

## 5. Accessories

### a. Stopped flow

- i. *Description.* The Olis U.S.A. stopped flow is a pneumatically driven, easy to use device which mounts into the sample chamber in place of the hanging cuvette or Peltier cell. Reagents are loaded through the use of ceramic valves and high speed mixing is achieved through the use of a Ball-Berger mixer. The cell is typically a 4 mm x 4 mm cell for fluorescence, although the 4 mm x 20 mm cell can also be employed for fluorescence measurements. Valves are manual controlled by the use of large, easy to use handles. Sensors are present on the front and back syringes (FS and BS), front and back fill valve handles (FH and BH), stopping syringe (SS), and exhaust handle (EH).
- ii. *Installation of stopped flow*

1. Remove the lid on the sample chamber by first removing the four screws holding the cover. These will either be 3/32" Allen screws or thumbscrews. Place the lid aside.



2. If a Peltier cell or turret is present, this must be removed prior to installation of the stopped flow. If no cell holder is present, proceed to the next step. Remove the screws holding this to the base plate. Remove the 3/32" four screws holding the side panel on the sample chamber. Place the whole assembly aside. Obtain the sample chamber side panel to be used with the stopped flow.

Attach it to the side position with the four 3/32" Allen screws.

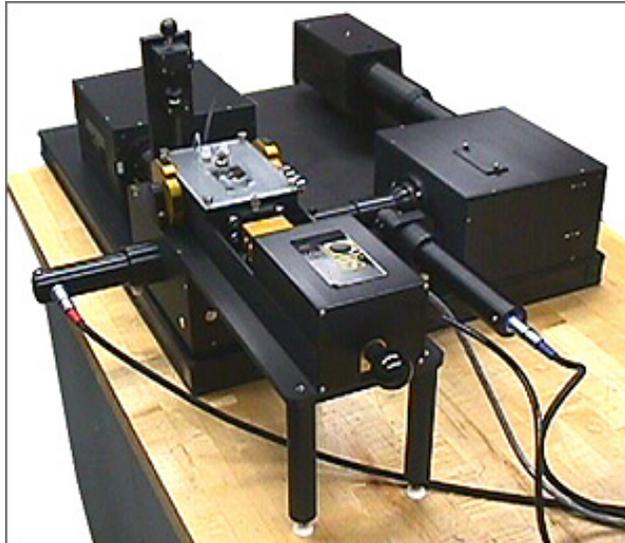


3. Locate the stopped flow assembly. Grasping the main assembly with your left hand and the ball of the drive plunger with your right hand. Note the stopped flow may or may not have legs attached to the right side (under the drive plunger). If no legs are present locate these prior to moving the stopped flow.

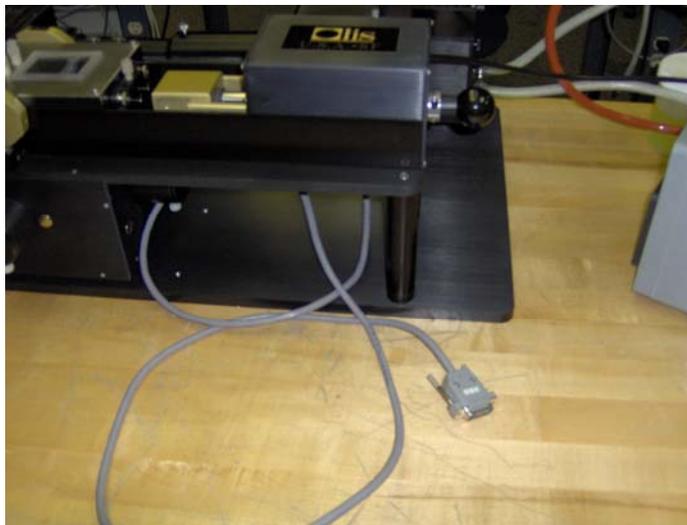


4. Lift the assembly into the sample chamber so that it fits snugly into the chamber. Gently wiggle the stopped flow to assure it is securely in place. Fasten the stopped flow to the chamber using four 3/32" Allen screws. If the stopped flow legs were not present on the apparatus, attach these now by sliding the legs under the right side of the stopped

flow and placing a 5/32" Allen screw through the holes on the right side of the apparatus. Tighten the screws to hold the legs in place.



