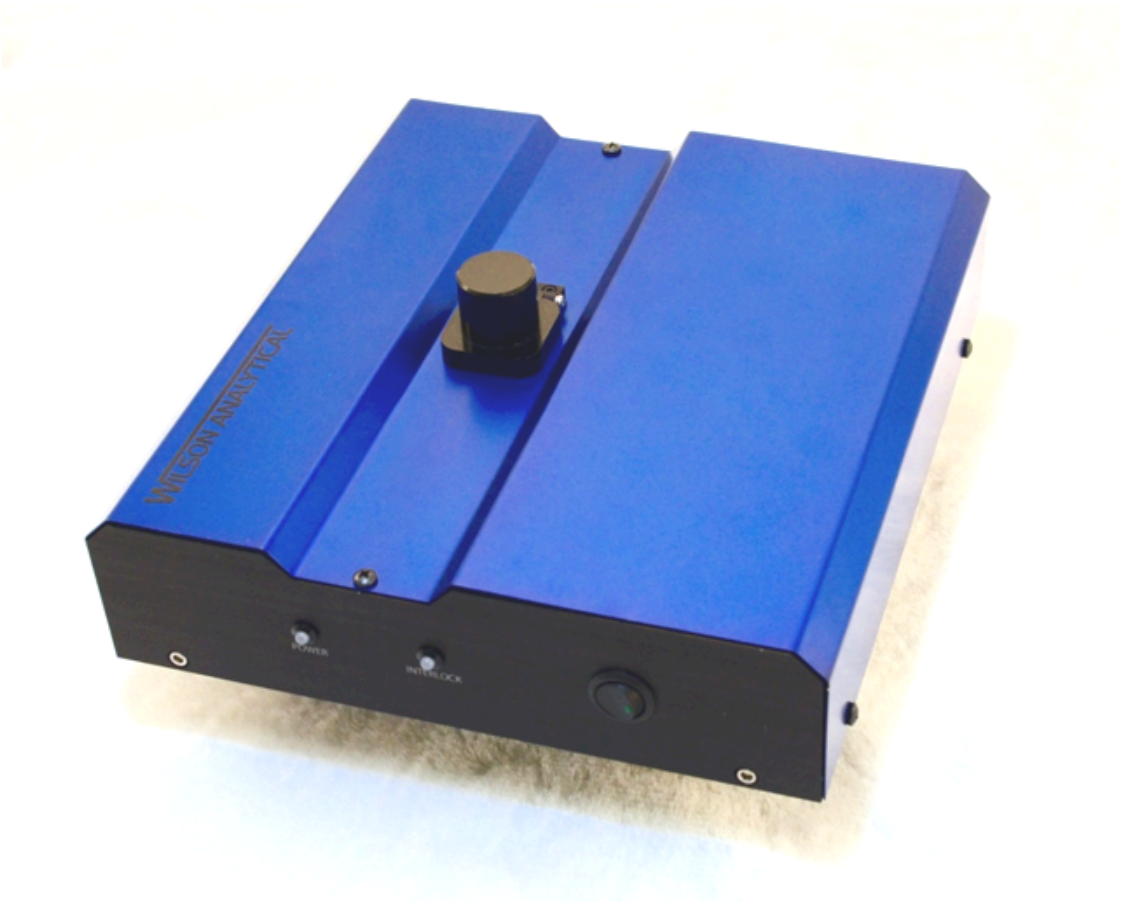
WILSON ANALYTICAL

LabBox User's Manual

Fluorescence or Absorbance Spectrometer



WILSON ANALYTICAL

Table of Contents

Contact Information

Wilson Analytical Services Inc.
#2, 215 Carnegie Drive, St. Albert,
Alberta, Canada. T8N 5B1

Email: support@wilsonanalytical.com
Phone: (780) 702-0610
Toll free: 1-866-3wilson (394-5766)
Website: www.wilsonanalytical.com

WILSON ANALYTICAL

Introduction to the LabBox

The LabBox is a uniquely designed fluorescence or absorbance spectrometer that provides laboratory-quality analytical data at any indoor location. Wilson Analytical's reputation for rugged instrumentation meets enhanced versatility with the LabBox's dual internal spectrometer positions (for fluorescence or absorbance measurements) and a range of high quality light sources to choose from.

