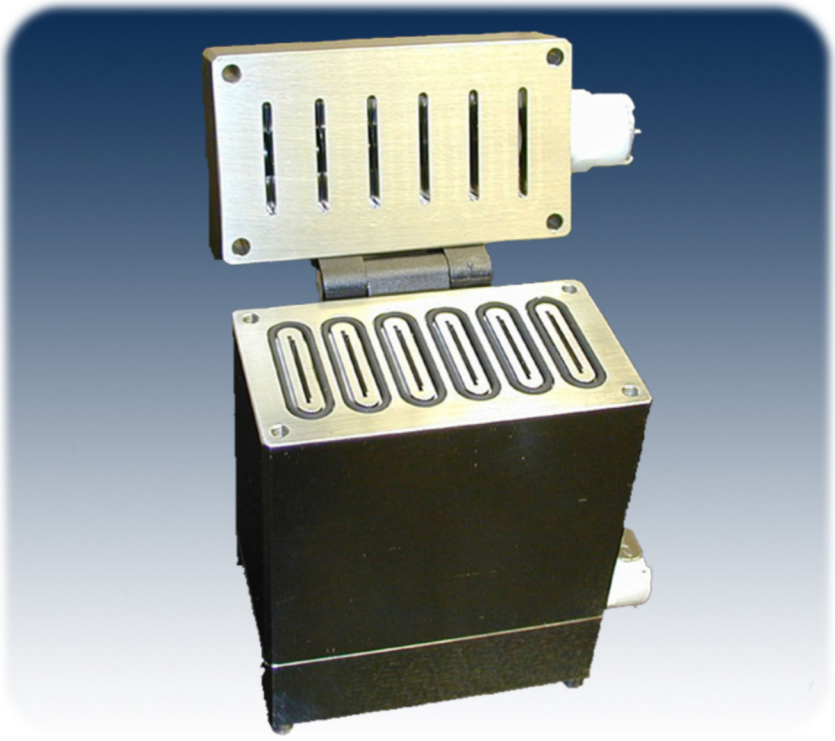
USER MANUAL

Streamer® System STR-4000



05-12-17
Rev 6.0

Culturing Cells in a Mechanically Active Environment™
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TABLE OF CONTENTS

1. GETTING STARTED

INTRODUCTION

Fluid-induced shear stress occurs in every tissue in the body as a result of interstitial fluid movement. Tissue deformation by compression, tension or shear forces results in the movement of interstitial fluid around cells. Fluid movement acts as a transport vehicle for ions, proteins, carbohydrates and other molecules capable of movement within the matrix. As the fluid moves past cell membranes, a shear stress (τ) is generated. If one assumes that laminar flow occurs through a parallel-plate flow chamber, fluid-induced shear stress values can be determined with the following formula:

$$\tau = 6\mu Q/bh^2$$

where τ is the shear stress in dyne/cm^2, μ is the viscosity of the fluid in dynes/cm^2, Q is the flow rate in ml/s, b is the width of the flow channel in cm, and h is the height of the flow channel in cm. Shear stress in the vascular system may vary from less than 1 to more than 35 dyne/cm^2. Fluid shear stress in canaliculi of bone may vary from 1 to 20 dyne/cm^2, while in cartilage it may be in the range of 1 to 5 dyne/cm^2.

The Streamer® is a parallel-plate flow system that is used to apply fluid-induced shear stress to cells grown in a monolayer. The system includes a six-chamber laminar flow device designed to hold 75 x 25 x 1 mm Culture Slips® (Fig. 1). Cells are cultured on these matrix-coated glass slides. StreamSoft™ software controls a peristaltic pump, thereby regulating the flow rate into the chamber and the magnitude of shear stress applied to the cells. Shear stress values from 0 to 35 dyne/cm^2 can be achieved depending on the tubing size used. This six place flow chamber can be used to assess RNA and protein expression by cells in response to fluid-induced shear stress, and production of secreted molecules into the perfusate.

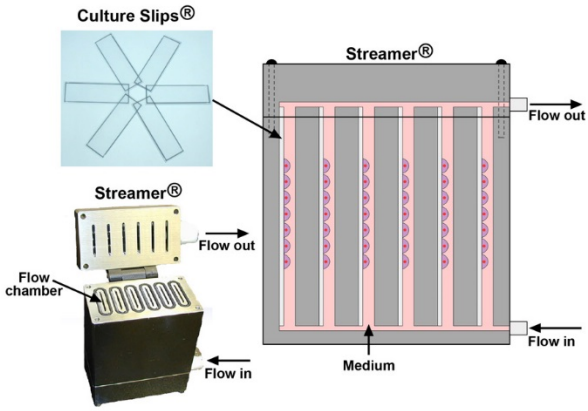


Figure 1. Image and schematic of Streamer® device with cells cultured in monolayer on Culture Slips®.

STREAMER® COMPONENTS

- Streamer® Device
- Streamer® Tubing (includes quick disconnect fittings)
- Masterflex® L/S Peristaltic Pump
- RS232 to USB Connector Cable
- Pulse Dampeners (2)
- 12 Culture Slips®
- StreamSoft™ Software V4.2
- 500 ml Culture Medium Collection Reservoir (includes quick disconnects and filter)
- Dell Inspiron Notebook Computer (optional)

STREAMER® SETUP AND ASSEMBLY

The following instructions are for the full Streamer® system. Once fully assembled in the incubator, the system should resemble the one pictured in Figure 2. **Always check the tubing for cracks or leaks prior to use.**

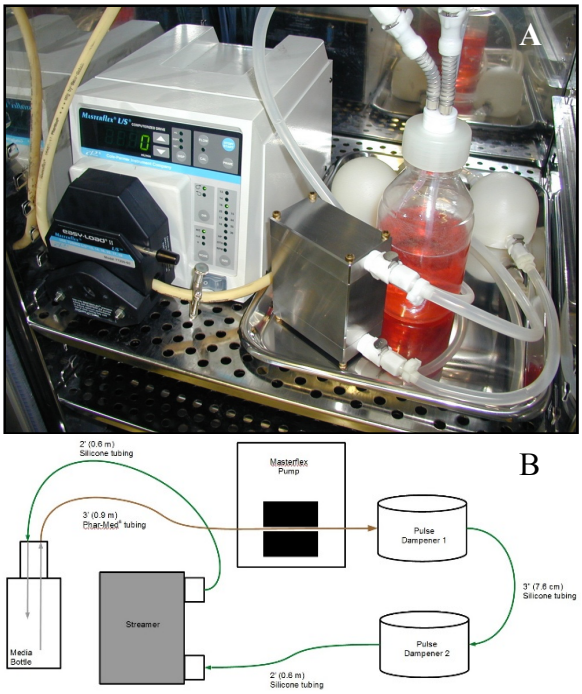


Figure 2. A) Streamer® system setup on the shelf in a standard incubator. B) Schematic of Streamer® system setup.

1. Connect the medium collection reservoir to the first pulse dampener with the 3' (0.9 m) long piece of beige Phar-Med® tubing. On the medium reservoir, the quick disconnect connected to the long tubing extending to the bottom of the bottle should be used. **Do not use the quick disconnect leading to the bent tubing in the bottle for this connection.**

2. Move the clamp mounted onto the Phar-Med® tubing to the end closest to the pulse dampener. Place the middle of this tubing segment into the pump head. Rotate the lever to the left to open the pump head for tubing placement, then rotate the lever to the right to secure the tubing into the pump head. **When not doing an experiment, the pump head lever should be rotated to the left to eliminate pressure on the tubing.**

3. Connect the first pulse dampener to the second pulse dampener with the 3" (7.6 cm) long segment of silicone tubing.

4. Connect the second pulse dampener to the inlet port (bottom) of the Streamer® with one of the 2' (0.6 m) pieces of silicone tubing.

5. Connect the outlet port (top) of the Streamer® to the quick disconnect on the medium bottle that is connected to the short, bent tubing in the bottle, using the other 2' (0.6 m) long piece of silicone tubing.

