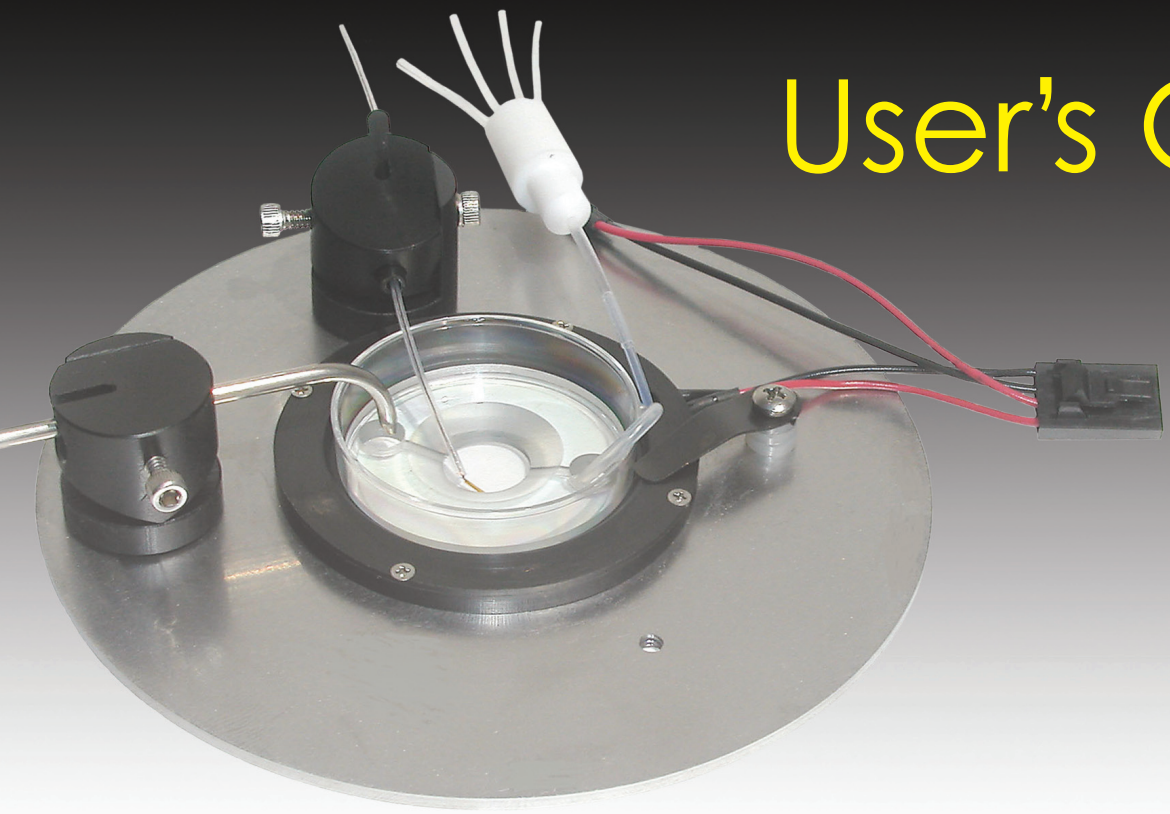
Temperature Control

User's Guide



Cooling Stages & Heaters

- **Precise Temperature Control throughout the experiment**
- **Conditions similar to** *in vivo*
- **Compatible with any perfusion system**
- **Heating stages for any microscope**
- **Compatible with Imaging systems**

Bioscience Tools

Ph: 877-853-9755
www.biosciencetools.com

Contents

CE

Specifications

Range -80 to 150ºC
Accuracy 0.1ºC
Stability 0.01ºC, required for sensitive applications: nano & piezo positioning, TIRF & AFM

No electrical noise suitable for electrophysiology
No vibrations no internal fan
Temperature sensors
built-in STAGE sensor
optional external probe BATH (0.87mm)
Feedback
from STAGE
adjustable DC and AC GAINs, self-adjusting

Overheating protection
Output 8A max (max 35V)
Size (Controller) : 8Wx4Hx9D in.
Power Supply
100-240VAC 230W

Introduction

The complete temperature control system includes a controller, an external temperature probe (optional), and a connecting cable to a cooling/heating element. All heating/cooling elements include a temperature sensor built inside the element - STAGE sensor. This internal sensor is used for FEEDBACK. It is also used to prevent accidental overheating of the sample. Most heating elements can be used as inline pre-heaters, if connected to a perfusion systems. If used with a microscope stage, a microscope adapter might be required to fit the cooling/heating elements to your microscope table. The following are an illustrated installation guide and example configurations of temperature controlled setups.

Installation Guide

1 Connect power cable. Plug the power cable into wall outlet. Plug the heating stage cable into the output connector on the back of the controller - DB-9 female connectors