Multiple unique features make the LabBox a valued addition to any lab or process control location:

- Optically stabilized LED Light source provides high brightness and long life
- Integrated USB spectrometer and cuvette holder
- Choose to have the unit factory-set for fluorescence or absorbance (transmission) work
- Fluorescence excitation enhanced by factory-selected light sources and optical filters
- Fluorescence detection anywhere within the visible spectrum using the built-in high-sensitivity spectrometer
- Absorbance - select either single wavelength LED or white-light LED illumination
- Absorbance detection anywhere in the high UV or visible using the built-in high-sensitivity spectrometer
- Individual LED wavelengths available at 365 nm (UV), 405 nm (violet), 450 nm (blue), 520 nm (green), 850 nm (near IR) or white-light polychromatic
- Sapphire windows and drain system built into the cuvette holder protect the instrument from spills
- Unit externals are entirely made of anodized aluminum to resist spills and damage
- LED interlocks for system safety and status information indicators

The LabBox can be controlled for fluorescence quantitation work using Wilson Analytical's *LightPilot* software. For information on how to install and run the *LightPilot* Software, refer to the *LightPilot* Software User's Manual. Control of the LabBox for absorbance and transmission measurements uses Ocean Optics' OceanView software, which is also available through Wilson Analytical.

WILSON ANALYTICAL

Set-up & Quick Start

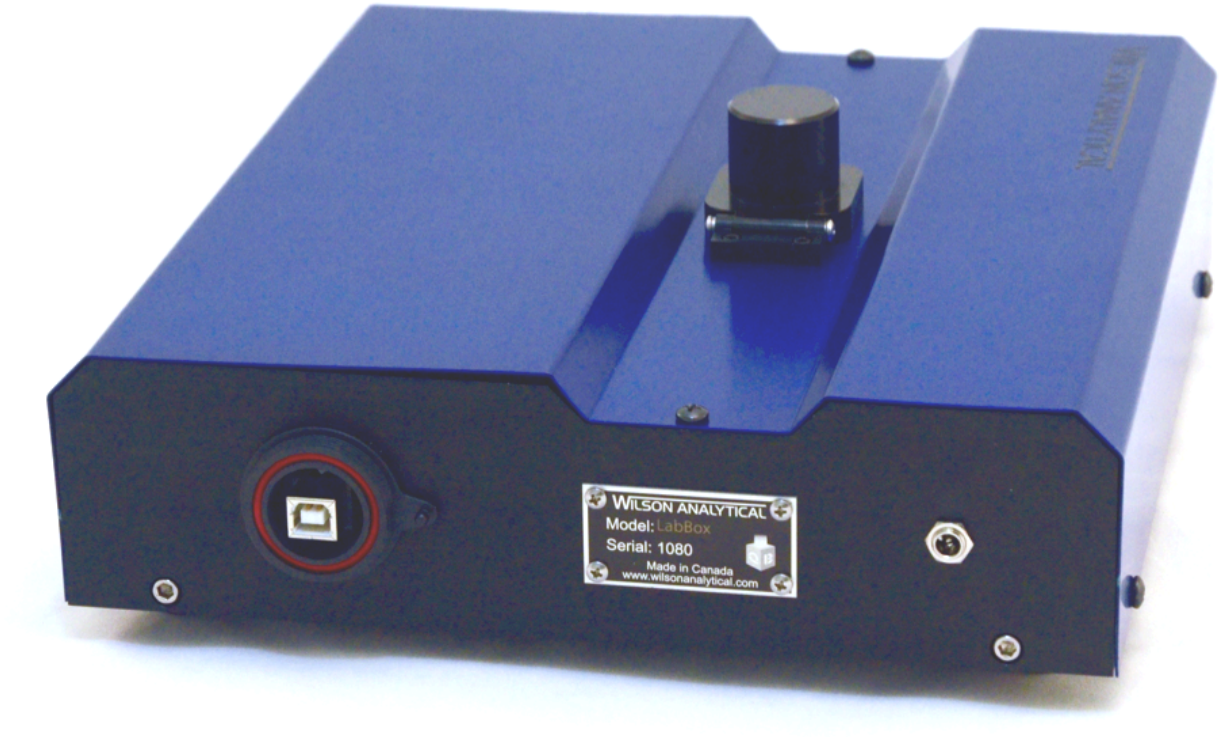


Figure 1. Image of the back of the LabBox showing the USB data port (left) and 12 V power port (right).

