

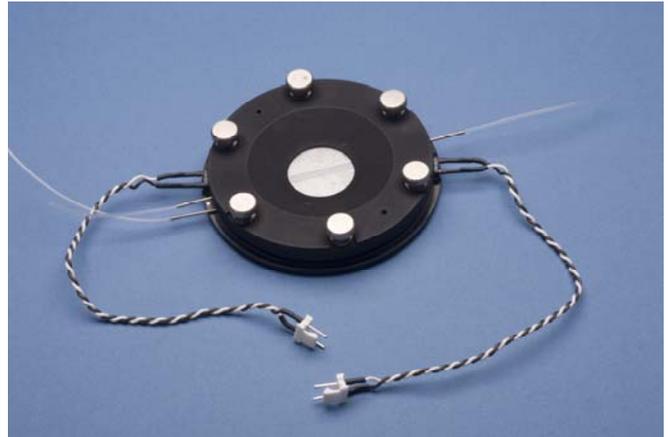
WARNER SERIES 30 IMAGING CHAMBERS

A feature in common with Warner **RC-30 Series** chambers is the use of glass coverslips as the top and bottom of the chamber. In many cases, the bottom coverslip will contain the imaging sample. When viewed with an inverted microscope, samples are visualized through a glass thickness of 0.16-0.19 mm (#1.5 thickness).

THE RC-31 CHAMBER

The **RC-31** is a low profile chamber incorporating special features for confocal imaging. These features include user defined bath geometry and volume, and a large viewing area.

The **RC-31** uses glass coverslips to form both the top and bottom of the chamber. This feature facilitates its use with both inverted and upright microscopes. Furthermore, the two-piece design and the six-point attachment screws of the **RC-31** allow for easy assembly and disassembly while applying uniform pressure across the gasket.



Variable Bath Volume/Fast Exchange - The chamber bath side wall is formed using a silicone gasket sandwiched between the upper and lower coverslips. Wall height (or spacing between the upper and lower coverslips) can range from 50 μ m to >350 μ m and is defined by the number and thickness of the gasket(s) used. Pre-cut gaskets are supplied with the chamber; one cut wide to allow full use of the large diameter aperture window, and a narrow, slotted version targeted towards fast solution exchange. Blank gaskets are also supplied and allow the development of customized bath geometries as required by your application

Large Viewing Area – The viewing aperture in the chamber housing is 23 mm in diameter. The chamber bottom is beveled to permit greater access to the bottom coverslip.

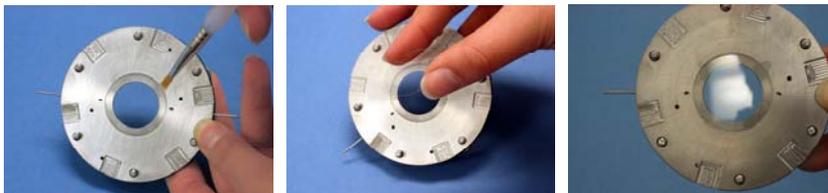
Heated Platform – The **RC-31** includes a pair of resistive heater elements mounted into the chamber base. Heater elements are compatible with Warner's TC-324B or TC-344B Temperature Controllers.

ASSEMBLY

The general procedure for assembling the **RC-31** is to first mount a 30mm glass coverslip to the platform TOP PLATE. This is followed by mounting the CHAMBER FORMING GASKET onto the coverslip in the TOP PLATE. Next, a sample-containing coverslip is mounted onto the PLATFORM BASE. Finally, the TOP PLATE and BASE are assembled together resulting in a single, sealed unit. The assembled chamber is mounted onto the microscope, usually via a stage adapter.

1. Begin by placing a 30 mm coverslip into the TOP PLATE.
 - a. First, locate the 30 mm indentation on the underside of the TOP PLATE.

b. Using a small paintbrush, apply a thin layer of vacuum grease to the outside edge of this indentation.