5. Connect the 15 pin cable coming from the stopped flow to the back of the instrument control box. The connector will be labeled 'SF.'



6. Locate the stopped flow firing box and connect the yellow cable to the control box.



7. Connect the black gas tubing from the apparatus to the stopped flow firing box. Push the tubing into the port labeled 'to SF'. Pull gently to assure a proper connection.



8. Connect the stopped flow firing box to a gas source capable of achieving 75 psi of pressure. Push one end of the provided back tubing into the red connector on the firing box labeled 'Gas in.' Gently pull the tubing to ensure a proper connection is made.
9. A brass connector with a 3/8" fitting is provided with the instrument. This fitting can be used with most US regulators. Metric fittings are also available when appropriate. Screw this fitting using a wrench into the regulator. Note that often a valve must be removed on the regulator to access the fitting.



10. The gas must be turned on to a pressure from 75 psi to 90 psi for use of the stopped flow. Do not exceed 90 psi as this may damage the stopped flow cell. Check the stopped flow for any leaks.
  
11. Turn the instrument on and open the software. Locate the sensor map on the instrument control box. Move each of the three valves on the stopped flow and observe the indicator lights on the map. Placing the valve handle in the FLOW position should cause the light to go off. Pull the drive shaft handle back from the drive syringe plungers. The indicator lights on the map should turn on when the plunger is not in contact with the plungers. Finally, with all valves in the FLOW position, push the drive shaft in to manually fire the stopped flow. As the stop syringe moves to the up position, the SS sensor light should turn on.



12. Note that if photon counting is used in steady state mode, it will be required that the photon counting module be replaced with an analog PMT as described above.

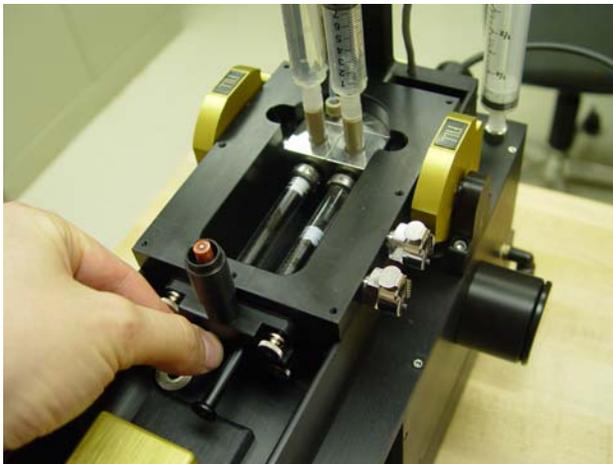
*iii. Operation of the stopped flow*

1. It is recommended that all buffers used in the stopped flow be filtered and degassed prior to use in the stopped flow.
2. With the drive syringe valves (FH and BH) in the FILL position attach two disposable luer lock syringes to the reservoir positions. Move the drive syringe plungers to empty the contents into the reservoir syringes. Remove the syringes.

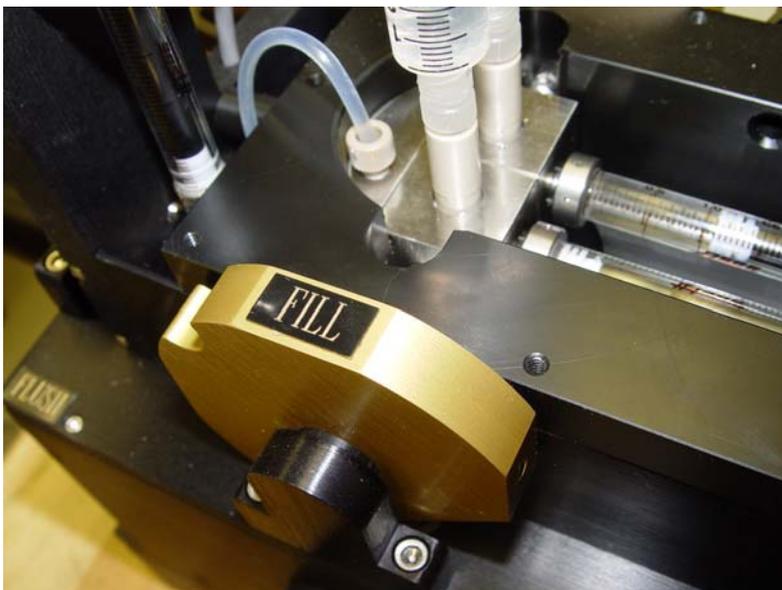


3. Replace the reservoir syringes with syringes containing the desired solvent or buffer.
4. Draw the syringes back to fill them and flush back and forth a few times to remove any bubbles from the syringe.

