

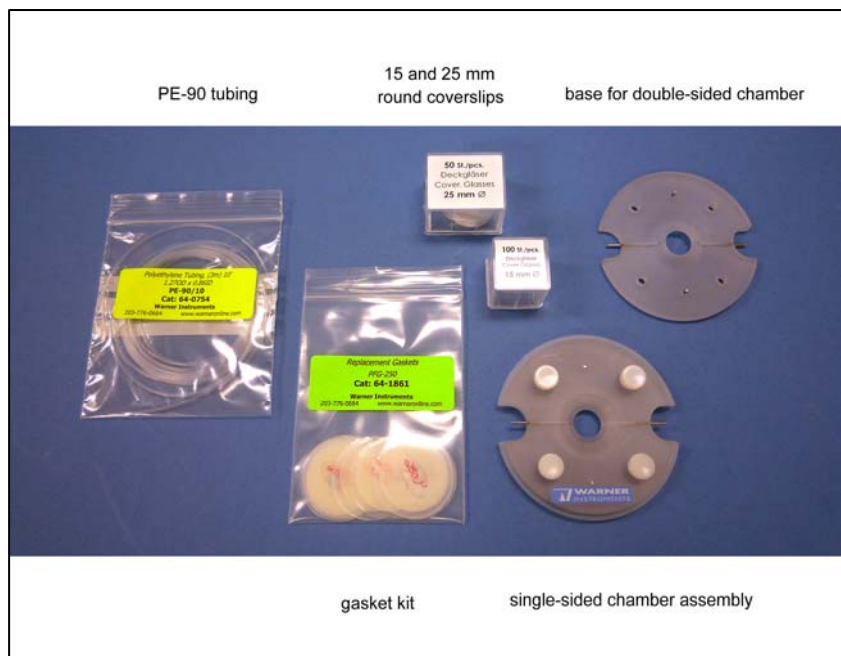
The **PFC-1** ProFlow chamber is a unique chamber/gasket system designed specifically for studies involving the effect of shear flow on cultured cells or tissue sections. The chamber comes with two different bottom units to facilitate studies using either single- or double-sided flow.

The bounding geometry of the ProFlow bath is defined by gaskets specifically designed to optimize the flow dynamics within the bath. This optimized design results in a uniform flow velocity across the *entire bath width* and allows the shear force to be quantitatively measured or calculated.



The ProFlow chamber is capable of being assembled either as a single-sided or double-sided chamber. The single sided assembly is well suited for studying the effects of shear force on cultured cells, and the double-sided assembly is well suited for studying the effects of shear force on a suspended tissue section.

The ProFlow comes with all required components for assembling each chamber type and includes the parts shown to the right. All replacement components, including the specially designed gaskets, are available from Warner.



## ASSEMBLY

As mentioned above, the **PFC-1** can be assembled either as a single-sided or double-sided chamber. We will begin by assembling a single sided chamber.

### Single-sided chamber assembly

The single-sided chamber can support cultured cells either on the top 15 mm or bottom 25 mm coverslip. The general procedure for assembling a this chamber is to first mount the smaller coverslip and gasket to the top plate, and then mount the larger coverslip to the bottom plate. The two halves are then assemble together.

1. Begin by mounting the 15 mm round coverglass and gasket onto the top plate.

a. First, locate the top plate. It can be identified by the presence of embedded perfusion channels and a Warner label. The top plate is the leftmost component shown in the photo immediately to the right.



b. Locate the 15 mm indentation on the underside of the top plate. This indentation closely surrounds the central opening in the top plate and is where the 15 mm coverslip will be placed. Note that the top plate also has a larger surrounding indentation designed to accept the gasket.