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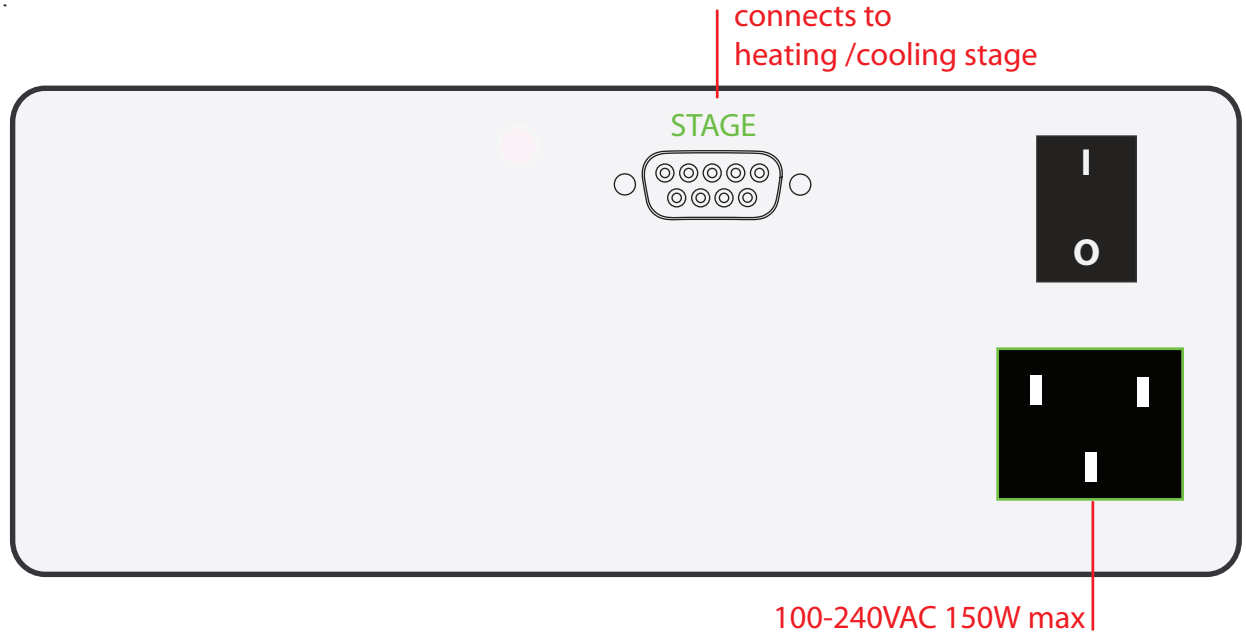
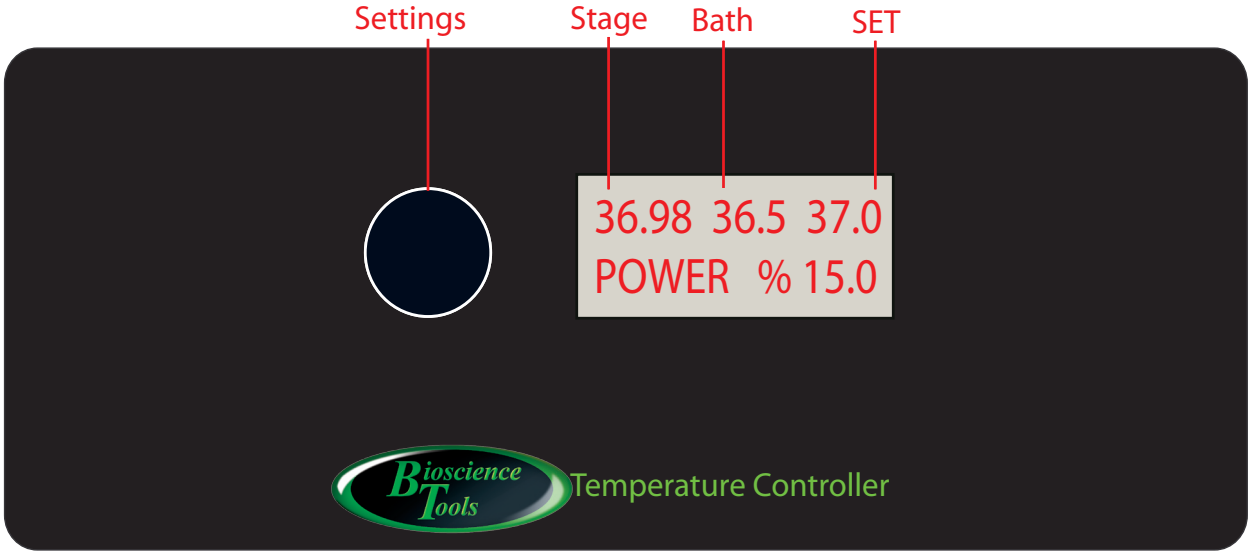
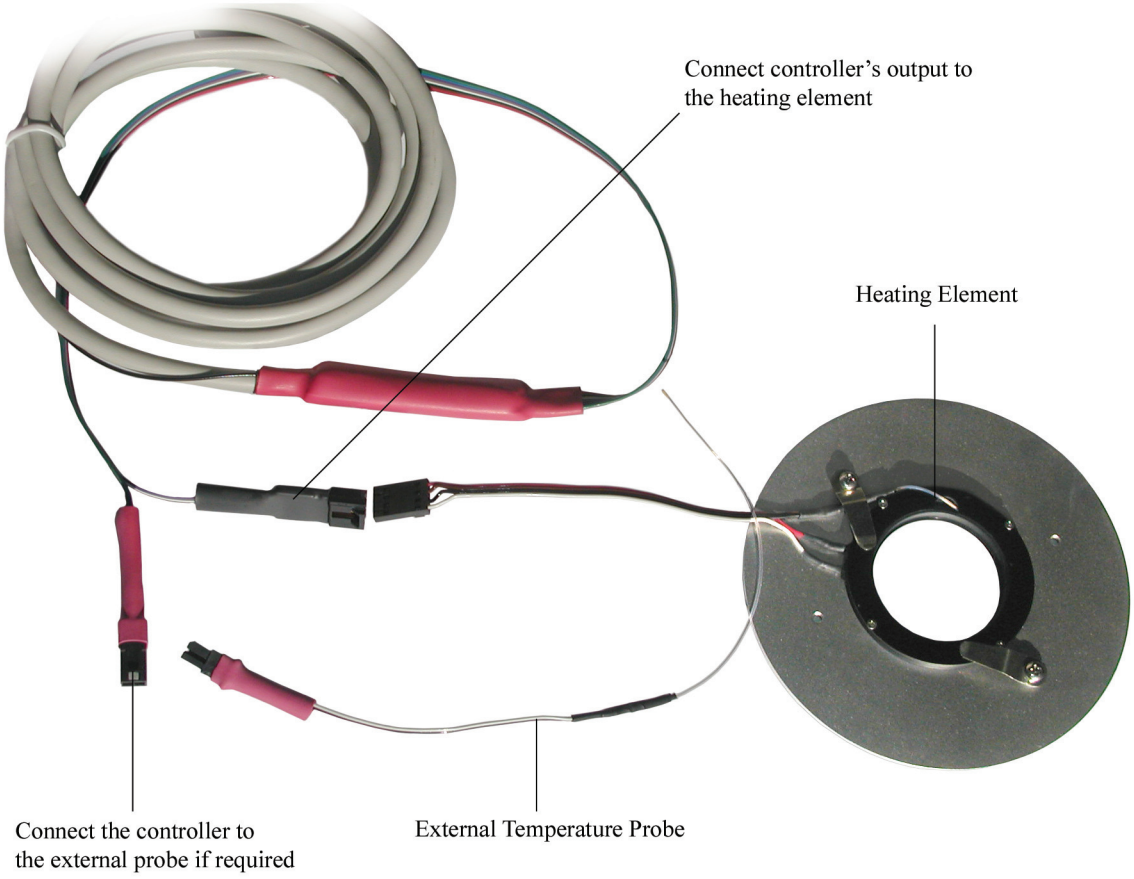
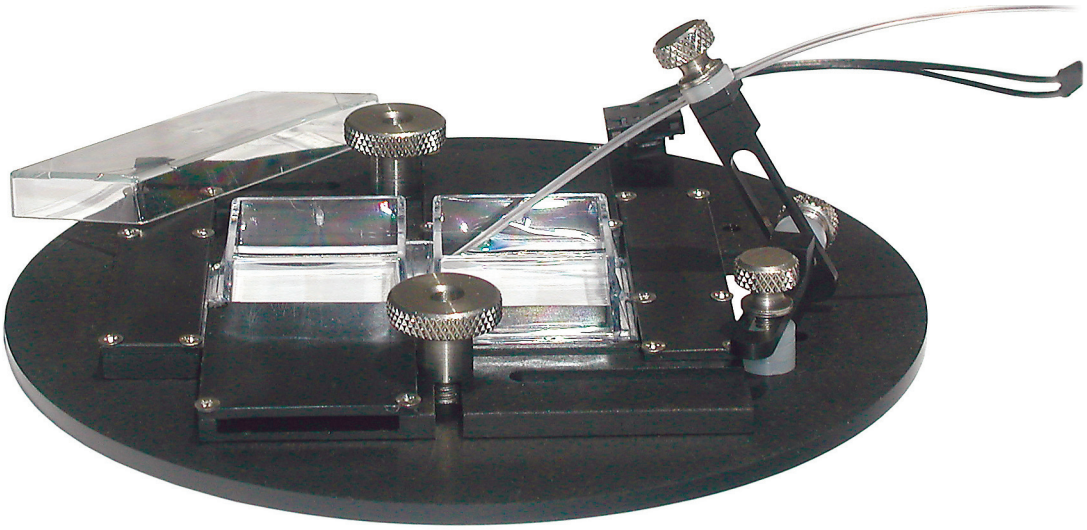




