Operating Manual
Fluorescence Lifetime Micro Oxygen Monitoring System

Manual version 3.1
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INSTECH

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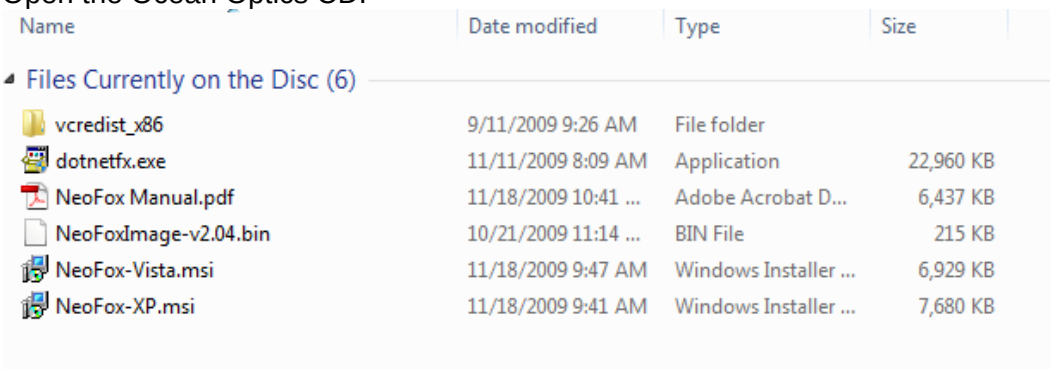
Set Up Hardware and Install Software

1. Unpack all parts.
2. If you have ordered more than one channel, you will receive a 10 port USB hub to connect all NeoFoxes to your PC. More channels can be added without changing the hub. The small end of the USB should be connected to the rear of the hub. The other end to an unused USB input on your PC. It will not be necessary to attach the hub power supply for only 2 channels. The computer should recognize the hub and automatically install it.
3. For single channel units, no hub is required and the USB cable should be connected directly from the NeoFox to the PC.
4. Open the Ocean Optics CD.



5. The NeoFox Manual.pdf is rather lengthy and printing of the entire manual is not necessary. Keep the file on your desktop for reference.
6. Install the software by running the appropriate .msi file. Make sure **not** to run the Viewer before the USB drivers have been installed.
7. Attach each Neo to the hub with cables provided and power on the Neos.
8. New hardware message should come up and accept the Neos.
9. Once the units have been recognized as USB ports, it is best to Check the Device Manager in My Computer—Properties-Hardware for the presence of Ocean Optics Neofox in the USB list. Once verified, then it is OK to start the viewer.
10. When the Viewer is started, tabs corresponding to the serial number of the Neos should be seen above the display as it cycles through and detects them.
11. You switch between them by clicking the top tabs (active one is the lighter color).
12. Instech will have installed the latest NeoFoxImage bin file-Now at v2.05.

Set NeoFox Parameters

The Instech chambers can come with two different fluorescent patch materials.

FOXY patches are best for general purpose applications where exposure to alcohol can be avoided. Alcohol cannot be used in the chamber without seriously degrading the FOXY patch coating. Signals from this material will be high with lowest noise.