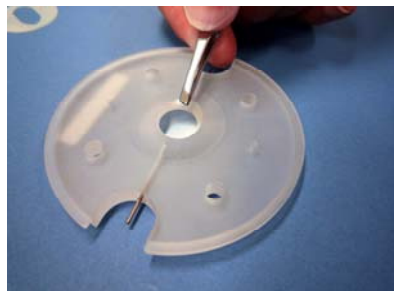
c. Carefully paint a thin coating of vacuum grease completely around the lip of the coverglass indentation.



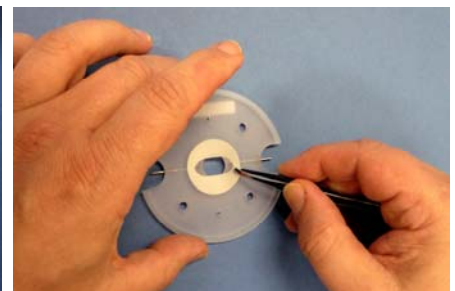
d. Also paint a thin film of vacuum grease within the gasket indentation immediately to each side of the coverglass indentation. This film does not need to cover the complete gasket area since it serves only to hold the gasket in place during assembly. Do not get grease into the flow ports or chamber bath area.



e. Set the 15 mm coverglass into place.



f. Then set the gasket into place above the coverslip. Take care to position the gasket so as to not block the flow ports into the chamber. The port openings should align with the corners of the gasket cutout.



2. Next, mount the 25 mm round coverglass onto the bottom plate.

a. Locate the single-sided bottom plate. It can be identified as the only component lacking integral perfusion channels. This plate supports a 25 mm coverglass and, as such, it has a 25 mm lip surrounding its central opening.

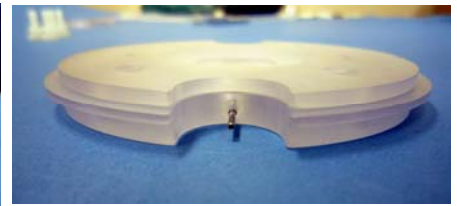
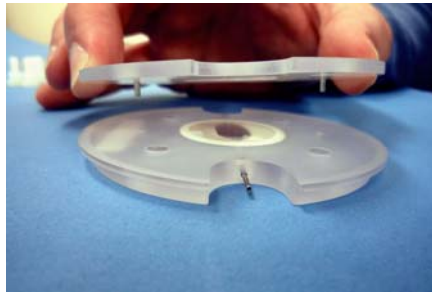


b. Carefully paint a thin coating of vacuum grease around the lip of the coverglass indentation and set the associated coverglass into place.



3. Assemble the chamber.

- a. Begin by placing the chamber bottom plate onto the gasket previously mounted to the top plate. The locating pins will serve as a guide for proper placement. The result of this assembly step will be an upside-down chamber resting on your workspace.



- b. Carefully pick the upside-down chamber up and flip it over. Insert and tighten the nylon screws to complete the assembly.



**Double-sided chamber assembly**

While the double-sided chamber is designed to support a tissue or bone section between the two flow paths, it can also support cultured cells on either the top or bottom coverslip. (This, of course, will require the insertion of a barrier between the two flow paths.)

The general procedure for assembling this chamber is to first mount coverslips and gaskets onto both the top and bottom plates. Then, the tissue or bone section is positioned onto one plate. Finally, the two chamber halves are assembled together.



1. Locate the assembly parts. The components for the double-sided chamber are shown to the right. Note that, in common with the top plate, the bottom plate also has embedded perfusion channels.
2. Mount the coverglass and gasket to the chamber top plate.