6. Before the first use, run deionized water through the entire system to make sure there are no leaks.

Note: *Any of the tubing lengths above can be shorted or extended according to your setup needs.*

STERILIZING THE STREAMER®

All components of the flow system (except the pump and computer) can be effectively and safely sterilized in an autoclave at standard autoclave temperature, pressure and time period (120 °C, 15 psi, 15-20 minutes). When autoclaving, leave all system components connected together. Release the clamp on the Phar-Med® tubing used between the medium collection reservoir and the first pulse dampener. Open the top of the Streamer® slightly so that steam can reach the inside of the device. Also, do not place any system component on top of the pulse dampeners, as the pulse dampeners may deform under load at high temperatures. The pulse dampeners should be placed on top of all system components in the autoclave.

Note: *After autoclaving, check the threaded quick disconnect fittings on the Streamer® inlet and outlet and on the pulse dampeners for tightness. If any of the fittings have become loose, turn them until they are ¼ turn past finger tight.*

If you do not have access to an autoclave you can use ethylene oxide gas treatment with subsequent vacuum treatment. 70% ethyl

alcohol can also be pumped through the system for cleaning, however, this will not completely sterilize the system.

STREAMER® PLACEMENT IN THE INCUBATOR

The Streamer® system should be placed into a temperature-controlled, CO_2 incubator for experiments. We recommend that the Streamer® be kept in the incubator for at least 20 minutes before starting an experiment to assure that the device is at a stable temperature for cell culture. The pump is placed into the incubator with the rest of the system. A containment tray is placed underneath the system to provide a means to transport the system to the cell culture hood, and to catch any fluid should a leak occur. ***The computer must not be placed into the incubator.***

USING THE PUMP

1. Plug the pump into a power outlet (110 V for North America, 220 V for Europe and Japan).

2. **If using the flow system manually (i.e. without computer control)**, ensure that the correct tubing size is selected on the pump and the clockwise flow direction. The standard tubing included with the Streamer® system is MasterFlex® L/S 17. Therefore, you will want to press the "size" button on the bottom right of the pump face until the green light is beside the number "17".

NOTE: *Once you reach the tubing size "25", the green light will remain on the same level for two depressions and the second one will cause the "HP" LED (near the bottom of the indicator light column) to light up. The "HP" button specifies the tubing numbers on the right column of the size listings. Therefore, when the green light is beside the number "17" you will want the "HP" light to be off.*

   Once this is set, the display on the pump will read the flow rate for that particular tubing as the pump is running. Consult the appendix or the data following this manual to find which flow rate corresponds to the desired level of shear stress. Use the arrow keys on the top left of the pump face to select the required flow rate. Press the blue start/stop button to initiate and stop flow.

3. **If using the system with computer control**, connect the male end of the RS-232 cable into the back of the pump and the USB end into a USB port of the computer. Turn on the power and start the software. When the software controller is functional, "PO1" will appear on the pump display. Use the software to create a regime with your desired flowrate(s). See pages 5-18 for further instructions on using the StreamSoft™ software.

QUICKSTART INSTRUCTIONS

1. Set up the entire system in an incubator. See page 1 for the system setup.
2. Sterilize the Streamer® and system components. See page 2 for sterilization instructions.
3. Connect the cable from the pump to the serial port of the computer. Turn on the computer and pump and open up the StreamSoft™ software program.
4. Select the *Operate* menu, then select *Users*. Add your name as a user by clicking *Add User*, then click the *Return* button.
5. Select the *Operate* menu again, then select *Configure Regimes*. Type a new name in the *Regime Name* field and click on *Insert Step* to insert a step into the regime. Create a regime by entering values in one or more steps. Once complete, click on *Save Regime*. Click *Return* to exit.
6. On the main screen, click on *Configure*; this will open the *Pre-Test Configuration* window. Select the appropriate *User*,

Regime and *Hardware*, then click *Update*. The regimen is now ready to start.

7. Culture cells on six Culture Slips®. Be sure that you culture on the side with the Teflon® rim printed around the borders. Be careful to plate cells only within this rim. We recommend allowing cells at least 48 hours to attach to slides before beginning your flow experiment.

After cells have attached to slides:

8. Be sure that the Streamer® is closed (the top lid should be flush with the body of the device).
9. Place 500 ml of PBS into the medium container and pump through the system to flush out impurities. This can be done by starting your regime or using the *manual mode* under the *Operate* menu in the software. If you are not using the software, set the pump to the appropriate tubing size, set the flowrate at 300 ml/min and press the start button.
10. After pumping for several minutes, remove the PBS from the medium container and replace with 500 ml of sterile tissue culture medium.
11. Pump the culture medium through the system to flush out remaining PBS. Remove the medium and replace with 500 ml of fresh sterile tissue culture medium.
12. Pump the tissue culture medium through the entire system. Once the system is full, tilt the pulse dampeners, one at a time, at an angle of approximately 20 degrees, such that the direction of the flow is going from the vertex of the angle to the open end of the angle. Leave the pulse dampener in this position until the fluid comes through the outlet fitting again, then lay the pulse dampener down horizontally. This process will allow the pulse dampener to fill to a level slightly higher than the fittings, thereby creating a bubble trap for any air bubbles that may accidentally enter the system. Do the same with the second pulse dampener. Once this process is complete, allow flow to continue and go to the next step.
13. As the flow continues, check for any air bubbles visibly trapped within the tubing. Also check the walls of the medium container to be sure that no air bubbles have formed on the sides. If so, swirl the medium around to release air bubbles from the side walls.
14. Once the tubing and flow device are filled with medium and all air bubbles are eliminated, reverse the flow direction on the pump to draw the medium level back to the flow chamber and down past the head of the chamber, then stop the pump. The fluid level will have to be estimated once the fluid can no longer be seen in the tubing coming from the head of the Streamer®.
15. Tighten the small clamp on the Phar-Med® tubing just to the right of the pump head so that the flow path in the tubing is completely closed off.
16. Turn the lever arm on the MasterFlex® pump all the way to the left to release the tubing and remove the tubing from the pump head. Carefully move the tray containing the Streamer® device, tubing, pulse dampeners, and fluid collection reservoir to the tissue culture hood.
17. Remove the Streamer® screws and open the hinged top.
18. Transfer your cells from the incubator to the tissue culture hood.
19. Using forceps and/or your fingers with sterile gloves, pick up each Culture Slip® and place it into each one of the slots in the flow device. ***Be sure that the side of the slide with cells attached is facing the flow area adjacent to the slot, not the closed wall of the slot***. Gently slide each Culture Slip® downward until it reaches the bottom of its chamber. Be careful that the Culture Slip® glass is not chipped against the stainless steel surface during this process.

All six slots must be filled to ensure proper flow rate readings*. *If you do not wish to use all six Culture Slips® with cells, use blank Culture Slips® for the remaining slots.

20. Close the top of the device, turn the bolts by hand, then tighten them with the hex wrench provided with the system.
21. Move the tray with the system components back to the incubator. Put the Phar-Med® tubing back into the MasterFlex® pump head and clamp the head down.
22. Unscrew the small clamp on the Phar-Med® tubing to open the flow path to full capacity.
23. Click the *Start* button in the software (or set the pump to the desired flowrate and depress the start button). Your regimen will start and a green light will go on at the top right corner of the screen.
24. The expected shear stress and actual value will be displayed on the graph in real time. Periodically monitor the flow system for leaks during the protocol.
25. When the flow regimen is over and the pump has stopped, remove the Streamer® system from the incubator as before. Open the top and remove the slides for processing.
26. Clean the Streamer® and system with deionized water. ***Never leave culture media in the Streamer® device after an experiment, as this will corrode the stainless steel finish over time.***

See the instructional video, ***Streamer® Assembly***, on Flexcell®'s website (http://www.flexcellint.com/videos-instruct.htm) for a demonstration of how to assemble the Flexcell® Streamer® and associated tubing to run with a MasterFlex Peristaltic Pump.

