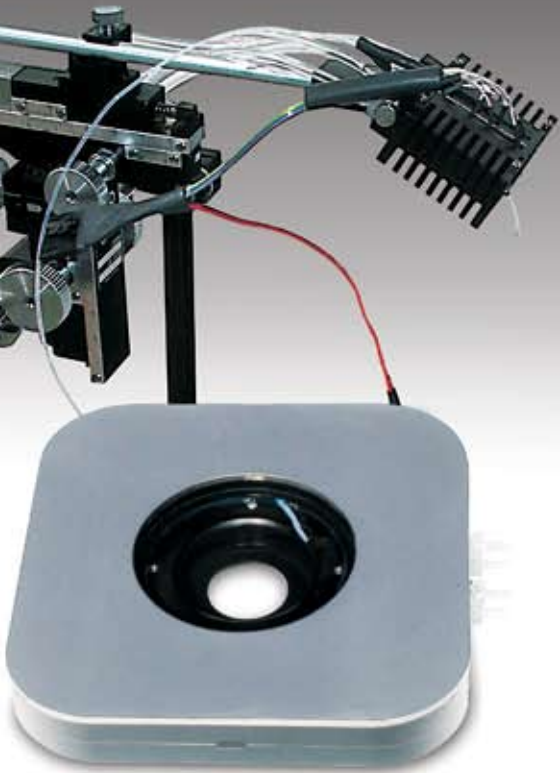


Temperature Control

User's Guide



Heating & Cooling

- Precise Temperature Control throughout the experiment
- Conditions similar to *in vivo*
- Compatible with any perfusion system
- No electrical noise during operation
- Compatible with Imaging systems and Electrophysiology stations



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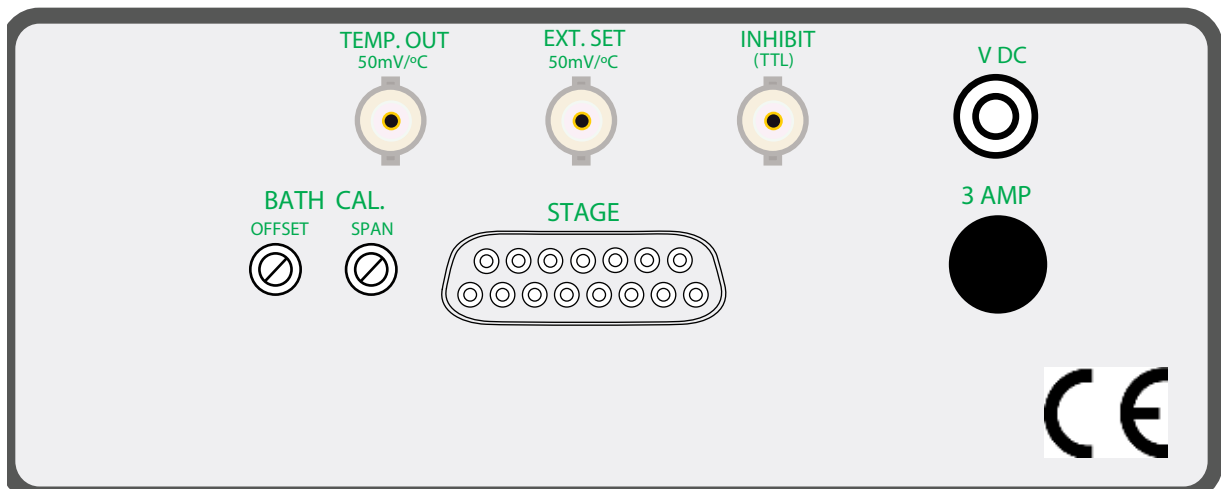
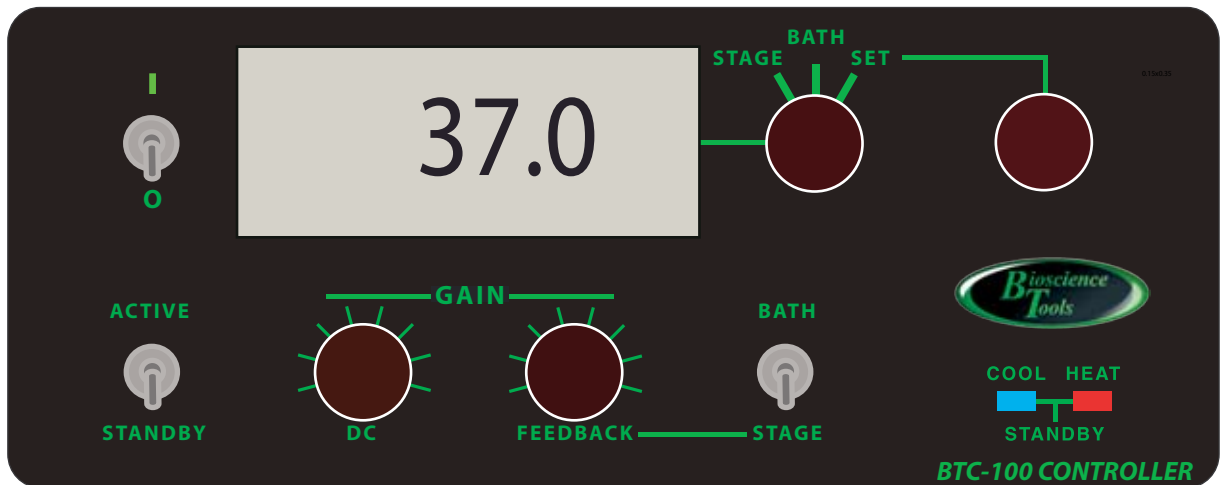
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Introduction

