Instech Oxygen Flow Cell Setup with FOXY OR PHOSPOR PATCH MATERIAL

Manual Version 3.82

Set Up Hardware and Install Software

1. Unpack all parts,
2. If you have ordered more than one channel, you will receive a multiple 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 cable 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. Install the software by unzipping and running setup from the thumb drive. Make sure **not** to run the Viewer before the USB drivers have been installed.
5. Attach each Neo to the hub with cables provided and power on the Neos.
6. New hardware message should come up and accept the Neos.
7. 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.
8. 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.
9. You switch between them by clicking the top tabs (active one is the lighter color).
10. Instech will have installed the latest NeoFoxImage bin file.

Hardware Setup

The inlet and outlet fittings are modular and can ordered with Luer or barb fittings for different size tubing. Luer fittings are shown below.

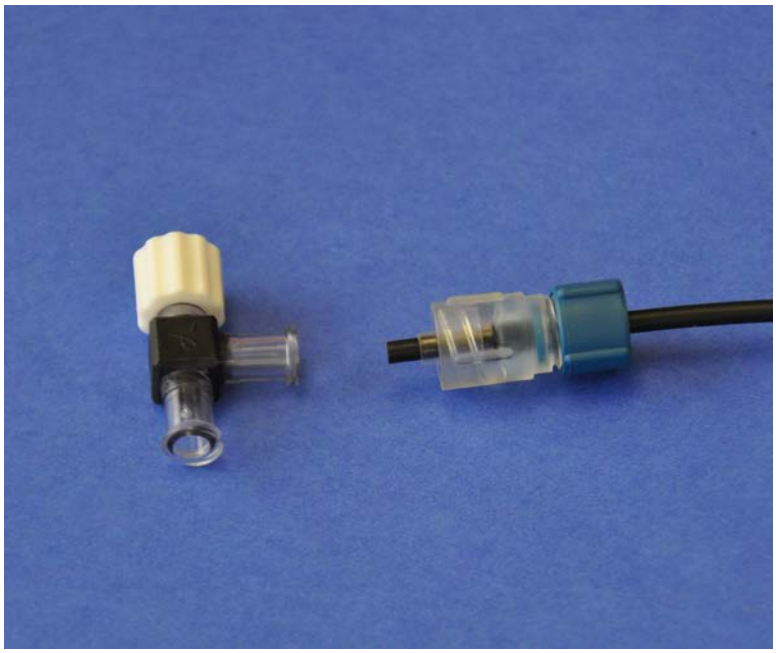

Figure 1 Flow cell with extension cable coupler

Figure 2 Coupler attached

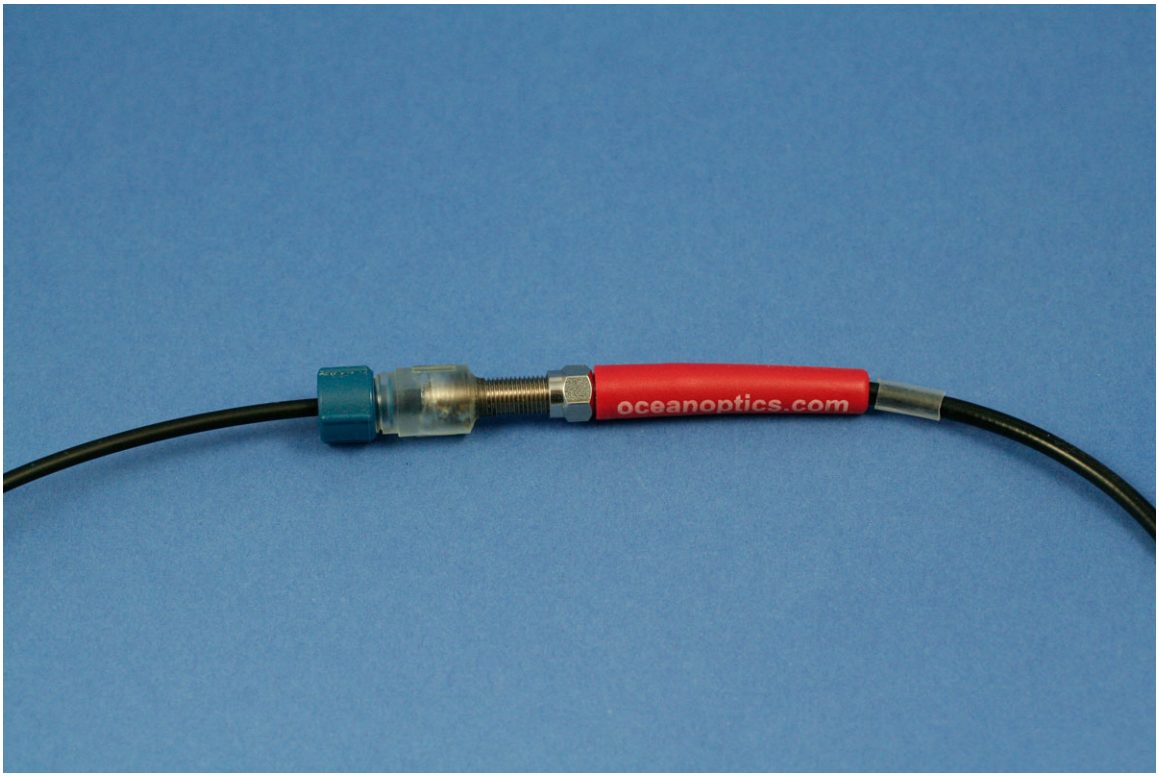

Figure 3 Extension cable attached to Bifboro bifurcated bundle

Attach the double end of the Bifboro cable to either of the connectors on the NeoFox unit. Do not pull on the extension cable where it enters the SMA coupler since it is extremely difficult to glue to the black sheath. If it becomes necessary to reseat the black coupling fibers, loosen the blue nut, push the fiber in until it hits bottom and firmly retighten the blue nut. Low signal levels can be the result of black fibers not seated to the bottom of the Touhy connector. Loosen blue cap and reseat if low.

Set NeoFox Parameters

FOXY patches are the most common on older systems and best for general purpose applications where exposure to alcohol can be avoided. Alcohol **cannot** be used in the flow cell without seriously degrading the FOXY patch coating. Signals from this material will be high with lowest noise even with 10 feet of extension cable. *If PHOSPOR patch material is installed they are more resistant to alcohol.*
