'Save As' box) and select the location in order to fill in the company and location information.

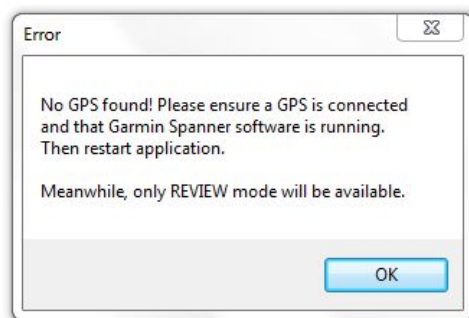


In order for the WhereBox to work, it must be in a location from which a GPS satellite radio signal can be reached. As such, it is best to place it out in the open; if indoors, it is best placed as near to the door as possible, especially if you are below ground. The 16 foot USB connection cable provided aids in this, allowing you to place the WhereBox anywhere within a 16 foot radius from where the laptop is set up.

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The bottom of the WhereBox is also magnetic, allowing it to be stuck to any magnetic surface, such as the roof of a truck or wall of a metal shed. This helps to improve the range of places the WhereBox can be set up, allowing for the best GPS satellite radio signal possible.

If you choose not to use the WhereBox an error message may appear prompting, you to connect the GPS device.



You can change it by simply opening the 'tools' menu on the toolbar and selecting "Change Setup". This will open a dialog box prompting you to enter the current password (the default password supplied to you, to access *Control* mode). After you have done this, a new window will open up, called "Setup". Ensure the "Require GPS" box at the bottom of the "setup" pop up is deselected.

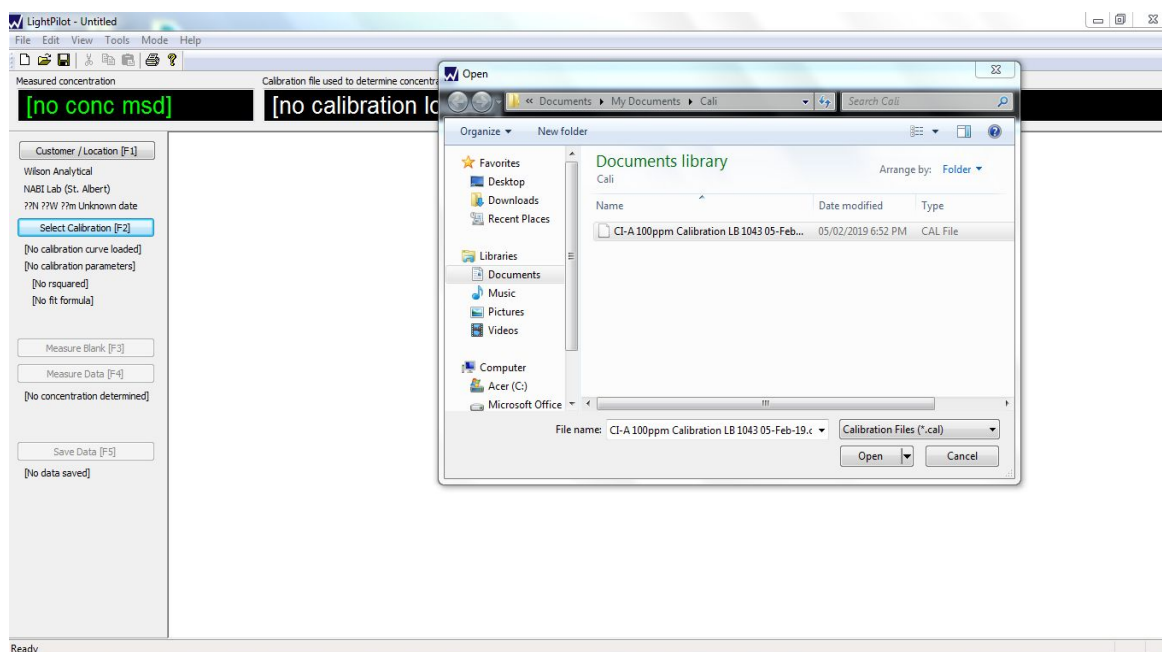
Note that if the WhereBox unit is not attached, the GPS field will just contain default letters and question marks that can be safely ignored.

Selecting a Calibration Curve

As discussed earlier, the user must select a calibration curve that *LightPilot* can use to calculate the chemical concentration in the unknown sample. This is straightforward to do by selecting "Select Calibration [F2]". A list of files with the ".cal" extension will be displayed. Simply select the calibration file that you wish to use, and *LightPilot* will use that particular calibration curve to determine the concentration of chemical in the unknown sample(s).

The name of the currently selected calibration curve is displayed at the top right hand side of the Acquisition mode screen, and is also saved in the sample data files that are created using that particular file. In that way, it is always possible to reconstruct after the fact the calibration that was used when measuring a particular sample.

