Warner Instruments

Two Dimensional Heating/Cooling Flow Chamber for Yeast Cell Imaging

Model YC-2

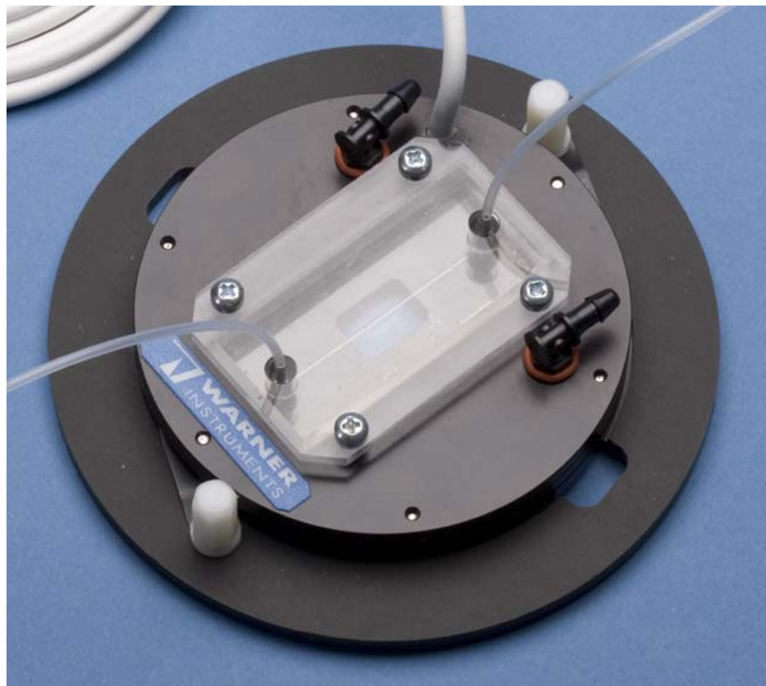

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Table of Contents

DESCRIPTION ... 3
INTRODUCTION ... 4
  PDMS Coated Coverslips ... 4
  The cellulose membrane and media switching time ... 4
  Image quality ... 5
SETUP ... 5
  Cellulose membrane preparation ... 5
  PDMS coverslip preparation ... 6
  Initial assembly ... 6
  Final assembly ... 7
YC-2 SYSTEM SETUP (including **TCM-1** and **CL-100**) ... 7
  General procedure ... 7
  Connecting the Thermal Cooling Module ... 7
SPECIFICATIONS ... 9
APPENDIX ... 9
  Cleaning/Maintenance ... 9
  Service ... 9

DESCRIPTION

Research in cell biology often requires the microscopic study of individual live cells under various conditions such as at different temperatures or in the presence of differering growth media. One particular interest is to monitor individual cell growth using time lapse microscopy while seamlessly and rapidly exchanging the liquid environment.

The **YC-2** Flow Chamber has been specifically developed to permit observation of bi-dimensionally proliferating cells over multiple cell cycles. The chamber design overcomes a common problem encountered when using standard time-lapse technology; namely, the media can now be quickly exchanged during the experiment since the cell progeny is no longer subject to wash-out by the flow.

The **YC-2** has been successfully tested with both yeast and bacterial cells. The chamber secures the cell environment and prevents cells from growing on top of each other. As a result, one can monitor the proliferation of single cells for many generations in a well-controlled, perfusable environment.

Peltier heating and cooling of the **YC-2** base is provided by Warner's **CL-100** or **CL-200** Bipolar Temperature Controller and **TCM-1** Thermal Cooling Module. In-line heating/cooling of perfusate can be directly achieved using our **SC-20** Dual In-Line Solution Heater/Cooler and a **CL-100** or **CL-200** Bipolar Temperature Controller.

Features of the **YC-2** include:

- ✓ Cells do not move during imaging yet no adherent coating is needed
- ✓ The cells' fluid environment can be changed rapidly without disturbing or moving the cells
- ✓ Cells only grow in two dimensions, thus providing a single focal plane for data collection
- ✓ Experiments can be performed over long time scales and for many cell divisions
- ✓ Temperature control enables environmental conditions between 5-50°C

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED FOR USE ON HUMAN SUBJECTS

INTRODUCTION

The **YC-2** principle of operation is that a permeable barrier, namely a cellulose membrane, separates the cells from the main flow chamber but readily allows nutrients to diffuse into the cell space.

The apparatus is assembled into a platform in the following manner:

1) A droplet of cells is placed onto a PDMS coated, 24x50 mm coverslip.
2) A cellulose membrane is placed on top of the coverslip.
3) The reusable PDMS flow chamber is secured on top of the cellulose membrane.
4) The chamber is connected to a media source.
5) The whole system is clamped onto a temperature controlled platform to prevent leakage and mounted onto a microscope.