2 Connect the output cable to the heating element/stage and external temperature probes, if used.



3 Prepare the sample chamber, petri dish for example, by filling the chamber with water. Using optional adjustable holder, position the external temperature probe inside the chamber - optional BATH probe. 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. Turn the controller ON - power switch on the back of the controller.



4a The controller has one LCD display, and SET dial, which allows you to adjust the reference temperature by rotating the dial - BLACK knob on front. The same knob is used to switch the controller from STANDBY mode to ACTIVE CONTROL mode. The controller ships with setting adjusted to provide stable operation at 37 C. The controller can store settings for two different heating elements/environments or temperature levels. These two sets can be selected by rotating the knob on the front panel: the display will show:

"Settings-------->1/2"

By pressing the knob, you will select either number 1 settings or number 2 settings. This can be done while the controller is in ACTIVE mode, to generate fast temperature steps by switching from first setting with lower temperature to another with higher temperature (from SET temperature 35ºC to 45ºC for example).

To switch the controller from STANDBY state into ACTIVE state and observe on the temperature monitor how the controller regulates the temperature of the heating stage: press the knob once and rotate clock-wise to switch CONTROL ON.

24.5 37.0
CONTROL ON

The display will show POWER provided to the heating element.

36.9 37.0
POWER % 2.5

Push the knob twice to adjust SET temperature. The display will show:

24.5 37.0
SET tºC

rotate the dial to change the settings. After a few seconds after selecting SET level, the display will return to the original screen.

To switch the controller back to STANDBY mode, push the knob for display to show

CONTROL ON

and rotate anti-clock-wise to switch CONTROL OFF. In a second the display will show:

35.1 37.0
STANDBY

In order to change the gains from feedback, push the knob three time to show AC% level:

AC % 10

and rotate to change feedback sensitivity from 0 to 100%: Push the dial again to show DC% level:

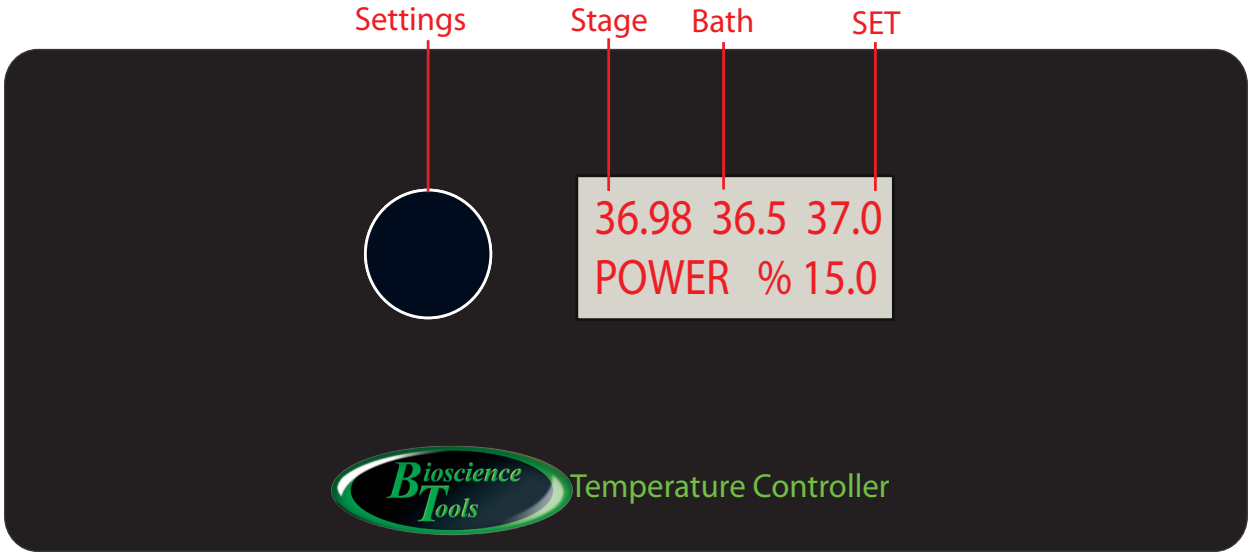
DC % 15.2

and rotate to change DC level, which is used to tune the controller for precise control. Although it is faster to adjust the controller by changing DC and AC levels manually, the controllers are self-adjusting to different environments if necessary: changing the heating elements for example, changing volume/mass of the sample or wide temperature fluctuations in the room. The controller can store settings for two different heating elements/environments. These two sets can be selected by rotating the knob on the front panel, after the controller is turned ON: the display will show “Settings-------->1/2”.

To prevent temperature overshoot and overheating, AC level can be adjusted to optional lower values, less than 1% (in 0.1-0.9% range, 0.9% is usually enough for the objective heaters TC-HLS). These low AC levels ensure slower temperature increase upon turning heating ON, in comparison to higher AC levels (up to 100%). These AC levels will also prevent overheating above SET temperature level, if the heater is not properly attached to the objective.

Additional built-in feature associated with low AC levels (<1%), is extra overheating protection if temperature gets above SET level more than 1ºC. This threshold can be always decreased even lower by adjusting HOT tºC level.

The low AC levels (0.1-09%) are optional to use, and can be changed to higher values if necessary.



You can set HOT temperature threshold level, if using AC above 1%, by pressing the dial to display:

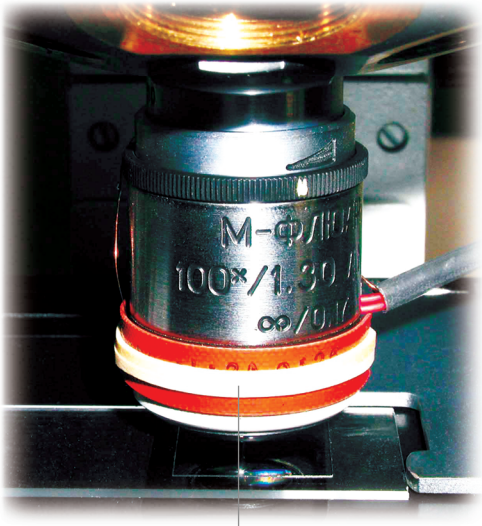
HOT tºC 45.0

After this, the controller will turn heating OFF, if STAGE overheats above the threshold.