2. STREAMSOFT™ V4.2 SOFTWARE

INSTALLATION INSTRUCTIONS

1. Insert the *StreamSoft™ V4.2* DVD into the DVD-ROM drive on the computer.
2. Double click *My Computer* (Windows XP) or *Computer* (Windows Vista/Windows 7).
3. Double click the *DVD-ROM drive.*
4. Double click the *Setup* installer.
5. The installer will now open and run.
6. On the *Product Notification* screen, click *Next.*
7. On the *Destination Directory* screen, click *Next.*
8. On the *License Agreement* screen, click *I accept the License Agreement* and then click *Next.*
9. On the next *License Agreement* screen, click *I accept the above 2 License Agreement(s)* and then click *Next.*
10. On the *Start Installation* screen, click *Next.*
11. Installation of the required National Instruments and StreamSoft™ software will now begin.
12. Once the installation is complete click *Finish* and restart the computer.
13. Installation of StreamSoft™ V4.2 is now complete.

***NOTE:** When the **Select Pump to Use** window appears when opening the StreamSoft™ V4.2 software, select the pump named **MasterFlex Peristaltic Pump** to ensure correct function of the equipment.*

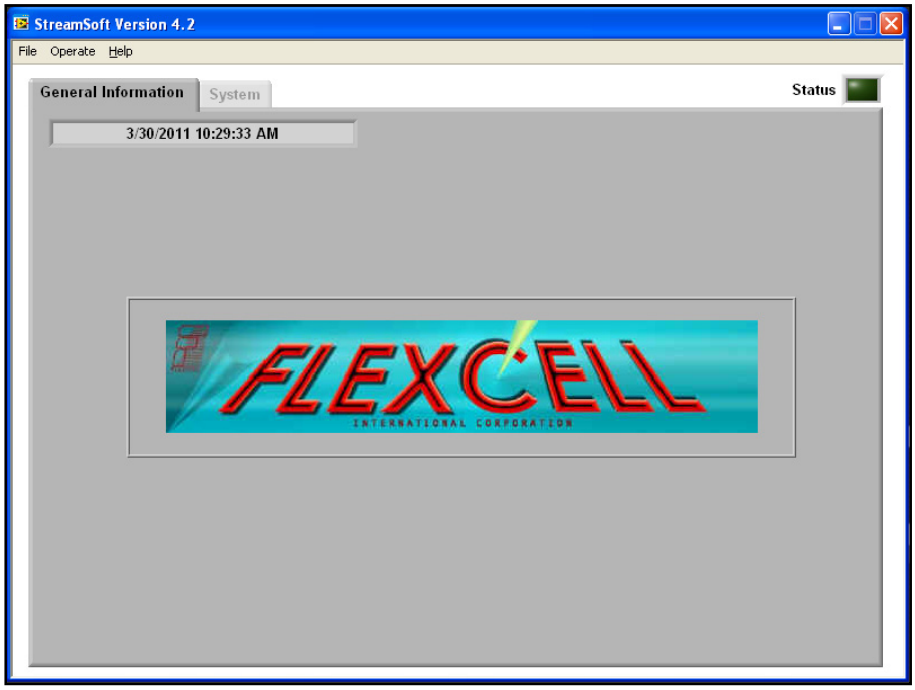
SETTING UP PARAMETERS IN STREAMSOFT™ V4.2

Specific parameters will need to be set up in StreamSoft™ V4.2 to customize it for your particular device and system. ***Setting up these parameters is extremely important to***

ensure accurate flow results for your system. For instructions on setting up these parameters, see *Configure Testing Apparatus* and *Configure System Variables*, pages 15-17. Complete this setup before proceeding with any experiments.

MAIN PANEL

GENERAL INFORMATION TAB

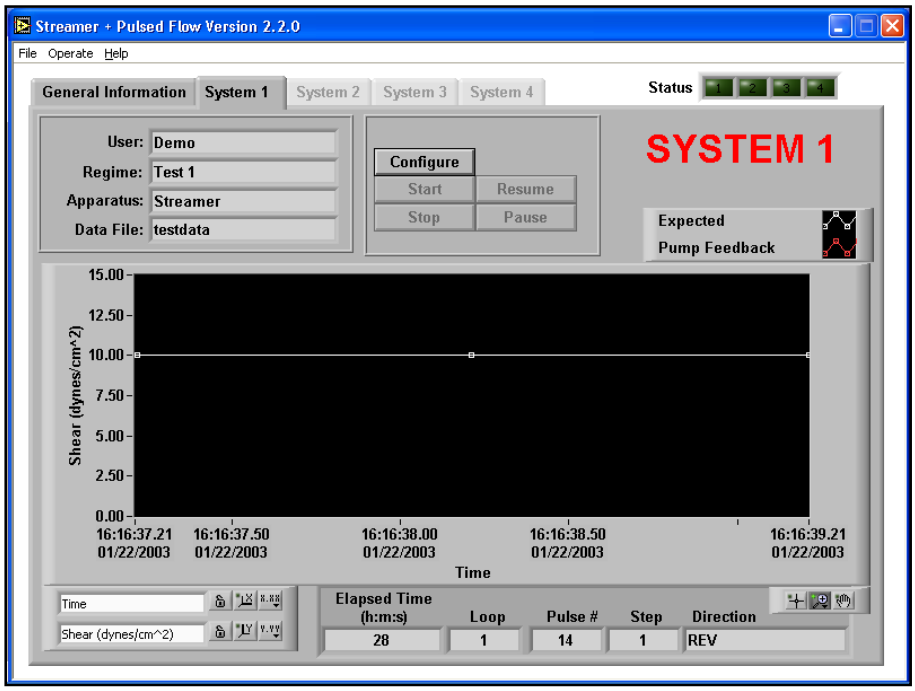


Function: The default main panel allows the user to verify that the system is running and to stop the tests at any time.

Buttons and Fields

Status	Number is bright green when an experiment is running
System 1,2,3,4	These tabs will automatically become highlighted according to the number of pumps connected to the computer (1 - 4).
General Information	Current date and time

SYSTEM TAB

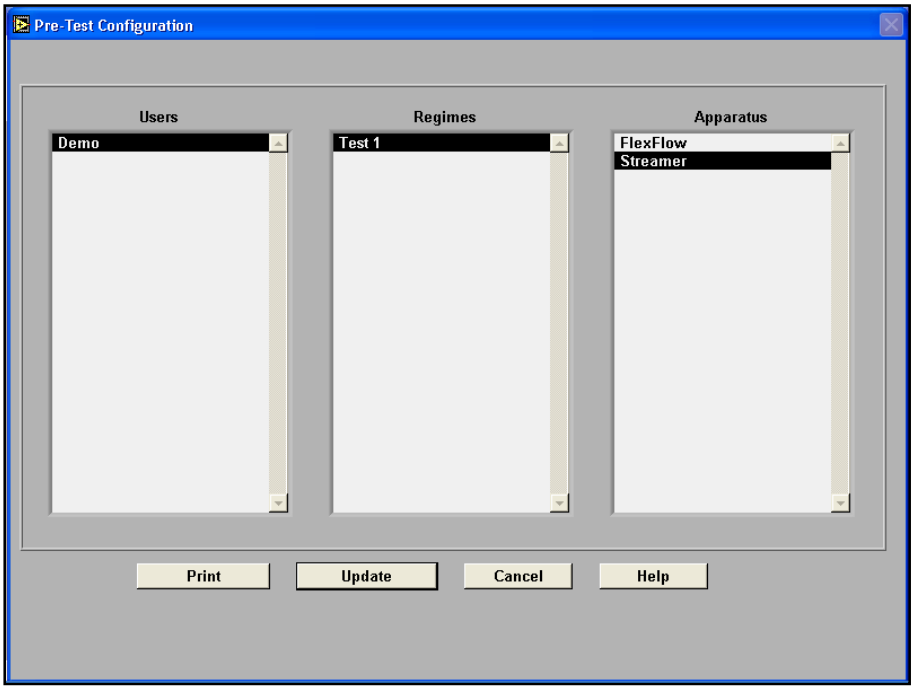


Function: This panel is used to run the experiments. Each ***System*** tab is identical.