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Following the earlier example of the calibration curve that was created for 'C-100', in this case we would click on "Load Calibration Curve", and then select the file named "C-100.cal".

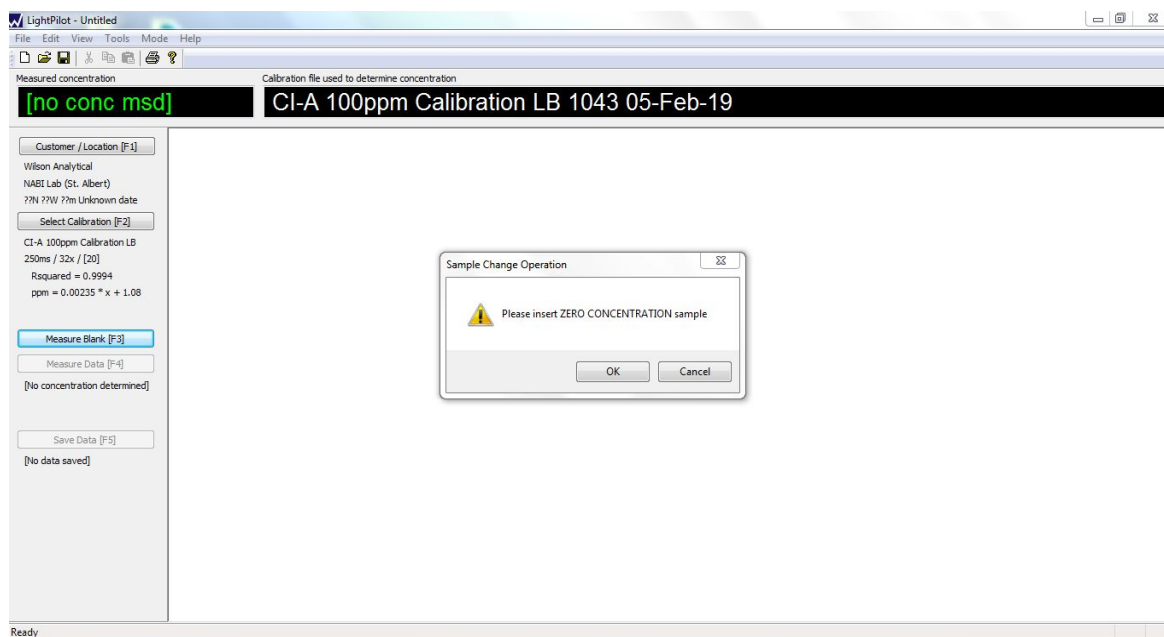
Once a calibration file has been selected, *LightPilot* reads the three-spectroscopy parameters that were used to create that calibration (Integration Time, Scans to Average and Boxcar Width) and uses them for the subsequent measurements of the unknown. The user cannot change the spectroscopy parameters from *Acquisition* mode. This is because it makes no sense for measurements on unknowns to be interpreted using a calibration curve obtained with different spectroscopic conditions. As *LightPilot* forces measurements to be taken under the same conditions as the calibration, the user doesn't have to check back or worry about ensuring there is a match.

Measuring a Blank Sample

Once the user has entered information about the sample and selected a calibration file, the next step is to take a measurement with a 'blank' sample. This gives the instrument a baseline response (from the cuvette, solvent, etc.) that is subtracted from the subsequent sample measurement. As discussed earlier, if possible the blank sample should use the same solvent matrix as the calibration standards.

It will only be possible to acquire a spectrum from the blank sample if the instrumentation is in operational mode, as indicated by the status LED's on the front of the instrument being green. Insert the blank sample into the cuvette holder and close the cover. The *Interlock* LED should now also be green. If it is red or, orange the sample cover is not closed.

