WEBINAR: What is hyperoxia, normoxia and hypoxia to cells:

Questions and answers from the January 23, 2019 webinar titled "What is hyperoxia, normoxia and hypoxia to cells: Why researchers should care about environmental oxygen and how it influences results"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address.

1. **If you have to take your cells out of the chamber to take an oxygen measurement doesn't that defeat the purpose?**

   The OxyLite system can be used within any hypoxia chamber but has been specifically designed to be used within the HypoxyLab itself. Inside the HypoxyLab a port system allows for direct use of the sensor within. Another thing to keep in mind is that unlike other probe-based sensors the OxyLite is very accurate at low oxygen levels and thus is a perfect fit with the HypoxyLab.

2. **I use a chamber that uses % O_2 right now, are you saying that it cannot compensate for altitude and weather-related barometric changes?**

   In short, no it cannot. While % measurements are still a far better solution than not compensating for oxygen at all, % measurements will have variability that absolute measurements like mmHg or kPa will not have. Further, it is easier to replicate absolute measurements, ensuring they are precise from day to day and from location to location making them the "gold standard" in measuring O_2 levels.

3. **How do you transfer cells from your O_2 regulated working environment to your workstation?**

   In short, as quickly as possible. Changes in HIF1 can be seen in minutes. Alternatively, an incubator and workstation like the HypoxyLab will remove this step entirely and allows one to complete an experiment from start to end without having to leave the chamber.

4. **What about long-lived cells that have been growing in normal air for years. Will hypoxia still Influence them?**

   Yes, even these cells are being affected by the high oxygen levels. These long-lived cells, even if they have been grown in normal air for years, have been shown to produce erroneous results. GSH and redox status, for example, may be significantly different compared to when cells are grown in physiologically relevant conditions. Therefore, I would still suggest regardless of cell type, all researchers pay attention to the oxygen their cells see and try and do their best to compensate for it.

5. **Could we use a chemical method, like Cobalt Chloride, to induce hypoxia? What's the difference between the chemical and oxygen regulating method?**

   It is difficult to compare chemicals and hypoxic chambers. In short, while chemicals like Cobalt Chloride have been shown to mimic hypoxia by inducing HIF-1A, it has also been shown to affect other genes which may also influence results. Further, using these chemicals lacks the suitable control one will get within an oxygen-controlled chamber. If a researcher is only examining HIF proteins, chemicals may suffice. However, a hypoxia chamber is still a better alternative to chemical stimulation. Lastly, chemicals are unable to control and maintain physoxic conditions.

6. **Going back to the HypoxyLab chamber you showed during the presentation, how do you transfer cells from you O_2 regulated working environment to your workstation?**

   The HypoxyLab has an easy entry flap on the front. Unlike a transfer hatch it allows for quick and easy access to put items into the chamber and take them out. Once the hatch is opened the system purges with nitrogen and also creates negitive pressure not allowing external oxygen to ingress the system. It works phenomenally well.

7. **How would one create oxygen concentration gradients in medium?**

   First it is important to understand more about your specific conditions, like is the medium 'static, unstirred'. Even still, it is not a simple answer. However, starting with a 'static' media concept you could play with the media used. As mentioned in the presentation the O_2 dissolvability is based on the Bunsen constant, which is influenced by temperature and liquid composition. Assuming operation at 37°C you could affect the media composition to create layers of different O_2 gradients.

8. **How may the O_2 levels may differ between adherent vs. suspension cell lines?**

   O_2 levels will differ based on several factors. will stirring/motion of suspended cell culture (in which case Henry's law will apply) take place and will one be working with a 'near' homogenous solution with regards to O_2 content. There will also be less variation in average O_2 exposure for the cells in suspension versus the adherent cells. Often for adherent cells, you would need to rely on Fick's law, where media height and cell density will be the major contributors with regards to the pericellular O_2 exposure levels. This means larger variation in levels both spatially as well as temporally. Spatial variation will be due to cell density and location, e.g. the liquid meniscus creates an addition height difference. Temporal variation will be due to variation in cell density and to a smaller part the compositional change in the culture media. On average in the same incubator the pericellular O_2 content exposure for adherent cells would be lower then for suspended cells.

9. **How are the partial pressures of the different tissues in the body measured?**

   These have been measured through different means, I would suggest using Keeley *et al.* 2019 as the base for looking this up for your specific tissues of interest. https://www.ncbi.nlm.nih.gov/pubmed/30354965. Of course, one could use the OxyLite system to measure oxygen level in tissues.

