Precise oxygen measurement in cell cultures and tissues

Non-invasive and real-time measurements, obtained within seconds

Ideal for in vitro hypoxia conditions

Oxygen Measurement

The ibidi OPAL Optical $O_2$ Measurement System allows for quantitative, real-time monitoring of absolute oxygen values in both the extracellular and intracellular environments of cells, tissues, and spheroids. The system uses phosphorescence lifetime to measure the concentration of oxygen by way of oxygen sensitive fluorophores in the form of cell-impermeable polystyrene beads or cell-permeable nanoparticles.

During live-cell imaging assays, a stage-top incubation system is used to control the oxygen and carbon dioxide levels; however, oxygen concentration is typically lower near or in cell clusters than in the incubation chamber (Figure 1). Knowledge of the precise oxygen levels in or near an experimental sample is often imperative for an accurate understanding of cellular activity.

Figure 1. Controlling and measuring of oxygen levels are critical factors for many research applications as the levels are much lower in the physiological environment of cells and tissues than in air.
Principle: Optoelectronical Measurement of Oxygen-Dependent Change in Phosphorescence Lifetime

The ibidi OPAL System is easily integrated into any fluorescence microscope. Extracellular or intracellular oxygen measurement is achieved using CPOx-Beads or NanO$_2$ phosphorescence lifetime probes, respectively.

**OPAL Detector Unit**
- Computer
- OPAL LED Light Source
- OPAL Filter Cube
- ibidi OPAL Controller

**ibidi OPAL Controller**
- Powered by Colibri Photonics
- ibidi OPAL Optical O$_2$ Measurement System
- Light Source
- Temperature Sensor
- Error Detector Active

**Intracellular Oxygen Measurement**
- CPOx-Beads have an oxygen-reactive fluorophore
- The 50 µm polystyrene beads are non-permeable and remain in the cell media for monitoring extra-cellular oxygen levels
- Results are obtained optically using a fluorescent microscope with an integrated OPAL System
- CPOx-Beads are also compatible with phosphorescence-lifetime imaging microscopy (PLIM)

**Extracellular Oxygen Measurement**
- NanO$_2$ Nanoparticle Reagent contains an oxygen-reactive fluorophore
- Cell-permeable nanoparticles enable monitoring of intracellular oxygen levels via endocytosis
- Results are obtained optically using a fluorescent microscope with an integrated OPAL System
- NanO$_2$ Nanoparticle Reagent is also compatible with PLIM

**Properties of the CPOx Beads**

<table>
<thead>
<tr>
<th></th>
<th>CPOx orange</th>
<th>CPOx red</th>
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<tbody>
<tr>
<td>Composition</td>
<td>50 µm beads</td>
<td>50 µm beads</td>
</tr>
<tr>
<td>Ex</td>
<td>530 nm (510-550 nm)</td>
<td>540 nm (500-550 nm)</td>
</tr>
<tr>
<td>Em</td>
<td>600 nm (570-670 nm)</td>
<td>650 nm (640-670 nm)</td>
</tr>
<tr>
<td>Lifetime (37°C)</td>
<td>4.0 µs (21% O$_2$)</td>
<td>20 µs (21% O$_2$)</td>
</tr>
<tr>
<td>Application</td>
<td>Normoxia/hyperoxia</td>
<td>Hypoxia</td>
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</table>

**Properties of the NanO$_2$ Nanoparticles**

<table>
<thead>
<tr>
<th></th>
<th>Nanoparticle reagent</th>
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<tbody>
<tr>
<td>Ex</td>
<td>400 nm (390-405 nm)</td>
</tr>
<tr>
<td>Em</td>
<td>650 nm (640-670 nm)</td>
</tr>
<tr>
<td>Lifetime (37°C)</td>
<td>23 µs (21% O$_2$)</td>
</tr>
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</table>
The CPOx-Beads were seeded with cells \(3 \times 10^5\) cells/ml in a µ-Slide VI (Cat. No. 80606). The oxygen concentrations in the incubation system were set from 5.1 kPa to 19.5 kPa. Site-resolved measurements with the OPAL System revealed that the oxygen level in the cell culture media did not equal the set values of the incubation system. The oxygen consumption of the cells in the culture media was too great for equilibrium to be established between the gaseous and liquid phases.