Restricting cells to the space between the coverslip and membrane allows the achievement of two crucial objectives: (1) As required for long term imaging, cells grow strictly in two dimensions over multiple cell cycles, and (2) the bathing solution can be exchanged within seconds without washing cells away.

Yeast cell proliferation is unaffected by the above stated objectives. The typical division time is on the order of 75 minutes for a wild-type (WT) mother cells and 85 minutes for WT daughter cells. These division times are in agreement with well accepted liquid culture growth rates.

PDMS Coated Coverslips

The supplied 24x50 mm coverslips are coated with a 40 μm thick layer of PDMS. This coating ensures rapid and reliable cell growth as cells do not grow well when sandwiched directly between glass and cellulose. PDMS is 100 times softer than a yeast cell and therefore cushions the cell, perhaps explaining this effect.

The cellulose membrane and media switching time

The solution dead-volume within the **YC-2** perfusion flow path is on the order of 20-40 μl, so fluid switching times are within seconds. Generally speaking, the effective time to exchange media *at the cell* is limited by the diffusion rate and pore size of the cellulose membrane. More specifically, the diffusion rate is influenced by the membrane thickness (which is approximately 30 μm), and the pore size imposes a molecular weight cutoff of 14 kDa.

In test measurements, the rate of fluorescence washout in the chamber following a switch from fluorescein to water is on the order of 30-40 seconds, which gives an estimate of the diffusion time.

Image quality

Acquiring sharp phase contrast and fluorescent images is easily achieved using the **YC-2** chamber and the design allows for accurate cell boundary segmentation. Fluorescent imaging of subcellular structures is generally improved over images made using traditional agar confinement methods, but at the expense of a higher background signal due to the wavelength-dependent autofluorescence of the cellulose membrane. The use of software-based background subtraction methods to remove the membrane contribution is recommended.

SETUP

The following assembly instructions may include images of the ***YC-1*** *(a heat-only version of the* ***YC-2****). Where this occurs the assembly method for both will be the same.*

Cellulose membrane preparation

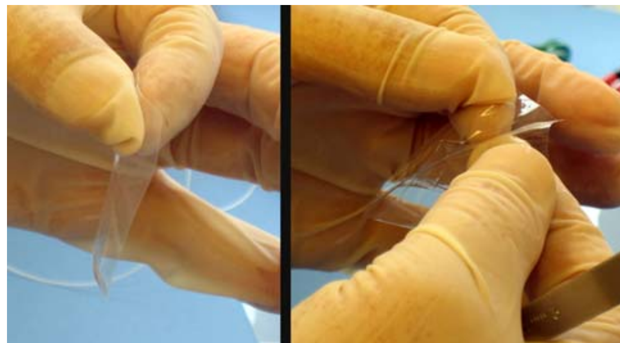
Materials and lab supplies required for preparation:

- Sodium carbonate (Na_2CO_3), 2% w/v, 500 ml
- TE buffer (10 mM Tris, 1 mM EDTA), pH 8.0 with HCl, 500 ml
- Petri dishes, 100 mm diameter, for membrane storage
- Hot plate, for boiling membranes
- Pre-cut cellulose membrane blanks (supplied)
- Optional: EDTA, for long term membrane storage

The pre-cut cellulose membrane blanks are made from collapsed dialysis tubes. As such, each membrane blank is comprised of two independent membranes that must be separated before use. Transfer a number of membrane blanks into a Petri dish of deionized (DI) water and soak for several hours to separate the membrane halves.

Hereafter, soaked membranes must be keep wet at all times.

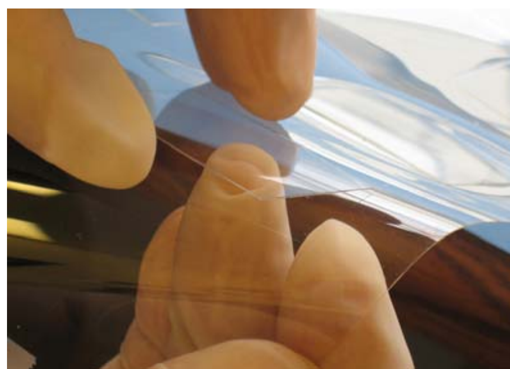
To separate the two halves, hold the membrane blank between your thumb and index finger and rub gently. The two membrane halves will separate and you can then peel them away from each other.