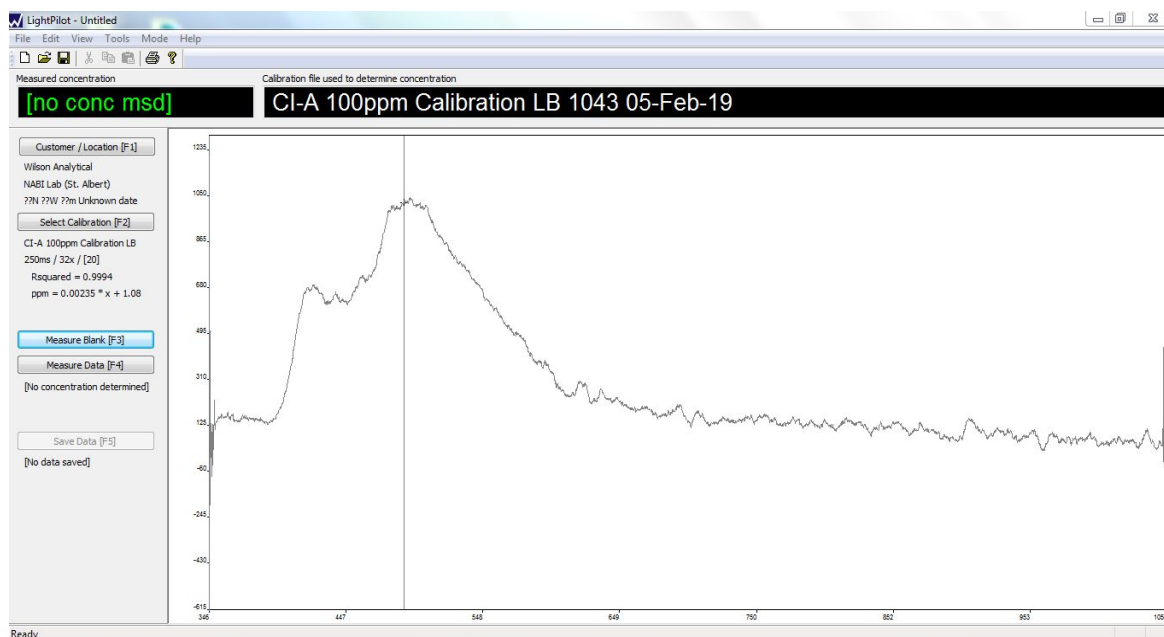
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Once the LED's on the front panel are green you are ready to take measurements. Click "Measure Blank Sample", and the software prompts you to ensure that the blank sample (zero concentration sample) is in place before proceeding. After clicking on ok wait until the *Power or data* LED changes from blue back to green, and then remove the sample.

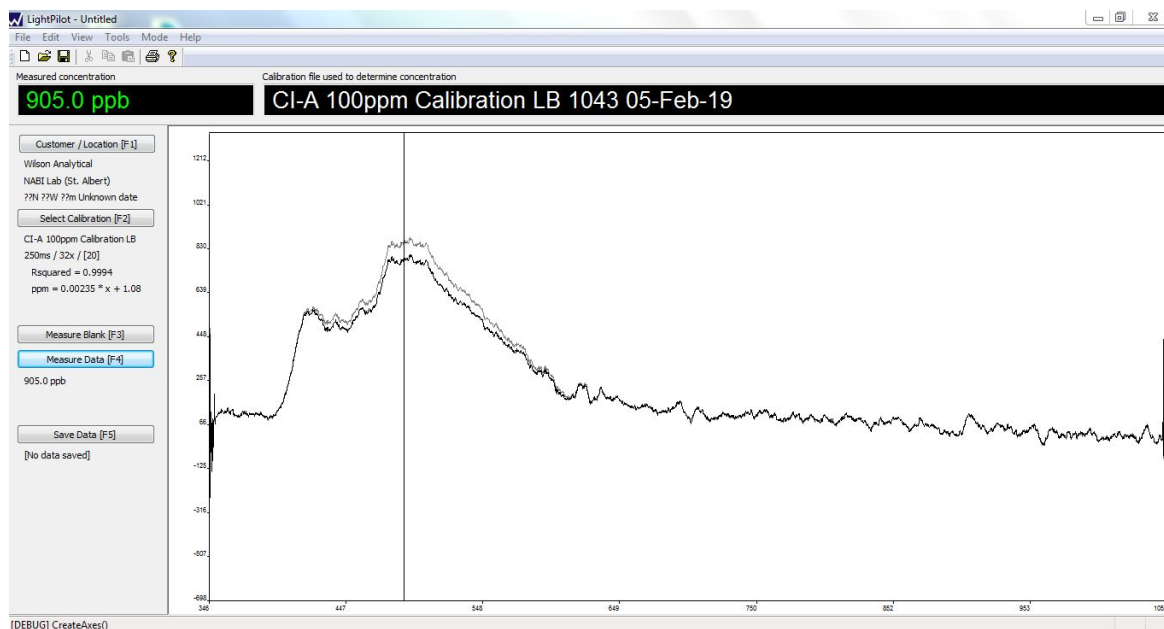
The fluorescence spectrum of the blank sample will be displayed on the computer screen. It is likely to be relatively uninteresting (flat) as it is a control sample. If the spectrum does contain peaks or features, there is a good chance that either the wrong sample has been selected (i.e. the sample isn't really a blank), or that the sample matrix contains fluorescent material that could interfere with the measurement.

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Before measuring the unknown sample, it is advisable to run the blank itself as a sample, in order to ensure the instrumentation is running properly. This is done in the manner described below, in the 'Measuring the Unknown Sample' section.

If the blank returns a fairly straight line (ie matches the blank measurement) then it is safe to run the unknown sample. However, if there are noticeable discrepancies between the blank spectrum taken previously and the blank measured as an unknown, you should either start over (ie redo the blank measurement) with the same blank, or prepare a new blank, to ensure the best results.