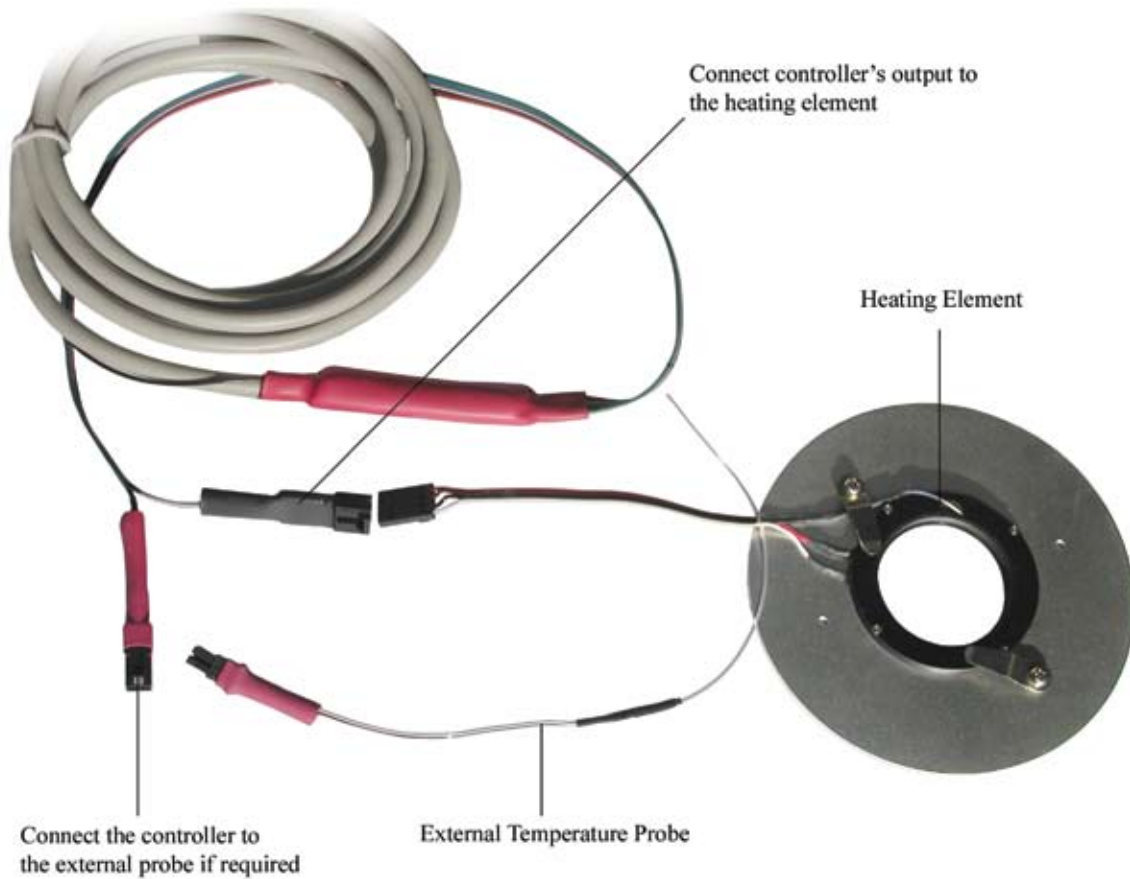
The complete temperature control system comes with a temperature controller, an external power adapter, a microscope adapter with a heating stage and 8 ft. connecting cable, a perfusion chamber, plus a magnetic holder for the temperature probe, and a holder with suction tubing. Note: some systems might not include not required accessories like suction tubing or perfusion chamber. All heating/cooling elements incorporate a s temperature sensor built inside. Most heating elements can be used as inline pre-heaters, if perfusion is required. The included microscope adapter is used to fit the stage on your microscope. The following are an illustrated installation guide and example configurations of temperature controlled setups.

Installation Guide

- 1 Connect power cable to the external DC power adapter. Plug the adapter to the power jacks on the back of the controller. Plug the heating stage cable into the output connector on the back of the controller.



2 Connect the output cable to the stage and external temperature probes, if used. For cooling systems: a source of cold distilled water needs to be connected to barbs on the cooling unit. CFPS-1U66 flow control unit can provide up to 22ml/min flow rates to prevent the colling unit from overheating. Plug the power cable into wall outlet.



3 Prepare the sample chamber, petri dish for example, by filling the chamber with water. Using provided adjustable holder, position the external temperature probe inside the chamber. You do not have to do this initial setup procedure while the heating stage is on the microscope. Use a desktop instead. You can transfer the heating stage on the microscope after you are familiar with the system. If cooling is used, you need to provide cold water flow through the cooling unit using CFPS-1U unit. Turn the controller ON.



4a

Before using the system, you need to know how to adjust temperature settings. Each controller has at least one LCD temperature monitor, and a switch, which allows you to choose the required temperature for display: set/reference temperature, stage temperature, or bath temperatures. Choose SET temperature, and adjust the reference temperature by rotating SET dial. You can also choose which probe to use to provide FEEDBACK to the controller. Choose STAGE feedback. Then, switch the temperature monitor to display STAGE temperature.