1. Using the provided power supply, connect the round 12 V power plug to the back of the LabBox (see Figure 1), and the main power cord to any convenient 120 V socket.
2. Power up the computer and the LabBox (see Figure 2).
3. Connect the computer to the USB port on the rear of the LabBox (see Figure 1) using the supplied USB cable.
4. For fluorescence quantitation work, double click on the blue **W** icon to start the Wilson Analytical *LightPilot* software. Refer to the *LightPilot* Software User's Manual for more information on how to run samples using *LightPilot*.
5. For absorbance or transmission work, use the Ocean Optics OceanView software.

WILSON ANALYTICAL

LED Status Indicators

Depending on the light source chosen, the LabBox can emit intense ultra-violet light. Without a cover for the sample chamber, it would theoretically be possible for this UV light to escape from the LabBox and enter the eyes of the user. As a safety precaution, the LabBox is equipped with an interlock, such that the light source will not operate unless the hat (lid) of the cuvette holder is closed.

Assuming the computer is on and its USB cable is connected to the LabBox, the *Interlock* LED on the front panel of the LabBox will show red when the hat is open, and green when the hat is in closed.

If the computer is not turned on, or if a working connection between the LabBox and the computer has not been established via USB cable, the *Interlock* LED on the front panel of the LabBox will show red. Once the computer is turned on and the USB connection has been made, the *Interlock* LED will show green (unless the cuvette hat is open). Table 1 below explains the indicator colour codes, while Figure 2 shows the front of the LabBox where the LED indicators reside.

Table 1. Interlock and Power LED Colour Codes.

Interlock LED	Power LED	Strobe/Lamp Enabled	Light Source	Conditions
Red	Green	No	Off	System not ready. Computer is off, USB cable is not connected, or cuvette holder hat is open.
Green	Green	No	Off	System ready. The light source is off. Computer is on, USB cable is connected, and cuvette holder hat is closed.
Green	Blue	Yes	On	System measuring. The light source is on. Computer is on, USB cable is connected, and cuvette holder hat is closed.

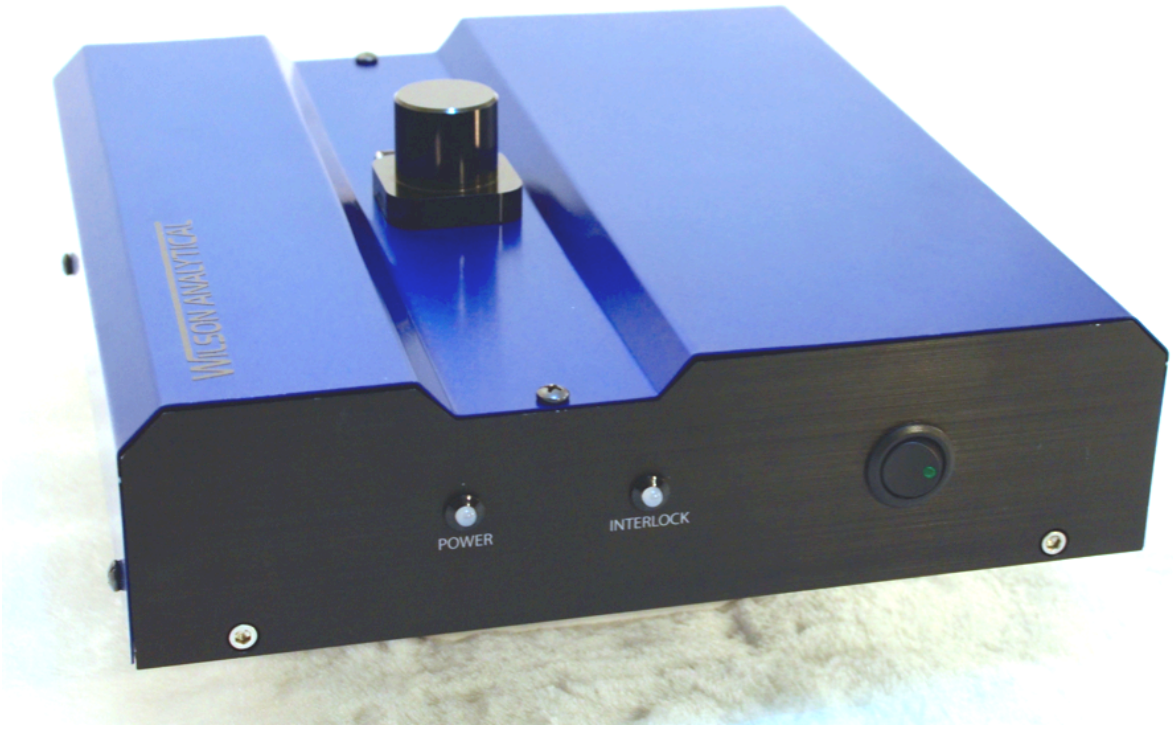


Figure 2. Image of the front of the LabBox, showing the power switch and the LED indicators, and the hinged hat (lid) on the top of the unit, which covers the cuvette holder opening used for samples.