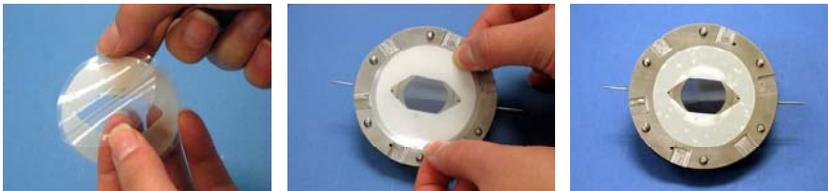


c. Next, secure a clean, 30 mm round, #1.5 thickness coverslip into the indentation making sure there is no excess grease at the edges.

NOTE: Total coverage is not important since the vacuum grease serves only to hold the coverslip in place during the assembly process.

2. The next task is to place the CHAMBER FORMING GASKET on top of the coverslip nested into the underside of the TOP PLATE.

a. Select the gasket to be used and remove the polyethylene cover from the side that will face the TOP PLATE.



b. Position the gasket so that the perfusion inlet and outlet ports, as well as the alignment holes, are centered within the gasket cutout.

c. Press the chamber forming gasket into place. The silicone gasket material should make a clean seal with the coverslip without the need to use grease. Take a moment to press out any trapped air bubbles.

d. Carefully remove the polyethylene cover from the exposed bottom of the gasket taking care to not disturb the existing seal

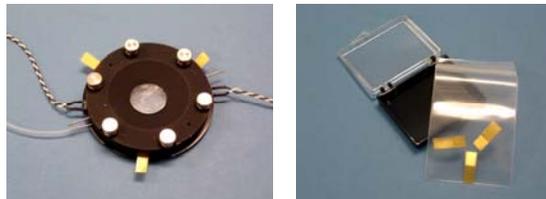
3. Next, locate the 40 mm indentation on the PLATFORM BASE. As described above, apply a thin film of vacuum grease to the outer edge of this indentation and secure your 40mm diameter culture-containing coverslip into place. Leave a small drop of buffer on the coverslip to cover your sample.



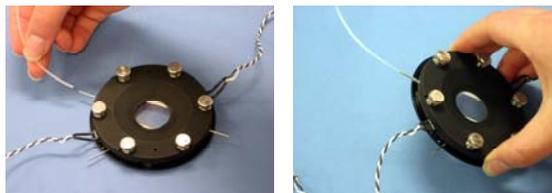
4. Place the coverslip TOP PLATE, with attached gasket, into the PLATFORM BASE while aligning the heater block cutouts (indentations) with the heater blocks in the base. Check for proper fit.



5. For applications requiring a gasket thickness less than 100 μm , two sets of metal shims (75 μm and 50 μm thick) have been included with the RC-31 chamber. Assemble the chamber as usual (steps 1-4) using a 100 μm gasket. Insert 5mm of a metal shim between the top and bottom halves of the chamber at 3 locations equal distances apart. Tighten the pressure plates so that the shims are secured into place. The gasket thickness is now the same as the thickness of the shims used.

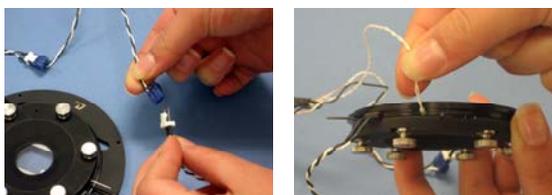


6. Screw the pressure PLATE screws, loosely at first, onto the assembly using a criss-cross pattern technique similar to tightening a tire to a car. (In other words, work your way around the perimeter, skipping every other screw until all screws have been tightened.)
7. Connect the input perfusion tube to the chamber input port and completely fill the bath with solution. Tilt the chamber output port up while filling to help avoid the occurrence of a trapped air pocket in the bath. Once the chamber is filled, wipe away any excess moisture and place the chamber onto a flat surface. Check for leaks and tighten the TOP PLATE as necessary.
8. Once the chamber has been verified as liquid-tight it may be mounted onto the microscope. Connect the perfusion outlet tube before mounting the chamber to the microscope to avoid spilling solution on the optics.



