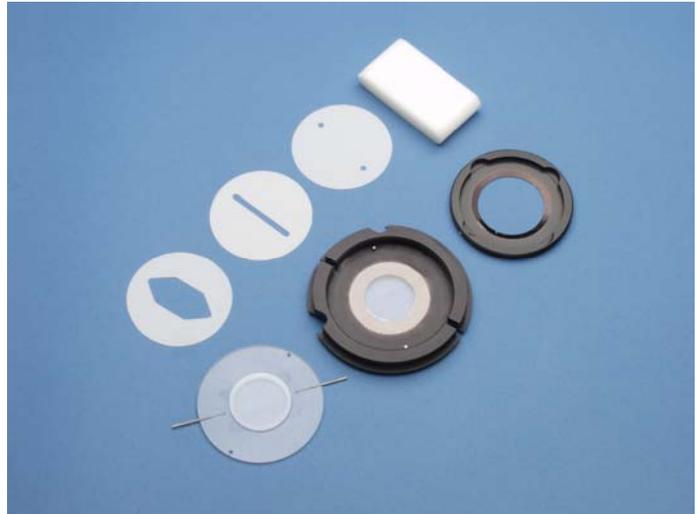


## WARNER IMAGING CHAMBERS

A feature in common with Warner **RC-30** Series chambers is the use of #1 glass coverslips as the top and bottom of the chamber. In many cases, the bottom coverslip can contain the imaging sample. When viewed with an inverted microscope, images are visualized through a glass thickness of 0.13-0.17 mm.

### THE RC-30WA CHAMBER

The **RC-30WA** is a low profile chamber incorporating special features for confocal imaging. These features include a user defined bath geometry and volume, and a large viewing area. The **RC-30WA** differs from the **RC-30** in that it has a wider aperture (yielding a larger working area) and uses round coverslips rather than the rectangular ones used in other models. Glass coverslips (sized #1) form both the top and bottom of this chamber allowing it to be inverted in the mounting platform. This feature facilitates its use with both inverted and upright microscopes. An optional heater model is also available.



Variable Bath Volume/Fast Exchange - The side wall of the chamber bath is formed using a silicone gasket sandwiched between the upper and lower coverslips. Wall height (or spacing between the coverslips) can range from 250-1000  $\mu\text{m}$  and is defined by the thickness of the gasket(s) used. Blank gaskets are supplied permitting the development of customized bath geometries as required by your application. Pre-cut gaskets are also 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.

Large Viewing Area – The viewing aperture in the chamber housing is 17.7 mm in diameter. The chamber bottom is beveled to permit maximum access to the bottom coverslip.

Reversible – The chamber can be reverse mounted in the stage adapter to accommodate upright microscopes.

Heated – The **RC-30HV** includes a pair of resistive heater elements mounted to the chamber base. Heater elements are compatible with all Warner Heater Controllers.