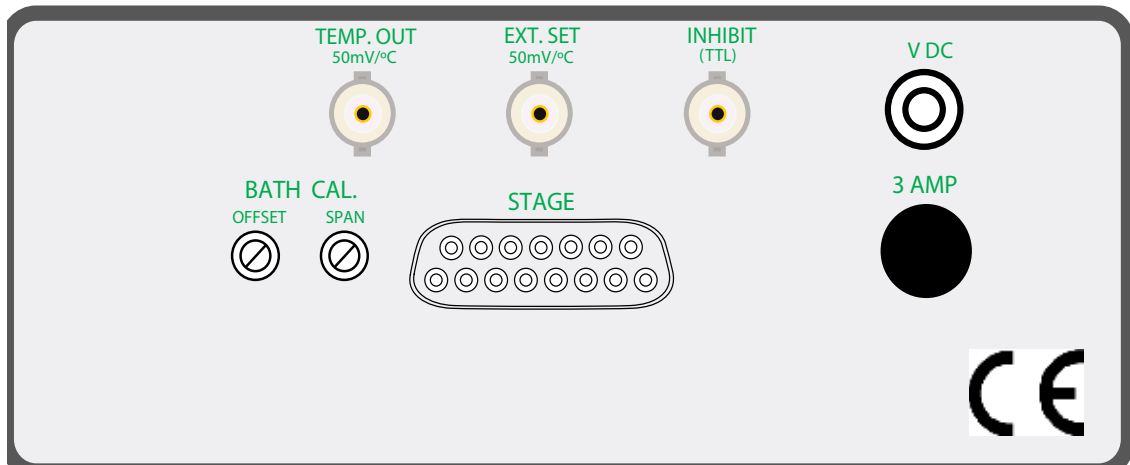
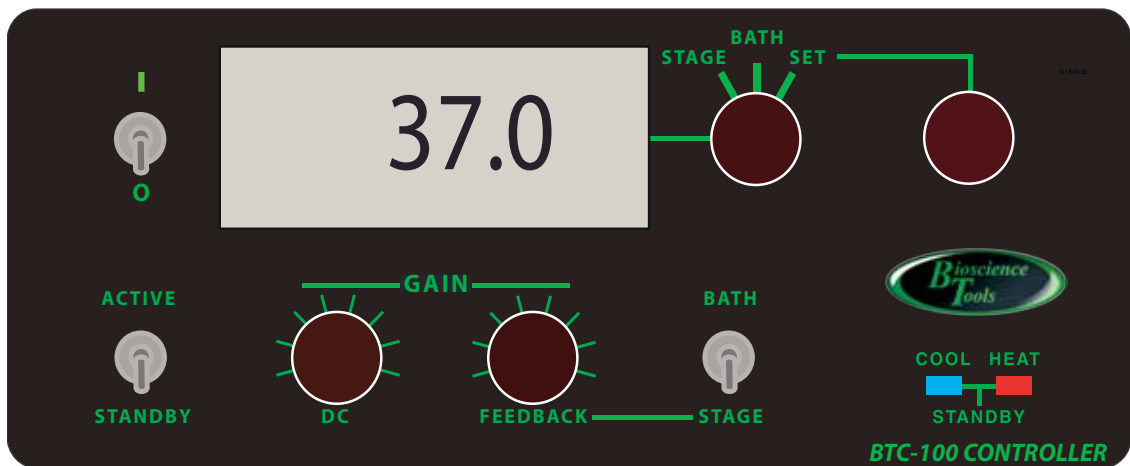
Now you can switch the controller from STANDBY state into ACTIVE state and observe on the temperature monitor how the controller regulates the temperature of the stage. GREEN LEDs on the front panels will indicate that the controller is functional.

This simple setting procedure will make systems with temperature sensors built-in inside heating/cooling elements functional within a few seconds. Setups with heating/elements elements distant from your sample, heaters for petri dish or chambers for coverslips for example, might require additional steps to achieve the correct temperature around your sample.

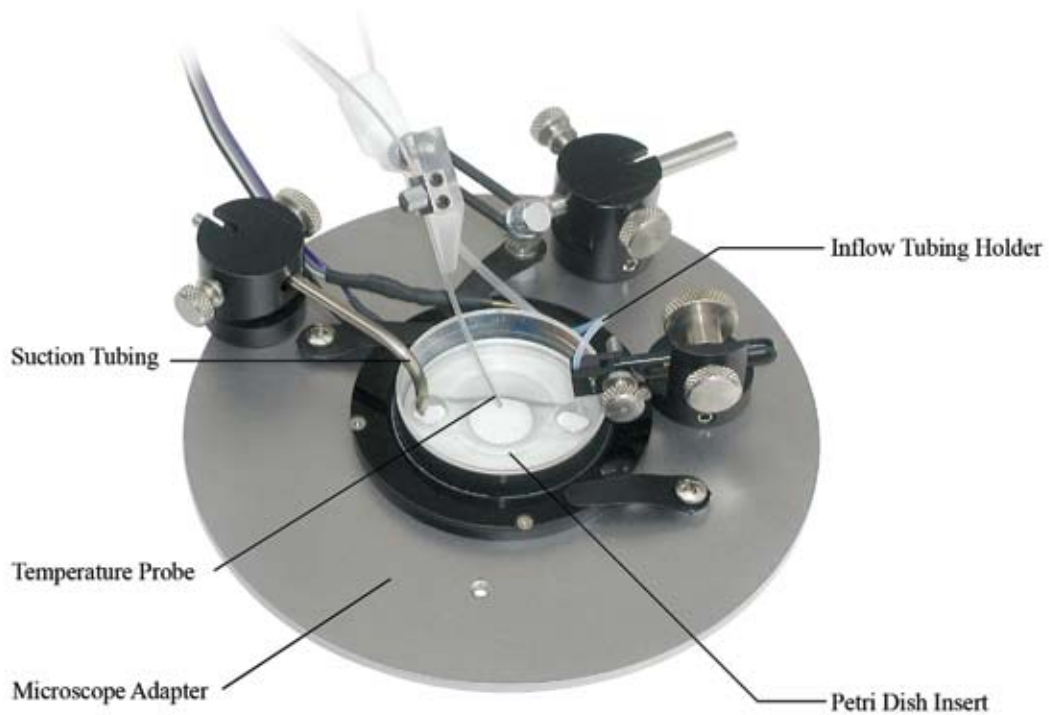
Since samples in the petri dish are located at some distance from the heating/cooling element, the temperature around the sample will be different from the temperature inside the heating element - this is called “temperature gradient”. If you switch the monitor to display BATH temperature, you will see this difference. A simple way to achieve the correct temperature around your sample is to switch the feedback of the controller to BATH temperature probe. This might result to temperature fluctuations, however. The fine tuning of the system is described in the following paragraphs.