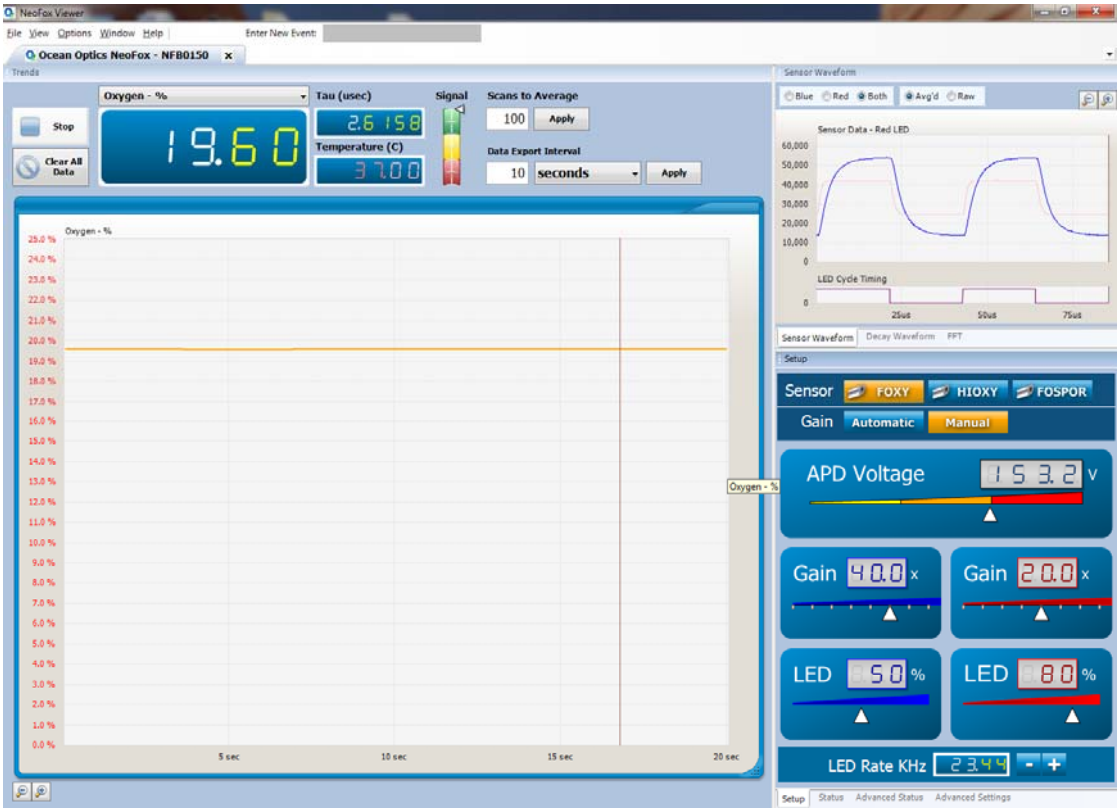
HIOXY patch versions will be resistant to organic solvents but will result in lower signal levels and slightly higher noise levels. This material is recommended for mitochondrial studies where it is necessary to rinse out inhibitors with alcohol. If water soluble inhibitors are used then the FOXY material is preferable. Since the gains have to be set high for this material, it makes it more susceptible

to room light interference and will add to the noise. Covering the top of the glass plug will reduce this effect.

Parameter Setup for each channel is required prior to calibration and to set the system for the patch in the chamber and will be different depending upon the patch material. Changing these parameters after calibration may affect the readings.

FOXY SETUP

1. In the View pull down select Advanced Settings.
2. Select FOXY for the sensor
3. Select Manual Gain.
4. Use the checkout sheet provided and set the values to match the sheet for initial operation.
5. Check Blue amplitude signal level by displaying Advanced Status. It should be in 10,000 range. The small arrow on the Signal level bar graph should be in the green area.