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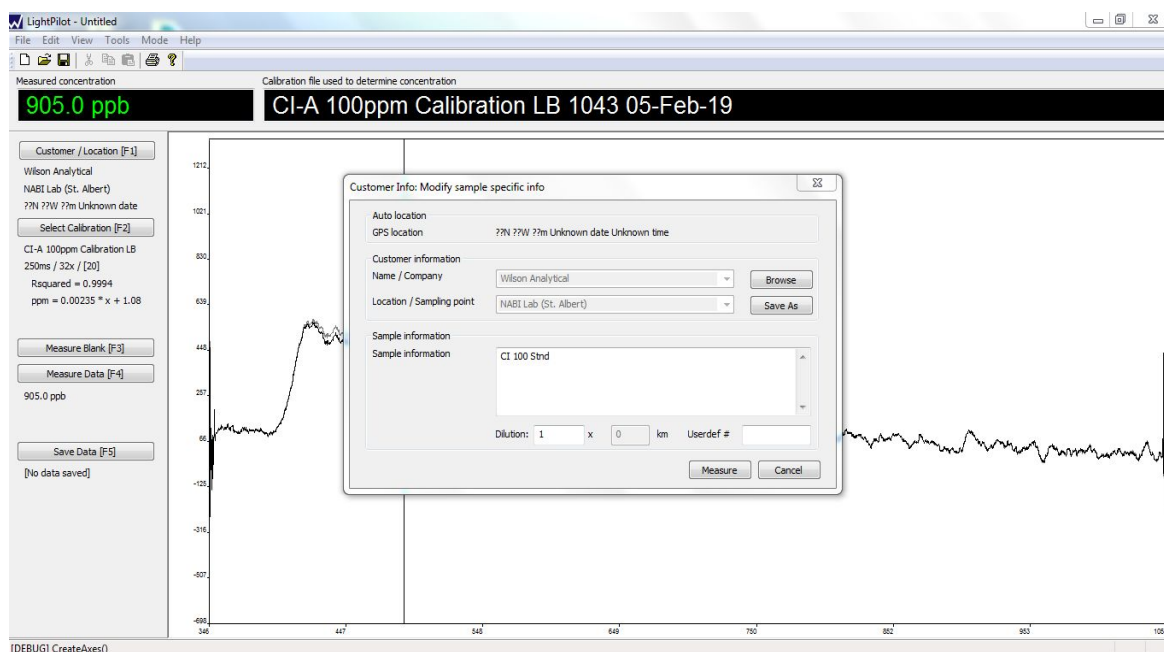
While not a necessary step in running a sample, running the blank as a sample can tell you early on if there is something wrong with your blank, or if the instrumentation is misreading the sample. Doing this serves as a sort of safeguard, and ensures the best results each time.

Though the default view on the software is to display the data, the raw spectrum and the baseline you do not have keep this setting. In order to change the data spectrum that is displayed simply open the 'view' menu on the toolbar, hover over 'Data Spectrum' and deselect the datapoints you do not want displayed. It is important to remember that the default setting will be displayed each time the program is closed and reopened, meaning you will have to change the view each time you open the software.

Measuring an Unknown Sample

Insert the unknown sample into the cuvette holder and close the cover. Proceed as before until the status LED's on the front panel are all showing green. Click "Measure Data" and correct any sample information as needed. The next step is to type in the dilution factor for that particular sample in the text box provided. For example, if the original sample was diluted by a factor of x100 prior to measurement, the user should type "100" (without the quotes) into the "Dilution Factor" text box. If no dilution was used, ensure that the dilution factor is set to "1".

