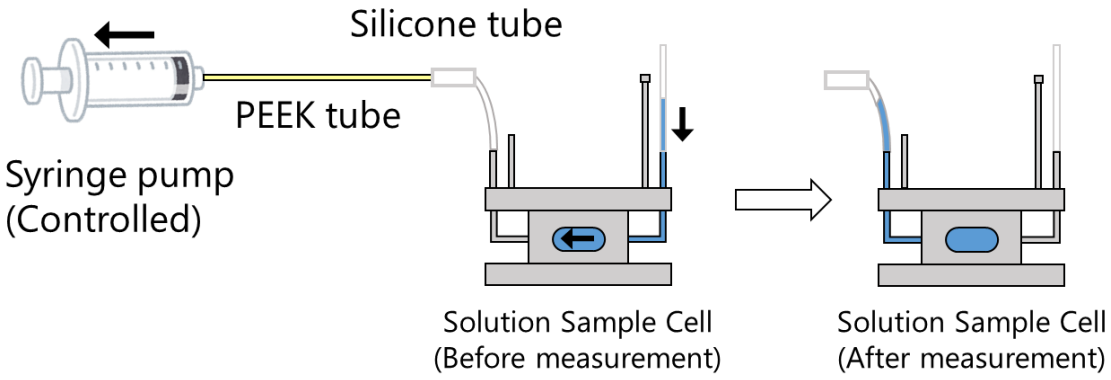
Manual for Sample Flow System (Unisoku) 2020.05.29

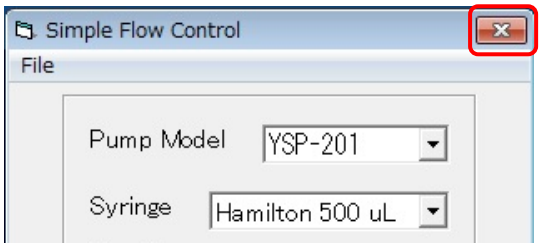
Sample Flow System is the equipment which move the solution sample in the solution sample cell by the syringe pump to avoid damage during X-ray exposure.
Users need to read only Section 6~13.



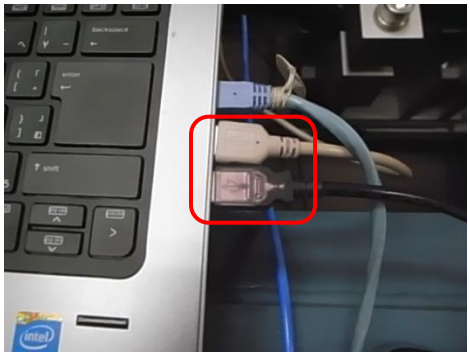
Usage of Sample Flow System (For Users)

CAUTION
If Sample Flow System and SEC-SAXS (UV spectrophotometer　Ocean Insight) are connected to a notebook PC simultaneously , malfunctions (stoppage of spectrophotometer software operation) may occur.
Users need to switch between Sample Flow System and UV spectrophotometer by themselves.

[In case of switching from Sample Flow System to SEC-SAXS system.]
Close “Simple Flow Control” application on notebook PC.

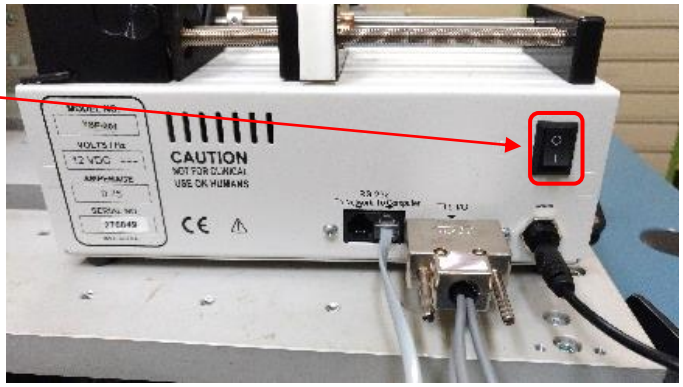


Unplug the black USB cable of syringe pump from notebook PC.
And then connect the cream USB cable of UV spectrophotometer to notebook PC.



Turn off the syringe pump.

Power switch of syringe pump



Refer to the SEC-SAXS manual for instructions on how to start up UV spectrophotometer.

EXAMPLE

Exposure time: 5 sec, Exposure interval: 5.01 sec, No. of images: 60

Operating time of syringe pump:

5.01(exposure period) x 60(No. image) +0.1 (delay) +0.1 = 300.8 sec

Delay 0.1sec

(Because it takes about 0.07sec to open X-ray shutter and move solution sample actually from start signal pulse.)

（To ensure moving the solution sample until the end of the measurement,

The extra 0.1 sec is added to the operating time of the syringe pump.）

When using a standard sample cell (volume approx. 19 ul) with a sample volume of 40 ul,

The flow rate is calculated as described below.

(20 ul /300.8 sec) x 60 = 3.99 ul/min.