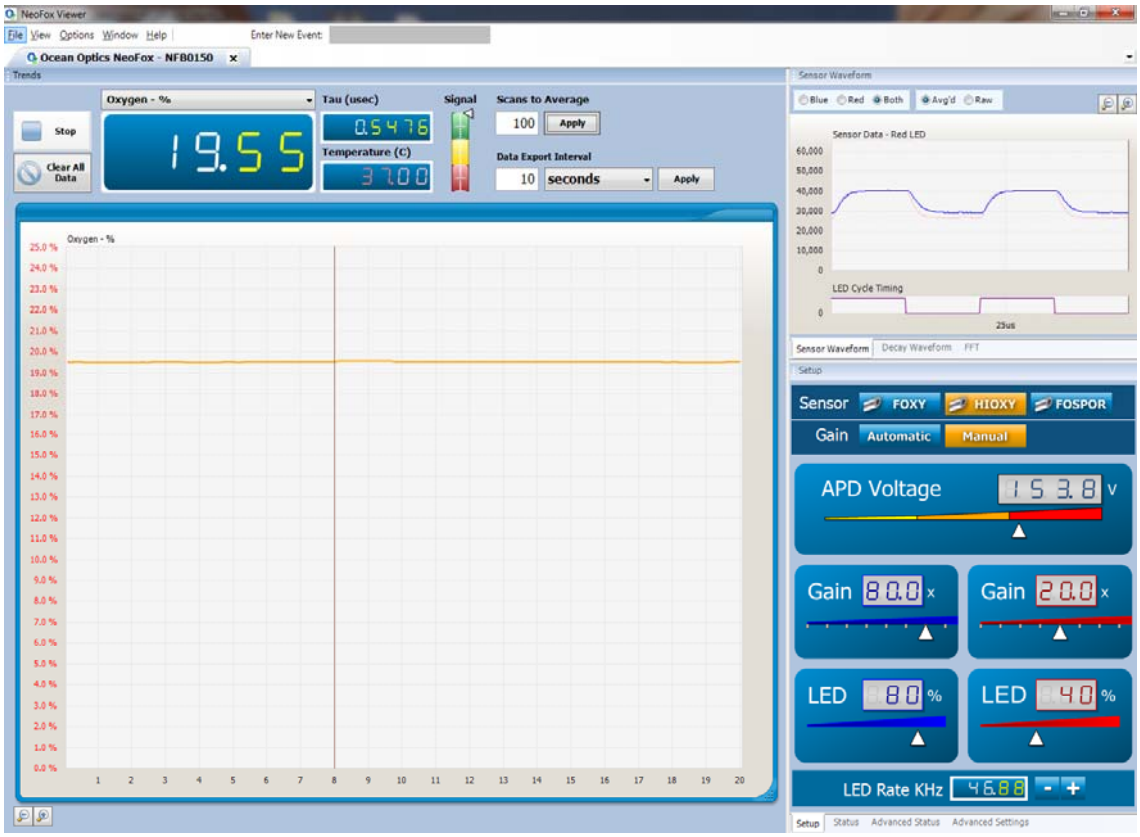


Typical FOXY Setup

HIOXY Setup

1. In the View pull down select Advanced Settings.
2. Select HIOXY for the sensor
3. Select Manual Gain.
4. Change LED rate down to 23.44 by hitting the – button.
5. Alter Blue gain and LED and possibly the APD voltage, until blue sensor signal looks like that shown below in the sensor waveform panel.

6. Check Blue amplitude signal level by displaying Advanced Status. It should be in 3000 or greater. The small arrow on the Signal level bar graph should be in the green area.



Typical HIOXY Setup

The Auto mode in Gain setting does not always select the optimum values and once set in Manual mode it can be left in that condition.

Scans to Average should be set around 100, each scan is 10 msec. or a 1 second averaging time. This will reduce noise without degrading data dynamics of data acquisition since the patch response time is longer than 1 sec.

Temperature Probe

If you have a temperature probe, insert it into the block and attach the cable to the rear of the NeoFox mini-phono input.

It is not necessary to have more than 1 probe, even for multiple chambers as long as they are in series from a single circulating water bath.

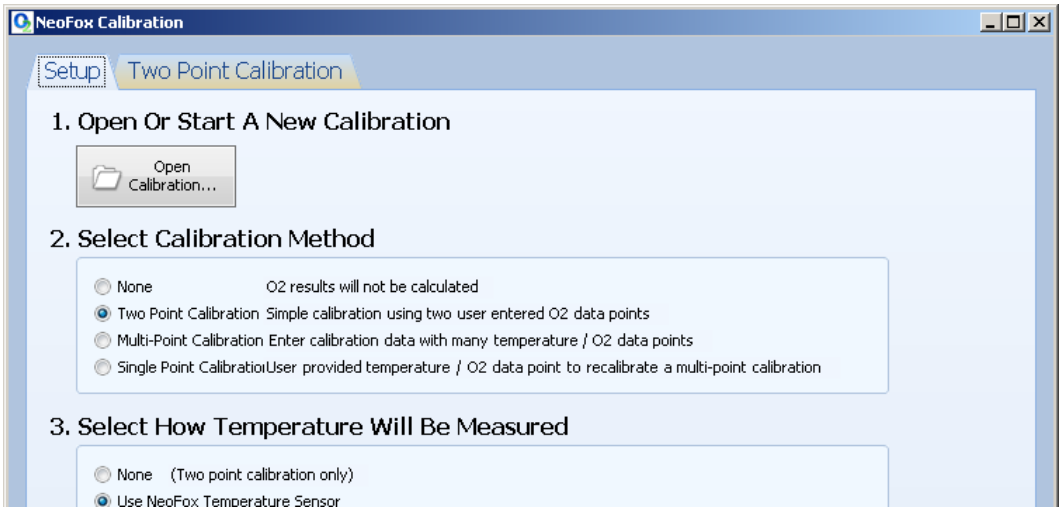
In the Calibration screen there is a place to use the probe as the temperature input. Select that for the Neo that has it attached.

Once that temperature is known, select “Use fixed temperature” on the others and manually enter the operating temperature.

By doing this, the “micromoles/liter” display will be compensated for temperature, no other units will be effected.

Calibrating Probes

Meaningful Oxygen values will be displayed only after a Two Point Calibration has been performed for each channel.