Parameter Setup for each channel is required prior to calibration and to set the system for the patch in the flow cell and might be slightly different from cell to cell. 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** and match settings to setup sheet provided. If the Neo has been powered down it reverts to the default settings of the Neo. If this occurs, go back to step 1.

Set PHOSPOR Parameter Parameters

1. Use the checkout sheet provided and set the values to match the sheet for initial operation.
2. Set to Phospor Material
3. Note that you may need to reduce the LED rate to 5.86 Khz.by hitting the Minus button The main difference in Setup is the lower value of LED rate, typically 5.86 KHz instead of 23.44 KHz. Illustrations may show either rate and material.
4. Adjust blue gain using slider and LED % if required.
5. Check Blue amplitude signal level by displaying Advanced Status or by placing the cursor over the top of the blue trace. It should be in 10,000 range or greater. The small arrow on the Signal level bar graph should be in the green area. Do not allow the signal to be so large that it saturates (gets flat on the top when exposed to zero oxygen).
6. Set the gain on the Red channel to approximately match the screen below.

Xx SCREEN PICTURE

Temperature

No temperature probes are provided for the flow cells.
"Use a Fixed Temperature" in the calibration screen and manually insert your temperature.
Do not select None. The temperature is the fluid temperature in contact with the sensor patch installed in the Tee.

Calibrating Flow Cells

Meaningful Oxygen values will be displayed only after a **Two Point Calibration** has been performed for each channel. For paired flow cells, they can be attached to each other in series and allowing both cells to be calibrated together with ambient solution then zero solution.