Buttons and Fields

User	Current user in currently configured regime
Regime	Regime currently configured
Apparatus	Device being used with the software (Streamer® or FlexFlow™)
Data File	Name of file to which data is being saved (if appropriate)
Configure	Configure (load) the experiment. The *Pre-Test Configuration* window will appear.
Start	Start the experiment.
Stop	Terminate the experiment. This button is only active when an experiment is running.
Pause	Suspend the experiment. The pump will stop, but the test regimen is kept in memory.
Resume	Resume a paused experiment.
Graph	This graph shows the expected and actual shear stresses during the experiment.
Elapsed Time (h:m:s)	Elapsed time in the current experiment
Loop	Current loop in the current step or series of steps
Pulse #	Total number of pulsations (square wave) or oscillations (FWD/REV) produced by the valves in this regime
Step	Current (active) step in regime
Direction	Current flow direction (FWD/REV)

PRE-TEST CONFIGURATION



Function: This panel allows the user to configure the parameters of an experiment. It appears when the user presses the *Configure* button on the System panel. The information selected here is transferred to the *User*, *Regime* and *Apparatus* fields on the *System* panel.

Buttons and Fields

Users	List of all users. Select users with the mouse.
Regimes	List of regimes created by the previously selected user. Select from list by using the mouse to highlight the desired regime.
Apparatus	List of configured flow devices. Select the device that will be used for the experiment. ***Important: Be sure that all parameters have been properly set for your device in the Configure Testing Apparatus window (see page 15).***
Print	Print the current panel to a printer or HTML file.
Update	Use the current selections to run the experiment.
Cancel	Cancel any new selections and use the previously configured setup for the experiment.
Help	Online help (not currently available)

PULL-DOWN MENUS

This section summarizes the function of each item in the three pull-down menus.

File	*Operate*	*Help*
-Print	-Manual Mode	-Help
-Exit	-View Data	-About LabVIEW
	-Users	
	-Configure Regime	
	-Configure Apparatus	
	-Configure System	
	-Reinitialize Hardware	

File

Print Allows user to print a copy of the current panel. This system is configured such that printing sends a copy of the panel being viewed to a printer or to an html file. If there is no printer connected to the computer, an error message from the Windows default printer queue will appear when the user tries to print.

Exit Allows user to close the program. If the pump is operating at the time of exit, it will continue running. The keyboard short-cut is **Ctrl-Q**.

Operate

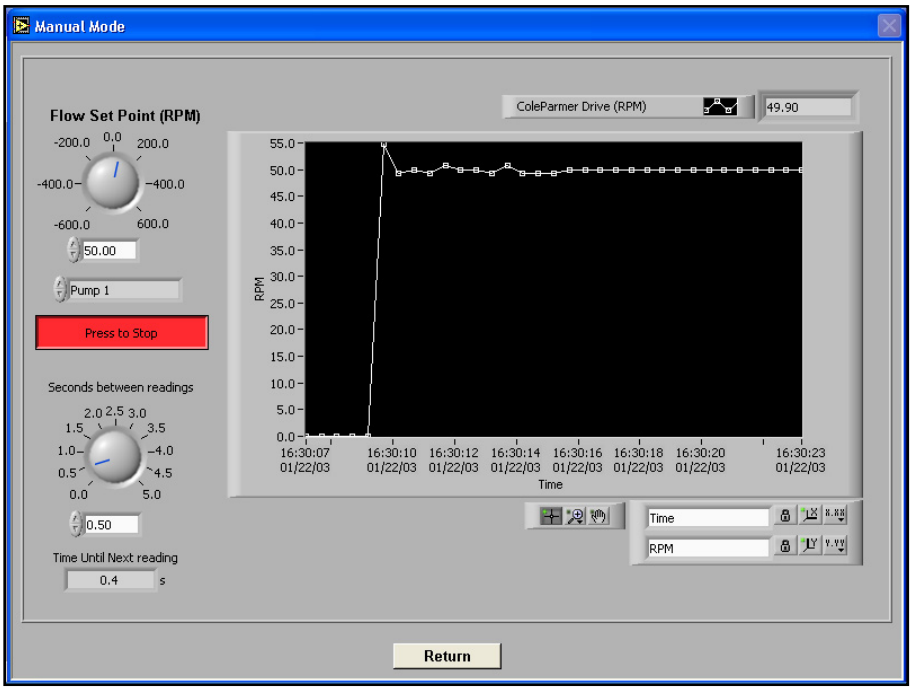
Manual Mode	Manually control the pump without setting up an experimental regimen.
View Data	View shear stress data from a previous experiment.
Users	Add and remove user names.
Configure Regime	Create an experimental protocol.
Configure Apparatus	Configure parameters of the flow device so that the software can assign the flow rates corresponding to the desired shear stress. **These parameters must be set correctly to ensure that the proper shear stress values are shown. See the manual of your device for the appropriate values.**
Configure System	Configure the system parameters such as data saving, the Com port used and the presence or absence of valves in the system (Osci-Flow®).
Reinitialize Hardware	This will reinitialize the software to connect the pump and Osci-Flow® (if present) in the event that a cable is disconnected or the pump is turned off.

Help

Help	Online help (not currently available)
About LabVIEW	Software version information

OPERATE MENU

MANUAL MODE

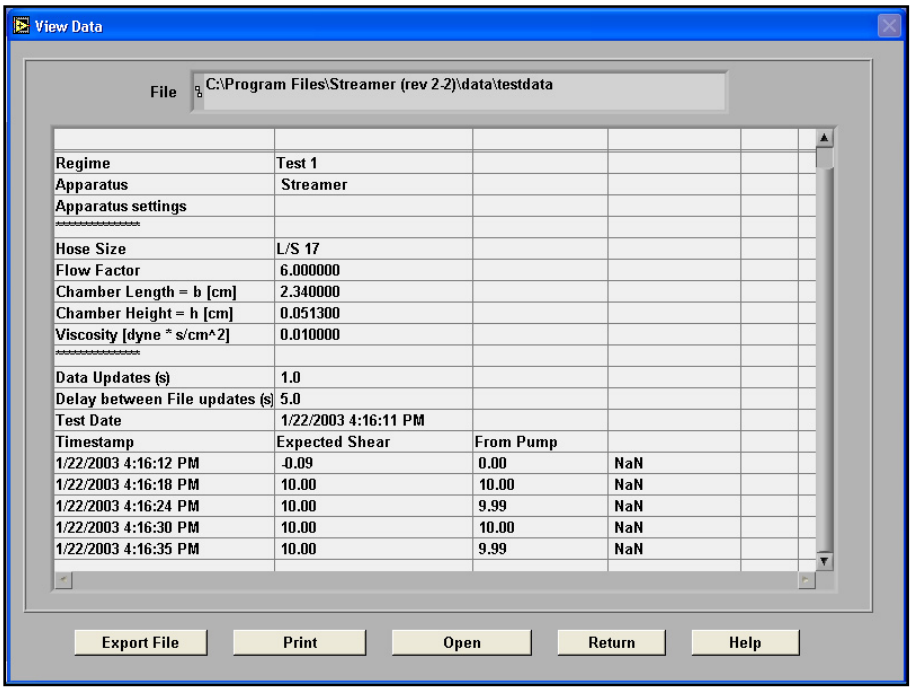


Function: This panel allows the user to manually control the pump. The actual flow rate and speed of the pump (RPM) are shown on the graph when the pump is working. Manual mode may be used to troubleshoot the pump operation. The shear stress value is not shown on this panel since it will depend on the tubing size and flow chamber used.

Instructions

1. Enter the flow set point (pump speed) either by entering a number in the box or using the mouse to drag the dial to the desired level.
2. Adjust the seconds between readings to a number between 0 and 5. This is the time between each update of the pump data on the graph.
3. Click on *Press to Start*.
4. Click on *Press to Stop* when ready to stop.
5. Click on *Return* when done.