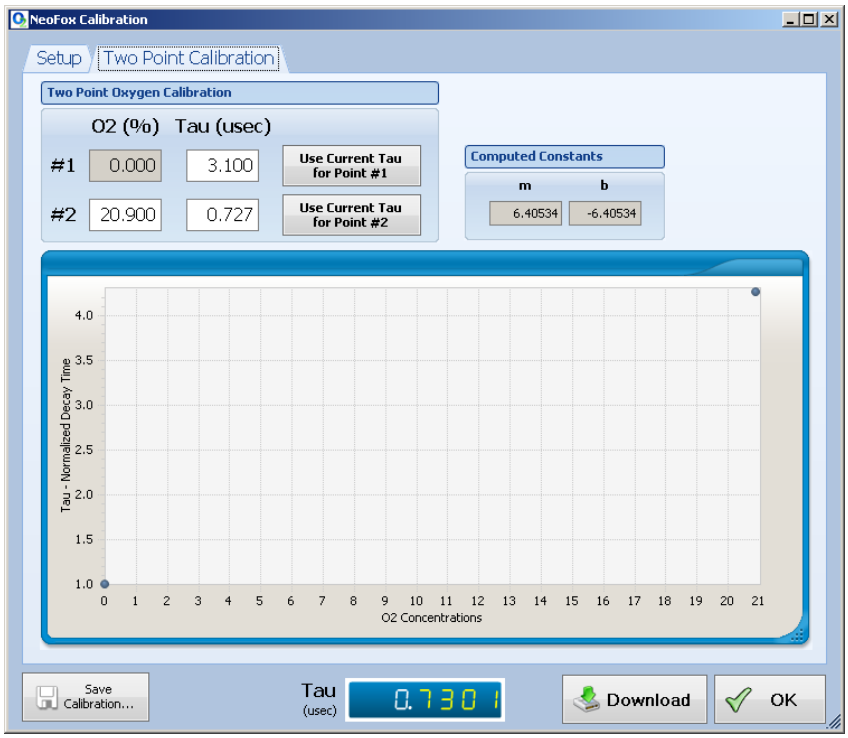


Calibrations should always be performed at your final operating temperature which is the reason the circulating water bath connections. Oxygen values are temperature sensitive. Start with the chamber that has the temperature probe installed. Record that temperature and insert the value into the temperature field for all other chambers in their respective calibration screens and select No temp probe installed. This will result in the proper conversion to micromoles/liter.

1. Remove the plug and place air equilibrated buffer into the chamber, making sure it is at temperature. Take care with this step. Place buffer in a vial in the water bath with an air space above the fluid and shake frequently.

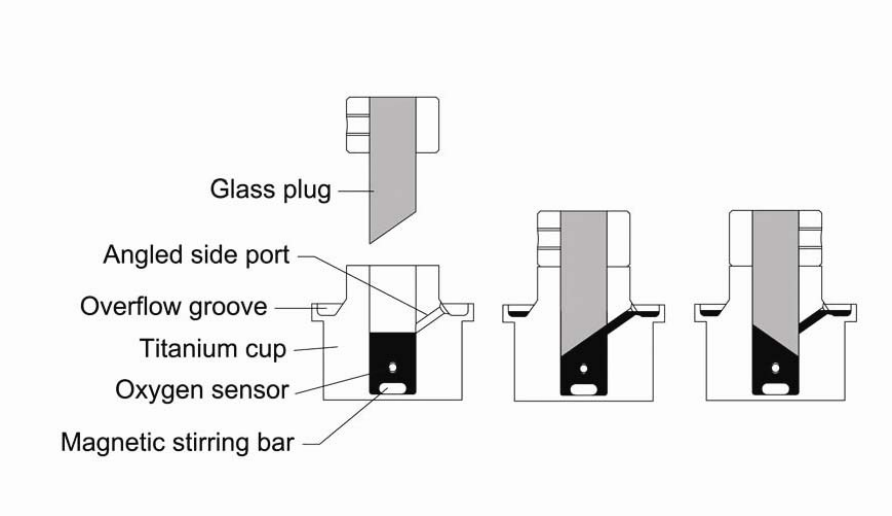
   Remember you will start in % Oxygen units. The percent oxygen is *not directly 20.9%.* This is only true when measuring gaseous oxygen. The partial pressure in mmHg is .209*(760mmHg-Vapor pressure of water at your operating temperature* see table in Appendix 1). Percent will be this value divided by 760. For example, at 37C partial pressure should be 148.9 mmHg and 19.6%. Use this value as your ambient sample calibration value.

