

## FAQs

### What are the unique features of the *OPAL* system?

- **OPAL** is intended to be linked to common fluorescence microscopes, but can also be used as OEM unit with bioreactors and fluorescence readers.
- A special two frequency phase modulation technique allows masking the contribution of disturbing fluorescence background. Extra small-sized probes can be used in real “biological” ambiance in combination with a supersensitive photomultiplier.
- **OPAL** can be configured to work with lifetime based oxygen sensors from third-party suppliers. For intracellular oxygen measurements soluble Nano O<sub>2</sub> Sensors (Luxcel, Ireland [www.luxcel.com](http://www.luxcel.com)) can be used.
- **OPAL** also supports detectors and light sources from third-party suppliers.
- The **OPAL** hardware can be embedded into custom-developed software.

### How do I install the *OPAL* system?

Please follow this [youtube link](#) to watch an exemplary installation procedure of our system.

### What are CPOx-Beads?

CPOx-Beads are polystyrene beads with diameters from 10 to 50 µm. CPOx-Beads contain and a phosphorescent sensor dye and photo stabilizers. CPOx-Beads are intended to be used within cell tissue. They are not intended to be used within the cells, since they are too large to be assimilated from cells.

### What is NanoO<sub>2</sub>Sensor?

NanoO<sub>2</sub> Sensor is a soluble, nanoparticle-based oxygen sensor to stain the cells. It penetrates the cell membrane by its own and accumulates inside the cells. Therefore, Nano O<sub>2</sub>Sensor is used to determine the oxygen content within cells.

### What are the assets and drawbacks of CPOx-Beads and NanoO<sub>2</sub>Sensor?

- CPOx-Beads glow much brighter than cells stained with NanoO<sub>2</sub>Sensor. The test result generated with CPOx-Beads will usually be less noisy.
- Samples with CPOx-Beads can be measured using an ocular adapter for the detector, if no camera port is available. Using signal-weak NanoO<sub>2</sub>Sensors the detector has to be linked to a camera port, which provides 100 % of the collected signal.
- Using signal-weak Nano O<sub>2</sub> Sensors, any sources of background fluorescence (e.g. fluorescent ingredients of culture media, fluorescent covers or ground plated off well plates) should be removed as much as possible.
- The polymer matrices of the CPOx-Beads absorb cytotoxic singlet oxygen, which unavoidably is formed during the detection process. CPOx-Beads within samples can be exposed to continuous illumination for hours or even days. In contrast, cells, which are stained with Nano O<sub>2</sub> Sensor, become sensitive to light. They may only be exposed to bright light for a short time-span. Otherwise, the singlet oxygen generated by Nano O<sub>2</sub> Sensors within the cells will cause stress and even damage. To measure the oxygen content of cells stained with Nano O<sub>2</sub> Sensor, the excitation light should only be switched on for a few seconds just before taking a measurement point. To prevent cells, which are stained with Nano O<sub>2</sub> Sensor, from damage, all microscopic examinations should be carried out using dimmed light.

### Should I use solid CPOx-Beads or soluble NanoO<sub>2</sub>Sensor?

Using Nano O<sub>2</sub> Sensor:

- You measure O<sub>2</sub> inside the cells.
- You can measure O<sub>2</sub> at any position of your cell sample, since all cells will be more or less stained.
- The signal will be rather weak, which makes the measurement susceptible to background signals.
- Cells stained with Nano O<sub>2</sub> Sensor will be damaged if exposed to high light intensity for a longer time-span.

Using CPOx-Beads:

- You measure O<sub>2</sub> inside the cell tissue, if the cells have overgrown the CPOx-beads.
- You can measure O<sub>2</sub> in your sample only at the positions of the CPOx-beads.
- High signal strength. Usually no interference by background signal.
- Based on the CPOx-beads, long-time exposure to the excitation light will not cause damage.

For more fails-safe measurements, we recommend to use CPOx-Beads.

### **How to prepare samples with NanoO<sub>2</sub>Sensor?**

Replace culture medium with normal growth media containing dissolved NanoO<sub>2</sub>Sensor and return to culture overnight. Prior to measurement, replace media with prewarmed fresh media without NanoO

<sup>2</sup>  
Sensor.

### **How to prepare a sample with CPOx-Beads?**

Prepare a suspension with CPOx-Beads in culture medium or PBS. Put a certain amount of this suspension to your culture or to your cell carrier gel solution.

### **Why is green light used for exciting the sensors?**

Common culture media contain riboflavin, which generates singlet oxygen during illuminating with violet or blue light. The generation of singlet oxygen falsifies the measurement results and may cause damage to the cell tissue. In the green spectral range, riboflavin is completely transparent and will not singlet oxygen.

### **How can CPOx-Beads be sterilized?**

Most simple way is to put CPOx-Beads in a small amount of ethanol for a short time span. Alternatively, a stock suspension of CPOx-Beads can be autoclaved up to 120 °C and 1 bar overload pressure. Recalibration may be required after autoclaving. Branch off a fraction of the stock solution to check calibration values.

### **How can the *OPAL* system be embedded into custom software?**

The opal system comes with a DLL file containing the control commands. The software is written in Visual Basic 2008, using the Microsoft .NET Framework 4. On request, Colibri Photonic may provide the source code.

### **Is there a fiber-optic version of the *OPAL* system available?**

On request, Colibri Photonics provides an accessory kit to connect a fiber-optic reflection probe. This reflection probe can be used to excite and to collect the signal of sensor spots. Sensor spots, which can be adhered to inner faces of glass jars, tubes etc. are available from third-party suppliers.

### **What microscopes are compatible?**

Generally, all types of fluorescence microscopes, which allow access to the lamp port, to the camera port, and to the filter cubes are suitable. This is the case for common types of Leica, Zeiss, Nikon and Olympus. Where necessary, adapters can be customized. Models of Keyence are inadequate, since all components are inaccessible within a closed housing. Please contact Colibri Photonics to clarify, if your microscope is compatible.

### **Can the camera and the standard fluorescence light source remain at the microscope?**

Dual adapters with flipping mirrors are available for the lamp port as well as for the camera port. These adapters are available from the microscope manufacturers and allow simultaneous connections of camera, fluorescence lamp and the *OPAL*'s components. Exceptions are microscopes with a fiber-optic fluorescence lamp. In this case the light guide has to be replugged.

### **Can a microscope with a fiber-optic fluorescence light source be used?**

Yes, it can be used. In this case the light guide has to be replugged from the microscope's lamp to the *OPAL*'s light source. Colibri provides a LED light source with an appropriate light guide receptacle.

### **How shall the CPOx-Beads be stored?**

Simply keep them at room temperature in dry form in a vial. Usual ambient light will not interfere with the beads, but do not expose them to full sunlight or to a bright lamp.

### **Can *OPAL* record a lifetime image?**

Not directly, since the detector is a photomultiplier without spatial resolution. But you can reconstruct the oxygen distribution within a sample by focusing single CPOx-Beads and measuring the oxygen concentrations step by step and taking down the respective x, y, z-positions of the microscope stage.

### **I don't want to buy the *OPAL* hardware. Can I use CPOx-Beads in my own setup to measure oxygen contents via intensity changes?**

If your samples show rapid changes of their oxygen content, you will surely detect corresponding intensity changes of the CPOx-Beads' phosphorescence signals. But according to experience, drawing conclusions from the intensities on actual oxygen concentrations is difficult. In contrast to the lifetimes, the phosphorescence intensities of single beads differ, are dependent of the illumination intensity, are affected by scattering and absorption of the sample and may show some drift due to photo bleaching.