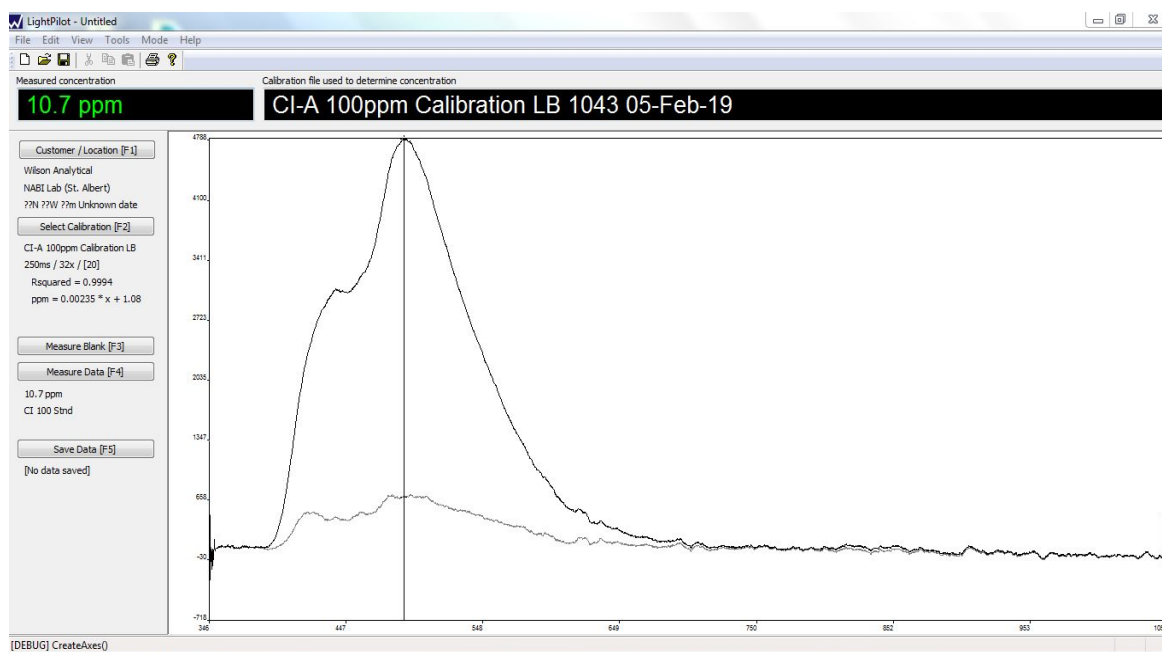
Finally, click on the "Measure" button to collect the sample data. Wait until the *Power or data* LED changes from blue back to green, and then remove the sample from the instrument.



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The fluorescence spectrum of the unknown sample will be displayed on the computer screen. The spectrum display will show a vertical line at the wavelength selected for the calibration calculation. Typically this will correspond to a peak in the fluorescence spectrum from the test sample. Note that the calibration wavelength cannot be changed at this point; it is displayed for reference only.

LightPilot will now calculate the actual concentration of the target chemical in the original sample, based on the dilution factor and the calibration curve that has been selected. The result is displayed at the top left hand side of the screen, and will be written into the data file when it is saved. For example, if the result of the measurement is 3.27ppm, and the dilution factor is x100, the result written at the top of the screen will be [$3.27 * 100 = 320\text{ppm}$].

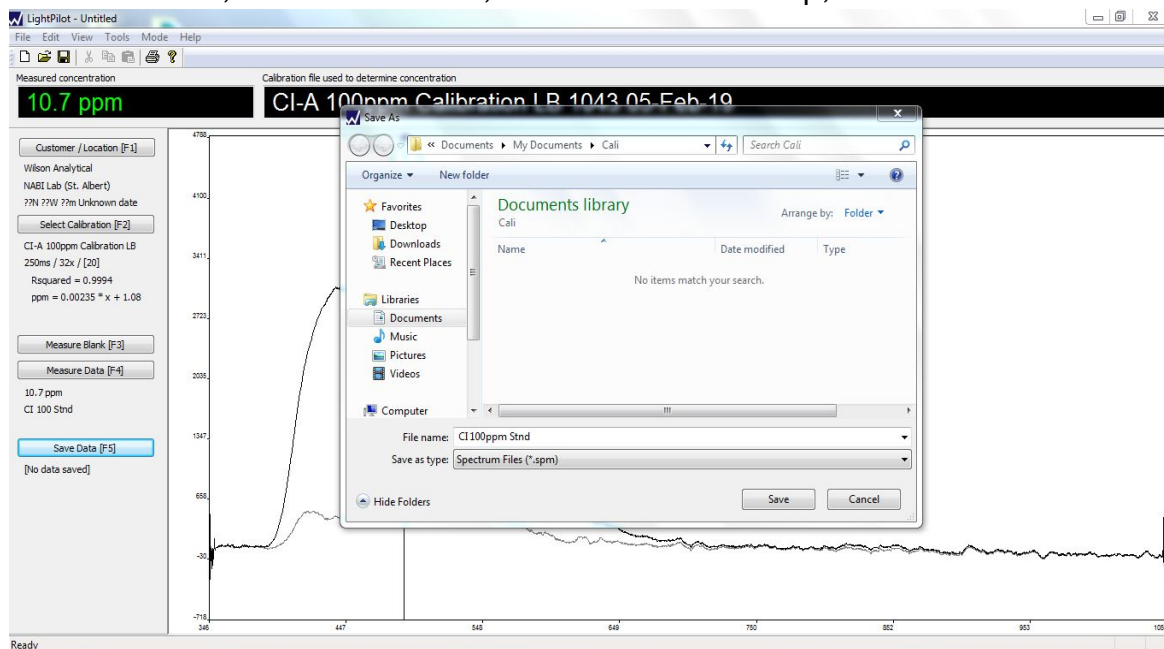


Saving the Data File

The final step in acquiring a new measurement is to save the information in a data file. This is done very easily by clicking on the "Save Data File" button at the bottom left hand side of the screen. Saving the file is not automatic, as it may be that the user notices some problem with the sample, the measurement, is just running a routine test, or is running a non-critical test just to get a feel for the results. However, if the user tries to leave the *Acquisition* mode without saving the data, or overwrites the current data without saving, he or she will be prompted by the software to save the new result.