Running Samples

1) To run a sample, open the hat (lid) to access to the cuvette opening (see Figure 2). The *Interlock* LED on the front of the LabBox will turn red while the hat is open (see Table 1).

2) Insert a 10 mm sample cuvette into the cuvette holder opening. The cuvette should be at least half full of liquid as the measurement takes place in the bottom third of the cuvette. The sample must be clear to avoid optical scattering. Any floating solids must be removed by filtration or centrifugation before analysis. A final polish filtration of the solution with a 0.2 micron syringe filter directly into the sample cuvette is often useful.

3) Close the hat. This will turn the *Interlock* LED back to green, and allow measurements to take place (see Table 1).

4) Use the spectrometer software on the computer to acquire data on the sample. The *Power* LED on the front of the LabBox will turn blue while the light source is on, and will return to green once the light source is off (see Table 1).

WILSON ANALYTICAL

Cuvettes 101

The LabBox works very well with disposable plastic cuvettes, which make cell cleaning a thing of the past. These cuvettes are typically 10mm x 10mm x 40mm in size, and are available in various designs and types of plastic, only some of which are suitable for fluorescence measurements, especially at UV wavelengths. Good quality fluorescence cuvettes (with caps) suitable for 365 nm UV excitation are available in boxes of 100 from Wilson Analytical.

Figure 3. Wilson Analytical Disposable Cuvettes

Be aware that the light travels through the lower half of the cuvette, and so when taking measurements, please ensure that cuvettes are always filled to at least the halfway mark (2 mL). It is also a good idea to handle cuvettes only at the top, because grease, smears, dirt and fingerprints on the outside of the cuvette will interfere with the accuracy of the measurements. We suggest writing sample information on the top 1/3 of the cuvette in order to distinguish between samples. Remember that all cuvettes look alike! It is also important to ensure that the outer surfaces of cuvettes are dry before they are placed into the LabBox sample holder. Drops or films of water or liquid will refract and scatter light, reducing the accuracy and repeatability of the measurements.

Finally, please note that plastic sample cuvettes are incompatible with many organic solvents, including alcohols. If you are planning to use solvents in your work, we strongly recommend that you

ensure beforehand that the specific plastic of the cuvettes (Wilson cuvettes are PMMA) will stand-up to the chemicals in question. If not, it is possible to obtain glass or quartz cuvettes that are obviously far more chemically robust, but will need to be cleaned between samples.

Using the Wilson Solid State Reference (SSR) QC Sample

A solid-state reference (SSR) sample is supplied with each LabBox set up fluorescence determinations with 365 nm excitation. The reference material is encased in an anodized aluminum holder that fits snugly inside a regular plastic 10mm x 10mm cuvette. The reference is obviously designed for re-use, and so the plastic cuvette acts as a protective housing. If and when the external plastic cuvette becomes scratched, damaged or uncleanable, simply slip the reference out of the old cuvette and place it in a new one.

Figure 4. Solid State Reference Standard

The Wilson SSR works as a “relative” fluorescence reference sample, which is well suited to its purpose as a Quality Control (QC) check to ensure that the instrument is running correctly and is calibrated properly prior to measuring samples. Since the concentration value obtained for the SSR is dependent on the calibration curve used to run it, providing a “certified” ppm value for the SSR is not possible. Its “value” is always determined experimentally for each calibration curve produced. See Figure 5 for a plot of the spectrum obtained on the Wilson SSR when run in a LabBox with 365 nm excitation.

Once a calibration curve is completed for a particular fluorescent material measured by the LabBox, the QC value for the SSR should be determined (assuming that the wavelength selected for calibration works with the SSR. The SSR is useful from approximately 400 to 550 nm. See Figure 5). The new curve should be used to determine the numerical concentration value of the Wilson SSR for at least 30 measurements, preferably collected over the course of a week or so. The average value and standard deviation of the measurements are then calculated and recorded as the QC value (the average) and range (+/- 2 std. dev.) for the SSR for that particular calibration curve. At the beginning of each session

(or whenever the user wishes to check that the LabBox is operating normally) simply select the appropriate calibration file, run a blank determination, then insert the SSR standard and acquire a spectrum. If the LabBox returns a value within two standard deviations of the accepted QC value for the SSR when using the specified calibration curve, then it is operating normally. Remember that a different QC value (the average) and range (+/- std. dev.) will be obtained for each calibration curve. However, if machine performance is all that is being verified by the QC measurement (not each calibration curve individually), then the same calibration curve can safely be used each time to ensure that the instrument is functioning correctly.