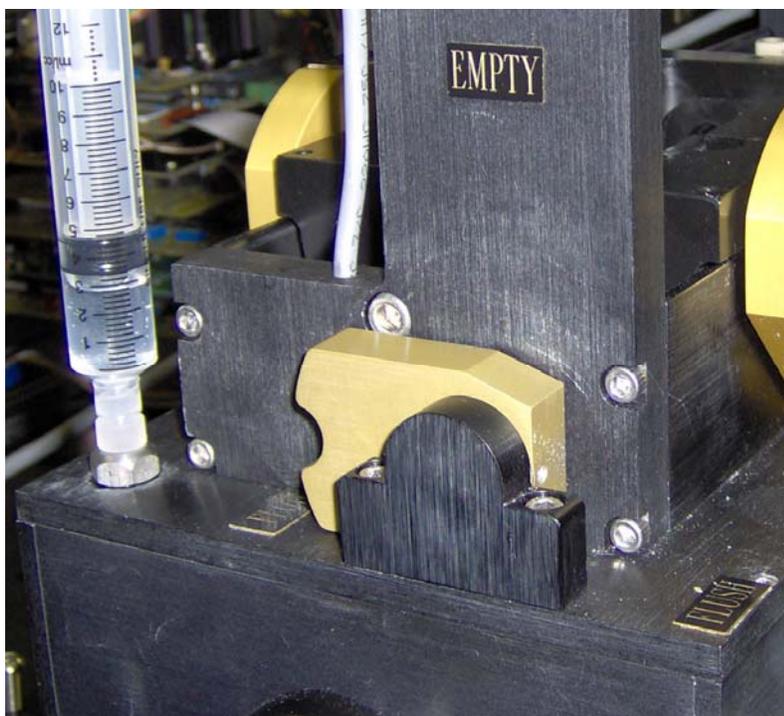
Place the syringe plungers in contact with the drive syringe block. The FS and FH indicator lights on the stopped flow sensor map should be off. If not, reposition the syringe plungers against the block.



5. Move the drive syringe valves to the FLOW position.



6. Ensure that a waste syringe is present in the waste position. Place the Exhaust valve (EH) in the empty position and push down on the stop syringe shaft to ensure the stop syringe is empty. Move the exhaust handle back to FLOW.

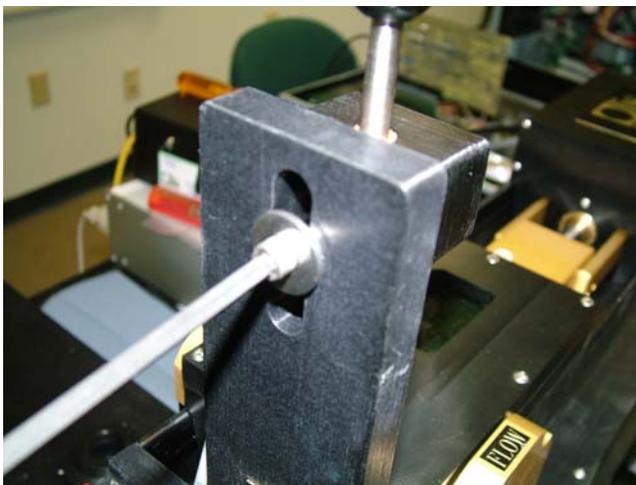


7. Turn the instrument, software, and gas pressure (75 psi – 90 psi) is on.
8. Manually fire the stopped flow by either lifting the manual fire lever on the stopped flow firing box or clicking Collect Data in the software.