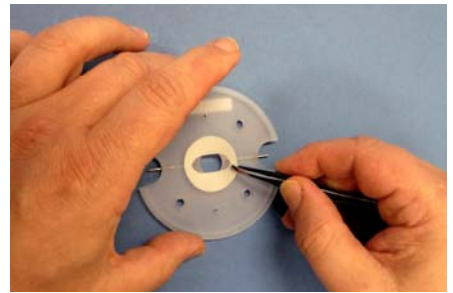
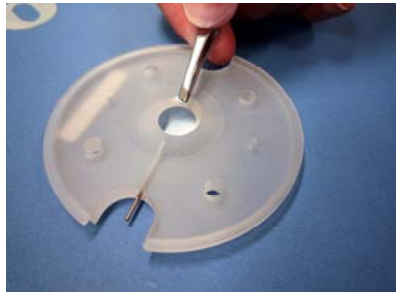
- a. First, locate the top plate. It can be identified by the presence of a Warner label.
- b. Locate the 15 mm indentation on the underside of the top plate. This indentation closely surrounds the central opening in the top plate and is where the 15 mm coverslip will be placed. Note that the top plate also has a larger surrounding indentation designed to accept a gasket.





c. Carefully paint a thin coating of vacuum grease completely around the lip of the coverglass indentation. Also paint a thin film of vacuum grease within the gasket indentation immediately to each side of the coverglass indentation. This film does not need to cover the full gasket area since it serves only to hold the gasket in place during assembly. Do not get grease into the flow ports or chamber bath area.

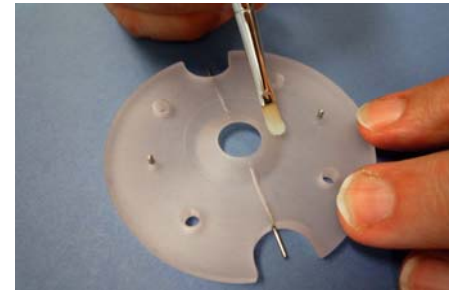
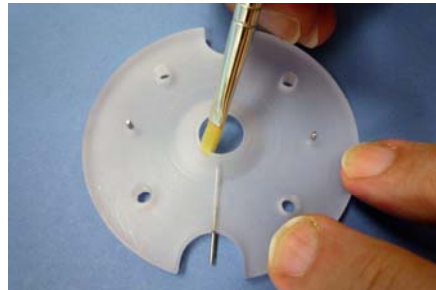
d. Set the 15 mm coverglass into place. Then set the gasket into place above the coverslip. Take care to position the gasket so as to not block the flow ports into the chamber. The port openings should align with the corners of the gasket cutout.



e. Set the top plate aside.

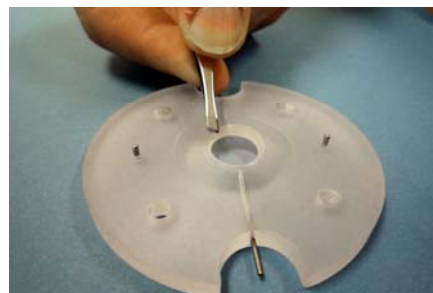
3. Next, locate the chamber bottom plate. This plate is similar to the top plate except it lacks a Warner label.

a. As before, locate the 15 mm indentation on the underside of the bottom plate. Note that the bottom plate also has a larger surrounding indentation designed to accept a gasket.

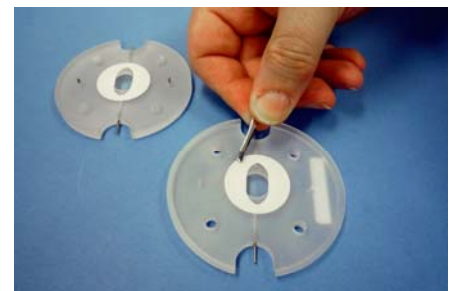


b. Carefully paint a thin coating of vacuum grease completely around the lip of the coverglass indentation. Also paint a thin film of vacuum grease within the gasket indentation immediately to each side of the coverglass indentation. This film need not cover the full gasket area since it serves only to hold the gasket in place during assembly. Do not get grease into the flow ports or chamber bath area.