If the reference check returns an out of range value for that curve, the most likely causes are a dirty or damaged SSR outer plastic cuvette, and/or dirty windows in the sample holder. If the cuvette holding the reference standard appears to be clean and undamaged, proceed with cleaning the windows in the cuvette holder and then re-measure the reference. The LabBox should then return a reading within the accepted value if operating normally. If the received signal is still not within the specified range, repeat the process. If this does not work, contact Wilson Analytical.

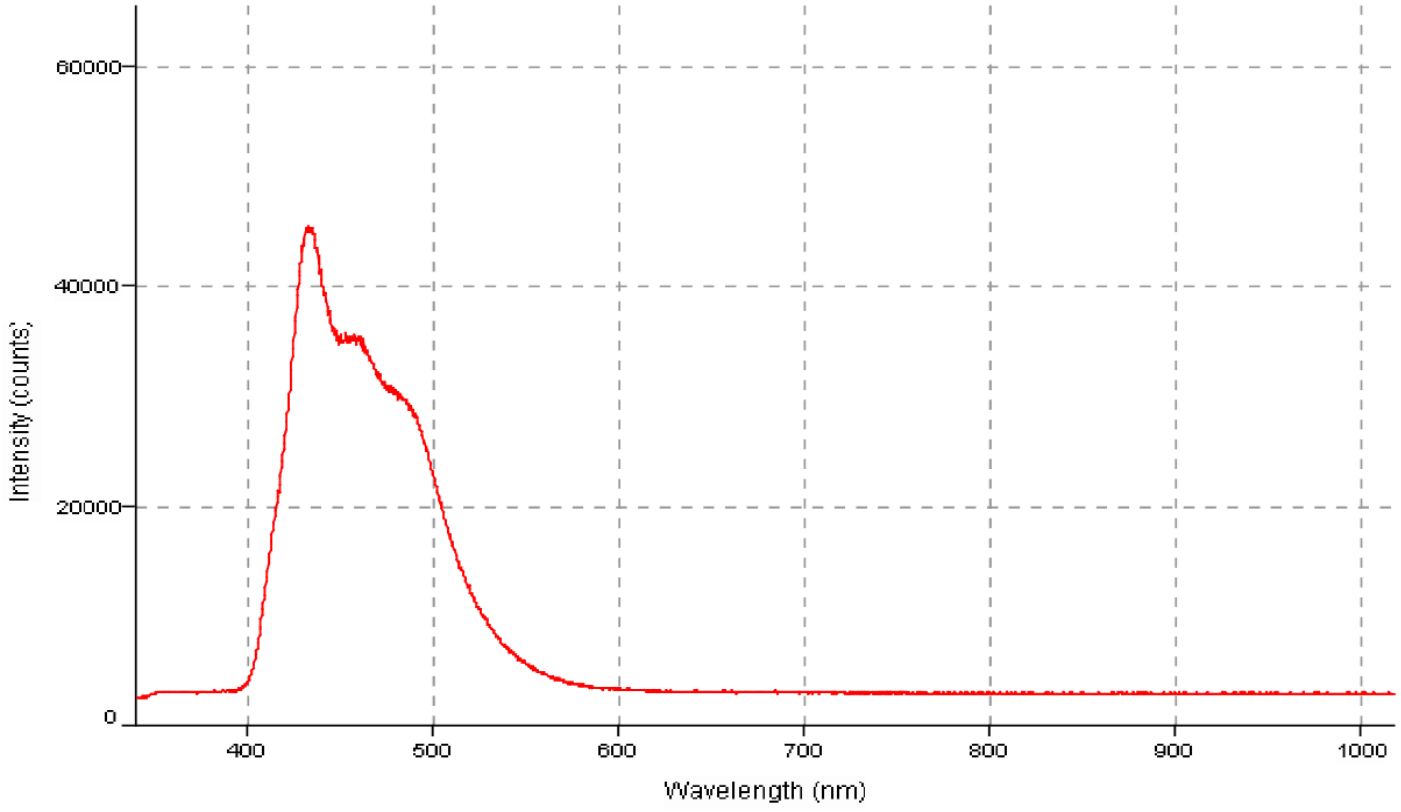


Figure 5. Fluorescence spectrum obtained with 365 nm excitation of the Wilson SSR QC Test Sample

Cleaning the Sapphire Windows in the Cuvette Holder

The LabBox is an optical measurement system, so dust, dirt or spills inside the cuvette holder may affect the performance of the unit. If the Wilson SSR QC sample returns a low value when run, it is likely that the cuvette holder windows need to be cleaned.