VIEW DATA

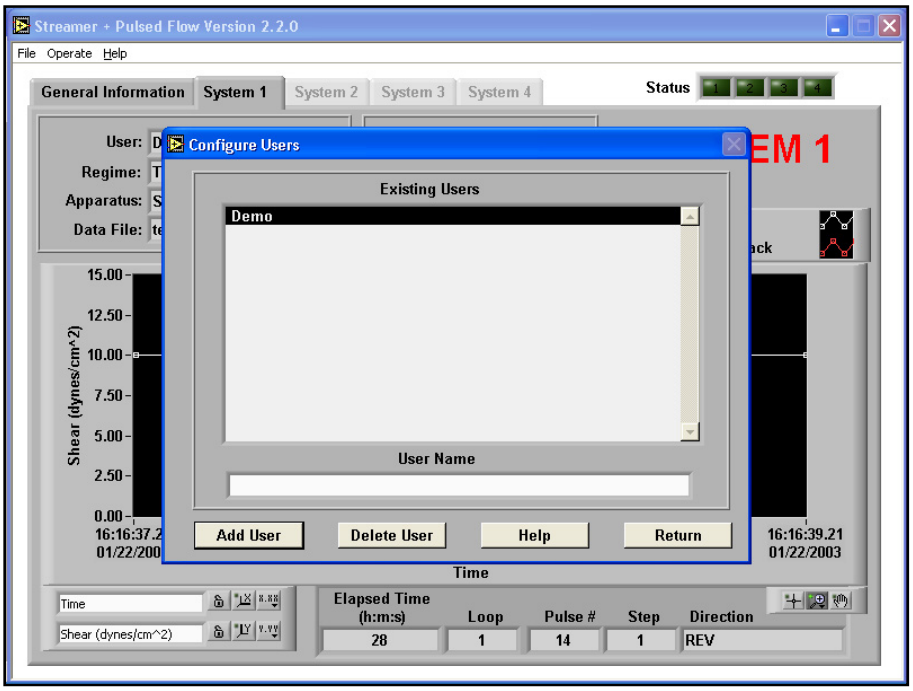


Function: This panel allows the user to view previously collected experimental data in a table format.

Buttons and Fields

File	The complete file path to the data file being viewed
Table	Contents of the experimental data log file
Export File	Export data to a spreadsheet-compatible format
Print	Print a copy of this panel to the Windows default printer or write a copy to an HTML file.
Open	Open a data file.
Return	Close this panel and return to the Main panel.
Help	Online help (not currently available)

CONFIGURE USERS



Function: This panel allows the user to create or delete users.

Buttons and Fields

Existing Users	Lists all current users of the system
User Name	Field used to enter new users
Add User	Add new users to the system.
Delete User	Delete users from the system.
Help	Online help (not currently available)
Return	Exit this panel and return to the Main panel.

Instructions

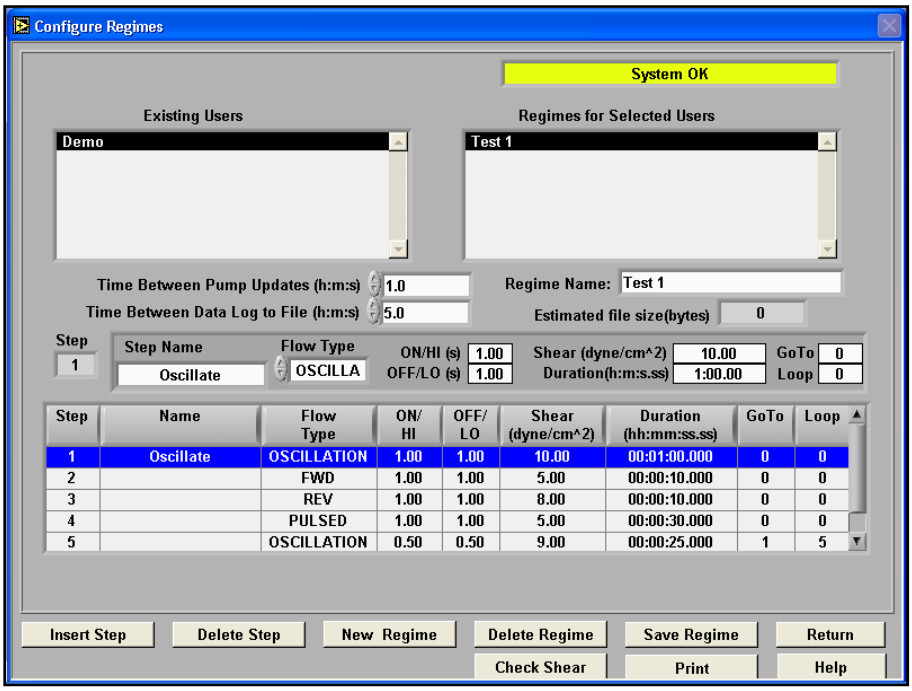
To add a user:

1) Type the name into the *User Name* field.
2) Press the *Add User* button.

To delete a user:

1) Using the mouse, select the user from the list of *Existing Users*.
2) Press the *Delete User* button. If the user has any stored regimes and data sets, the operator will be prompted to confirm the deletion.

CONFIGURE REGIMES: SETUP PARAMETERS



Function: This panel allows the user to configure (create) a regime.

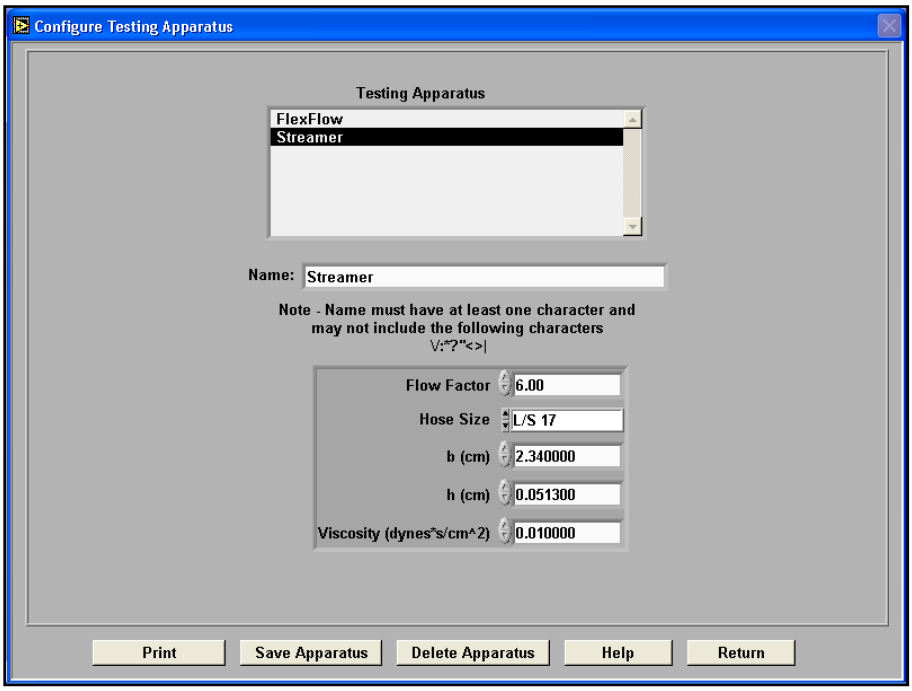
Buttons and Fields

Existing Users	List of all users; select a user from the list using the mouse.
Regimes for Selected Users	List of regimens created by the current user. Selecting from this list will load that regimen and allow the user to view and/or modify that regime.
Regime Name	Name of the current regimen; if creating a new regimen, enter a name in this field.
Time Between Pump Updates	Time elapsed between computer updates of the pump parameters; default is 1 second.
Time Between Data Log to File	Time interval between each computer sampling of the experimental flow data. Default value is 10 seconds. For an extremely long test, increase this interval to reduce the size of the data file. ***NOTE**: this function only applies when the data saving option is selected in the Configure System Variables window (see page 17).*
Estimated file size	This is an estimate of how large the data file would be given the total test length and the time between data. ***NOTE:** this function only applies when the data saving option is selected in the Configure System Variables window (see page 17).*
Step	Current step number selected or being modified
Step Name	Name of the currently selected step

