WEBINAR: The 4 W's of Microcirculation and Tissue pO2: An in vivo Approach in Animal Models for Preclinical Research

Questions and answers from the August 12, 2020 webinar titled "The 4 W's of Microcirculation and Tissue pO2: An in vivo Approach in Animal Models for Preclinical Research"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address. Questions have been grouped into relevant categories.

OxyLite and OxyFlo Questions

1. **We generally think of blood oxygenation in terms of oxygenated hemoglobin, but the OxyLite is detecting free oxygen. What is the correlation between those? I did not think there was much free oxygen in the body/blood?**

   In general, a high hemoglobin saturation usually leads (in healthy tissues) to a higher free/dissolved oxygen content; however, in some cases, the disassociation from hemoglobin is impaired and thus high blood saturation does not always mean high tissue oxygenation. In all tissues, access to oxygen is only possible when it is disassociated from hemoglobin as the bound oxygen cannot diffuse out of the blood.

2. **For pO2, can the sensor be applied on top of the skin or it needs to be 'inserted' into a tissue?**

   The OxyLite sensors must be inserted into tissue such that the tip of the sensor is located at the desired measurement location. This avoids any environmental oxygen contamination.

3. **What is the maximum depth that can be measured? For example, for brain studies can you get measurements in deep structures like the thalamus?**

If you can place the sensor in the tissue of interest, it can measure there. The sampling region for most OxyLite sensors is about $1mm^3$ however there are some larger OxyLite sensors that have larger sampling regions. It really is application dependent. The OxyFlo also has a $1mm^3$ sampling region for surface sensors and invasive sensors.

4. **What is the total area that can be measured with the OxyLite?**

   Depending on your sensor choice, you can measure $0.25mm^3$ to $8mm^2$ with each OxyLite sensor. Depending on how many sensors you plan to use, that area can increase. Most of the sensors are used to measure pO2 in microregions and areas of interest in certain tissues. Please reach out to our application specialists and they can direct you towards the best sensors for your application.

Application or Model Specific Questions

1. **Can we use the OxyFlo to measure blood flow in mouse skin?**

   Yes absolutely, our OxyFlo surface sensors can be found on our OxyFlo Sensor page here: https://www.scintica.com/products/oxford-optronix/blood-flow-monitors/ and head to the "Probes" tab.

2. **Can you suggest any references for using the OxyLite in mouse muscle?**

   Here is a list of all our citations for the OxyLite and OxyFlo systems. You can find many articles that use muscle as their tissue of interest: https://www.scintica.com/wp-content/uploads/2020/02/References.pdf

3. **Can the OxyLite be used in the lumen of the GI tract?**

   Absolutely, please consult with our application specialists for more information about which sensors are appropriate for your work!

4. **Can pO2 be normal in peripheral blood while the brain is hypoxic during gaseous anesthesia of the animal?**

This is a good question. In some applications I am sure that this is possible; however, I personally do not have any experience with this. You would have to try this with an OxyLite to confirm.

5. **Can the OxyLite and OxyFlo systems be used with cardiac catheterization, or do they work better if the body is opened in a more-invasive sense?**

   They work best when the tissue of interest can be visualized for correct insertion. Many of the probes cannot be threaded down catheters that are small enough for rodent/small animal hearts. Large animal models are a bit easier, but it is best to consult with our application specialists to create a solution that will work for your application.

Sensor Specific Questions

1. **Can you give us more details regarding sensor size?**

   All sensors can be found on our website in one of three places:
   1. OxyLite ONLY sensors here in the "sensors" tab:
   https://www.scintica.com/products/oxford-optronix/oxygen-monitors/
   2. OxyFlo ONLY probes here in the "probes" tab:
   https://www.scintica.com/products/oxford-optronix/blood-flow-monitors/
   3. Combo (dual and triple parameter sensors) here in the "sensors" tab:
   https://www.scintica.com/products/oxford-optronix/combined-systems/

2. **Can the OxyLite sensor be used to track chronic pO2 changes?**

   Yes, we do have chronic implantable sensors for neurology applications and for large animals. You can actually see an example of chronic sensor implantation using the standard sensors in large animals if you watch our webinar from May 2018: https://insidescientific.com/webinar/measuring-tissue-perfusion-po2-conscious-animals-organ-failure/

3. **Does the sensor get clogged? How do you keep it clear?**

   The sensors are not hollow like needles but are thin glass fibres or solid stainless-steel casings. Keeping them clean requires delicate patting with alcohol and non-lint wipes. If you are interested in more information about sensor cleaning procedures and how to care for them, please reach out to our application specialists for more information.

4. **Are the sensors/probes disposable, or is there a cleaning and sanitation process?**

   The sensors for both the OxyLite and OxyFlo are reusable and information about their reusability and consumability can be found on our website in the "Probes" and "Sensors" tabs mentioned above. They can generally be cleaned with 70% alcohols and non-lint wipes, and for sterilization protocols please see the end of this document.

5. **What method can be used to sterilize the sensors if they are intended to be re-used?**

   Recommended Sensor Sterilization Protocols:

   <u>STERRAD® technique:</u>
   STERRAD® 100S
   Sterrad® is a sterilization method that uses low-temperature, hydrogen peroxide gas plasma technology to sterilize instruments and medical devices safely and effectively. This method protects the instruments, users, patients and is less harmful to the environment unlike the ETO gas method.
   The process has 5 phases once instrument enters the machine:

   1. Vacuum Phase - Chamber evacuates pressure
   2. Injection Phase - Liquid peroxide is injected into the chamber, vaporizing the hydrogen peroxide solution, and disbursing it into the chamber.
   3. Diffusion Phase - Hydrogen Peroxide vapor permeates the chamber, exposing all instruments to the vapor cloud. At completion of this phase the chamber pressure is reduced, and the radio frequency plasma discharge is initiated.
   4. Plasma Phase - Electromagnetic field is created and hydrogen peroxide vapor breaks apart, Plasma cloud forms in ultraviolet light and inactivates all remaining bacteria, rapidly sterilizing all instruments and materials with no toxic residues.
   5. Vent Phase - Filtered air is drawn into the chamber to equalize pressure so that the door can be opened. No need for aeration or cool down due to the low-temperature technology. Instruments are ready for immediate use.

   It is a 55-minute cycle with a cycle temperature of 40°C to 55°C (104°F to 131°F)

   <u>ETO sterilization protocol:</u>
   Here are some of the EtO parameters that have been tested and we are fairly confident that the sensors function after processing:
   - Preconditioning: 16 hours at 45°C

- Sterilisation at 45°C and 60% Relative Humidity.
- Dwell time for EtO: 4 hours
- Nitrogen washes: 2 with a vacuum hold for 20 mins.
- Aeration at 45°C for 4 days.

We tested 20 sensors for EtO sterilisation and looked at the effects of sterilisation on calibration and stability. The results were very good but obviously this is a limited data set and we cannot make any claims based on the results.