The optical windows inside the LabBox cuvette holder are manufactured from single crystal sapphire, making them extremely resistant to scratches and chemicals. Nevertheless, any damage or staining to these windows will adversely affect the results from the LabBox, and so care should be exercised during the cleaning process. Never insert sharp objects into the cuvette holder.

The sapphire windows, which are located near the bottom of the anodized aluminum cuvette holder, are waterproof and are gasketed in place. This makes the entire cuvette holder assembly internally watertight. In addition, there is a drain in the bottom of the cuvette holder, which exits through the bottom of the LabBox. This design allows the windows to be cleaned while still inside the fully assembled LabBox.

To clean the windows, it is only necessary to open the cuvette holder lid, and to use a test-tube brush or cotton swab with soapy water to clean the inside of the cuvette holder. Gentle pressure on the surface of the windows at the bottom of the cuvette holder is quite acceptable, but do not attempt to clean them by abrasion. The excess cleaning solution will drain out the bottom of the LabBox, as will any rinse water used to remove residual soap solution from the windows and cuvette holder after cleaning. Dry the windows as efficiently as possible using a Kimwipe wrapped around the end of a cotton swab, then check with a flashlight to ensure that no Kimwipe pieces remain in the cuvette holder. A final rinse with methanol can be used to speed drying, as can a flow of warm air through the cuvette holder. Leaving the lid open will also enhance drying.

After cleaning, insert the Wilson SSR, select the appropriate calibration file and acquire a spectrum. The LabBox should now return a reading within $^{+}$/- 2 std. dev. of the accepted value for that calibration curve to be operating normally. If the received signal is still not within the specified range, repeat the process. If this does not work, contact Wilson Analytical.

Exterior Maintenance

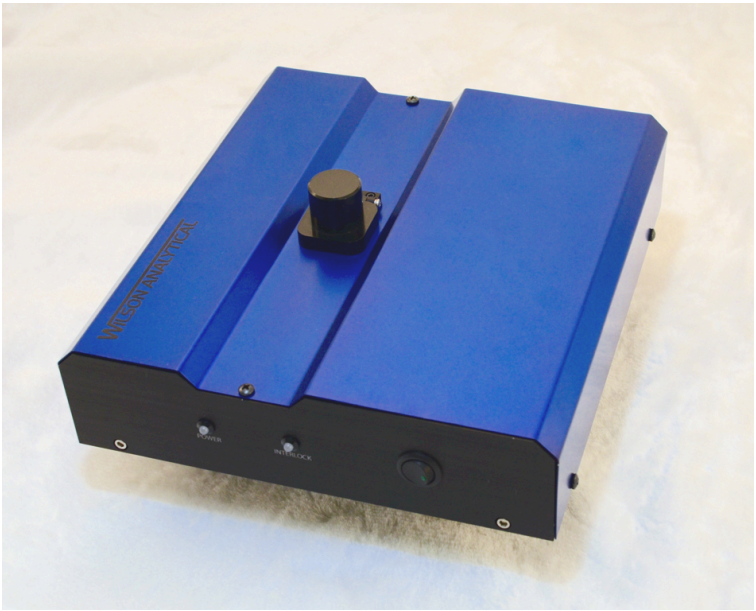
The most damaging chemicals to the sample holder, and indeed to the LabBox itself, will be strong acids or alkalis, because the outer case and sample holder are manufactured from aluminum. Although all the accessible LabBox components have been anodized or coated to provide chemical

and corrosion resistance, the lifetime and accuracy of the unit will be enhanced if its surfaces are kept clean and chemical-free by wiping the exterior as needed.

Technical Support

If you are experiencing problems with your LabBox, please refer to the relevant section of this user manual for advice and troubleshooting suggestions. For example, low measured values may be due to dirty optical windows. If referral to the manual and the remedial action suggested has not solved the problem, please contact Wilson Analytical. If using email, please remember to include your e-mail address and phone number in any correspondence.