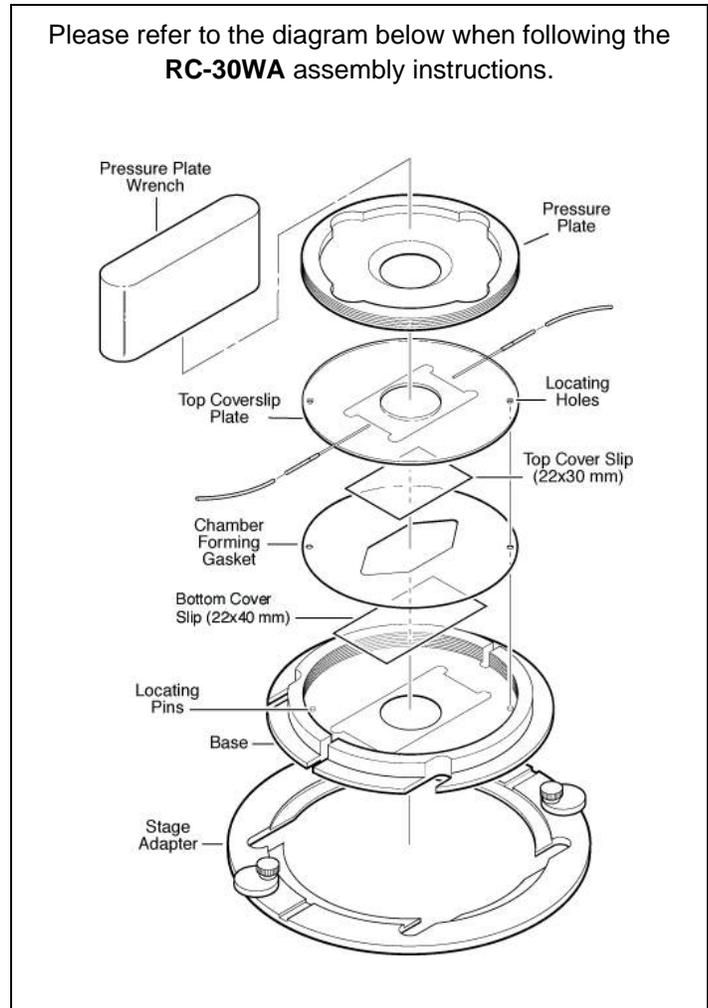
## ASSEMBLY

The general procedure for assembling the **RC-30WA** is to first secure a coverslip to the platform **COVERSLIP TOP PLATE**. This is followed by mounting the **CHAMBER FORMING GASKET** onto the **COVERSLIP TOP PLATE**. Next, a sample containing coverslip is mounted onto **PLATFORM BASE**. Finally, the various components are assembled resulting in a sealed, single unit. The assembled chamber can then be mounted onto the microscope, usually via a stage adapter.

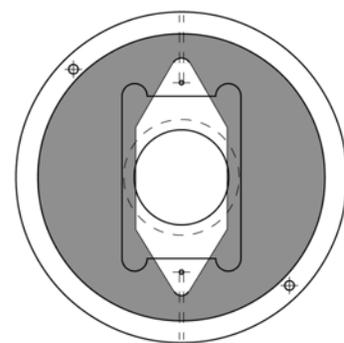
1. Begin by mounting a 20x30 mm coverslip onto the COVERSIP TOP PLATE. Locate the 22x30 mm indentation on the underside of the COVERSIP TOP PLATE and apply a thin film of vacuum grease to this indentation. Secure a clean 22x30 mm coverslip into place making sure there is no excess grease.

**NOTE:** Total coverage is not important since the grease only serves to hold the coverslip in place during the assembly process and provides no sealing.

2. Next, secure the CHAMBER FORMING GASKET to the underside of the COVERSIP TOP PLATE. Select the gasket to be used and remove the polyethylene cover from the side which will face the COVERSIP TOP PLATE. Position the gasket so that the perfusion inlet and outlet ports, as well as the alignment holes, are centered as shown in the sketch at the bottom of this page. Press the chamber forming gasket into place. The silicone gasket material should make a clean seal with the plastic coverslip top plate. Take a moment to press out any trapped air bubbles.



3. Carefully remove the polyethylene cover from the exposed bottom of the gasket taking care to not disturb the existing seal.
4. Locate the 22x40 mm indentation on the PLATFORM BASE. As described above, apply a thin film of vacuum grease to this indentation and secure your culture containing coverslip into place. Leave a small drop of buffer on the coverslip to cover your sample.
5. Place the COVERSIP TOP PLATE with the attached gasket into the PLATFORM BASE aligning the two holes in the plate with the corresponding pins in the base. Check for proper fit.
6. Screw the PRESSURE PLATE onto the assembly and tighten with the PRESSURE PLATE WRENCH. Check the quality of the assembly by gently wriggling the stainless steel perfusion ports on the TOP COVERSIP PLATE. There should be no movement.
7. Connect a perfusion line to the chamber input and completely fill the bath with solution. Tilting the chamber while filling will help avoid the occurrence of trapped air pockets. Once the chamber is filled, wipe away any excess moisture and place



the chamber onto a flat surface. Check for leaks and re-tighten the PRESSURE PLATE if necessary.

8. Once the chamber has been verified to be water-tight, it may be mounted onto the microscope. Connect the perfusion inlet and outlet tubes before mounting the chamber to avoid spilling solution on the microscope optics.

### Connecting to the heating elements

Prior to mounting the **RC-30WA** to 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 located on the underside of the **RC-30WA** platform. The white feedback thermistor is inserted onto the 2 mm hole located along the side of the **RC-30WA** platform.

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

### Mounting onto the microscope

The **RC-30WA** chamber can be mounted directly onto a microscope stage if the stage is both flat and has a cutout which fits the platform. In most cases, however, the stage cutout will be larger than the platform necessitating the use of a stage adapter. Warner Instruments stocks stage adapters for most popular microscopes (see Appendix A) and we will custom manufacture adapters for special applications. Contact our Sales Department for details.

### PERFUSION

Perfusate is delivered to the chamber through the supplied 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-30WA** chamber to be used with an in-line heater such as the **SH-27B**.

### Fluid control

The selection of solution sources and rate of delivery can be of either manual or automatic design and is left to the user. However, Warner Instruments manufactures several perfusion control systems (such as the valve-driven **VC-8** and **VC-8M Control Systems**) all of which can be used with this application.

The rate of solution delivery can be of a pumped or gravity feed design. While these approaches allow control of the flow rate, Warner Instruments also offers a dedicated solution flow regulator (Model **FR-50 Flow Regulator**).

