Webinar Q&A Report:

Measuring Tissue Perfusion and PO2 in Conscious Animals to Investigate Organ Failure

1. In what animal models has this system been used?

   The OxyLite™ and OxyFlo™ systems have been used in a wide variety of animal models, in a large number of anatomical locations.

   Animal models include rodents such as mice and rats, along with larger animals such as rabbits, dogs, sheep, and pigs. There does not seem to be a limitation on the size or species of animal used with these systems.

   Anatomical locations include the kidney, liver, heart, brain, spinal cord, muscle, tumors, gastrointestinal organs, skin, eye, bone marrow, etc. The sensors must be inserted into the tissue to provide measures of PO_2 and/or microcirculation.

   Research areas of interest include cancer/tumor, vital organs (kidney, liver, and brain), shock, resuscitation, infection/sepsis, ophthalmology, ischemia/reperfusion, middle cerebral artery occlusion, wound healing, as well as general physiology.

   Please refer to the reference list for a full listing of the peer reviewed journal articles that show the use of these systems (https://www.scintica.com/wp-content/uploads/2018/04/Combined-Systems_Citations.pdf).

2. Can this system be used in both animals and humans?

   The OxyLite™ and OxyFlo™ systems are not approved for use in humans nor are they FDA or CE approved. These instruments, however, can be used in a wide variety of animal models.

   Dr. Clive May has received special IRB (institutional review board) approval for use of the OxyLite™ system to measure urinary PO_2 levels in sepsis patients in the ICU (intensive care unit), and patients in the operating room on cardiopulmonary bypass. This is essentially a non-invasive procedure as the probe is passed through the catheter, which is already in place for these patients, such that the tip of the probe is able to measure the PO_2 levels in the urine that is expelled during the procedure.

3. Is the same probe used to perform measurements in different organs?

   Many of the sensors can be used in multiple organs and can be inserted with different surgical techniques depending on the organ of interest. Using these techniques, the user is able to get reliable and sensitive measurements of PO_2 and blood perfusion from a variety of organs including but not limited to; brain, spinal cord, heart, kidney, liver, skeletal muscle, skin, bladder, and many more.

   Dr. Clive May and his team will use a similar technique, to that which was shown in the webinar, for chronic implantation in the liver and spleen of larger animals. As he described in the webinar, the technique for implantation into the brain is slightly different, with the probes being inserted through a craniotomy and being held in place on the skull with dental cement.

4. Where does the probe/sensor acquire the signal – arterial or venous blood, or a mixture?

   The OxyLite™ and OxyFlo™ probe performs measurements from whatever tissue or vessel the probe is inserted into. In this way, if the OxyLite™ probe is placed within an artery, the PO_2 from the arterial blood will be measured, while probes placed in veins will capture data from venous blood. If the probe is placed within the tissue, then the measurement would be reflective of the microcirculation and tissue PO_2 within that microregion of tissue.

5. How are differences in blood plasma volume accounted for?

   The OxyLite™ probes, which measure PO_2, work on the principles of fluorescence quenching and therefore the fluorescent lifetime principle. In this way, the PO_2 measurement is independent of changes in the blood plasma volume.

   The OxyFlo™ probes measure flow using laser Doppler techniques, which are intended only for tissue microcirculation and perfusion measurements, and not for flow rates in the blood vessels.

6. How is the data analyzed?

   All Oxford Optronix systems provide access to the raw signals, via analog output connectors. These signals can be acquired by any data acquisition system, typically this is done through a BNC input. The OxyLite™ and OxyFlo™ Pro systems may be connected to a computer, via USB, running LabChart from ADInstruments (version 8 or higher).

7. How is the system calibrated?

   The sensors for the OxyLite™ system come pre-calibrated from Oxford Optronix directly. A unique 6-point *in vitro* oxygen/nitrogen calibration is performed on each sensor; this calibration is tested three times before they leave the manufacturer.

   The OxyFlo™ sensors purchased with the system originally come pre-calibrated from Oxford Optronix. Probes purchased at a later time can be calibrated using a one-step, automated calibration kit.

8. How are the chronic implantable sensors calibrated to ensure accurate measurements of PO_2 over the course of the study?

   The chronic implantable sensors are calibrated in the same way that all sensors are calibrated, to ensure long lasting accuracy and stability. These sensors use a fluorescence lifetime technique, called fluorescence quenching, which is not subject to accuracy loss due to drift

9. Do the probes have a battery, and if so, what is the battery life?

   None of the OxyLite™ and OxyFlo™ probes or sensors contain a battery. The external component of the chronic implantable sensors/probes are simply attached to an adapter cable when measurements are to be performed.