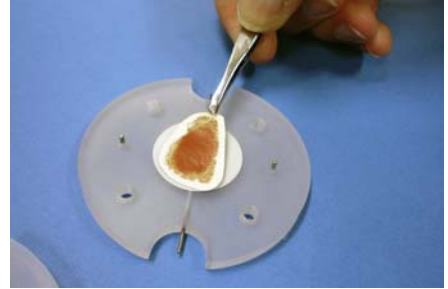
c. Set the 15 mm coverglass into place. Then set the gasket into place above the coverslip. Take care to position the gasket so as to not block the flow ports into the chamber. The port openings should align with the corners of the gasket cutout.



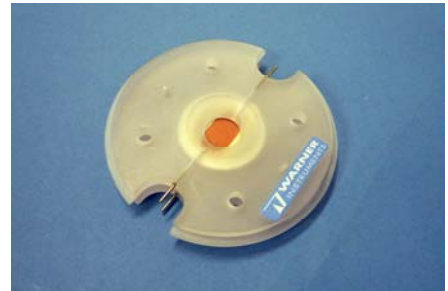
d. The completed top and bottom plates are shown to the right.



4. Finally, position the sample and complete the assembly.
- Position the sample onto the gasket on the bottom plate. Take care to completely cover the gasket cutout with the sample.



- Place the top plate onto the bottom plate. Use the guide pins to align the two halves.



- Secure the top and bottom plates together using the Nylon screws.



## Mounting onto the microscope

The **PFC-1** 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 diameter of the chamber. In most cases the stage cutout will be larger than the chamber necessitating the use of a stage adapter. Warner Instruments has stage adapters for most microscopes and 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 PFC-1 to be used with an in-line solution heater such as the SH-27B.**

## Fluid control

Selection of solution source 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.

Solution delivery to the chamber can be via a pumped or gravity-fed source. Since the chamber is designed for shear-flow studies, we assume the user will use a pumped delivery system since this allows a quantitative

determination of shear stress. Warner Instruments is a subsidiary of Harvard Apparatus, and Harvard Apparatus is a world leader in syringe pump systems. As such, we'll be glad to assist you in determining a suitable syringe pump for your application.

### **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 **PFC-1**. Air should be removed from each feed line by pre-filling it 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.

### **MAINTENANCE**

Cleaning of the top and bottom plates should be performed using a dilute detergent solution. Alternatively, Warner has developed a trisodium phosphate (TSP) wash protocol which is effective in cleaning plastic and anodized metal parts. Unfortunately, we do not have a suitable cleaning procedure for removing vacuum grease from plastic parts (without destroying them), and recommend rubbing the grease from the plastic surface with a soft cloth. Contact our Technical Support staff or download the TSP protocol in PDF format from our website: ([http://www.warneronline.com/pdf/whitepapers/cleaning\\_plastic.pdf](http://www.warneronline.com/pdf/whitepapers/cleaning_plastic.pdf))

## APPENDIX

### A. Shear Force Calculations

The following discussion is taken with modifications from Song, et al., PLoS ONE, 5:e12796.

A computational fluid dynamics model can be used to calculate flow velocity, pressure, and shear stress within the PFC-1 chamber. Flow characteristics are calculated from the continuity (1) and Navier-Stokes (2) equations and wall shear stress is derived from the wall strain rate (3).

We assume that the flow medium is incompressible and that flow is laminar at rates of interest for physiological relevance. These assumptions are appropriate, given that most flow mediums are similar to 0.9% saline, a Newtonian fluid with density comprising 996 kg/m<sup>3</sup>, at body temperature (310 K), and with laminar viscosity (0.001 kg/ms).