Although the heaters tested and the controller is adjusted before shipment, the controller might need to be adjusted again after installation (attaching the heater to the objective):

First, turn heating ON with factory adjusted settings. Heating is turned ON by pressing the knob on the front panel (display will show “CONTROL1/2 OFF”) and rotating the knob clock-wise to show “CONTROL1/2 ON”. The controller starts self-adjusting (tuning) to stabilize around SET temperature level. If tuning takes too long, at the end of the experiment, press the front knob to display DC% level (this will put new DC% adjusted level into the controller memory and will shorten significantly tuning the next time the control is ON). DC% level can be adjusted manually, if temperature does not reach SET level fast enough, or stabilizes above SET level: increase/decrease gradually DC level in steps not more than 0.5% until temperature stabilizes at SET level.

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



Objective Heater

Uniformly Heated Bottom

Since samples in the petri dish are located at some distance from the heating element, the temperature inside the sample will be different from the temperature of the heating element - this is called “temperature gradient”. If you use an external probe to display BATH temperature, you will see this difference, provided the external temperature probe placed inside the sample chamber. You can achieve the required temperature in the sample chamber by increasing SET reference level to compensate the offset temperature difference between heating elements and solution inside sample chambers.

Using an objective heater with oil or water immersion objectives will eliminate this temperature gradient. The second channel of the controller (or another controller) is usually used to regulate the temperature of the objective heater, which has a built-in temperature sensor and does not require too much of fine tuning.

The chambers with uniformly heated bottom, TC-MWPHB, TC-HP75x65 or TC-HPQ75x50 for example, usually do not generate undesirable temperature gradient.

The controller can also minimize temperature fluctuations measured by the external BATH probe located/placed remotely from the internal STAGE sensor. The fluctuations are observed sometimes due to room temperature changes, during overnight experiments for example. The new tracking feature will report observed fluctuations and suggests recommended changes in compensation level (from 0 to 10 ºC/per ºC room).

Press the front knob to show TRACK OFF, and rotate to switch tracking ON:

TRACK ON

Press again, and make sure that dt/ ºC value is set to 0.0 vale:

dt/ ºC 0.0

After working for long time, the controller will detect any fluctuations in ROOM and BATH temperatures (ºC if

any), which can be displayed by pressing the front knob several times:

MIN dBATH -0.1
MIN dROOM -0.2

After pressing the knob again, you can see MAX fluctuations during the experiment (ºC):

MAX dBATH 1.5
MAX dROOM 1.2

In order to compensate the changes in BATH temperature, that happened due to fluctuations in ROOM temperature: press the front knob several time until the display show

dt/ ºC 0.9
TRACK ON

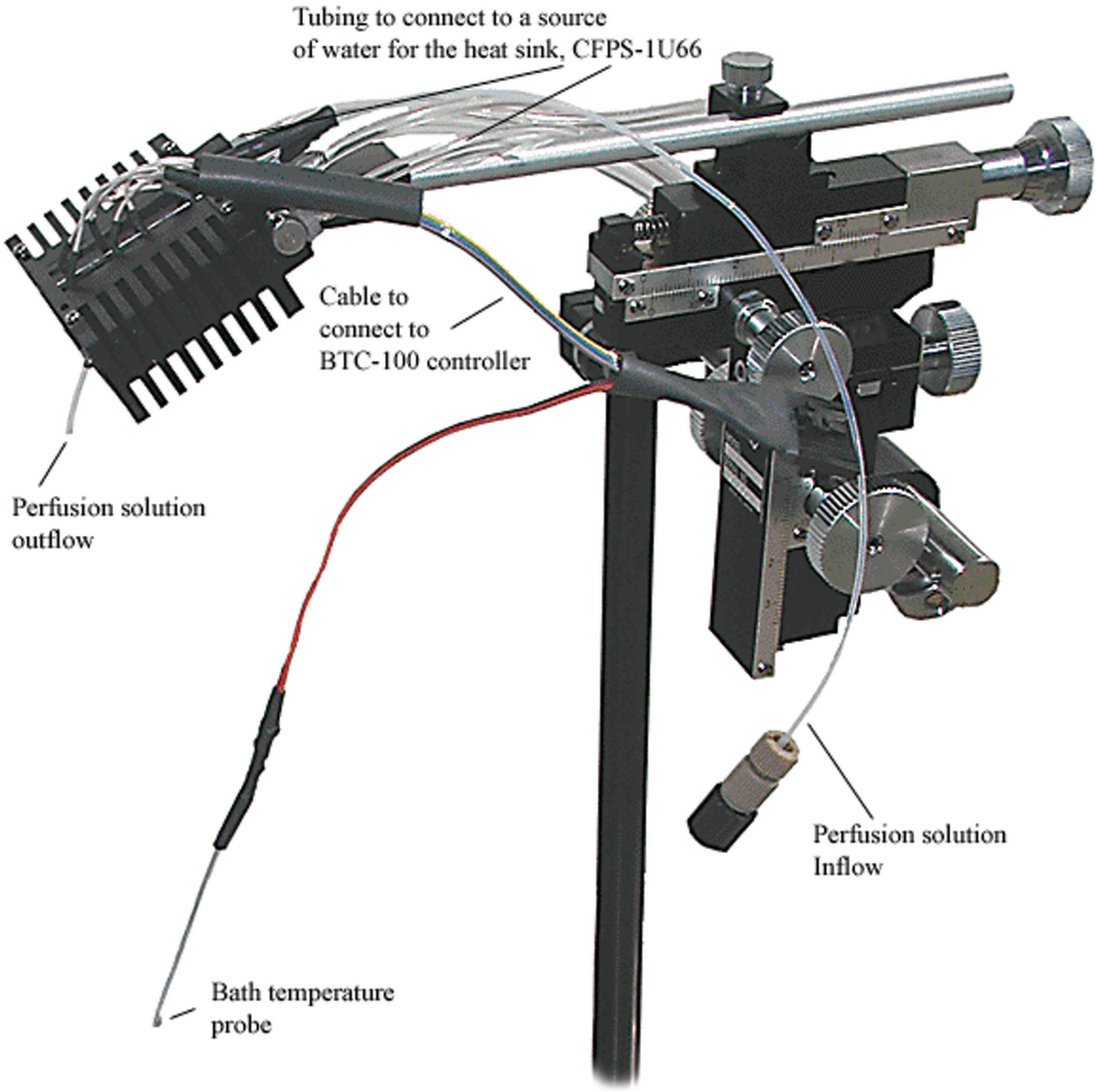
And make a note of the suggested value of 0.9 for dt/ ºC parameter. Then, press the knob again and adjust the value for dt/ ºC:

dt/ ºC 0.9

Note, that this is a suggested value, and might have to be adjusted after next experiment again. This new feature is similar to using feedback from BATH probe, but results in much more stable temperature control with minimal temperature fluctuations. This is also much safer in a sense, that it will nor result to overheating of your sample due to customer error - failure to position the external probe inside the sample for example.