10. What is the accuracy of the PO_2 system, in mmHg?

    The accuracy of the OxyLite™ system depends on the amount of oxygen present within the tissue and is dependent on the temperature remaining constant (±0.2°C):

Range of Tissue PO_2	Accuracy
0 – 7mmHg	±0.7mmHg
7 – 150mmHg	±10% of reading
150 – 200mmHg	±15% of reading

11. Do the techniques presented by Dr. May translate well into rodent models, and are conscious measurements possible?

    In principle these studies may be translated into smaller animals, such as rabbits, however there are challenges in doing so - mainly around the protection of the implanted sensors between measurements, these same challenges would apply to chronic implantation of the sensors in rodents as well. The external component of the sensors may be scratched at or chewed on by the animal or cage mates. This may damage the sensor, or cause the tip of the sensor to move, both of which are not desirable. These challenges are more common with smaller rodents, such as mice and rats.

    Implanted sensors are best used in areas where dental cement can be used to secure the external component of the sensor, and where the animal will not have access to scratch or chew. A good example of this is with the sensor placed in the brain and attached to the skull. When implanted, chronic measurements can be made in rodents.

    For conscious measurements the animal will be connected to the OxyFlo™ or OxyLite™ system using a tether, so the animal should be lightly restrained to prevent any disruption in the measurement created by movement.

12. Clinically we give vasopressors 24 hr after sepsis. Have you tested your parameters at this time point?

    In order to make his studies clinically relevant, Dr. Clive May has given vasopressors at 24 h of sepsis in sheep with established sepsis and acute kidney injury. See their papers on treatment in sepsis with noradrenaline (Lankadeva YR et al. Kidney International 90: 100-108, 2016) and Angiotensin II (Lankadeva YR et al. Critical Care Medicine 46: e41-48; 2018).

13. Do the Laser Doppler probes/software used to measure perfusion also provide measurements of RBC mass and velocity?

    The OxyFlo™ system provides measurements in Blood Perfusion Units, an arbitrary unit that gives information on microvasculature flow. Neither RBC mass nor velocity are measured using laser Doppler techniques.

14. Can this system be used to measure tissue oxygen consumption?

    Yes. The OxyLite™ system uses fluorescence quenching, an optical technology that does not consume any oxygen during measurements. This system can monitor tissue PO_2 changes over time.

15. How reproducible are the measurements from point to point in the tissue? If you put in multiple probes into a single kidney medulla, how similar or different would you expect the values to be?

    Dr. Clive May and his team have not put multiple probes in a single organ to examine the point to point variability within the tissue. However, they have implanted probes in the cortex and medulla of numerous animals, which has given them consistent levels across many groups of animals. This gives them confidence that provided the tips are in a similar area of an organ, the PO_2 and perfusion measurements are reproducible within the variability expected between animals.

16. Have you examined the kidneys for specific morphological changes in the medulla vs cortex in your model, and does it correlate with the observed changes in flow and oxygen tension?

    In the first paper in which Dr. May and his team described the chronic implantation of these probes, they presented a figure showing minimal damage around the tips of the probes that had been implanted in the renal cortex and medulla for 8 days (American Journal of Physiology 308: R832–R839, 2015). When the team removes the probes at the end of an experiment, they always check the position of the probes in the renal cortex and medulla and with visual inspection. Dr. May and his team have not seen any haematomas around the probe tips.

17. Are you able to sterilize the probes/sensors between uses? If so, how?

If appropriate for your animal protocol, you may clean the probes using 70-80% ethanol between uses.

Dr. May and his team place the sensors in 80% ethanol in saline for 3-4 minutes, rinse them in saline prior to implantation. They have seen no signs of infection throughout their studies.

If complete sterilization is required there are two techniques that can be used:

A. Ethylene Oxide (EtO):

Preconditioning Phase - 16 hours at 45°C

Sterilization Phase - 45°C and 60% RH
Dwell time for EtO - 4 hours
N_2 washes - 2 with a vacuum hold for 20 mins
Aeration Phase - at 45°C for 4 days.

B. Sterrad – this technique uses lower temperatures along with hydrogen peroxide gas plasma technology to sterilize instruments safely and effectively, and may be less toxic than ethylene oxide:

Vacuum Phase - Chamber evacuates
Injection Phase - Liquid peroxide is injected into the chamber, vaporizing the hydrogen peroxide solution and dispersing it into the chamber
Diffusion Phase - Hydrogen peroxide vapor permeates the chamber, exposing all instruments to the vapor cloud.
Plasma Phase - Plasma cloud forms in ultraviolet light and inactivates all remaining bacteria, rapidly sterilizing all instruments and materials with no toxic residues
Vent Phase - Filtered air is drawn into the chamber to equalize pressure so that the door can be opened

18. How would you apply your investigation method during a surgery to monitor the tissue perfusion, for example during an extracorporeal circulation in rats or during invasive blood pressure monitoring in septic rats (CLP or LPS model)?

The OxyLite™ and OxyFlo™ systems can be used in such a wide variety of applications that it would also work well in these types of experiments.