The Navier-Stokes equation is applied assuming that body forces are negligible and that the flow is steady in three dimensions, also appropriate assumptions for the length and time scale in this chamber. Hence,

$$\nabla \cdot v = 0 \quad (1)$$

$$\rho(v \cdot \nabla v) = \mu \nabla^2 v - \nabla P \quad (2)$$

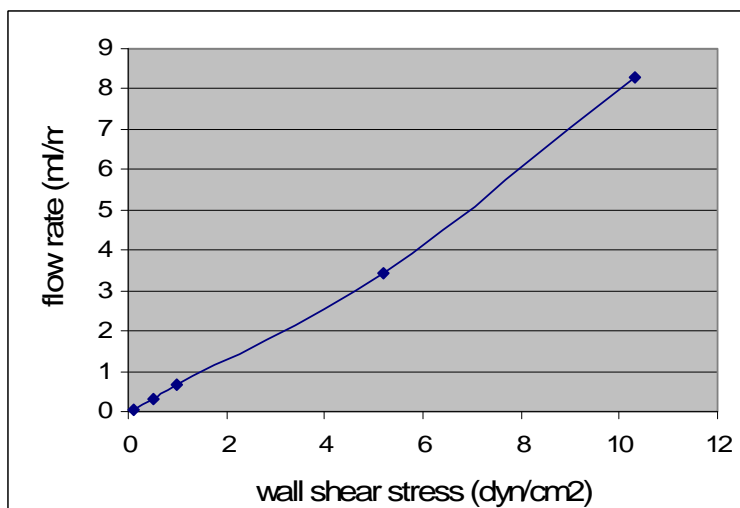
$$\tau_{cell} = \mu \left. \frac{\partial v}{\partial x} \right|_{x=10 \mu m} \quad (3)$$

where  $v$  is the velocity vector,  $\rho$  is the density,  $P$  is the pressure,  $\mu$  is the viscosity,  $\tau_{cell}$  is the shear stress at 10  $\mu$ m height,  $\frac{\partial v}{\partial x}$  is the strain rate, and  $x$  is the height from bottom of chamber.

As an example, a target shear stress of 0.2 dyn/cm<sup>2</sup> (0.02 N/m<sup>2</sup>) is induced using an input pressure gradient of 3.9 Pa which yields a flow rate of 0.13 ml/min in this chamber.

More practically, the graph to the right shows the linear nature of the wall shear stress as a function of flow rate into the chamber. **Taking the shear stress at a flow rate of 1 ml/min yields a unit shear of 1.5279 dyn/cm<sup>2</sup> for each ml/min of flow rate.**

As an example, a target shear stress of 2 dyn/cm<sup>2</sup> would require a flow rate of 1.3 ml/min. Alternatively, a given flow rate of 5 ml/min will induce a shear stress of 7.6 dyn/cm<sup>2</sup> in the chamber.



## **B. References**

1. In situ spatiotemporal mapping of flow fields around seeded stem cells at the subcellular length scale. Song MJ, Dean D, Knothe Tate ML., PLoS One. 2010 Sep 17;5(9). pii: e12796.
2. Design of tissue engineering scaffolds as delivery devices for mechanical and mechanically modulated signals. Anderson EJ, Knothe Tate ML., Tissue Eng. 2007 Oct;13(10):2525-38.
3. Open access to novel dual flow chamber technology for in vitro cell mechanotransduction, toxicity and pharmacokinetics. Anderson EJ, Knothe Tate ML., Biomed Eng Online. 2007 Dec 4;6:46.

## **C. Warner Stage Adapters**

The **PFC-1** chamber fits Warner's Series 20 stage adapters.

We carry an extensive line of stage adapters for our chambers and 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 on our website (<http://www.warneronline.com>).



## D. 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
<b>Coverslips</b>			
CS-15R15	64-0713	15 mm round coverglass, #1.5 thickness	100
CS-25R20	64-0722	25 mm round coverglass, #2 thickness	100
<b>Gaskets</b>			
	64-0330	ProFlow gaskets, 375 um thick	10
<b>Polyethylene Tubing</b>			
PE-90/10	64-0754	1.27 mm OD x 0.86 mm ID tubing	10 ft. (3.3 M)
<b>Multi-Perfusion Zero Dead Space Manifolds</b>			
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

- 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**).