Transfer the separated membranes into a beaker filled with 250 ml of the Na_2CO_3 solution and boil for 20 minutes. Remove the boiled membranes from the Na_2CO_3 solution and transfer to a Petri dish filled with DI water to cool.

Next, place the membranes into a beaker filled with 250 ml of the TE solution and again boil for 20 minutes. Remove the boiled membranes from the TE solution and transfer to a Petri dish with DI water for immediate use, or store in a refrigerator at 4°C for extended use. For long term storage, use an EDTA buffer in place of the DI water.

PDMS coverslip preparation

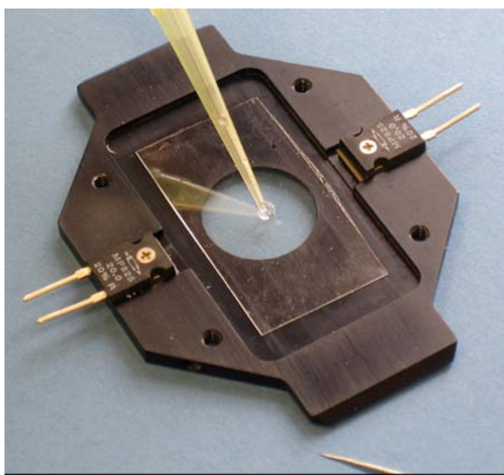


The 24x50 mm, PDMS-coated coverslips are shipped on an acetate carrier containing ten coverslips to a sheet.

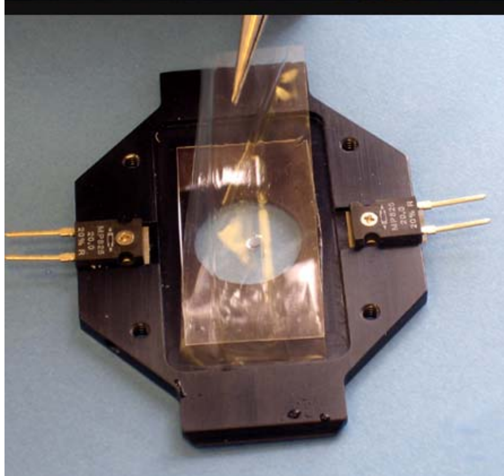
Coverslips can be separated from the carrier by gently bending the acetate away from the corner of a coverslip until the acetate separates from the PDMS. A gentle nudge from a scalpel may be required if the two does not separate easily. However, take care to not damage the PDMS coating on the coverslip.

Use a razor blade to gently trim any excess PDMS from the edges of the coverslip.

Initial assembly



Place a PDMS coated coverslip onto the platform, PDMS side up, and add 10 µl of logphase cells (at $2x10^6$ cells/ml) onto the coated surface as shown to the right.



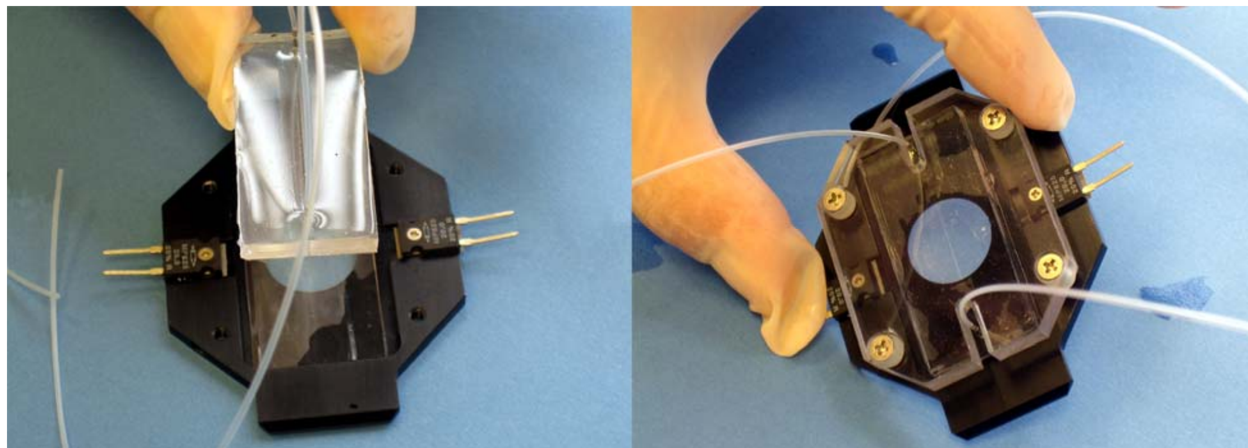
Next, place a clean, wet cellulose membrane onto the top of the coverslip as shown to the right.