2. Click the button to enter the current tau reading in point #2.



3. Add sodium dithionite crystals into the chamber, while stirring, until the tau stops increasing. Titrate for maximum tau. Remember this amount. Excess is permissible. Use that value for point #1. Remember to click in the graph area before downloading the calibration values to each Neo unit. Two blue dots will update. Calibrations are stored there, not in your computer, and will be recalled for the next use. If you do not click the graph first, the calibration will not update.
4. Rinse well and repeat for other chambers.
5. It is good practice to record the tau values for future comparisons.
6. At this time, oxygen values in excess of 100 will not be displayed in the oxygen window but will be correctly logged and graphed.
7. Temperature probes are for information and do not compensate any of the readings except for micromoles/liter. Again, only one sensor is needed for the string of chambers.

Operating the Chamber



1. Remove the plug/collar assembly by lifting straight up.
2. Add liquid to the chamber-slightly in excess of stated volume.
3. Orient the plug and collar assembly so that the high side of the bevel aligns with the angled fill port hole (leftmost illustration). Set screw should indicate the high side.
4. Slowly lower the plug while checking for bubbles and allow the excess to spill out into the cup
5. Seal the chamber by rotating the plug assembly 180 degrees (rightmost illustration).
6. To minimize air leakage, leave the small column of fluid in the angled fill port.
7. Additions may be made by rotating the plug to the fill position (center). Use a needle or narrow tipped pipette that allows excess fluid to pass by and spill into the cup ring. The long needle or tip is preferred to make the additions at the bottom of the chamber and the overflow is at the top.
8. Check that no air has been injected. Eppendorf pipettes will finish with an air bubble and are not recommended to be used in their normal mode.

The chamber volume is approximate and should be checked by the user.

Please try to minimize spills to prevent damage to the stirring motors.

Maintenance

No maintenance is required for the long life sensing patch as it is part of the chamber. If damaged, the cup will need to be returned for repair.

Cup Removal or Replacement

1. Remove the glass plug.
2. Remove the SMA fiber connection from the block coupler.
3. Loosen the setscrew across from the fiber attachment.
4. Lift the cup vertically out and remove the stir bar.
5. Smear the underside of the new cup with silicone grease to prevent spilled liquid from running under the cup lip and entering the stirring motor cavity.

6. Drop the cup into the block and twist to visually align the sensor hole with the coupling sleeve. This will distribute the silicone grease as well.
7. Replace the SMA coupler into the sleeve.
8. When properly aligned, the tip of the SMA connector will engage the recess in the titanium cup. Gently rotate the cup back and forth until rotation is stopped by this engagement. This assures final alignment is correct. Use the blue tubular wrench in this process.
9. Run the software for this channel to assure signal is maximized. When properly positioned retighten the cup hold set screw (front top of block).
10. Once parameters have been set for this patch material, switch view to show Advanced Setup and scroll down to Blue Intensity. It should be 3000 or greater. Much greater is OK If it is low, either the alignment is incorrect or parameters need to be adjusted.
11. Replace stir bar and glass plug.

Replacing Glass Plug/Valve

Chips may make it difficult to clear air bubbles.

1. Remove the plug.
2. Loosen the setscrew and remove the glass.
3. The correct position of the plug within the black collar is when the glass is flush with the top edge of the collar.
4. Align the setscrew with the highest point of the bevel to permit clearance of air bubbles.
5. Retighten set screw.

Installing Firmware Updates

1. Make to new Image file available e.g. on the desktop.
2. Stop data collection.
3. Select Options, then Firmware Update and follow prompts
4. Do not disconnect power during the update.
5. Repeat for each unit to be updated.

Appendix 1

Saturated Vapor Pressure, Density for Water

Temp (°C)	Temp (°F)	Saturated Vapor Pressure (mmHg)	Saturated Vapor Density (gm/m^3)	Temp (°C)	Temp (°F)	Saturated Vapor Pressure (mmHg)	Saturated Vapor Density (gm/m^3)
-10	14	2.15	2.36	40	104	55.3	51.1
0	32	4.58	4.85	60	140	149.4	130.5
5	41	6.54	6.8	80	176	355.1	293.8
10	50	9.21	9.4	95	203	634	505
11	51.8	9.84	10.01	96	205	658	523
12	53.6	10.52	10.66	97	207	682	541
13	55.4	11.23	11.35	98	208	707	560
14	57.2	11.99	12.07	99	210	733	579
15	59	12.79	12.83	100	212	760	598
20	68	17.54	17.3	101	214	788	618
25	77	23.76	23	110	230	1074.6	...
30	86	31.8	30.4	120	248	1489	...
37	98.6	47.07	44	200	392	11659	7840

Courtesy of hyperphysics.phy-astr.gsu.edu/HBASE/kinetic/watvap.html