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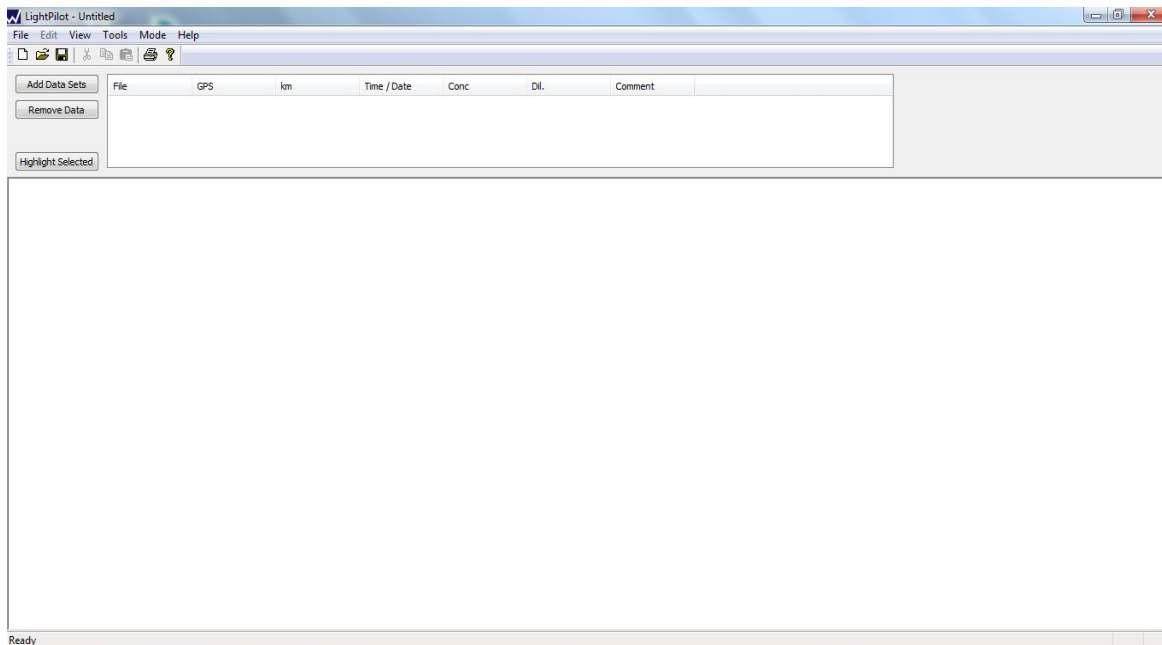
Data files are saved with a “.spm” extension. The files include all of the sample information entered by the user and the complete spectrum obtained from the unknown sample, plus information about the calibration file used, the GPS locators, a date and time stamp, and the unit number.



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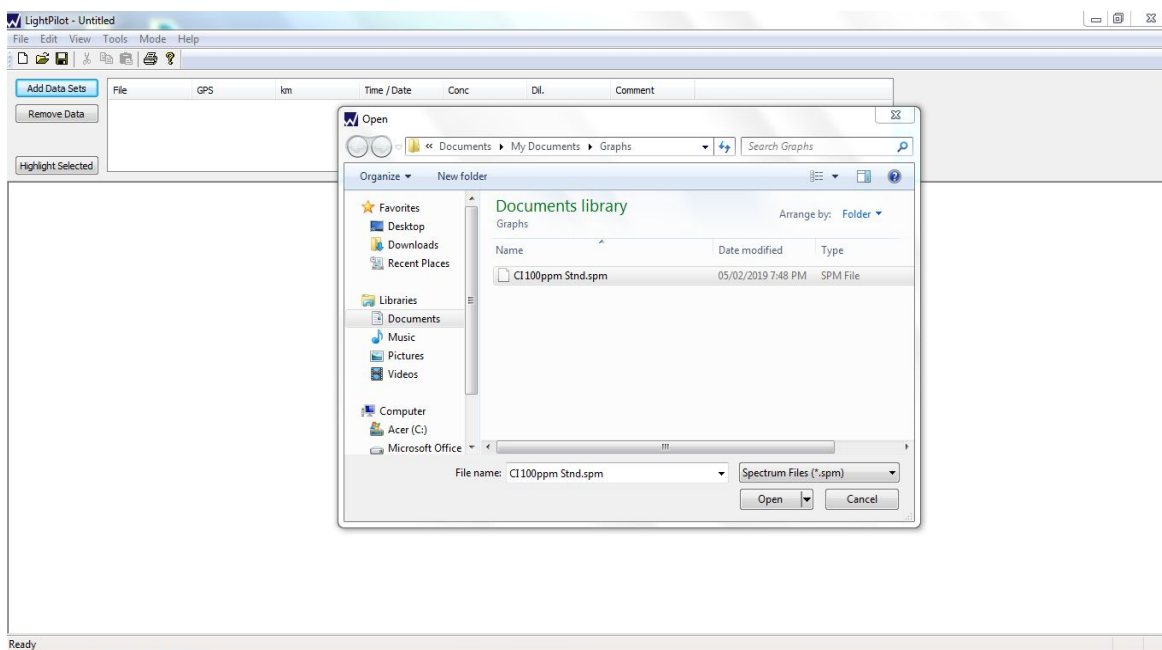
‘Review’ Mode Display and Print Data Files

