BIOPTECHS *Biological Optical Technologies*
Established Standards for Live-Cell Microscopy

Bioptechs Culture Cylinder & Delta T® Dish Samples

Bioptechs Improvements to Live Cell Imaging Environments
Contained in this packet you will find Bioptechs Culture Cylinders, a Delta T™ Dish, temperature Dots, and a brief introduction to a dependable Humidification System.

Culture Cylinder Description:
Culturing Cylinders are used to barricade cells or contain suspended specimens in a Delta T™ Dish; or to restrict and concentrate the growth and location of cells plated on a coverslip. An added benefit is that cells will plate faster within the confines of the cylinder because they don't have to expend as much energy re-equilibrating the entire dish worth of media. They are 5mm high and available in a variety of inside diameters including, 2mm, 4mm, 6mm, 8mm, 10mm, and 12mm. They have at least a 1mm wall thickness. The cylinders are made of Borosilicate glass, that are optically polished on the bottom surface to mate with and form a seal with other flat glass surfaces such as coverslips and Delta T™ Dishes. Culture Cylinders can be autoclaved for reuse.

Culture Cylinder Usage:
Place the Culture Cylinder, polished side down, onto a flat glass surface such as a Delta T™ Dish. Then pipette cells into the cylinder filling it about 1/2 to 2/3 full. Pipette additional media without cells around the Culture Cylinder to an equal level of the contents of the Culture Cylinder. Incubate until cells plate. The Culture Cylinder can then be removed after cells have plated. There will be no residuals such as grease or wax as with cloning rings. The cells can continue to proliferate or move at will. They are also used to isolate adjacent cells, growth media, inhibitors, chemo-attractants, etc.

Delta T™ Dishes:
Delta T™ Dishes are made of a polystyrene ring which has a specially coated clear glass substrate bonded onto the bottom surface forming a 2ml liquid containment structure having a 35mm OD which tapers down to a 23mm optical aperture. The glass substrate is available in two thicknesses: 0.5mm for low N.A. and #1.5 coverslip for high N.A. applications. The dish is also available with either black or clear perimeters. We recommend the use of black dishes for fluorescence applications.

Temperature Dots:
Temperature Dots can be placed on any flat surface or temporarily in an aqueous environment to visually indicate a temperature. They are yellowish-orange at 36, green at 37, and blue at 38 degrees. They look black past 38 degrees. They are a convenient way to test any flat surface for physiological temperatures.

High Numeric Aperture Imaging:
If you are using high numeric aperture immersion objectives typically with high N.A. dishes at physiological tempertures, it will be necessary to regulate the temperature of the objective as well. This is due to the fact that the optical coupling medium, oil, glycerin or water, also has a thermal coupling effect. In this case, a Bioptechs, Inc. Objective Heater and an Objective Heater Controller should be used to provide uniform temperature across the field. The Bioptechs Objective Heating System is highly efficient and regulates the objective temperature based on the heat propagation characteristics of the objective. It is not just a heating ring or wraparound warming blanket. It does not radiate unwanted, un-transferred heat as typical with velcro and heating ring types.

Delta T™ Live-Cell Micro-Environmental Control:
Bioptechs introduced and established The Delta T™ Culture Dish System with direct thermal transfer and dual mode temperature control for imaging applications in 1993. Bioptechs has now integrated numerous customer requests into its current generation Open Culture Dish Micro-Environmental Control System. Now the following new features are included on all systems:

- TTL switching interface with Mode Indicator (dynamic or imaging) on the front panel
- Heat-shock mode for nearly instantaneous heat shock protein activation
- Remote setpoint port allows external settings or cycling
- Temperature output (for analog recording, Temperature ÷10 in Volts 37°C = 3.7 Volts
- The ability to disable the alarm when it goes into alert mode
- User adjustable Heated Lid power supply

* Can be powered from a battery for remote or electrically quiet applications

- Numerous accessories to accommodate nearly any specimen type.

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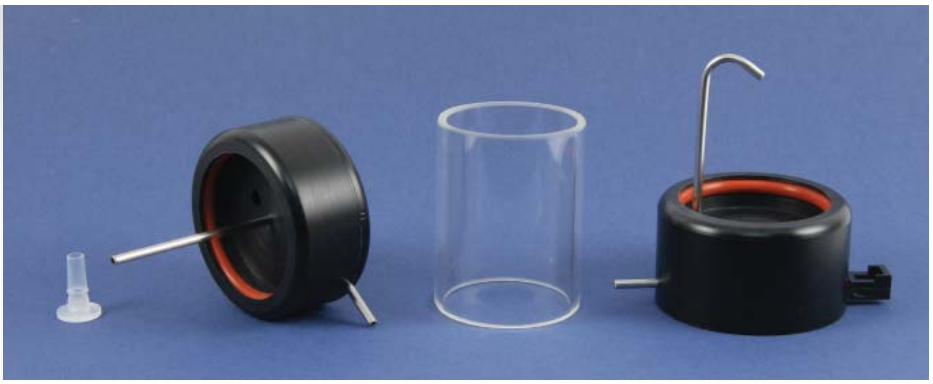
Humidity Control for Live-Cell Imaging:

Humidity control during live-cell, time-lapse experiments can be frustrating and lead to costly wasted time if you don't have the right equipment and set up properly. It is important that the gas delivered to the chamber actually arrives at 100% humidity and the flow rate is appropriate for the size of the chamber. For single well time-lapse chambers, the airspace to culture medium ratio is low. Therefore, as a rule the volumetric gas exchange rate (assuming it is done properly) should be on the order of 1 to 5 volumes per hour to prevent pH or osmolarity shifts. The humidity in a closed cell chamber or dish reaches 100% within seconds of closure. Therefore, if you are applying a specialized gas mixture or 5% CO2 in air to the existing 100% RH airspace the inflow must be 100% RH or it will not take long to cause the pH and osmolarity of the media to change. The Bioptechs Micro-Gas Humidifier is an efficient, reliable and convenient means to eliminate this obstacle for long-term imaging. It is economical and easy to setup. A demand type regulator is attached to a pre-mixed gas bottle. The gas is then transferred to a Bioptechs Humidification Vessel by means of a simple peristaltic pump. The humidified outflow is directly coupled to the Delta T™ dish covered by an o-ring sealed Heated Lid that prevents condensation from forming thus creating a high numeric aperture compatible, temperature controlled, "micro-incubator" environment on your stage. This technique is easy to setup, reliable and easily modified to accommodate protocols.



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