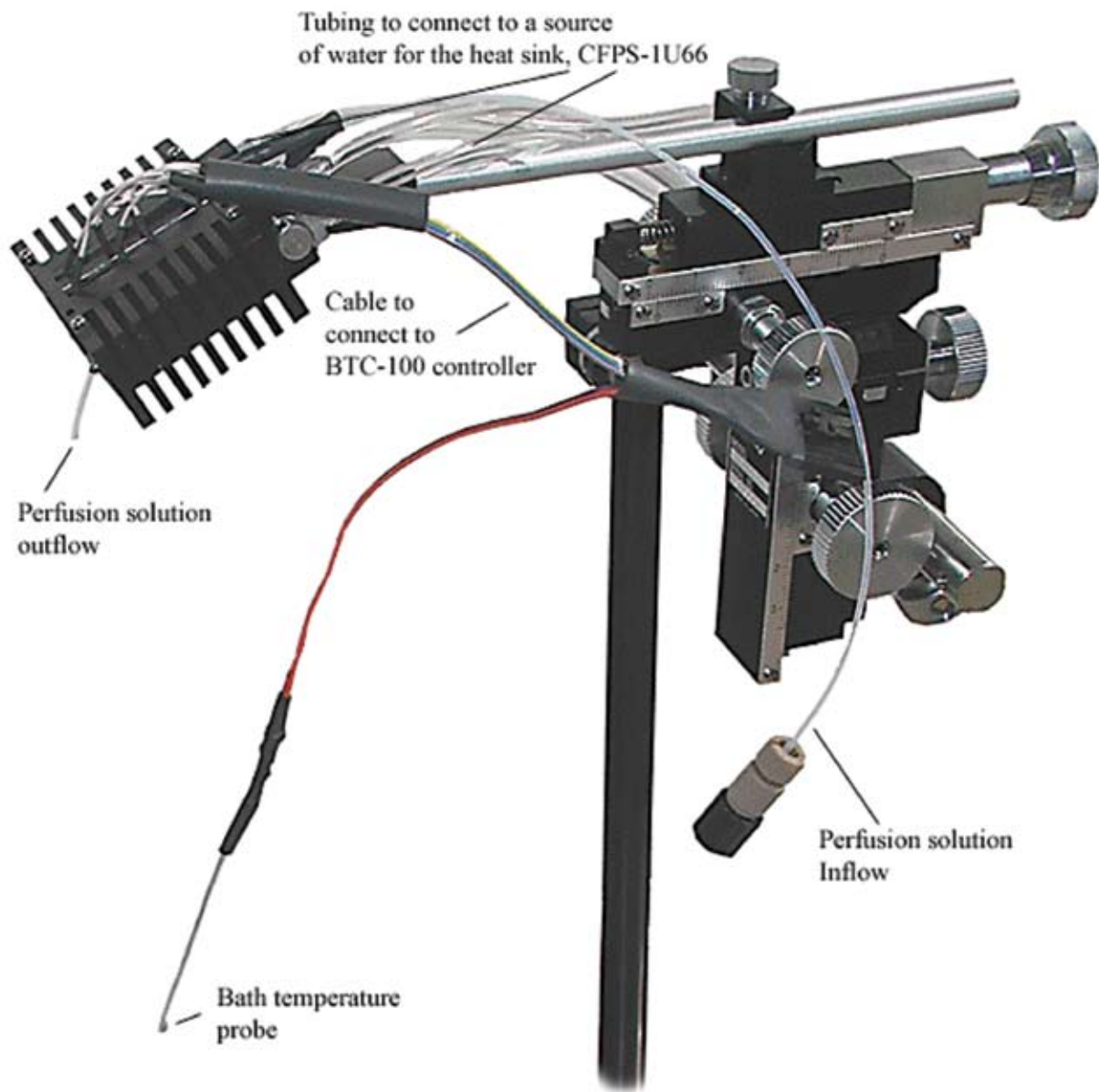
First, decrease FEEDBACK GAIN from the probe to zero. Then start increasing DC feedback GAIN until the temperature stabilizes near the SET level. This procedure might take up to an hour of your time. Just by doing this, you might achieve the acceptable level of temperature stability. This initial setup of providing enough DC power to the system is considered to be the first step of FINE TUNING procedure in general.

4b To make the controller follow the temperature fluctuations more actively, however, you need to increase FEEDBACK GAIN. If the system stabilizes at temperature above required SET level, decrease DC GAIN slightly until the required temperature is displayed. Note: day-to-day parameter fluctuations, volume of solution around your sample, for example, might affect stability of the settings, so some adjustments might be made by changing SET temperature as well, while still keeping the sample temperature at the required level.

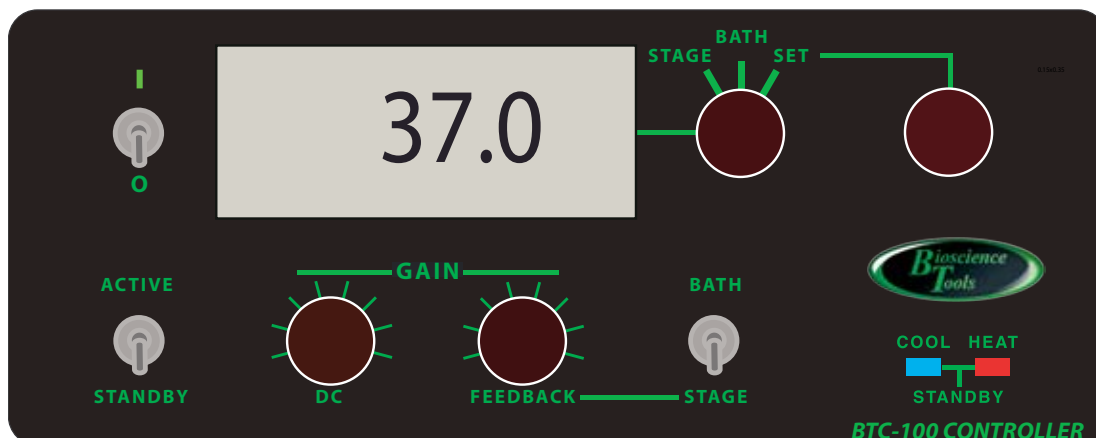


4c Using continuous perfusion of your sample helps eliminate the undesirable temperature gradient in the system. Note: if flow rate in the system does not change, temperature stability might be achieved by switching the controller back to feedback from STAGE probe, and adjusting SET temperature to a higher level, so that the BATH temperature is still at the correct point. This trick of using STAGE probe for feedback might be used without perfusion as well, especially with closed systems below. Using STAGE sensors for feedback usually provides more stable configuration with minimum temperature fluctuations.