The oxygen-sensitive nanoparticles have an inverse optical response to oxygen concentration. Low sample oxygenation produces a high response, and a high oxygen level produces a low response.

**Red**: Response at 0% oxygen.  
**Blue**: Response at 21% oxygen.

**Advantages:**
- Passive cell staining – simply add the reagent to the medium, incubate, wash, and measure  
- Low toxicity, long retention in cells, bright signal  
- Optimized for multi-parametric (multi-color) analyses

**Technical Specifications**

**Detector Unit:**
- Photocathode Area Size: Dia.8 mm (round shaped area)  
- Wavelength: 230 nm (short); 630 nm (peak); 920 nm (long)  
- Luminous Sensitivity Min.: 350 µA/Im (Cathode); 3.5 x 107 V/Im (Anode)  
- Luminous Sensitivity Typ.: 500 µA/Im (Cathode); 1.0 x 108 V/Im (Anode)  
- Radiant Sensitivity Typ.: 78 mA/W (Cathode); 15 V/nW (Anode)  
- Ripple Noise (peak to peak) Max.: 0.5 mV  
- Settling Time Max.: 10 s

**LED Light Source:**
- Radiation Class: Incoherent optical radiation  
- LED Color: Green (530nm)  
- Power: 300 mW

**Filter Set:**
- Emission: 630 / 92 nm or 607/70 nm  
- Excitation: 531 / 40 nm  
- Dichroic Mirror: 555 nm

**OPAL Controller:**
- Temperature Range: Ambient temperature to +55°C  
- Lifetime Range: 1 – 1000 µs
Control the Cell Imaging Environment with the ibidi Heating & Incubation System

To provide the optimal conditions to perform cellular oxygen level studies, the ibidi system consists of the following components:

- Gas mixer for optimal CO₂ and O₂ levels
- Humidifying column humidifies the mixed gas as it exits the controller
- Temperature controller for stable and uniform consistency
- Stage-top chamber with heated plate eliminates condensation for excellent phase-contrast imaging

Research Focus and Applications of the ibidi OPAL O₂ Measurement System

- Cancer Research – Tumor Environment and Growth
- Stem Cell Research – Multipotency, Embryogenesis, Gene Expression
- Immunology – Cancer Therapy
- Zoology, Cell Biology, Internal Medicine, Anatomy, Biomedical Research, Neurology

Ordering Information:

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>74001</td>
<td>ibidi OPAL – Optical O₂ Measurement System: Optoelectronic hardware and software for generating and processing of oxygen-dependent phosphorescence lifetime signals. Controller, Detector Unit, LED Light Source, Adapter Set, Filter Cube, PC software</td>
</tr>
<tr>
<td>74051</td>
<td>Adapter Set: for mounting OPAL to different microscopes, customized for your microscope and your requirements</td>
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<tr>
<td>74002</td>
<td>OPAL Mirror Box</td>
</tr>
<tr>
<td>10918</td>
<td>ibidi Heating System, Universal Fit, for 1 Chamber: ibidi Temperature Controller, Heated Plate in Multi-Well Format for 1 Chamber, 1 Heating Insert, Heated Lid</td>
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<td>11922</td>
<td>ibidi Gas Incubation System for CO₂ and O₂: ibidi CO₂ and O₂ Gas Mixer, Humidifying Column</td>
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<tr>
<td>74101</td>
<td>CPOx-Beads, red: fluorescence lifetime probe for extracellular O₂ measurement, Ø 50 µm, 3 mg, 5 mg/ml, (10-100 assays)</td>
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<tr>
<td>74102</td>
<td>CPOx-Beads, red: fluorescence lifetime probe for extracellular O₂ measurement, Ø 50 µm, 10 mg, 5 mg/ml (30-300 assays)</td>
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<td>74111</td>
<td>CPOx-Beads, orange: fluorescence lifetime probe for extracellular O₂ measurement, Ø 50 µm, 3 mg, 5 mg/ml (10-100 assays)</td>
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<td>74112</td>
<td>CPOx-Beads, orange: fluorescence lifetime probe for extracellular O₂ measurement, Ø 50 µm, 10 mg, 5 mg/ml (30-300 assays)</td>
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<tr>
<td>74151</td>
<td>NanO₂ fluorescence lifetime nanoparticle reagent for intracellular O₂ measurement, 100 µg (10-100 assays)</td>
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