4b Using continuous perfusion of your sample helps to eliminate the undesirable temperature gradient in the system.

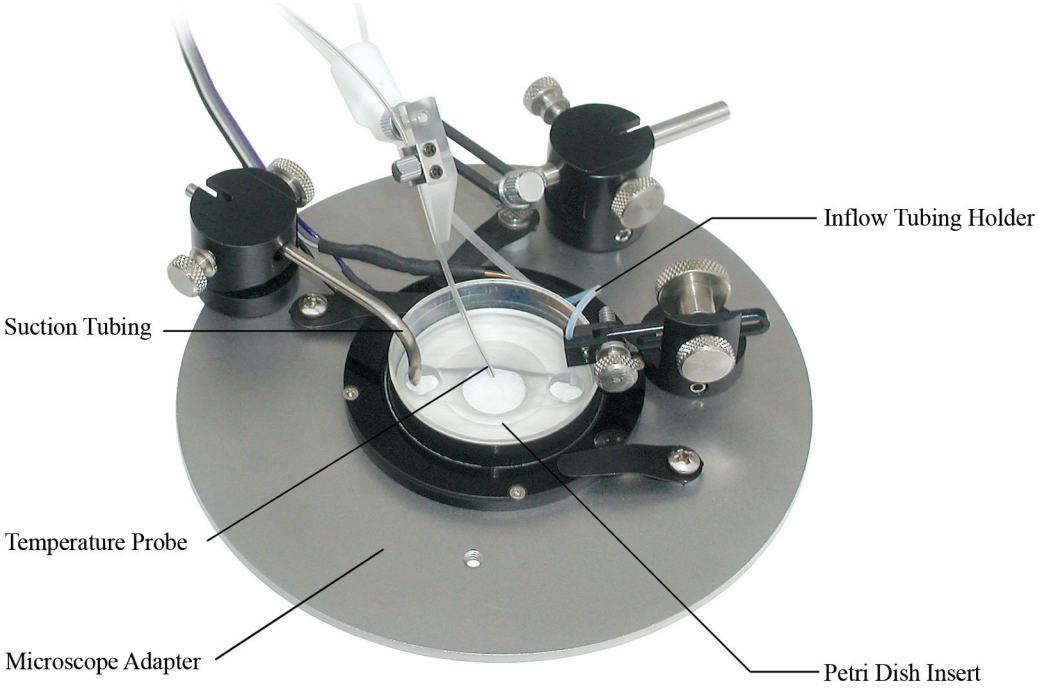
This miniature perfusion unit TC-RD (on the right) is used as a preheater or cooling unit during sample perfusion. It connects to the second channel, or another controller



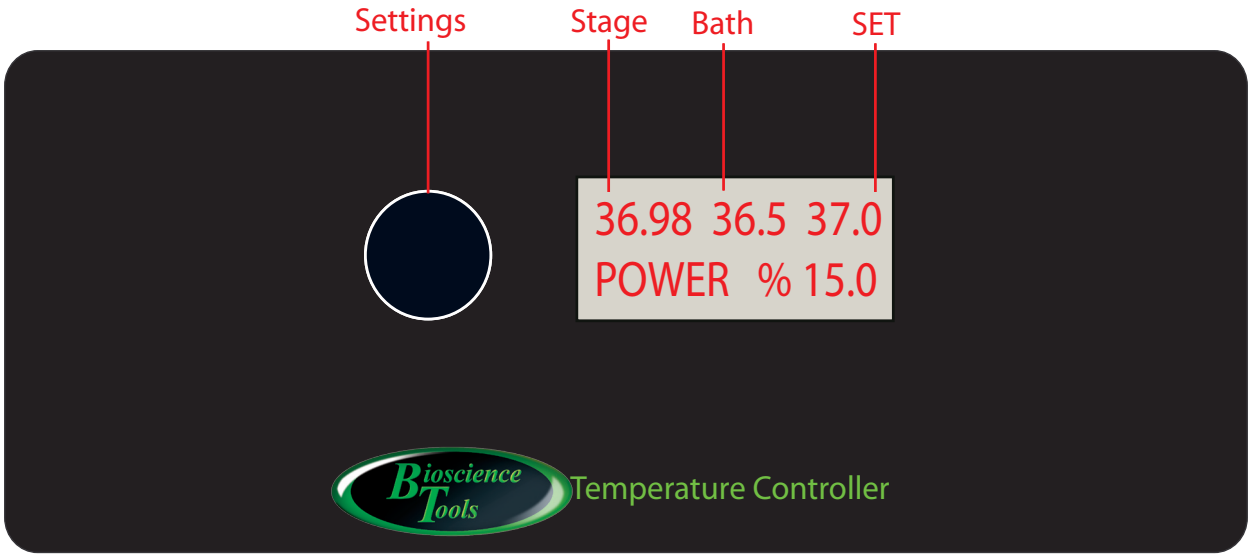
4c If a cooling stage is used for very low temperatures, you might need to provide cold water flow through the cooling unit heat sink using a CFPS-1U/66 unit. Two of positioned vertically ports need to be connected together, while the remaining two ports are used for inflow and outflow heat sink water

4d During cooling operation the stage heat sink can overheat, which will inhibit bringing the temperature down too much. In order to help the stage to reach low temperatures, a BTC-W water cooling unit should be used. Simply connect INPUT to a water source and OUTPUT to the sink port on the stage. NOTE: some stages have separate sinks: so either two BTC-W units should be used, or one sink should be connected to the other in sequence.

This water cooling unit can be also used with TC-RD miniature perfusion unit. TC-RD is used as a preheater or cooling unit during sample perfusion.