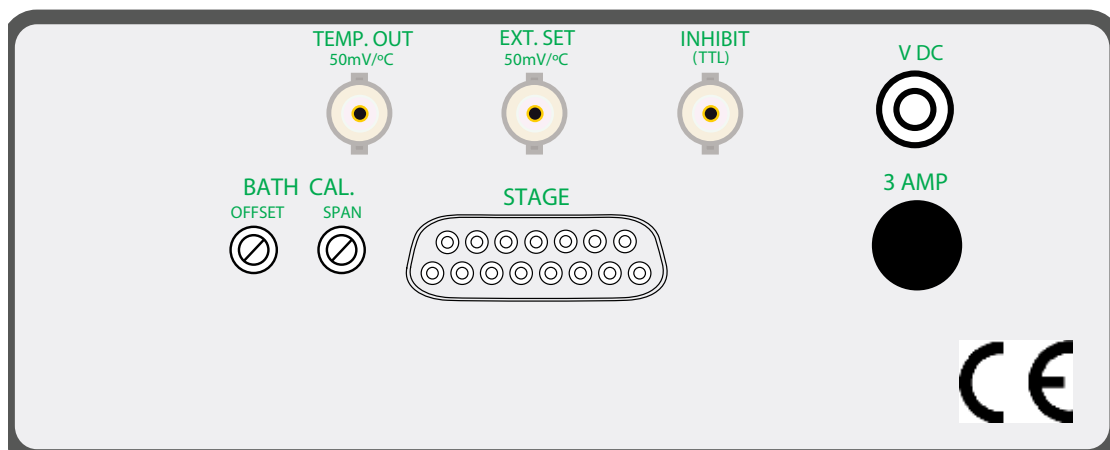


BTC-100 Front Panel Controls



Front Panel Controls	
I O switch	Turns POWER ON.
Red & Blue LEDs	Indicates COOL, HEAT and STANDBY modes.
STANDBY/ACTIVE switch	Turns the controller into ACTIVE (operational) mode.
LCD monitor	Displays temperature in degrees of Celsius.
STAGE/BATH/SET switch	Selects temperature to display on LCD monitor.
SET dial	Sets the reference temperature.
BATH/STAGE switch	Selects a temperature probe to use for the feedback to the controller.
GAIN dials	DC gain sets the level of power sent to the heating/cooling stage independent on readings from the feedback temperature probe. FEEDBACK gain sets the level of sensitivity to difference between SET temperature and the actual temperature obtained from the feedback temperature probe. Higher gain makes the controller to respond faster, but the level of fluctuations might be increased in some conditions.
Green LEDs	Indicate the level of POWER provided to the heating stage.

BTC-100 Output and Back Panel



Outputs	
STAGE DB-15 connector	Connects to the stage cable. Provides power to the heating stage and temperature readings to the controller.
TEMP OUT BNC	Used to monitor temperature reading from the external temperature probe, usually BATH temperature. 50mV/°C

Back Panel Controls	
POWER jack	Connect to the provided external DC power adapter or batteries.
3 AMP	Replacement fuse.
EXT. SET BNC	Used for temperature setting, using external sources, data acquisition systems, for example. 50mV/°C.
Inhibit (TTL) BNC	Connects to an external source of TTL signal (+5V) to switch the controller to STANDBY mode. Can be used to stop electrical disturbance during sensitive recordings.
BATH CAL. POTs	Although the controller is shipped calibrated, these internal potentiometers can be used to adjust the calibration settings. First, SPAN is adjusted to set the correct difference between temperature readings from two samples (usually ice water and hot tap water). Then, OFFSET is adjusted to set the correct temperature at the middle point (around 37°C, for example). Note: a high quality and high sensitivity temperature meter is required to calibrate the controller.

Noise and Grounding

Noise

50/60-Hertz power line noise may be encountered because of:

1. Improper grounding of probes, microelectrodes, bath or instrument chassis.
2. Radiation from transformers of adjacent equipment.
3. Power noise from attached equipment, stimulators, etc.
4. Antenna effects of cable or wire.
5. Potential difference between various components of electronic set-up (due to the distance electronics are from one another, or different earth grounds).

50/60 Hz noise is not the only electrical signal likely to cause interference problems, some others are:

1. Remote switches, such as in refrigerators or heaters.
2. Voltage pulses emanating from adjacent microelectrodes.
3. Broadcast interference from TV/Radio.

Instrument Grounding

Chassis Ground

The controller's case, Chassis, is connected to the system ground. The Instrument Chassis can be grounded manually by connecting to other system grounds.

Heating stage cable

The connecting cable is shielded, but the shielding is not connected to the heating stage. The shielding can be connected to the microscope stage or your system ground inside Faraday cage using the connector marked with COL-ORED handle. Grounding the shielding separately will help to eliminate loops made through shielding.

Specifications

Range

0-100°C

Accuracy

0.1°C

Temperature probes

built-in STAGE probe

external BATH probe

Feedback

from STAGE or BATH probes

adjustable DC and AC GAINS

Analog Input

Input analog voltage to SET temperature; rear panel

BNC; 5V/100°C resolution

Analog Output

To monitor temperature (5V/100°C)

Size (Controller) : 6Wx2.5Hx9D in.

Power Supply

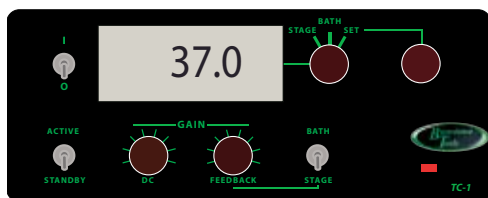
94 to 234 V AC, 50/60 Hz, External 12-24VDC

adapter

Warranty

This product is warranted to be free from defects in material and workmanship for the duration of one year. Normal wear, or damage resulting from abuse, accident, alteration, misuse, service by an unauthorized party or shipping damage, are excluded from this warranty and are not covered. Bioscience Tools will repair or replace the defective product covered by this warranty free of charge if it is returned, postage prepaid, to Bioscience Tools, 4527 52nd Street San Diego, CA 92115, ph: 1-877-853-9755.