A representative of Wilson Analytical will contact you as soon as possible to discuss the problem. If the situation cannot be remedied remotely, a Return Authorization Number (RAN) will be issued. At that point, the unit must be returned to us for repair. Unless the instrument is under warranty, it should be shipped pre-paid, and the enclosed documentation should include the RAN that was issued to you, your contact details and shipping address, and a summary of the problems you have encountered with the unit.



www.wilsonanalytical.com

WILSON ANALYTICAL

Appendix-- Wilson Analytical LabBox

Best Practices for Measuring Corrosion Inhibitor Residuals with the LabBox

Measuring Sample Concentrations in the Linear Range

The way to get the most accurate results from any analytical technique is to make sure there is a “linear relationship” between the concentration of the chemical that is being measured, and the output of the measuring device. In other words, if a concentration of 50 ppm gives a signal, or response, of 1000 counts, a sample with 100 ppm of the same chemical should give a response of 2000 counts. When this is true for a given system, we say we are working in the “linear range”.

Things are no different with fluorescence, and for most fluorescent chemicals, there is a natural range where a doubling of the concentration gives a doubling of the detector response. The exact range will depend on the chemical being used, but typically, concentrations below about 100 ppm will give a linear response.

Above this natural “linear range”, what happens is that an increase in sample concentration gives less than the expected increase in detector response, and great care must be taken not to underestimate the amount of chemical truly present. This phenomenon is called “quenching” of the fluorescence.

As 100 ppm is less than the required dose for many field chemicals, the phenomenon of quenching means that samples obtained directly from tanks, flowlines or other process equipment must usually be diluted before being measured. For example, if the expected concentration of chemical in a process stream is 500 ppm, a dilution of at least 10x, and perhaps 100x is necessary to ensure that measurements are being taken in the “linear range”.

The LabBox uses an intense light source to make accurate fluorescence measurements possible down to 1 ppm, or in some cases even lower. This means that sample concentrations of 100 ppm (already on the edge of the linear range) can be further diluted by a factor of 100x if necessary and still be measured accurately. This level of dilution has the additional great advantage of reducing matrix effects, such as extreme salinity, to insignificant levels.

Measuring Sample Concentrations as Quickly as Possible

Chemicals that can be measured by fluorescence are detectable because they absorb light. Sometimes this light is in the visible range, but often it is in the ultra-violet, invisible to the naked eye, and of much higher energy (It is over-exposure to ultra-violet radiation that causes sunburns). The fluorescence process is very fast indeed, with the LabBox collecting data in less than a minute, and this speed of measurement is an advantage over other techniques, such as chromatography, that typically take 30 minutes or more to complete.

However, the absorption of light, as well as making fluorescence possible, can destabilize the molecule being detected, and even cause it to chemically change and degrade. This is a much slower process than fluorescence (hours to days), but this photo-sensitivity means that samples that are stored in glass or clear plastic containers will exhibit lower and lower fluorescence signals over time. If the amount of light (especially bright sunlight in the summer) being received by the sample is uncontrolled during transport and storage, then the concentrations measured in those samples will often be significantly lower than the true (original) values. Care taken to avoid excessive light exposure during the transport and storage of samples will of course help alleviate this problem, as will rapid transport and analysis of the samples.

Be Aware of Interferences and Remove Them

Fluorescence is an optical analysis technique, and is adversely affected by solids and oil droplets in the water samples being tested. Water samples must be thoroughly filtered or centrifuged prior to analysis by the LabBox until no visible oil or solids are present. Unless the original sample was full of oil, two filtrations are usually enough. Use a fresh filter for each filtration for increased filtration efficiency, and to avoid sample-to-sample contamination. A final polish filtration of the solution with a 0.2 micron syringe filter directly into the sample cuvette is often useful.

Many organic chemicals will absorb light and exhibit fluorescence, and sometimes there are naturally-occurring substances in water samples that can mask, or interfere with the fluorescence signal from the chemical of interest. This can happen for example when the water sample comes from an oil or gas producing formation, in which case the produced water can contain water soluble organic substances such as naphthenic acids, or polyaromatic hydrocarbons that have strong fluorescent responses.