9. Confirm the stopped flow has fired and observe the volume filled by the stop syringe. This total volume can be

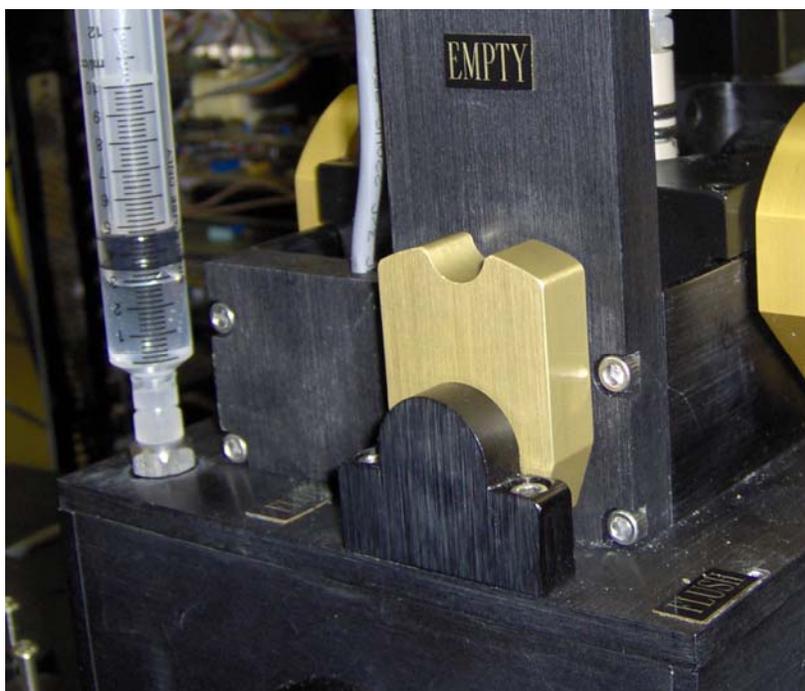
changed by using a 5/32" Allen wrench to loosen the bolt holding the stage in place. Lower the stage (with the exhaust valve on EMPTY) until the desired volume of the stop syringe is desired. Do not go below 150-200  $\mu\text{L}$ . Retighten the Allen screw. Move the exhaust valve back to FLOW. Note that the stopped flow will not fire unless all valves are in the Flow position, the stop syringe is down, and the plungers are in contact with the piston block.



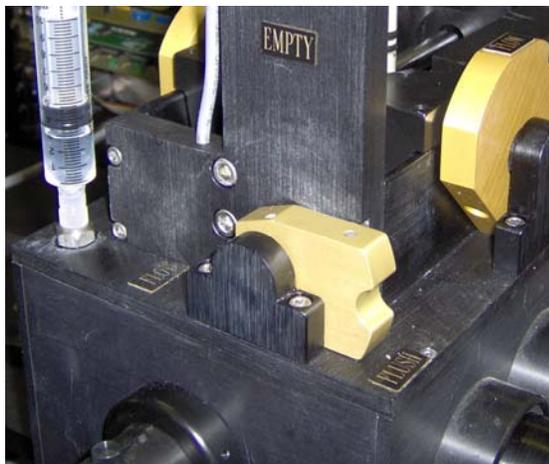
10. Using the reservoir syringe fill one of the syringes with the fluorescent reactant.
11. Move the wavelengths to the proper settings for the fluorescence measurement and click on Live Mode to see streaming data.
12. Using the manual fire lever on the stopped flow firing box, fire the stopped flow. Observe the fluorescence intensity of the product and adjust the PMT HV accordingly. Empty the stop syringe (first put the exhaust valve to EMPTY) and push the reagents one more time. Again, adjust the PMT HV if necessary. Note that slitwidths may also be changed to increase or decrease signal.



13. Fill the second drive syringe with the second reagent. Note that if a fluorescence enhancement is expected upon mixing, the PMT HV will have to be set at a lower setting to accommodate the new signal.
14. Set the Collection Time, and press Collect Data to collect the stopped flow shot. It may take one or two shots to completely flush the system of the previous reagents.
15. After each shot, move the exhaust handle to EMPTY and push the stop syringe down.



16. When the experiment has been finished, flush the system with water or another solvent of choice. The Flush position of the stopped flow may be used to push large volumes through the cell and directly into the waste syringe, bypassing the stop syringe. Both the drive syringe valves must be in the FLOW position to do this. If the system is to be used again in a few days, this is sufficient for storage. Olis recommends storing in ethanol for longer term storage.