Flow Type	Specifies the direction or type of flow for this step (forward, reverse, pulsed (square wave), oscillation)
ON/HI (s)	When using pulsed (square wave) or oscillatory flow, specifies how long the valves remain in a position to allow the fluid flow to continue unhindered or flow in the forward direction, respectively. For normal forward or reverse (unidirectional) flow, this value remains at 1.00.
OFF/LO (s)	When using pulsed (square wave) or oscillatory flow, specifies how long the valves remain in a position to stop the fluid flow to the device or cause it to flow in the reverse direction, respectively. For normal forward or reverse (unidirectional) flow, this value remains at 1.00.
Shear (dyne/cm^2)	The value of shear stress to be applied to the cells in this step.
Duration (h:m:s.ss)	Time to spend in this step (hours:minutes:seconds.milliseconds)
GoTo	To create a loop, indicate which step to go back to. The *GoTo* step must always be a step number before the current step.
Loop	Indicates how many times to loop between the *GoTo* step and the current step.
Summary Table	This table is a listing of the current steps in the regimen. Selecting a row from this table will allow the parameters of the step to be viewed and modified.
Insert Step	Insert a step into the regimen before or after the current step.
Delete Step	Delete the currently selected step.
New Regime	Clear all parameters and start a new regime. Type in a new name under *Regime Name* and select *Insert Step*.
Delete Regime	Delete the currently selected regime.
Save Regime	Save a new or modified regime.
Return	Exit this panel and return to the *Main Panel*
Check Shear	Check the shear stresses entered in your regime to see if they are achievable with the apparatus, pump and tubing size that you are using.
Print	Print the current panel to a printer or an HTML file.
Help	Online help (not currently available)

Instructions on how to set all the parameters for an experiment are included in the *Doing an Experiment* section of this manual.

CONFIGURE TESTING APPARATUS



Function: This panel allows the user to create, modify or delete a testing apparatus (Streamer® or FlexFlow™ flow chamber).

As each Streamer® and FlexFlow™ device is manufactured to strict dimensional specifications, the values for the height and width of the chambers must be entered into the software for each individual device.

These values are measured for *your specific device* and must be correct for accurate shear stress measurement. The values can be found in the appendix of the manual for your device.

Buttons and Fields

Testing Apparatus	List of all flow devices available
Name	When a testing apparatus is selected, this field (and the parameters) will be updated.
Flow Factor	A factor that accounts for any parallel paths in the flow stream. This number is 6 for the Streamer® and 1 for the FlexFlow™.
Hose Size	Hose size determines how fast the pump must move to achieve the desired flow rate and shear stress level. The sizes listed are standard for Masterflex® tubing. Select the hose size that you are using with your system.
b	Width of the flow area (cm) in a single chamber of the Streamer® or FlexFlow™ device. This number is found in the back of the manual for your device listed as Flow Area Width (cm).

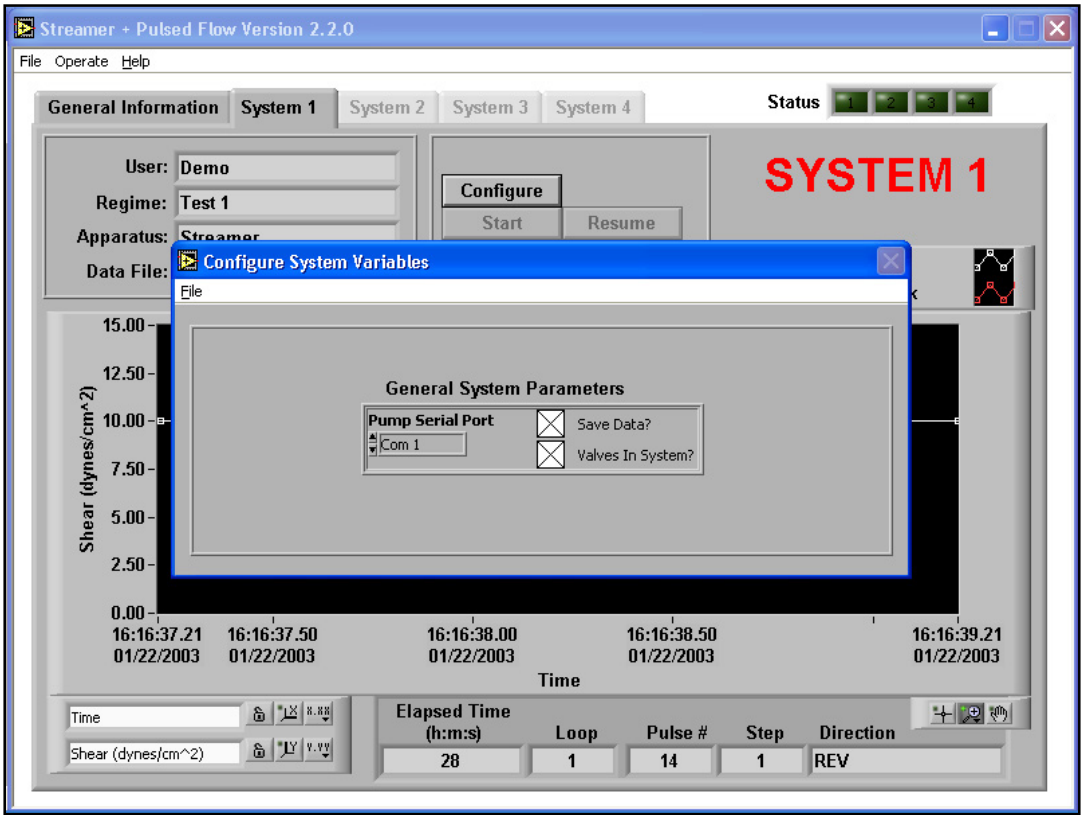
h	Height of the flow area (cm) in a single chamber of the Streamer® or FlexFlow™ device. This number is found in the back of the manual for your device listed as Flow Area Height (cm).
Viscosity	Viscosity of the perfusate/media used in the experiment. The standard value is 0.01.
Print	Print the current panel to a printer or an HTML file.
Save Apparatus	Save changes to the apparatus listed in Name.
Delete Apparatus	Deletes apparatus listed in Name.
Help	Online help (not yet available)
Return	Exit this panel and return to the Main panel. Any changes that have not been saved will be discarded.

HOW TO ENTER THE PROPER VALUES FOR YOUR DEVICE

Please check the Appendix of the manual for your device for the proper *b* and *h* values.

1. Select the *Testing Apparatus* being used for the experiment or enter a name for a new apparatus in the *Name* box.
2. Enter the correct flow factor for your device. This specifies the number of parallel flow chambers in your device.
3. Select the correct *Hose Size* for the type of Masterflex® tubing being used in the experiment.
4. Enter the proper *b* and *h* values for your device.
5. Enter the *Viscosity* of the perfusate fluid used in the experiment. The default value is 0.01 dynes*s/cm^2.
6. Click *Save Apparatus* button, then click *Return* to exit this screen.