The third mode of the *LightPilot* software is *Review*, used to display, examine and print data files obtained from the various test samples. From the toolbar, select the ‘*Mode*’ menu, and then ‘*Review*’. No password is required, and the *Review* screen will be displayed.

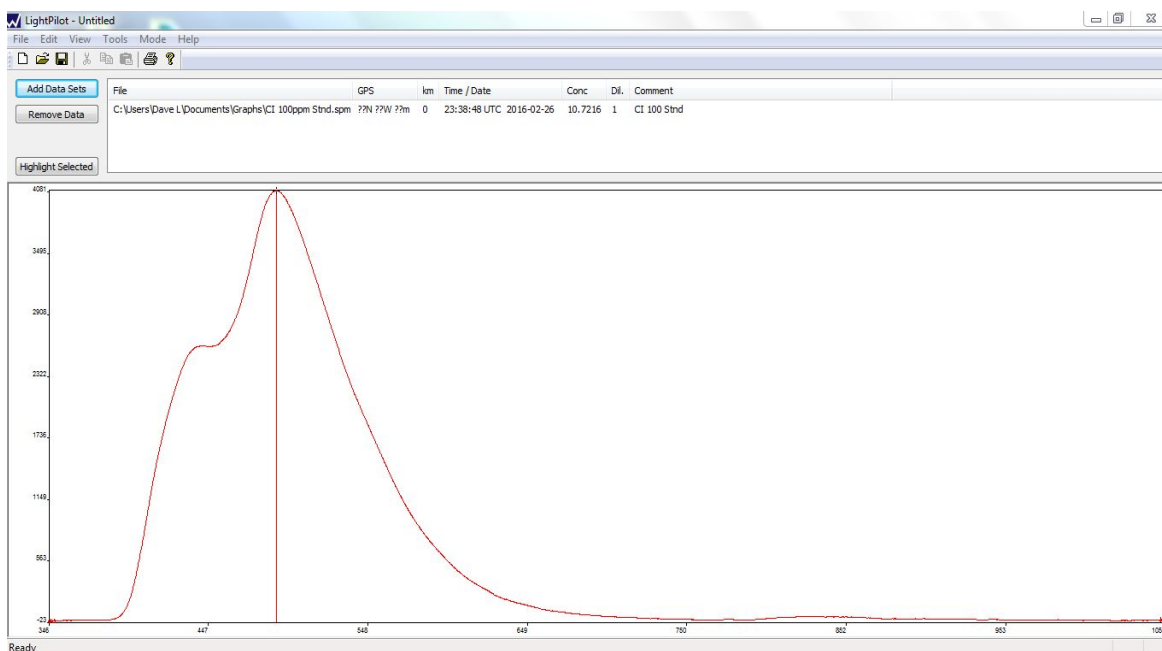


Click on “Add Data Sets”, and a dialogue box appears with a list of all the available “.spm” files. Once selected (double-click), the file opens and the spectrum is displayed in the lower right hand portion of the screen. In this way, the user can display and review multiple spectra.