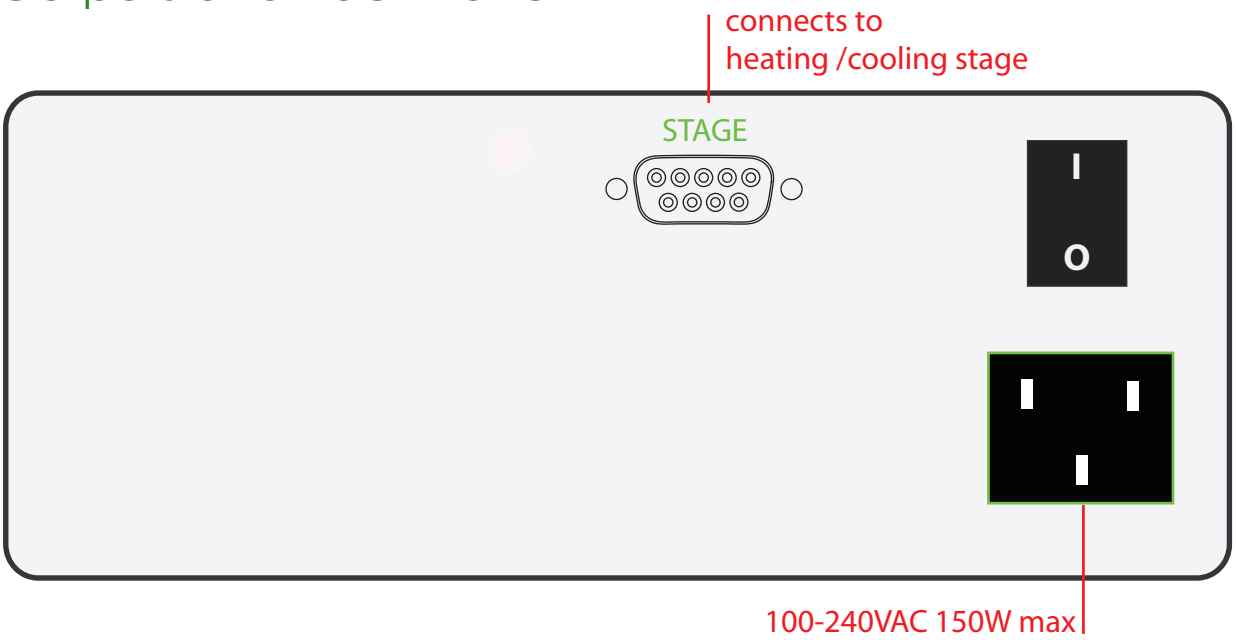

Inflow Tubing Holder
Suction Tubing
Temperature Probe
Microscope Adapter
Petri Dish Insert

BTC-1-100 Front Panel Controls



Front Panel Controls	
LCD monitor	Displays temperature readings from temperature probes, and shows SET temperature.
Display Switch (knob)	Switches the display to show settings by pushing the knob down: each click will switch to adjust different parameters in the following order: CONTROL ON/OFF, SET, AC %, DC %, t°C HOT, TRACK, dt/°C
SET dial (knob)	After display shows SET tC - sets the reference temperature
STANDBY dial (knob)	Provides POWER to the heating stages. After the display shows CONTROL, rotating clockwise, turns the controller into ACTIVE modes; anti-clockwise - into STANDBY mode when no power is provided to the heating stages.

Outputs and Back Panel



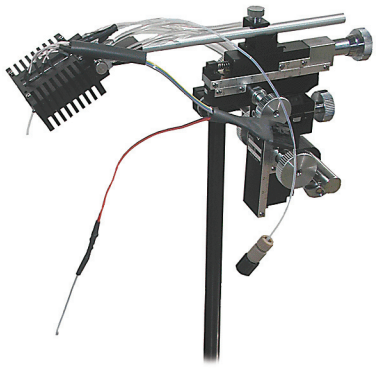
Outputs	
DB-9 Output	Connect to STAGE cables to provide power to the heating element and to provide temperature readings to the controller.

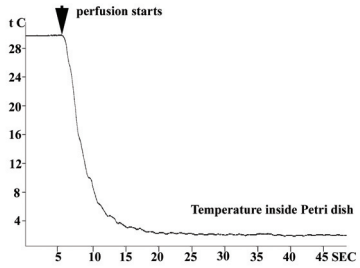
Back Panel Controls	
SWITCH	Turns the controller ON/OFF.
POWER jack	Connects to 100-240VAC power outlet.

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, ph: 1-877-853-9755.

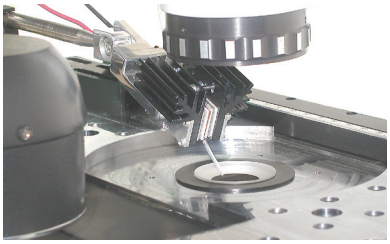
Cooling/Heating Elements





Miniature Perfusion Cooler/Heater Unit TC-RD

Controls temperature of perfusion solutions in the range from 0 to 150°C. A small heating/cooling element is designed to mount on a manipulator next to your sample to provide fast temperature changes by streaming the solution directly onto the sample. Monitoring and control temperature by data acquisition systems through BNC connectors on the back panel. Set temperature either manually or using your data acquisition interface, to generate ramps, for example. Optimized for patch clamp applications: no electrical noise.



On the right is an example of fast temperature change inside a petri dish. Experimental conditions: TC-RD system was set at 0°C; the petri dish was set at 30°C using another TC-1 controller and TC-PCP heating stage; two flow control CFPS-1U66 units were used - one for solution suction from the dish, through PDI insert inside the dish; and another CFPS-1U66 to cool heat sink of TC-RD unit; the third CFPS-1U unit was used to perfuse the dish; a slow temperature sensor was used to simulate temperature change in the whole dish; the actual temperature change in the point of solution application is much faster. Item#: TC-RD

- **Dimensions:** 1 x 2 x 2in
- **Temperature stability:** better than 0.1°C, built-in sensor
- **Sink:** water cooling for very low temperatures
- **Feedback:** Selectable Stage, or External sensor (Bath)

Cooling & Heating Microscope Stage for coverslips BTC-S-35

- **Dimensions:** 120x120x23mm
- **Optical aperture:** 22mm
- **Temperature stability:** 0.1°C, built-in sensor
- **Sink:** liquid cooling for very low temperatures, optional water cooler unit BTC-W
- **Solution Pre-heater/cooler:** Replaceable/Removable Teflon tubing, easy to wash
- **Microscope adapter:** Fits to 74mm cutout of standard microscope adapters

Can be used with: Standard 35mm disposable Petri dishes; CSC chambers for replaceable coverslip chambers. Requires BTC-100 controller. Requires a microscope adapter (specify microscope model). Item#: BTC-S

Cooling & Heating microscope stage for slides, BTC-SL

- **Dimensions:** 120x120x23mm
- **Temperature stability:** 0.1°C, built-in sensor
- **Sink:** liquid cooling for very low temperatures, optional water cooler unit BTC-W
- **Optical aperture: 40x**22mm
- **Microscope adapter:** Fits to 74mm cutout of standard microscope adapters