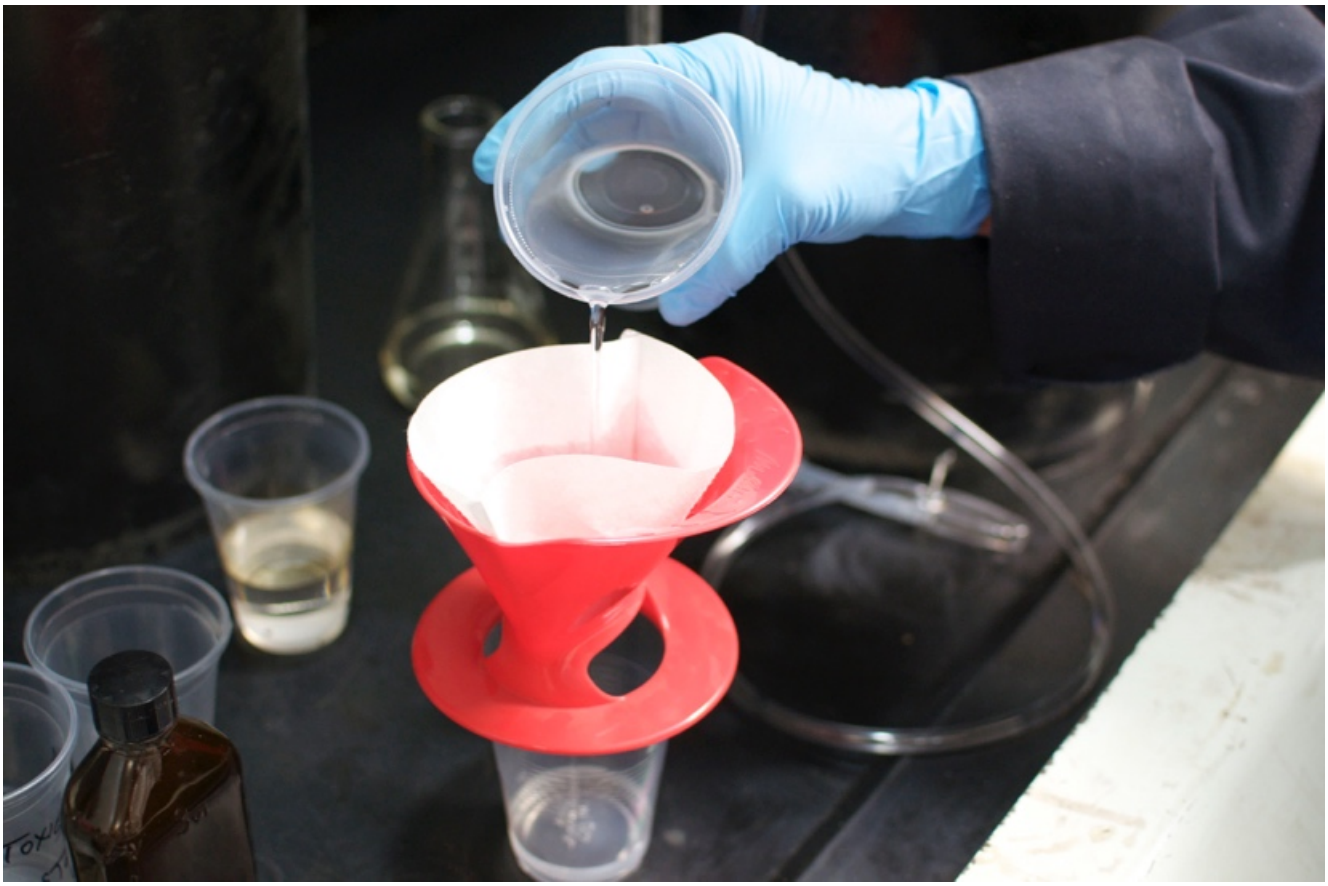


Figure 6. Sample being filtered prior to analysis on the LabBox

The LabBox is a full fluorescence spectrometer that shows the user the complete fluorescence spectrum of each sample. Even without being trained in fluorescence, it will be obvious to most users that the fluorescence spectrum from, for example, a corrosion inhibitor of interest, is the same time and time again; only the intensity of the spectrum varies with the concentration. However, if a fluorescent interference such as a naphthenic acid is present in the sample, the spectrum will look completely different, alerting the user to the contamination in the sample.

Once interferences such as naphthenic acids or polyaromatic hydrocarbons have been encountered, they must be removed from the samples before meaningful data can be obtained. The easiest way of doing this is to include an absorbent filter in the dilution step, such that the desired volume of sample passes through a solid phase extraction (SPE) cartridge, chosen to remove that specific interference.