*iv. Changing the syringes*

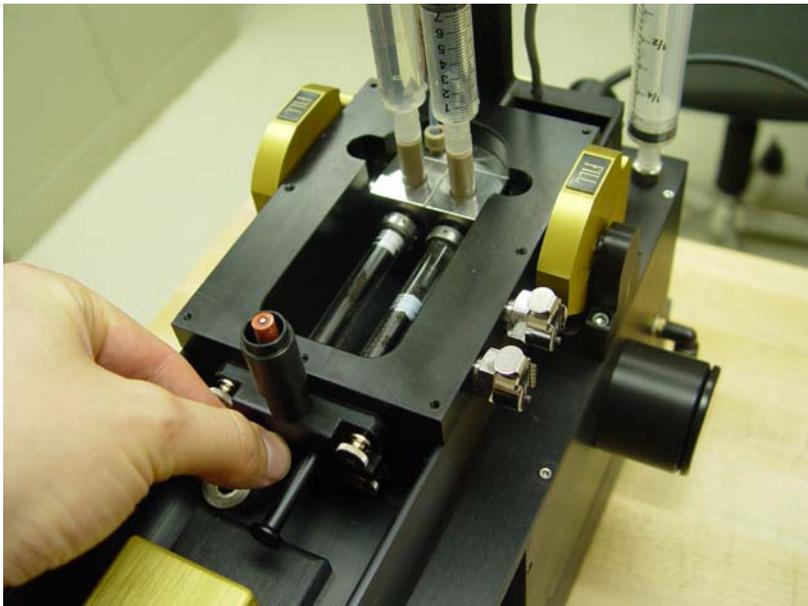
1. Grasp both the plunger block and shaft. Turn the handle on the shaft counter-clockwise block as shown until the shaft is removed from the plunger.



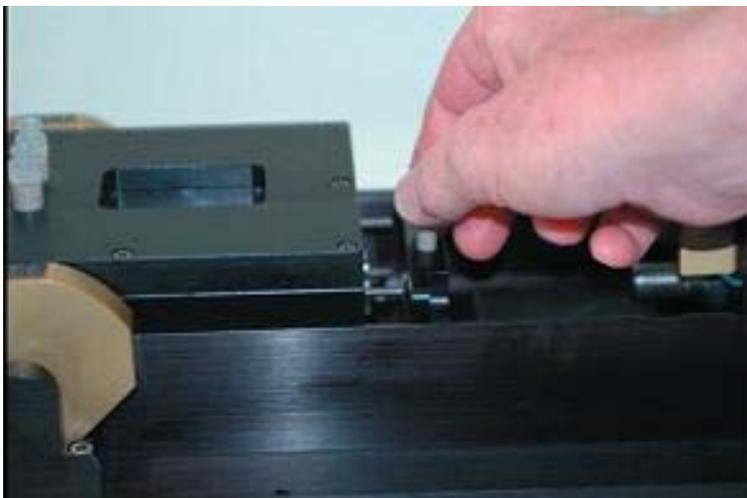
2. Lift the plunger block up and secure it to the shaft with a rubber band to keep the block out of the way.



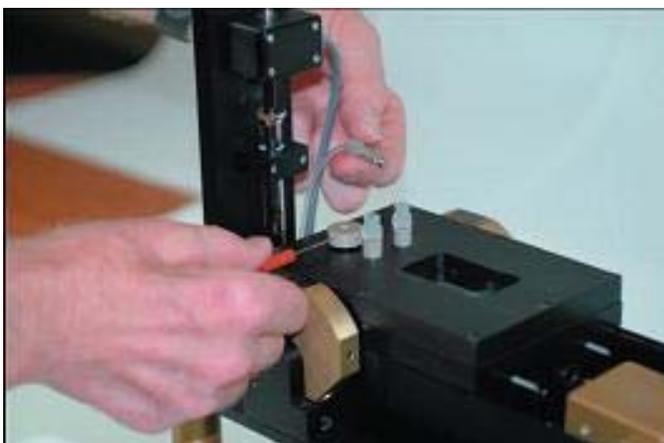
3. With the Fill/Flow valves in the Fill position, pull back on the plungers of drive syringes until they are removed from the stopped flow.



4. Remove the beige fitting above the bracket surrounding the syringe shafts. Remove the four thumbnuts and slide the bracket out.



5. Using the tool shown, loosen the fitting containing the tubing exiting the stopped flow cell. Continuing to use this tool to hold the fitting, loosen the tubing coming from this fitting. A pair of pliers may be required to do this.