Slides and Chambered Coverglasses Cooling & Heating stage, BTC-SLM

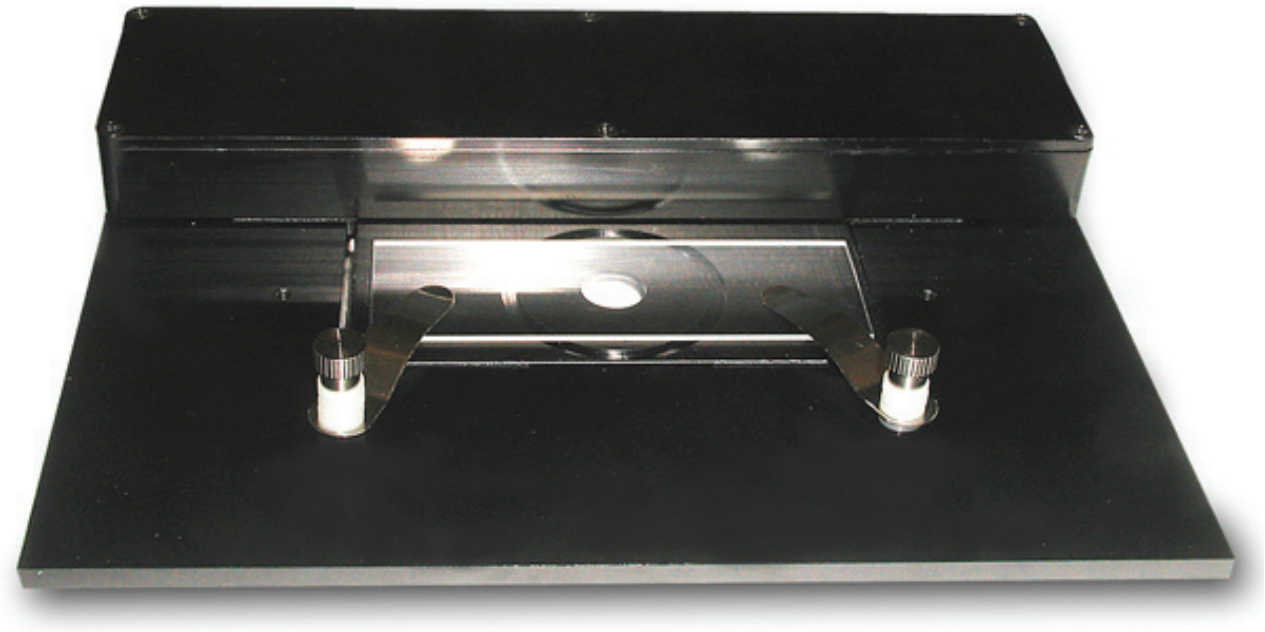
Can be used with: custom devices, disposable slides and coverglasses. Can cool the sample down to -5°C (or heat up to 150°C). Fits 160x110mm cutout of motorized stages, and type K Zeiss stages. The cooling area is an inside cutout 26x79mm (to fit standard slides), with 20x40mm aperture in the middle. The inside cutout is 17mm deep, with 1mm lip to hold the sample. Requires sink cooling and a temperature controller. **Item#: BTC-SLM**

- **Dimensions: 110x160x18mm, 26x79mm cooling/heating area**
- **Optical aperture:** 20x46mm
- **Objective working distance, minimum:** 0mm (for inverted microscopes)
- **Temperature stability:** 0.1°C, built-in sensor
- **Sink: optional** water cooling for very low temperatures, requires BTC-W unit
- **Microscope adapter:** Fits to 74mm cutout of standard microscope adapters IMA-74

Low-Profile Cooling & Heating plate, BTC-L

Can be used with: standard 35mm disposable Petri dishes, glass bottom dishes, and disposable slides and coverglasses. Can cool the sample down to -2°C (or heat up to 150°C). The cooling area is 40x80mm with 10mm aperture in the middle. The low profile of the stage allows easy access to your samples. Provided clamps will fix the sample in place. Can be placed on upright microscopes. Can be mounted on a microscope stage (specify dimensions of microscope stage cutout, 108mm diameter for Nikon for example). Requires sink cooling and a temperature controller. **Item#: BTC-L**

- **Dimensions:** 120x160mm, 80x40mm cooling/heating area
- **Optical aperture:** 10mm diameter
- **Objective working distance, minimum:** 0mm (for upright microscopes)/ 3mm (for inverted microscopes)
- **Temperature stability:** 0.1°C, built-in sensor
- Heat **Sink: optional** water cooling for low temperatures, requires BTC-W unit
- **Microscope adapter:** Fits to 74mm cutout of standard microscope adapters IMA-74



Low Profile Cooling & Heating stage for Slides and Chambered Coverglasses, BTC-SL-128x86

This low profile heating/cooling stage designed to fit inside 128x86mm holders for standard multi-well plates. Can be used with: custom devices, disposable slides and coverglasses. Positioned on both sides threaded #4-40 holes can be used to mount optional IMA-MH tubing and probes holders. Can cool the sample down to 0°C (in combination with BTC-W heat exchange unit) or heat up to 150°C. The cooling area is an inside cutout 29x79x1mm (to fit standard slides), with 20x40mm aperture in the middle. Requires a temperature controller. **Item#: BTC-SL-128x86**

- **Dimensions: 128x86mm, 29x79mm cooling/heating area**
- **Optical aperture:** 20x46mm
- **Objective working distance, minimum:** 0mm (for inverted and upright microscopes)
- **Temperature stability:** 0.1°C, built-in sensor
- **Sink: optional** water cooling for very low temperatures, requires BTC-W unit
- **Microscope adapter:** Fits to 128x86mm holders for standard multi-well plates

Cooling & Heating microscope stage for 50mm dishes, BTC-S50

Can be used with wider up to 59mm disposable dishes, including Willco 50mm glass bottom dishes. Comes with reducing adapter for 50mm dishes. Built in lines to cool heat sink for deep cooling. 30mm clearance. Click on image to enlarge. Consider a different cooling stage for rectangular slides. Requires a microscope adapter (specify microscope model when ordering), and a temperature controller. **Item#: BTC-S50**

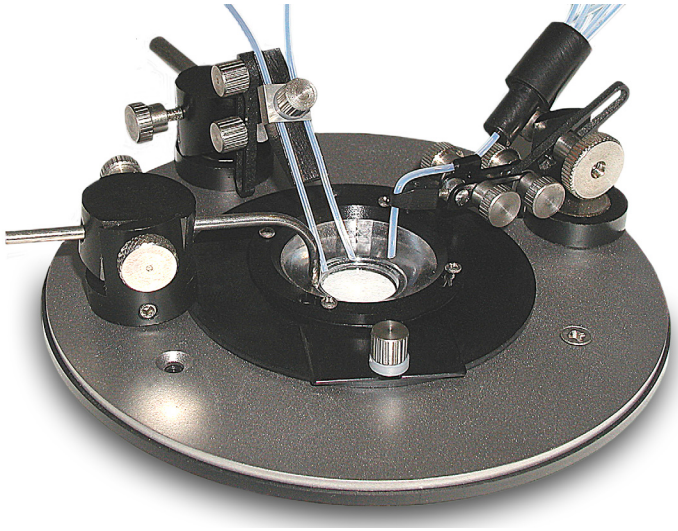
- **Dimensions:** 145x145x23mm
- **Temperature stability:** better than 0.1°C, built-in sensor
- **Sink:** water cooling for very low temperatures, optional water cooler unit BTC-W
- **Optical aperture:** 33mm
- **Microscope adapter:** Fits to 74mm cutout of standard microscope adapters

Cooling & Heating Microscope Objective, BTC-O

Can be used with any microscope objective (or any cylindrical object). Can cool the objective down to -6°C (or heat up to 150°C). The cooling area should be specified when ordering, for example 22.5mm diameter and 10mm wide for x40 Zeiss objective (technical drawings are required). Built-in clamp will fix the objective in place. Can be placed on upright and inverted microscopes. Requires sink cooling and a temperature controller Item#: **BTC-O**

- **Dimensions:** custom cooling/heating area (22.5x10mm for example)
- **Optical aperture:** custom
- **Objective working distance, minimum:** 0mm (for upright microscopes)/ 0mm (for inverted microscopes)
- **Stability: 0.1°C, built-in sensor**
- **Heat Sink: optional water cooling for low temperatures**

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.