CONFIGURE SYSTEM VARIABLES

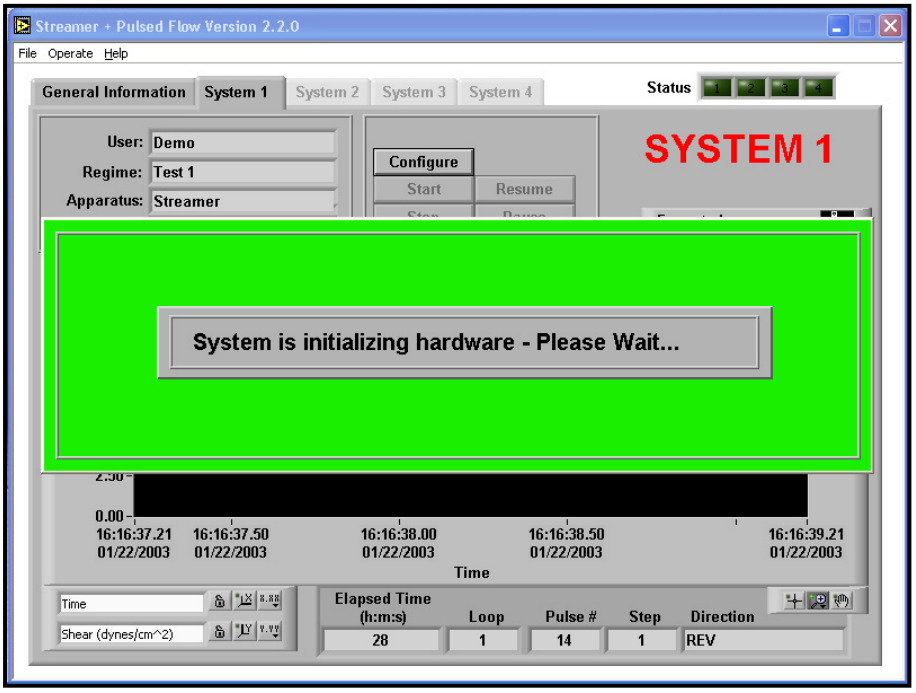


Function: This panel is used to select three system parameters – Communications port, data saving, and the presence of valves in the flow system.

Buttons and Fields

Pump Serial Port	Com 1 is the default port. This should be changed only if there is a conflict with this port on your computer.
Save Data?	Select this option if you want to save regime data files.
Valves in System?	Select this option if you are using the Osci-Flow® Flow Controller.

REINITIALIZE HARDWARE



Function: This panel will appear when the computer program is first started. It will also appear when the *Reinitialize Hardware* item is selected in the *Operate* menu. When the system is properly initiated, the pump will display PO1. If power to the pump is cycled during experimentation, or communication is lost, the user should reinitialize the hardware before turning off the program and starting it again.

STREAMSOFT™ V4.2 NOTES

- When running a regime in StreamSoft™ V4.2, do not run other applications on the same computer. The communication timing to the pump and Osci-Flow® requires full CPU availability. If another program or operation is running that requires CPU power, it is possible that the pump or valve timing could be interrupted. This effect may be noticeable when using the Osci-Flow® at a higher frequency than 2 seconds on, 2 seconds off, or when oscillating the pump speed to create pulsatile flow.

- When the *Select Pump to Use* window appears when opening the StreamSoft™ V4.2 software, select the pump named *MasterFlex Peristaltic Pump* to ensure correct function of the equipment.

3. DOING AN EXPERIMENT

OVERVIEW

There are two major components to running an experiment with the Streamer® system: configuration of the pump software and preparation of the flow chamber with Culture Slips® and cells.

We suggest that the following steps be done:

1. Culture cells on Culture Slips® and sterilize the Streamer® system components.
2. Configure a regimen in the StreamSoft™ program.
3. Prepare the Streamer® system with media, then place the Culture Slips® into the chambers of the Streamer® device in a sterile environment such as a laminar flow hood. Move the system to the incubator. ***Be sure that you have placed six Culture Slips® into the device, as any less will invalidate the shear stress values. Use blank Culture Slips® if necessary.***
4. Assign the user, regime and apparatus for the experiment.
5. Start the experiment.
6. When the experiment is finished, remove the Culture Slips® from the Streamer® flow device and proceed to analyze the cells.

The processes of creating a regimen and placing the slides into the flow device are described in further detail in this section.

CREATING A REGIME

1. From the main panel, select the *Configure Regimes* item in the *Operate* menu.
2. Click on an existing user name.
3. To create a new regimen, click on *New Regime*. Enter a name in the *Regime Name* field.
4. Click *Insert Step*; give this step a name in the *Step Name* field.
5. Click on *Save Regime*. The regime name should appear in the *Regimes for Selected Users* field at the top.
6. Specify the Flow Type (FWD, REV, PULSED, OSCILLATION), ON/HI & OFF/LO times (only when using the pulsed or oscillation functions; see pages 13-14 for more details), Shear, and Duration for this step. Click on *Save Regime* to save all information entered up to this point.

NOTE: *The Flow Type can only be specified when using the Osci-Flow® device-- without the 'Valves in System' selection used with the Osci-Flow®, the Flow Type will remain on FWD. See page 17 for more details.*

If you wish to add additional steps:

1. Click *Insert Step*. You will be queried as to whether this step should be inserted before or after the current step. Click on *before* or *after* according to your preference. Enter the preferred parameters as in #6. If this step was inserted after the step entered in #6, you can also use the *GoTo* and *Loop* options to loop through steps 1 and 2. Under *GoTo* in step 2, enter "1". Under *Loop*, enter the number of times that you would like to loop through steps 1 and 2.
2. Add additional steps as desired. Once the regime is complete, click on *Save Regime*.
3. **Optional:** Check the shear stress(es) in your regime to be sure that they are achievable with your apparatus, tubing size and pump. Click on *Check Shear* at the bottom of the Configure Regimes window while your regime is selected (see page 13). The Pre-Test Configuration window will appear (see page 8). Select a user, regime, and apparatus. Click on *Update*. The software will tell you if your shear stresses are achievable with this apparatus and the

tubing size and pump assigned to it. Modify shear stresses if necessary.
4. The regime is now ready to run.

SETTING UP AN EXPERIMENT

1. Set up the system in an incubator according to instructions on page 2.
2. Sterilize the Streamer® unit according to instructions on page 2. Close the lid and tighten screws until the lid is flush with the body of the device.
3. Place the Streamer® in the incubator with the remainder of the system. This will keep the temperature of the unit at 37°C.
4. Culture cells on 6 Culture Slips®. ***Be sure that you culture on the side with the brown Teflon® rim printed around the borders.*** Be careful to plate cells only within this rim. Allow cells at least 48 hours for full attachment to slides.
5. Create your regime in the StreamSoft™ software.

After cells have attached to slides:

1. Put one bottle of PBS into the system medium container.
2. Pump the PBS through the system to flush the tubing and Streamer® device, then discard the perfusate; this is done to remove any cytotoxic substances that may have accumulated during sterilization.
3. Put 500 ml of medium into the medium container (this may be adjusted later as you determine your system volume requirements).
4. Flow the medium through the system to flush out remaining PBS. Remove medium and replace with 500 ml of fresh sterile tissue culture medium.
5. Pump medium through the entire system to fill the flow device and tubing. Once the system is full, tilt the pulse dampeners, one at a time, at an angle of approximately 20 degrees, such that the direction of the flow is going from the vertex of the angle to the open end of the angle. Leave the pulse dampener in this position until the fluid comes through the outlet fitting again, then lay the pulse dampener down horizontally. This process will allow the pulse dampener to fill to a level slightly higher than the fittings, thereby creating a bubble trap for any air bubbles that may accidentally enter the system. Do the same with the second pulse dampener. Once this process is complete, allow flow to continue.
6. As the flow continues, check to be sure that no air bubbles are visibly trapped within the tubing. Also check the walls of the medium container to be sure that no air bubbles have formed on the sides. If so, swirl the medium around to release air bubbles from the side walls.
7. Once the tubing and flow device are filled with medium and all air bubbles are eliminated, stop flow, then reverse flow so that the medium is drawn down to about 80% of the Streamer® body. The fluid level will have to be estimated once the fluid flows past the Streamer® outlet fitting. When the fluid reaches this level, stop the flow again.
8. Tighten the clamp on the Phar-Med® tubing just to the right of the pump head so that the flow path in the tubing is completely closed off.
9. Turn the lever arm on the MasterFlex® pump all the way to the left to release the tubing and remove the tubing from the pump head. Carefully move the tray containing the Streamer® device, tubing, pulse dampeners, and fluid collection reservoir to the tissue culture hood.
10. Remove the Streamer® screws and open the hinged top.
11. Transfer your cells from the incubator to the tissue culture hood.
12. Using forceps and/or your fingers with sterile gloves, grasp a Culture Slip® at one

end. Be careful not to stimulate or crush any cells on the slide.