6. Remove the fitting from the water bath and place in a secure location.
7. Using a 3/32" Allen wrench, loosen the eight screws holding the lid on the bath. It is not advisable to completely remove the screws because they are not of the same length. Note the screws above the exhaust valve and the syringes are shorter than the others. Inserting the long screws into these positions may damage the bath.
8. Gently lift the bath lid and place it in a secure location.



9. If fluid is present in the bath remove enough to give access to the syringes. A large pipette is probably the best method for this.
10. Reach inside of the bath and push out the O-ring from the opening.



11. Insert the stopped flow tool into the base of the syringe and rotate the syringe counter-clockwise. Carefully remove the syringe by sliding it out the right side of the bath. Repeat for the second syringe.



12. Insert the new syringes into the bath. Screw them clockwise into the valve using the stopped flow tool. Tighten the syringes until they are snug. Exercise care not to over tighten the syringes. This may cause them to leak.
13. Locate the O-ring for the syringe bracket, and lay the O-ring evenly around the bracket.



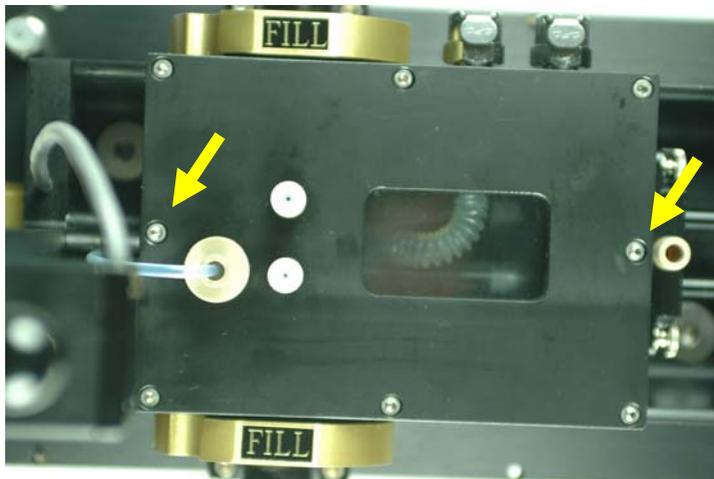
14. Press the bracket into position around the syringe barrels. Note that if the syringe sizes have been changed, a different sized bracket will be required for the new syringes.
15. Locate the O-ring shown. Place this O-ring over the barrel of one of the syringes. Use the black plastic O-ring tool shown to push the O-ring down the barrel until it is approximately in the middle of the bracket.



16. Replace the outer half of the syringe plunger bracket and replace the thumb nuts and purge fitting.

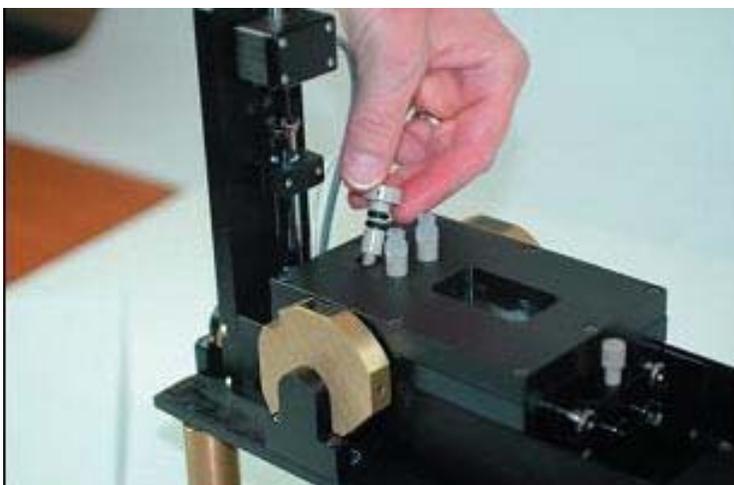
17. Insert the plungers carefully into the syringe barrels.

18. Replace the bath lid. Tighten the eight 3/32" Allen screws. Again, take care to place the shorter screws over the exhaust valve in the positions shown.



19. Insert the exit tube fitting into the bath. Turn clockwise and tighten using the stopped flow tool.

20. Screw the exit tubing into the fitting. Gently tighten with a pair of pliers.



21. Remove the rubber band from the plunger block. Screw the plunger shaft into the block by turning the shaft knob clockwise until the shaft is securely inserted into the block.



*v. Removal of stopped flow*

1. Ensure the gas pressure is off and use the vent release valve on the firing box to remove any excess pressure.