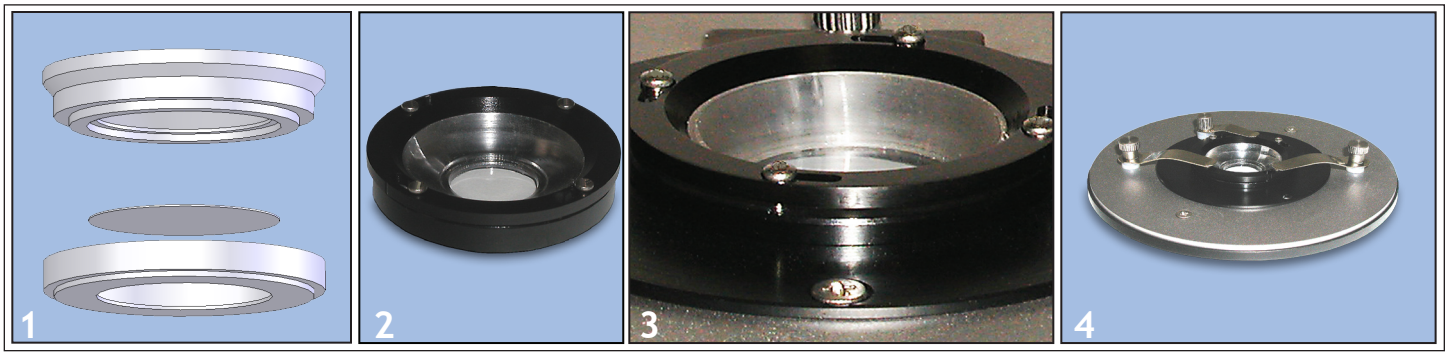
2. Fix the plastic insert with a metal ring from the top.

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

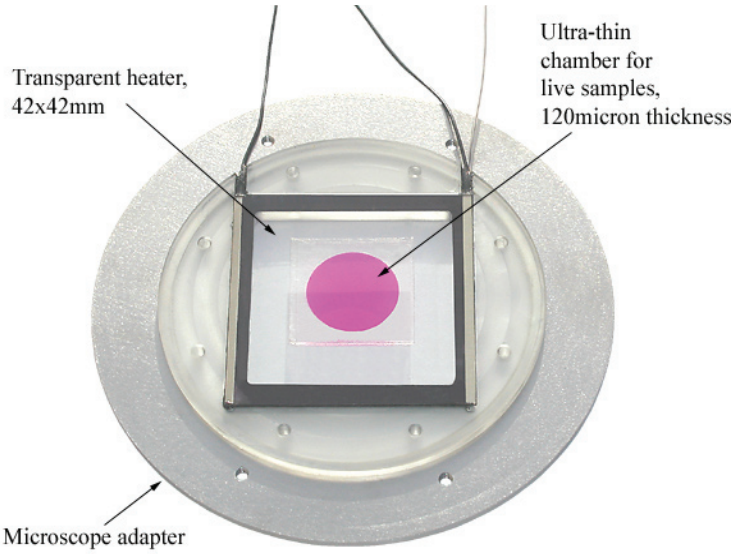
Catalog #	Features:
CSC	Chamber for replaceable round coverslips. Simply put a coverslip inside and seal by a snap-in action.
	Choose the right diameter to fit your coverslips.



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-HP65x75. Heated slides TC-GSH can be also used.

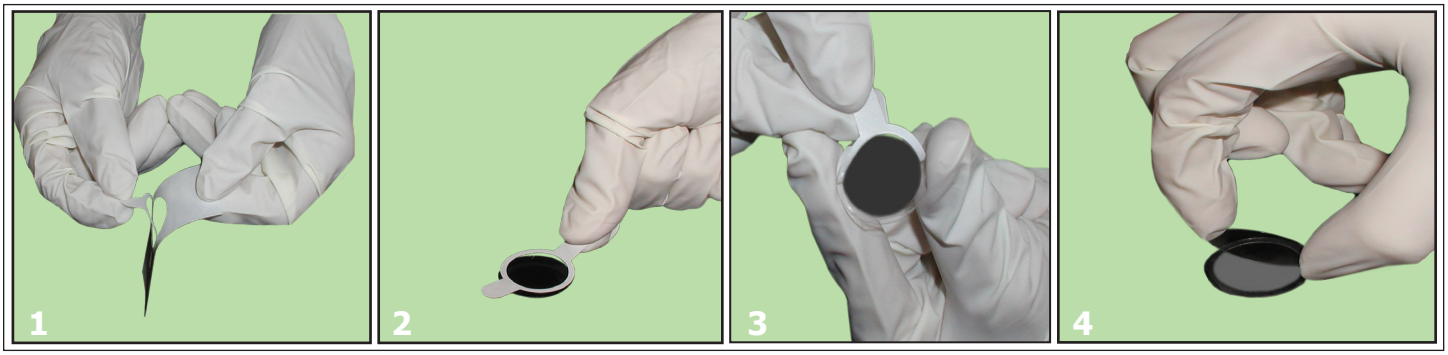
1. Remove protective liner from the bottom surface to expose the adhesive.

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 UTIC-11D UTIC-20-24x24 UTIC-13-24x24	Ultra-thin self adhesive chambers for high resolution imaging. Can be used with coverslips, and on any glass or plastic surface.
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.

Noise and Grounding

Noise

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

1. Improper grounding of probes, micro electrodes, bath or instrument chassis.
2. Radiation from transformers of adjacent equipment.
3. Power noise from attached equipment, i.e., 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 micro electrodes.
3. Broadcast interference from TV/Radio.

Instrument Grounding and System Ground

The chassis ground and the output cable shielding are internally connected to the system (circuit) ground. You can access the shielding ground by attaching a shorting wire connecting your system ground either to the screws of one of the OUTPUT DB-9 connectors, or to a small wire showing from the inside the cable closer to STAGE connector. If ground loops are experienced (objectionable 50/60 Hz), try placing the shorting wire to a different point (one of these two). If TTL or analog signals are used, the controller might be already connected to the system ground through the shielding of the cables.