Connecting to the heating elements

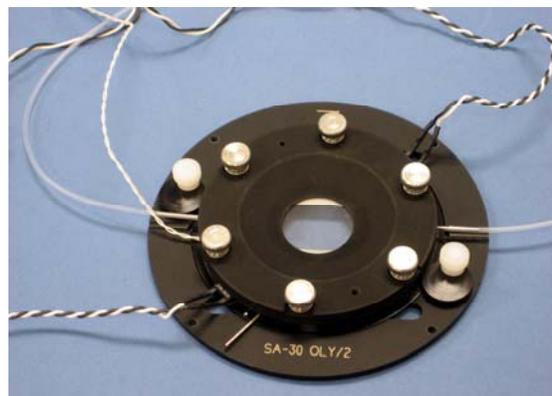
Prior to mounting the **RC-31** onto the microscope, you will want to attach the power and feedback lines from the heater controller to the platform. The two blue-ended heater cables simply connect to the two 2-prong heater blocks. The white feedback thermistor is inserted onto the 2 mm hole located on the 3 side of the platform base between tightening screws.



NOTE: Place a drop of oil (immersion or mineral), or alternatively a small amount of vacuum grease on the thermistor before insertion to aid in thermal transfer between the thermistor and platform.

Mounting onto the microscope

The **RC-31** can be mounted directly onto a microscope stage if the stage is both flat and has a cutout which fits or is smaller than the platform. In most cases, however, the stage cutout will be larger than the platform necessitating the use of a stage adapter. Warner Instruments has stage adapters for most popular microscopes (see Appendix A) and we can custom manufacture an adapter for a special application. Contact our Sales Department for details.



PERFUSION

Perfusate is delivered to the chamber through PE-90 tubing. We recommend pre-filling all perfusion lines before attachment to reduce the occurrence of bubbles in the flow path.

NOTE: PE-90 tubing fits neatly inside PE-160 tubing. This allows the **RC-31** to be used with an in-line solution heater such as the SH-27B.

Fluid control

Selection of solution source and rate of delivery can be of either manual or automatic design and is left to the user. However, Warner manufactures several perfusion control systems (such as the valve-driven VC-8 and VC-8M Control Systems) which can be used with this application. The solution delivery can be pumped or gravity-fed. While these approaches allow control over the flow rate, Warner also offers dedicated solution flow regulators (FR-50 and FR-55S) which can be used in conjunction with the VC Systems. Finally, a reference by Trese

Leinders-Zufall describing the advantages of different perfusion control systems is available for download from the **RC-31** product page on our website:

Multiple perfusion solutions

Warner Instruments' multi-port manifolds (MM, ML, MP, or MPP Series) can be used to connect from 2-8 solution lines to the **RC-31**. Air should be removed from each feed line by pre-filling with its appropriate solution. The manifold output tube is attached to the input port of the chamber. We recommend making the connection between the manifold and chamber as short as possible to minimize solution exchange times.

PLATFORM HEATING

A general discussion regarding issues surrounding heating of solutions and platforms is available for download from the **RC-31** product page on our website:

Monitoring the heat

Heat is transferred to the aluminum platform from a pair of 20 Ω power resistors mounted on the underside of the platform. The temperature of the platform is monitored and controlled by measuring the platform thermistor resistance and adjusting the voltage to the heaters.

Automatic heat control is achieved by using a Warner Temperature Controller (TC-324B or TC-344B). These devices allow the desired temperature to be set and automatically maintained at less than 1°C deviation. The maximum temperature rating of the supplied thermistor is 60°C.

MAINTENANCE

Cleaning of the TOP PLATE should be performed using a dilute detergent solution. Alternatively, Warner has developed a trisodium phosphate (TSP) wash protocol which is effective in cleaning anodized metal and plastic parts. Contact our Technical Support staff or download the protocol in PDF format from our website: (http://www.warneronline.com/pdf/whitepapers/cleaning_plastic.pdf)

APPENDIX

A. Warner Stage Adapters

Warner carries an extensive line of stage adapters for our chambers and we constantly add new adapters as microscope manufacturers add to or modify their product lines. Contact our offices if you do not find an adapter for your microscope below. You may also want to check our website (<http://www.warneronline.com>) to see if an adapter has been added since this manual was printed.