The appropriate probe/sensors to be used would need to be discussed based on the specific tissue of interest. Information pertaining to the physiological parameters required and tissue of interest would guide the choice of appropriate sensors. The study design would include assessment of sensor placement by adjusting inhaled oxygen concentration or by performing vessel occlusions to alter the tissue oxygenation.

19. Can these systems be used to assess tissue perfusion during CPR?

Yes, the OxyFlo™ and OxyLite™ systems have been used on CPR models recently. This data is not available for distribution however the results were promising and warrant further study.

As with many other models used, the systems are very capable of assessing flow and PO_2 in the brain and other vital organs.

20. Could this methodology be used to investigate placental failure in a mouse model?

    Although we don't have experience with this specific example, the OxyFlo™ and OxyLite™ systems should be able to be inserted into the placenta of mice to assess PO_2 and flow. As with the insertion of the probes/sensors into the cortex of the kidney, the sensor placement is of utmost importance.

21. How can you evaluate Tissue Perfusion and PO_2 in the heart, in models such as cardiac failure or diabetes?

    The OxyLite™ systems can measure PO_2 in the myocardium. In the case of coronary artery ligation or occlusion, in a rat for example, changes in tissue oxygenation can be measured. The motion of the heart is not an issue for these sensors.

    The beating heart moves at such a speed that the OxyFlo™ sensor is not able to assess tissue perfusion in this type of model.

22. Can meaningful quantitative measures of the functional parameters described in the webinar summary be gathered from different animals or groups of treated and untreated animals to yield means from which statistical differences can be determined?

    Yes, this system has been used across a wide variety of applications including various animal models and anatomical targets. Please see the list of references for many different examples (https://www.scintica.com/wp-content/uploads/2018/04/Combined-Systems_Citations.pdf).

    Specifically, you may also refer to numerous publications by Dr. Clive May and his team (American Journal of Physiology 308: R832–R839, 2015; American Journal of Physiology 309: R1226–R1233, 2015; Lankadeva YR et al. Kidney International 90: 100-108, 2016; Lankadeva YR et al. Critical Care Medicine 46: e41-48; 2018).

23. What is the effect of clot formation around the probe on tissue oxygen values?

    If a clot forms around the sensor tips, then the sensors will show a slower response time or may not read correctly. Histological evidence (as described in the webinar) shows that this usually will not occur unless the sensor is inserted into a blood vessel without Heparin.

    Dr. May and his team have not seen clot formation around the tips of the sensors when probes are removed 1 week after implantation.

24. Given the fact that pericytes can regulate renal blood flow, is it possible to study their interactions with endothelium with the presented method?

    Unfortunately, it is not possible to study the interactions of the pericytes with the endothelium using the OxyFlo™ or OxyLite™ systems. These systems are designed to study changes in flow or PO_2 levels and cannot differentiate which cells or interactions are modulating these values. However, if the flow or PO_2 is modulated by the pericytes or their interactions with the endothelium, these changes can be measured using these systems.

25. What is the best method to measure oxygen extraction in the cortex and medulla?

Oxygen extraction by the whole kidney can be calculated from the amount of oxygen delivered in the artery and the amount remaining in the renal vein. Dr. Clive May does not know of a way to determine oxygen extraction in the cortex or medulla specifically.

26. Can this system be used to assess heart rate, while also measuring tissue perfusion?

The OxyFlo™ is used to assess microcirculation; at this level of circulation the pulsatility created by the heart is minimal and would not be the best way to measure heart rate. The heart rate should be assessed by detecting the arterial pulse or ECG.

27. Which method do you consider as the gold standard for measurement of kidney tissue perfusion and PO_2 in mice?

The OxyLite™ and OxyFlo™ systems, and their predecessors, have been one of the most widely used probe-based methods to assess PO_2 and flow in the kidney and a wide variety of other organs (see the reference list for many examples: https://www.scintica.com/wp-content/uploads/2018/04/Combined-Systems_Citations.pdf).

There are of course other techniques, including a large body of work surrounding MRI (magnetic resonance imaging) sequences which may be used to assess perfusion (with or without exogenous contrast agents) as well as tissue oxygenation (^{19}F-MRI) in various organs.

28. How accurately can these techniques reveal the difference between *in vivo* and *ex vivo* O_2 consumption in rodent (mouse/rat) heart?

The OxyLite™ sensors may be inserted into the myocardium, in either *in vivo* or *ex vivo* models, allowing for measurement of PO_2. This application can be done in many animal models, including mice and rats. Use of the system in an *ex vivo* Langendorff preparation should also be possible.

29. What have been the challenges to achieve these results?

Dr. Clive May describes the main challenge was to develop a technique to fix the probes to the kidney so that they did not move over a prolonged period in a conscious moving animal and that this did not cause damage to the kidney.

If you have additional questions for Scintica Instrumentation regarding content from their webinar or wish to receive additional information about their products and laboratory services, please contact them by email or phone.

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