13. Place the Culture Slip® into one of the slots in the Streamer® device. ***Be sure that the side with cells attached is facing the flow area (the shorter slot parallel and adjacent to the slide slot).*** Be careful not to chip the glass against the stainless steel surface.
14. Repeat this for the other Culture Slips®, making sure the surface with the cells all face the proper direction in the flow device.

NOTE: *All six slots must be filled to ensure proper flow rate readings. If you do not wish to use all six Culture Slips® with cells, use blank Culture Slips® for the remaining slots.*

15. Once all Culture Slips® are in the Streamer® unit, close the lid and tighten the screws using the hex head tool provided with the system.

NOTE: *As you are moving the Streamer® from this point on, always position the device vertically such that the inlet connector is at the bottom and the outlet connector is at the top.*

If you wish to run the Streamer® on its side, first fill the remainder of the system with fluid so that all air is completely out of the Streamer® chamber.

Be aware that any air that accidentally enters the system may eventually form a dry area at the topmost slide so that these cells will no longer see fluid media and shear stress.

Be sure that your system does not regularly see additional air bubbles (after initial filling and air bubble elimination) before using the Streamer® on its side.

16. Move the tray with the system components back to the incubator. Put the Phar-Med® tubing back into the MasterFlex® pump head and clamp the head down.
17. Unscrew the clamp on the Phar-Med® tubing to open the flow path to full capacity.
18. If you are running the experiment manually, set the pump to the desired flow rate and press the start button. If running the experiment under software control, go to the *System* tab, click on the *Configure* button, assign the *User*, *Regime* and *Apparatus*. Click *Update*, then *Start*.
19. Once the experiment is over, move the Streamer® back to the tissue culture hood and remove the slides.
20. Once the slides are removed, place the Streamer® back into the incubator and run deionized water through the system to remove all remaining media. Refresh the deionized water and run a second or third time if necessary. ***Be sure to never leave the Streamer® with culture media inside as this will corrode the stainless steel finish over time.***

FILLING THE SYSTEM TO ELIMINATE AIR BUBBLES

Before using the Streamer® system with cells, all of the tubing must be filled with media and all air bubbles removed. To fill the system, create a regime with two steps, the first in FWD mode and the second in REV mode. Each step should be 2 minutes at a shear stress level ½ that of which your device is capable. This will give sufficient time for fluid to fill all of the tubing. As you notice air bubbles in the silicone tubing at different locations, shake the tubing to release the air bubbles.

POST-EXPERIMENT ANALYSIS

Upon removal of the slides from the flow device, many post-flow evaluations can be done.

- Cells on the Culture Slips® can be fixed with formalin then permeabilized and stained with rhodamine Phalloidin and DAPI to visualize cell alignment.

- Cells can be lysed with appropriate buffer to collect total RNA or intracellular proteins.
- The Culture Slips® can be returned to their original culture vessel for further incubation and subsequent collection of cell supernatant. The medium can then be assayed for released effector molecules.
- The cells can be trypsinized for replating or counting.

APPLICATION NOTES

CULTURING CELLS ON CULTURE SLIPS®

Culture Slips® are Teflon®-bordered 75 x 25 x 1 mm glass culture surfaces that are either untreated or bonded with peptides of collagen, elastin, fibronectin (RGD repeat as Pronectin F), laminin (as the YIGSR peptide). The Teflon® border provides a means to culture cells only in the flow area. Bonded peptides increase cell attachment.

Cells are plated on the growth surface at 10-25,000 cells/cm^2 in 3 to 5 ml of medium. ***Be sure to plate cells on the side where the Teflon® border is printed.*** Once the cells are attached, additional medium is added and the culture vessel placed into a CO_2 incubator at 37°C. Once the cells have grown to confluence (normally 48 hours), the Culture Slips® are removed and inserted into the Streamer® flow device for the experiment. Once the flow experiment is over, the Culture Slips® can be returned to their original culture vessel to allow the measurement of secreted molecules post-flow.

If you experience cell detachment problems during flow regimes, try the following protocol for better cell attachment to the Culture Slips®.

1. Plate ½ of the normal amount of cells on the Culture Slips®.
2. Reduce the media serum concentration (5% preferably) to slow the cell growth rate. This will give the cells time to make their own protein matrix which will improve attachment.
3. Allow the cells to grow to near confluency (4-5 days).

APPENDIX: PARALLEL STREAMER® SHEAR STRESS NUMBERS

Flow Area Height(cm)	Flow Area Width(cm)
0.0513	2.3396
Serial #	**Flow Factor**
SGS-1109	6
System Flow Rate (ml/min)	**Shear Stress (dyn/cm^2)**
0	0.0
37	1.0
74	2.0
111	3.0
148	4.0
185	5.0
222	6.0
259	7.0
296	8.0
332	9.0
369	10.0
406	11.0
443	12.0
480	13.0
517	14.0
554	15.0
591	16.0
628	17.0
665	18.0
702	19.0
739	20.0
776	21.0
813	22.0
850	23.0
887	24.0
924	25.0
961	26.0
997	27.0
1034	28.0
1071	29.0
1108	30.0
1145	31.0
1182	32.0
1219	33.0
1256	34.0
1293	35.0

WARRANTY INFORMATION

1. FLEXCELL INTERNATIONAL CORPORATION warrants to the original purchaser/customer all hardware components of the **Streamer® Shear Stress System** for a period of **one year** from the date of delivery to the purchaser/customer to be free from manufacturing defects in workmanship or materials with the following exceptions, terms and conditions:

a. ITEMS EXCLUDED FROM THE WARRANTY ARE: software, disks, manuals and external peripherals such as printers, mouse or track ball units, imaging devices, vacuum pumps, air tanks, electric voltage converters, compressors, surge suppressers and all other accessory equipment.

b. DURING THE WARRANTY PERIOD, the purchaser/customer must notify Flexcell of any warranty claim in writing, by telephone, fax transmission or email identifying each defective part or specifically describe the exact problem no later than the last day the warranty is in effect.

c. FLEXCELL AGREES to correct any defect in workmanship or material and supply new or rebuilt parts in exchange for defective parts upon completion and submission by purchaser/customer of a printed "Parts Return Authorization" form furnished by Flexcell. Parts must be properly packed in original container and shipped to our factory service center or distributor with all shipping costs prepaid if the unit is out of warranty coverage. If the original shipping box is not available, Flexcell will send the required protective shipping container. (Flexcell will recommend the insurance value for parts or equipment to be shipped.) Return carrier shipping costs will be paid by Flexcell from the service center. The purchaser/customer is solely responsible for payment of custom fees, taxes, holding fees or value added taxes.

d. THIS LIMITED WARRANTY only covers failures due to defects in materials or workmanship which occur during normal use. It does not cover damage which occurs in shipment or failures of original equipment due to products identified as add-ons not manufactured by Flexcell International Corporation or its distributors nor does this limited warranty cover damages or failures which result from accident or disaster such as fire, explosion, flood, wind, lightning, or earthquake or misuse, abuse, neglect, mishandling, misapplication, alteration, faulty installation, modification or service by anyone other than our factory or distributor. This warranty is extended only to the original purchaser/customer unless a transfer of ownership is approved by Flexcell in writing.

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2. GOVERNING LAW. The performance of the duties and liabilities of the parties under the terms and conditions of this Limited Warranty shall be governed in all respects by the laws of the Commonwealth of Pennsylvania, the United States of America.

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3. INTERNATIONAL CUSTOMERS. The full text of the foregoing limited warranty and all disclaimers is applicable to international customers/purchasers except when the purchase was made from an international distributor or reseller, the warranty will be covered through your distributor or reseller.

If technical advisory support service is not available through your distributor or reseller, for service contact warranty headquarters by phone or fax.

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Issued April 2011

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NOTICE