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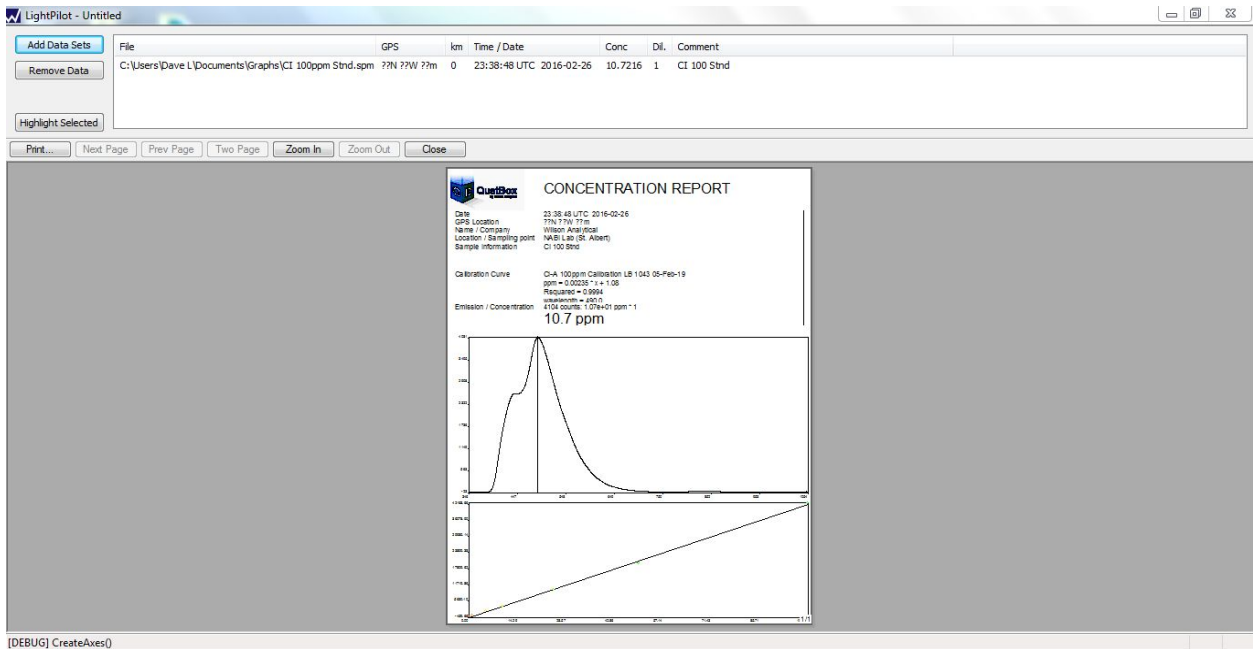
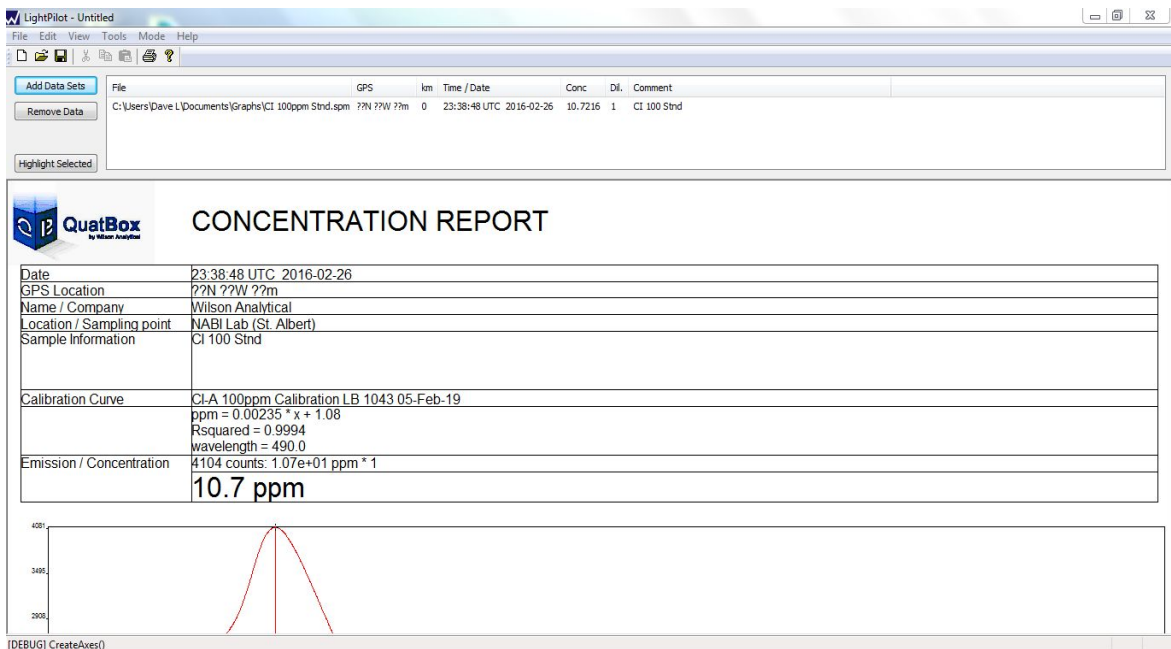


Once one or more spectra are displayed, the user can use the 'View' menu to change what is seen on the screen. The choices include the calibration data that was used to determine the concentration measured for sample being reviewed, as well as the "Raw", "Baseline" and "Data" spectra to see how well the blank subtraction is working for this determination.



Finally a report can be produced, previewed and printed using the graph type "Report".

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