Complete Temperature Controlled System



The complete functional temperature controlled setup includes the following components:

1

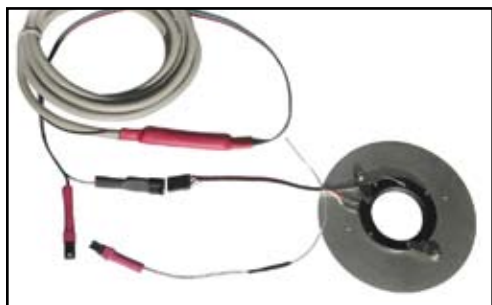
The main component of the system is a controller, which regulates the temperature of your sample by providing appropriate amount of energy to the heating/cooling elements.

The controller uses sensors to get the temperature feedback from the sample and compares these readings with required settings. There are two types of feedback: one is determined by the volume or thermal mass of solution around your sample, and the second is determined by difference between required temperature and the actual temperature of the sample. The first type is responsible for providing steady amount of power to the system to compensate thermal energy dissipation due to temperature gradient between your sample



and environment. It is regulated by DC GAIN. The second type is regulated by AC GAIN, and traditionally called FEEDBACK GAIN.

If heated bottom chambers TC-HB are used in the system, the sample is heated uniformly. The traditional chambers like petri dishes, however, are usually heated from sides, along the outer walls of the dish. This inevitably creates temperature gradient inside the dish with temperature being higher along the walls of the dish than temperature in the middle, where your sample is usually located. Using oil or water immersion objectives with regulated objective heaters effectively eliminates this undesirable gradient. Another way to eliminate the temperature gradient in the system is to use continuous perfusion, or flow of solution,



which is preheated by passing through the heating element. All our heaters can be used as solution preheaters.

2

Each system includes a set of connecting cables and temperature probes. The heating elements come with microscope adapters, and incorporate solution preheaters and temperature sensors.



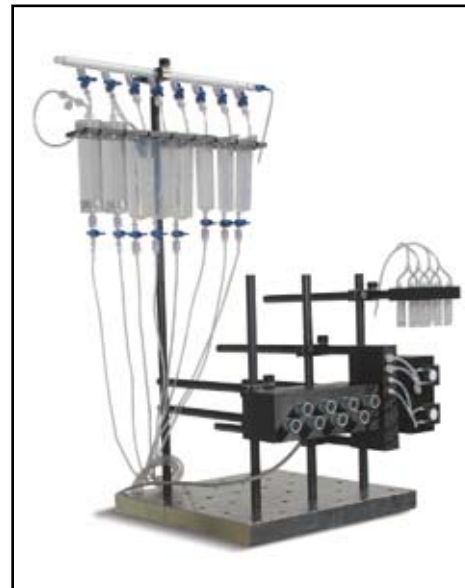
3

Closed controlled environment systems not only define gas composition and preserve moisture inside the system, but also help to prevent heat dissipation. In combination with heated top and heated immersion objectives, they provide effective way to control environment around your sample during long term experiments, time lapsed imaging for example. The closed systems also include a set of accessories to configure solution exchange, test substance application, or continuous perfusion of the sample. Petri dishes, chambers for coverslips, and chambered coverglasses can be used with these systems.

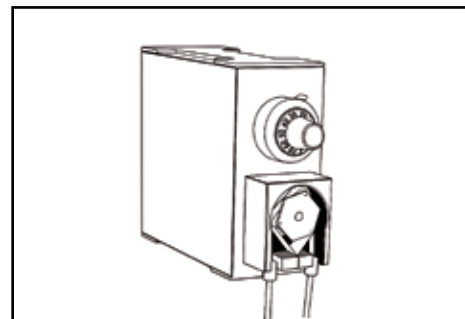


4

A set of perfusion accessories, from heated zero-dead volume manifolds to automatic solution switches, allow you to manipulate media around your sample and keep stable temperature control at the same time. Automatic flow controllers and simple gravity driven systems can be combined to switch and mix up to 320 different solutions. Computerized packages with software to program any solution exchange protocols will create flexible experimental environment to perform not only test substance applications during imaging, but staining your sample while still under the microscope.



To complete the heated perfusion system you need to configure solution outflow, which is required in the open systems - petri dish, for example. The controlled flow unit CFPS-1U66 can handle up to 22ml/min. Outflow starts automatically, every time perfusion is ON, to prevent accidental solution overflow in the system. Multiple flow control units can be used to control inflow rate as well.



Chambers for replaceable coverslips - CSC

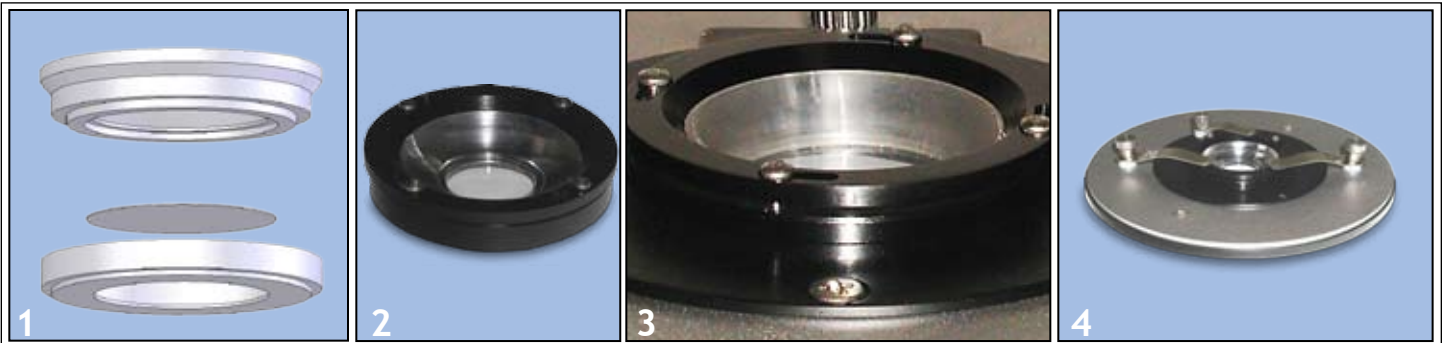


Example of using CSC chamber in a perfusion setup. Magnetic holders are arranged on a microscope adapter MA to provide solution inflow and outflow.