10. **Given that it's the pO_2 at the medium/cell interface that represents 'physoxia', and there are two gas transition phases between the controlled atmosphere and the cell layer, how would we set the atmosphere to deliver and monitor optimal pO_2 in practical terms?**

    Practically, in a controlled environment such as the HypoxyLab you would first set a strict SOP with regards to your cell plastics/dishes, cell handling and media addition to reduce starting variation as much as possible. For example, media height to account for diffusion in the media and starting cell numbers. If the media is already equilibrated to the to environment, for instance with the help of the HypoxyCOOL, this would reduce the 2-gas phases consideration significantly to achieve equilibrium and would then only need to supply cell consumed O_2. Next, one would then need to assess the practical O_2 levels (perhaps with the OxyLite) with repeated measures at several timepoints and after different handling points while varying the pO_2 within the system. Otherwise one can only revert to using an equation based on the circumstances and give an estimation. The best approach would be to first do the math followed by a measurement for the finetuning.

11. **How long must one expose cells to hypoxic conditions for the cell culture to be considered as hypoxic?**

    This is both A simple and complex question. The short answer is namely what a cell considers as hypoxia. This can arguably be defined as the point where or when a cell starts adapting/ reacting to the change in O_2 presence beyond what it would under physoxia. This will differ considerably between cell types.

    First, it is necessary to determine what physoxic conditions are for your cells and then assess how large a difference in your 'hypoxia' indicator is required to be considered a significant change. Of course, this could then be anything from a mild to extreme form of hypoxia.

12. What considerations do I have if I am trying to standardize a model of cyclic hypoxia?

The considerations are protocol dependant. The HypoxyLab is able to set up to cycle different oxygen profiles automatically and even loop these profiles if needed. Therefore, you should have the ability to mimic cyclic or intermittent hypoxia.

13. Ideally but also practically, what should be the depth of media above the cultured cell layer?

Preferably it wouldn't be higher than 200µm to allow for passive O_2 diffusion to be as optimal as possible. However, practically this would require you to change your cell culture media at a very high frequency as the carbohydrate consumption would lead to energy starvation especially in non-confluent culture dishes. This would also lead to repetitive disturbances with your cell culturing that needs to be accounted for. Therefore, one needs to provide cells enough media to avoid starvation but still allow for passive O_2 diffusion. There is no correct answer but researchers should be able to justify any decision they make.

14. Can the OxyLite be used in any fluid like CSF or amniotic fluid. Also, can we use the probe in tissue *in vivo*?

The OxyLite can indeed be used on fluids both *in vivo* and *in vitro*. It is important to consider the oxygen in environmental air when obtaining a measurement *ex vivo*. Thus, if possible, using the glass fiber probe and taking the measurement *in vivo* at the site where the fluid is found is ideal.

15. Can you place animals in the HypoxyLab or are there other options for that?

Animals cannot be placed within the HypoxyLab but Scintica does offer animal hypoxia options as well. For more information, please contact an applications specialist (+1 519-914-5495).

16. You mentioned two gas phases, outside and inside the cell culture plastic. How fast does diffusion occur between these two phases and what is the approximate oxygen inside a flask that is already located inside an oxygen-regulated incubator?

I would not be able to define what the O_2 concentration would be in the headspace of your flask, there are to many variables that will affect this e.g. location in the incubator, size of the headspace, bottleneck size facilitation the diffusion area, filtered cap or not, difference/gradient in O_2/CO_2 and H_2O levels in the air of the headspace and the incubator. Though assuming cell handling was performed outside an O_2 regulated environment it would be similar to 'room air' O_2 levels for some time. Further, although there is a filter on the lid of a flask, diffusion is relatively slow as there is no active air flow into the flask. One would need to think depending the gradient differences in term >15 minutes to even hours depending on the headspace. There is no simple answer and I would generally recommend a very stringent SOP depending on the importance of O_2 levels of your research related cell culturing. I would also recommend obtaining the O_2 concentration measurement to validate/confirm this all.

17. Can the OxyLite probes can be used in microfluidics experiments?

This will depend on the size of the channel and whether the probe will fit. If so, there is no practical reason the probes cannot be used in these experiments. For more information, contact an applications specialist to discuss your specific experimental set-up.