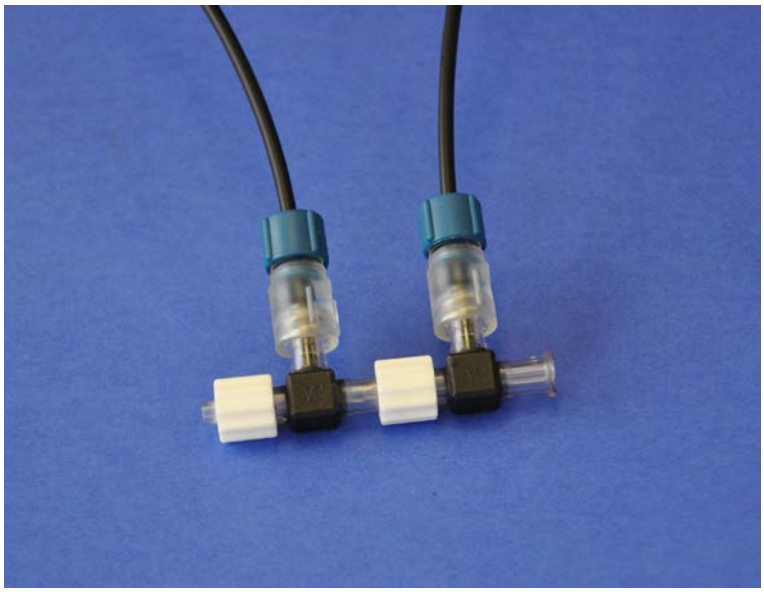

Figure 4 Flow cells in series for dual calibration

Typical Dual Calibration Procedure

Switch channels using the tabs at the top of the screen to view and enter calibration data.

1. Select cell 1
2. Go to Options-Calibration-select Two point calibration
3. With ambient equilibrated buffer in both cells
4. Transfer the tau into the line corresponding the value you have determined for ambient by hitting the transfer tau button (lower entry). Only one value should be entered at this time.
5. Select cell 2 calibration screen
6. Transfer the tau for ambient of cell 2
7. Shift solution in the cells to the zero value solution
8. Go back to cell 1 calibration screen
9. Transfer the tau into the top line (zero O2)
10. Both values have now been entered and you must click in the graph area to update the calibration for cell 1.
11. Click Download to transfer the information to the Neo unit where it is saved.
12. Select cell 2
13. With the zero solution still in the cells, transfer the tau to the top line by selecting the transfer button for the zero value of tau.
14. Repeat steps 10 and 11 for cell2.
15. Both cells have now been calibrated. Note- all taus for a given cell have been entered before downloading

Details

Calibrations should always be performed at your final operating temperature. Oxygen values are temperature sensitive. Select “Use a Fixed Temperature” and enter the perfusate temperature into the temperature field for all flow cells in their respective

1. Run air equilibrated buffer through the cell/s, making sure it is at temperature. Take care with this step. **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 by760. For example, at 37C partial pressure should be 148.9 mmHg and 19.6%. Use this value as your ambient sample calibration value**. If you are not working at 37C then the percent oxygen will have to be adjusted for the vapor pressure of your perfusate.
2. Click the button to enter the current tau reading in point #2. Record this value in case of any errors during the calibration. The tau values can always be manually entered.
3. Run a solution containing sodium dithionite through the cell/s until the tau stops increasing. Titrate for maximum tau. 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 in the Neo units, 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 the flow cell/s well between solution changes.
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.

Maintenance

No maintenance is required for the long life sensing patch as it is part of the flow cell. If damaged, the cell will need to be replaced.

Installing Firmware Updates when required

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

Some components are incorporated from Ocean Optics Inc.

Instech Laboratories Inc 5209 Militia Hill Road Plymouth Meeting, PA 19462
Phone 800 443-4227 Web: www.instechlabs.com