| Manufacturer | Microscope Model | Stage Adapter | Order Number |
|--------------|---|----------------------------|--------------|
| Nikon | Nikon Diaphot/TE200/TE300/TE2000 | SA-30NIK | 64-0322 |
| | Nikon TMS with 9 x 13 cm Cut-Out | SA-30TMS/9 | 64-0324 |
| | Nikon E400/E600/E800 Stage | SA-30UU | 64-0380 |
| Olympus | Olympus IMT-2/IX-50/IX-70/BX-50WI | SA-30/OLY2 | 64-0325 |
| Leica | Leica Galvo Z Inverted Stage | SA-30GALVZi | 64-1456 |
| | Leica Dmirb/E with 3-Plate Mechanical Stage | SA-30L3P | 64-0328 |
| | Leica Galvo Z Upright Stage | SA-30GALVOZ | 64-0377 |

| | | | |
|--------------|--|--------------------------|---------|
| Prior & Ludl | Prior & Ludl Motorized Stage | SA-30PLI | 64-0329 |
| Zeiss | Zeiss Axiovert 211 x 230 | SA-30LZ | 64-0326 |
| | Zeiss LSM510 Stage | SA-30UUZ | 64-0379 |
| | Zeiss Axiovert 85 x 130 Mechanical (K) Stage | SA-30KZ | 64-0327 |

NOTE: Warner Instrument **Series P** platforms are designed to fit the Zeiss 76x26 microscope slide frame (#471719) without a stage adapter. Heater platforms will require an insulating material between the platform and frame.

B. Chamber supplies/spare parts

We stock the following supplies for your convenience. Contact our Sales Department for prices.

| Part Number | Order Number | Description | Qty/pkg |
|---|--------------|--|----------------|
| Coverslips | | | |
| CS-30R | 64-1499 | 30mm diameter #1.5 Thickness | 90 |
| CS-40R15 | 64-1696 | 40mm diameter #1.5 Thickness | 50 |
| CS-15R | 64-0703 | 15mm diameter (fits RC-25F chamber) | 100 |
| CS-22S | 64-0704 | 22mm x 22mm square (fits RC-21B chamber) | 100 |
| CS-25R | 64-0705 | 25mm diameter (fits RC-21R chamber) | 100 |
| CS-22/30 | 64-0706 | 22mm x 30mm rectangle (fits RC-30 chamber) | 100 |
| CS-22/40 | 64-0707 | 22mm x 40mm rectangle (fits RC-22, 22C, 24, 24E, 26 & 26G, 30 chambers) | 50 |
| Gaskets | | | |
| GS30L/10 | 64-0330 | Large Bath Gasket 250 μ m | 10 |
| GS30L/15 | 64-0331 | Large Bath Gasket 375 μ m | 10 |
| GS30S/10 | 64-0332 | Slotted Gasket 250 μ m | 10 |
| GS30S/15 | 64-0333 | Slotted Gasket 375 μ m | 10 |
| GS30B/10 | 64-0334 | Blank Gasket 250 μ m | 10 |
| GS30B/15 | 64-0335 | Blank Gasket 375 μ m | 10 |
| GS-30B/4 | 64-0341 | Blank Gaskets 125 μ m | 10 |
| GS-30L/4 | 64-0342 | Large Bath Gasket 125 μ m | 10 |
| GS-30S/4 | 64-0343 | Slotted Bath Gasket 125 μ m | 10 |
| Polyethylene Tubing | | | |
| PE-90/10 | 64-0754 | 1.27 mm OD x 0.86 mm ID tubing | 10 ft. (3.3 M) |
| Replacement/Spare Parts for Heater Platforms | | | |
| RH-2 | 64-0274 | Heater elements | 2 |
| TS-60P | 64-0269 | Probe Thermistor | 1 |

| Multi-Perfusion Zero Dead Space Manifolds | | | |
|--|---------------------------|-------------------|---|
| MM-2 or ML-2 | 64-0203 or 64-0200 | 2 input, 1 output | 1 |
| MM-4 or ML-4 | 64-0204 or 64-0201 | 4 input, 1 output | 1 |
| MM-6 or ML-6 | 64-0205 or 64-0202 | 6 input, 1 output | 1 |
| ML-8 | 64-0199 | 8 input, 1 output | 1 |

C. Comments

- 1) Silicone vacuum grease (also called stopcock grease) is available from Warner Instruments (**Warner #111**).
- 2) Best temperature regulation is achieved by preheating the perfusion solution with an in-line heater (**SH-27B** or **SF-28**) in addition to warming the chamber.