Finally, a reference by Trese Leinders-Zufall describing the advantages of different perfusion control systems is available for download from the Support section of our website (<http://www.warneronline.com>).

### Multiple perfusion solutions

Warner Instruments multi-port manifolds (**MM** or **ML Series**) can be used to connect up to 8 solution lines to the **RC-30WA** chamber. 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 Warner platforms is available for download on our website. (<http://www.warneronline.com>)

## Monitoring the heat

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

Automatic heat control is achieved by using a Warner Temperature Controller (e.g., **TC-324** or **TC-344**). 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 COVERSLIP TOP PLATE should be performed using a dilute detergent solution. Alternatively, Warner instruments has developed a trisodium phosphate (TSP) wash protocol which is effective in cleaning plastic parts. Contact our Technical Support staff or download the protocol in PDF format from our website. (<http://www.warneronline.com>)

**NOTE:** Do not use alcohol, ether or other solvents on plastic parts. Solvents may be used on the anodized surfaces of the platforms. Aluminum chamber parts may be autoclaved.

## 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.

Microscope Manufacturer	Model No.	Order Number
Nikon Diaphot / TE200 / TE300 / TE2000	SA-NIK	64-0291
Nikon TMS with 8x12 cm cutout	SA-TMS/8	64-0292
Nikon TMS with 9x13 cm cutout	SA-TMS/9	64-0293
Olympus IMT	SA-OLY	64-0294
Olympus IMT-2 / IX-50 / IX-51 / IX-70 / IX-71 Burleigh Gibraltar™ stainless steel stage	SA-OLY/2	64-0295
Leica-MicroSystems DMIL with Object Guide Leica-MicroSystems DMIRB/E with plane stage Zeiss Axiovert with 211x230 specimen stage Zeiss Axiovert 25 / 35 / 100 / 200	SA-20LZ	64-0296
Zeiss Axiovert with 85x130 Mechanical Stage Zeiss Axiovert 100M, 200M	SA-20KZ	64-0297
Nikon E400 / E600 / E800 Olympus BX-40 / BX-50 / IX-81	SA-20UU	64-0298
Prior & Ludl Motorized Stage on upright scope	SA-20PL	64-0299
Prior & Ludl Motorized Stage on inverted scope	SA-20PLI	64-0300
Zeiss, Leica-MicroSystems DMIRB/E with 3-plate mechanical stage	SA-20L3P	64-0301
HAI 900 Inverted Microscope	-	64-0302
Zeiss Axioskop with 75x30 mechanical stage Zeiss Axioskop LSM 510	SA-20UUZ	64-0336
Nikon Eclipse TS100	SA-TS100	64-0340
Burleigh Gibraltar™ aluminum stage	SA-OLY/3	64-0386

**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	Description	Qty/pkg
<b>#1 Coverslips</b>		
CS-12R	12mm diameter (for RC-25 chamber)	100
CS-15R	15mm diameter (for RC-25F chamber)	100
CS-22S	22mm x 22mm square (for RC-21B chamber)	100
CS-25R	25mm diameter (for RC-21R chamber)	100
CS-22/30	22mm x 30mm rectangle (for RC-30 chamber)	100
CS-22/40	22mm x 40mm rectangle (for RC-22, 22C, 24, 24E, 26 & 26G, 30 chambers)	50
<b>Chambers</b>		
RC30	Chamber	
RC30HV	Chamber w/heaters	
<b>Gaskets</b>		
GS30L/10	Large Bath Gasket 250 $\mu$ m	10
GS30L/15	Large Bath Gasket 375 $\mu$ m	10
GS30S/10	Slotted Gasket 250 $\mu$ m	10
GS30S/15	Slotted Gasket 375 $\mu$ m	10
GS30B/10	Blank Gasket 250 $\mu$ m	10
GS30B/15	Blank Gasket 375 $\mu$ m	10
<b>Polyethylene Tubing</b>		
PE-90/10	0.97 mm OD x 0.58 mm ID tubing	10 ft. (3.3 M)
<b>Replacement/Spare Parts for Heater Platforms</b>		
RH-2	Heater elements	2
TS-60P	Probe Thermistor	1
<b>Multi-Perfusion Zero Dead Space Manifolds</b>		
MM-2 or ML-2	2 input, 1 output	1
MM-3 or ML-3	3 input, 1 output	1
MM-4 or ML-4	4 input, 1 output	1
MM-5 or ML-5	5 input, 1 output	1
MM-6 or ML-6	6 input, 1 output	1
MM-7 or ML-7	7 input, 1 output	1
MM-8 or ML-8	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 (**Warner SH-27B** or **SF-28**) in addition to warming the chamber.