To secure a tight contact between the membrane and the PDMS, wait about 15 minutes for the water layer between the PDMS and membrane to (mostly) evaporate. The evaporated membrane will stick to the PDMS.

Note: Monitor the drying process closely since excessive drying will damage the cells and, if allowed to over-dry, the membrane will un-stick from the PDMS.

Final assembly

Once the cellulose membrane has dried sufficiently, place the PDMS flow chamber onto the membrane. Next, screw the clear top plate into place to compress and seal the system. Finally, connect the flow chamber to your perfusion source. The supplied PE-160 flow lines attach to the stainless steel connection ports in the PDMS flow chamber.



YC-2 SYSTEM SETUP (including **TCM-1** and **CL-100**)

Peltier heating and cooling of the **YC-2** is achieved using a **CL-100** or **CL-200** temperature controller and a **TCM-1** thermal cooling module. Feedback control for the temperature controller is provided via a thermistor which is embedded into the **YC-2** platform.

Operating instructions for these devices can be found in their respective user's manuals, available on the related product page in the Warner website (www.warneronline.com).

General procedure

The general assembly procedure is to attach the **TCM-1** to the **YC-2**, away from the microscope, and to check for a secure, leak-free connection. This is followed by positioning the **YC-2** into the appropriate Series 20 stage adapter. A connection is then made between the **YC-2** and **CL-100**.

Connecting the Thermal Cooling Module

Note: This step should be performed away from the microscope.

1. First review the assembly instructions for the **TCM-1** Thermal Cooling Module. We will use a modified procedure.
2. The goal is to connect flow lines from the *fill reservoir* of the **TCM-1,** to the **YC-2**, and back to the fill reservoir.

3. Begin by connecting the **WATER JACKET CONNECTING TUBES** (supplied with the **TCM-1**) to the upper and lower fluid flow ports on the rear of the **TCM-1** as shown to the figure above.
4. Now cut the ¼ inch Tygon® tubing (supplied with the **TCM-1**) into two equal length pieces. This tubing will run from the **YC-2** to the **TCM-1**. Make the length as short as needed.
5. Attach these cut tubes to the **YC-2** perfusion flow ports as shown in the figure to the right.
6. Join the open end of one of the tubes in step 5 to the upper **WATER JACKET CONNECTING TUBE** which you attached to the **TCM-1** flow port in step 3. Join the open end of the other tube to the lower **WATER JACKET CONNECTING TUBE**.
7. You should now have a complete and closed tubing loop from the **TCM-1** to the **YC-2** and back.
8. Next, remove the screw from the top of the **TCM-1** to reveal the fill port for the internal fluid reservoir. Add the supplied algaecide (included with the **TCM-1**) to the fill reservoir. Refill the bottle with water and add to the reservoir until the system is completely full. (Look for the bubbles in the flow lines to disappear).
9. Power the **TCM-1** and check for leaks.
10. Once the chamber has been assembled, it can be easily mounted onto your microscope stage using any of our Series 20 Stage Adapters.

    Note: Due to the dimensions of the **YC-2** chamber, the traditional "button clamp" assembly on the stage adapter must be replaced by a spring clamp assembly (provided). These spring clamps will hold the **YC-2** securely in the stage adapter.

SPECIFICATIONS

Materials:	Platform: black anodized aluminum Top cover: polycarbonate Flow cell: PDMS (50 x 23 x 4.5 mm, L x W x H)
Bath Dimensions:	40.0 x 0.6 x 0.1 mm (L x W x D)
Bath Volume:	2.4 µl
Coverslips:	24 x 50 mm, #1 thickness with 40 µm thick PDMS coating
Cellulose Membranes:	24 x 50 mm x 30 µm thick
Perfusion Ports:	1.27 mm OD

APPENDIX

Cleaning/Maintenance

The **YC-2** platform is constructed of anodized aluminum and the clear top plate is made from polycarbonate. The platform and top plate can be gently cleaned using common laboratory detergents. Do not scrub the top plate.

The flow chamber is made from PDMS and may be used more than once, but the PDMS coated coverslips are strictly single-use. Between uses, the flow chamber can be rinsed with H_2O and should be discarded when conditions warrant. Note that while the perfusing media contacts the flow chamber, your cells do not.

We do not recommend autoclaving any components in this system.

Service

We recommend that all technical questions be referred to our Technical Support Department. Normal business hours are 8:30 AM to 5:00 PM (EST), Monday through Friday.

Our offices are located at 1125 Dixwell Avenue, Hamden, CT 06514. We can be reached by phone at (800) 599-4203 or (203) 776-0664. Our fax number is (203) 776-1278. E-mail us at support@warneronline.com or through the web at http://www.warneronline.com/contact.html.