1. Position the bottom part of the 2-parts chamber on a flat surface. Put a cover slip inside the groove in the bottom part. Put the top part inside the bottom part.
2. Fix the plastic insert with a metal ring from the top.
3. Put the assembled chamber inside microscope adapter or the temperature controlled stage.
4. Use provided clamps to fix the chamber in place, this is especially useful if oil immersion objective is used with an inverted microscope.

Arrange magnetic holders with inflow manifold and

Catalog #	Features:
CSC	Chamber for replaceable round coverslips. Simply put a coverslip inside and seal by a snap-in action.
	Choose the right diameter to fit your coverslips.

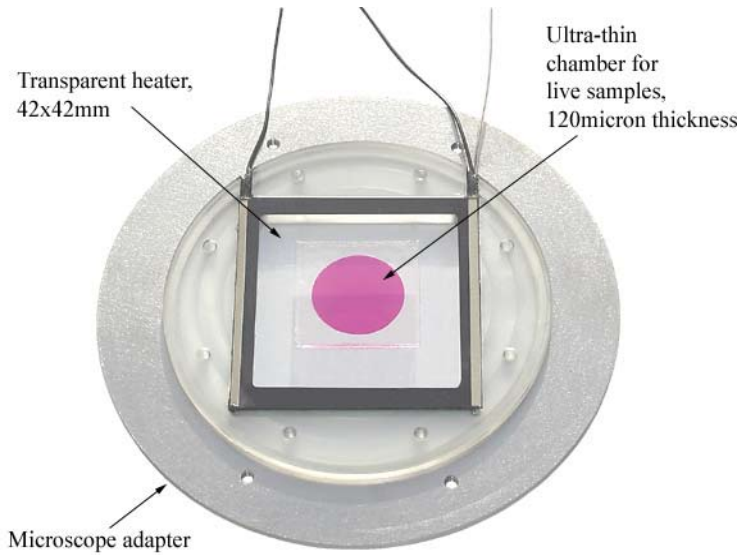


suction tubing around the chamber. While using the cover slips with cultured cells, excess of solution on the cover slip might result in bad seal and cause leakage. Try to leave only a minimum amount of liquid on the cover slip before assembling the chamber.

Note: Although the plastic CSC-10P chamber is tight enough, you can further improve the seal against solution leak by putting a thin layer of silicone grease or mineral oil (or Vaseline) inside the bottom part of the chamber, especially along the edges of the groove for the cover slip. Using provided clamps helps to seal the chamber as well.



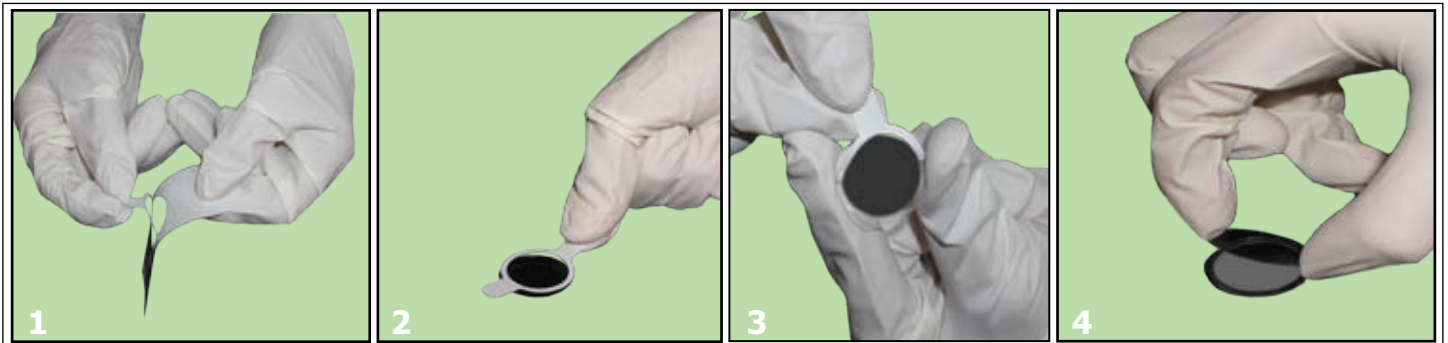
Ultra-thin imaging chambers - UTIC



Ultra-thin chamber formed on top of a heated glass plate TC-HP50x50. Heated slides TC-GS can be also used.

1. Remove protective liner from the bottom surface to expose the adhesive.
2. Apply the adhesive side down onto the surface of a coverslip, glass slide, or on the bottom of a chamber. Press gently to seal.
3. Remove the remaining protective liner. Aliquot a small amount of media into the chamber, or place your sample inside and fill the chamber with additional medium.
4. Place another coverslip on the top. Press gently but firmly to seal the chamber.

Catalog #	Features:
UTIC-21	Ultra-thin self adhesive chambers for high resolution imaging. Can be used with coverslips, and on any glass or plastic surface.
UTIC-11D	
UTIC-20-24x24	
UTIC-13-24x24	
quantity	Pack of 100 layers.



5. Place the sealed chamber into metal holder UTIC-25, which fits microscope adapters MA and heating stages, TC-E35. An open chamber can be also formed using a plastic holder, PCCS1 for example.
6. The holder and glass surface can be cleaned after use by removing residual adhesive with a scalpel. Adhesive Removal solutions are also helpful.



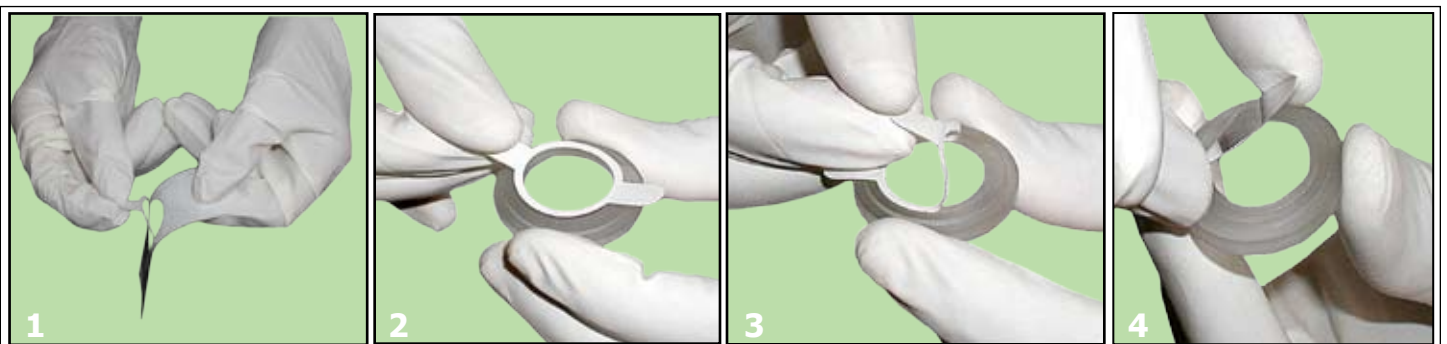
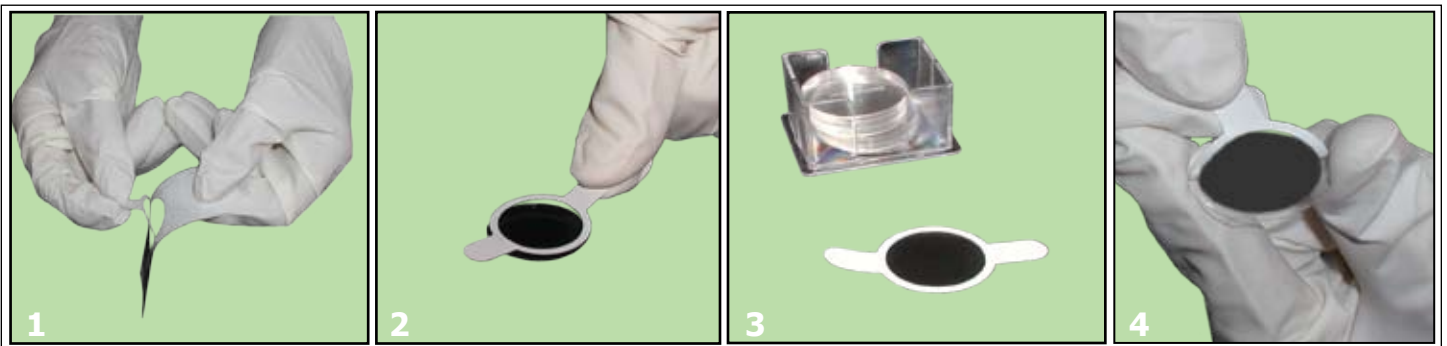
Low-profile Laminar Perfusion Chamber - PCCS2



Low-profile chamber inside microscope adapter MA. Miniature magnetic holder with suction tubing MTH-S and zero-dead volume manifold ZMM are positioned around the chamber to form a perfusion system. PCCS2 chamber has separate compartments for inflow and outflow. This prevents bubbles from entering the working volume and provides smooth perfusion. Laminar profile facilitates solution exchange.

1. Remove protective liner from the bottom surface to expose the adhesive.
2. Apply the adhesive side down onto the surface of a coverslip. Press gently to seal. This can be done even if the coverslip contains media with your sample.
3. Add media and/or sample onto the coverslip if some incubation is necessary. Otherwise proceed to step 4.
4. Remove the remaining protective liner, and put the plastic holder on top of the coverslip. Press gently to seal.

Catalog #	Features:
PCCS1	Low-profile plastic chamber-holder for 25mm coverslips.
PCCS2	Low-profile plastic perfusion chamber-holder for 25mm coverslips. Separate compartments for inflow and outflow to prevent bubbles to enter the working volume.
UTIC-21	Replacement adhesive layers, x100
UTIC-11D	Replacement adhesive layers for PCCS2 chamber, x100



Alternatively, if the sample does not have to be cultured on the coverslips, for example, you can place adhesive layer onto the plastic holder first, and then attach the coverslip to the holder:

1. Remove protective liner from the bottom surface to expose the adhesive.
2. Apply the adhesive side down to the bottom of the holder. Press gently to seal.
3. Remove the remaining protective liner.
4. Position a coverslip on the holder. Press gently to seal.
5. Position your sample inside the chamber.
6. Arrange inflow tubing, suction tubing and reference electrodes or probes around the sample using magnetic holders.
7. Adjust the angle of suction tubing using screws in the holder.

The chamber can be used with both stainless magnetic MA and universal IMA microscope adapters. The microscope adapters provide adjustable clamps, which can be used to fix the chamber firmly in place. This is especially useful if oil immersion objectives of inverted microscopes are used.

Both types of chambers PCCS1, PCCS2 and PCCS1-S, PCCS2-S are reusable.

Polycarbonate PCCS1, PCCS2 chambers can be used with any adapter for 35mm dishes. They provide not only low-profile to access your sample with electrodes and other accessories, but a flashed bottom surface available for immersion optics. After use, the coverslip should be removed by breaking and the chamber needs to be cleaned from residual adhesive. Use a scalpel or blade to pick up the adhesive, and wash with a Adhesive Remover, or Paint Remover solution available from hardware stores (Home Depot).

Silicone PCCS1-S, PCCS2-S chambers require PCCS-21 holder with 21mm optical clearance, which fits MA or IMA microscope adapters. The chamber can be also used with hating microscope stages TC-PD-15, which have 15mm optical clearance. The adhesive can be easily removed from these chambers without destruction of the coverslip. Simply lift the chamber, while pushing the coverslip down with an appropriate tool, forceps for example.



Example of using Low-profile chamber together with miniature holder MH-2. This multi-holder is capable of positioning several tubing around the chamber to form a perfusion system.