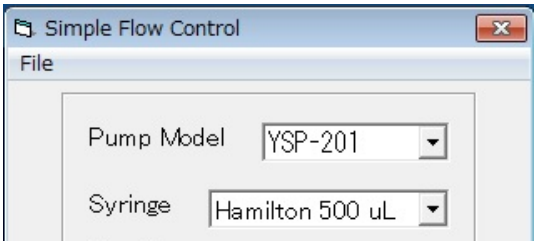
Check the limit of exposure time with no radiation damage in scattering intensity without Sample flow system.
The flow rate should be set to a rate such that the solution is replaced completely within that limit of exposure time
Adjust the exposure time, number of images and amount of sample as you need.

6. Setting of Syringe pump

6.1. On the remote desktop window of Measurement PC, click on the Unisoku software icon (Unisoku S&K). (If it has started already, proceed to 6.3.)

6.2. ※Note※ If The small window “エラー　RS232C ポートのオープンに失敗しました。ERROR Failed to open RS232C port.” appears
USB connected syringe pump are not correctly identified.
Click “OK”
A new dialogue box will open, enter “4” as COM port number and click “OK”.
If “4” is correct, “Simple Flow Control” window appears immediately.
In case the small window “エラー　RS232C ポートのオープンに失敗しました。ERROR Failed to open RS232C port.” appears again, enter 5, 6, and 7 as COM port number.

6.3. Settings of “Simple Flow Control”
Select Pump Model: YSP-201 and Syringe: Hamilton 500 ul. (Even if they are selected correctly, re- select from the pull-down menu again.)



6.4. Confirm that the monitor area of "Simple Flow Control" window is grey.

1) Monitor area color: Grey

(Syringe pump is not in operation.)

Proceed 6.5.

2) Monitor area color: Green

(Syringe pump is running with Self Start/Stop mode or waiting for pulse signal with Ext. Start/Stop mode.)

Click stop button in the control area of "Simple Flow Control" window.

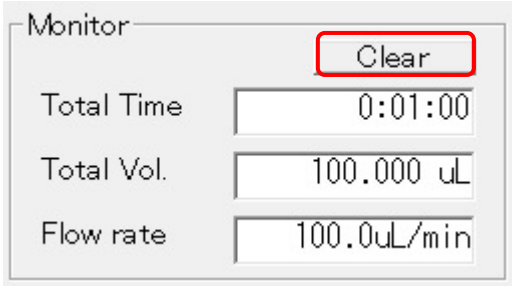
3) Monitor area color: Pink

(Syringe pump is running with Ext. Start/Stop mode)

Wait until the monitor area color changes to green and then click stop button in the control area of "Simple Flow Control" window.

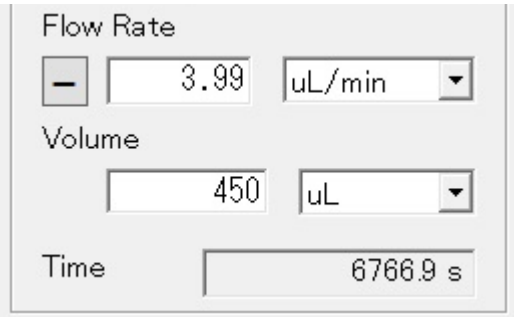
6.5. Confirm that the syringe plunger's position is at 20~30ul. If not, press the white locking button of syringe pump to release it and adjust the position.

6.6. Click Clear button in the monitor area of “Simple Flow Control” window to set the current position is 0ul. Use the range of 0~-450ul from this point.



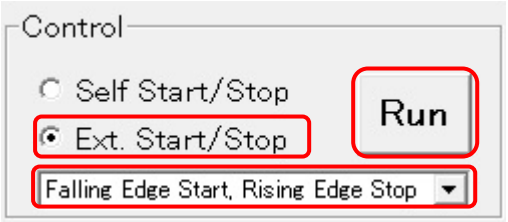
6.7. Set Flow rate and Volume parameters.

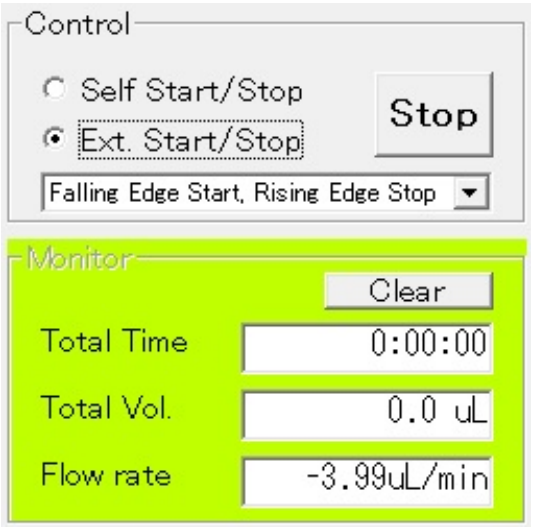
Flow Rate：-3.99 ul/min （Flow rate is NEGATIVE value）, Volume：450 ul



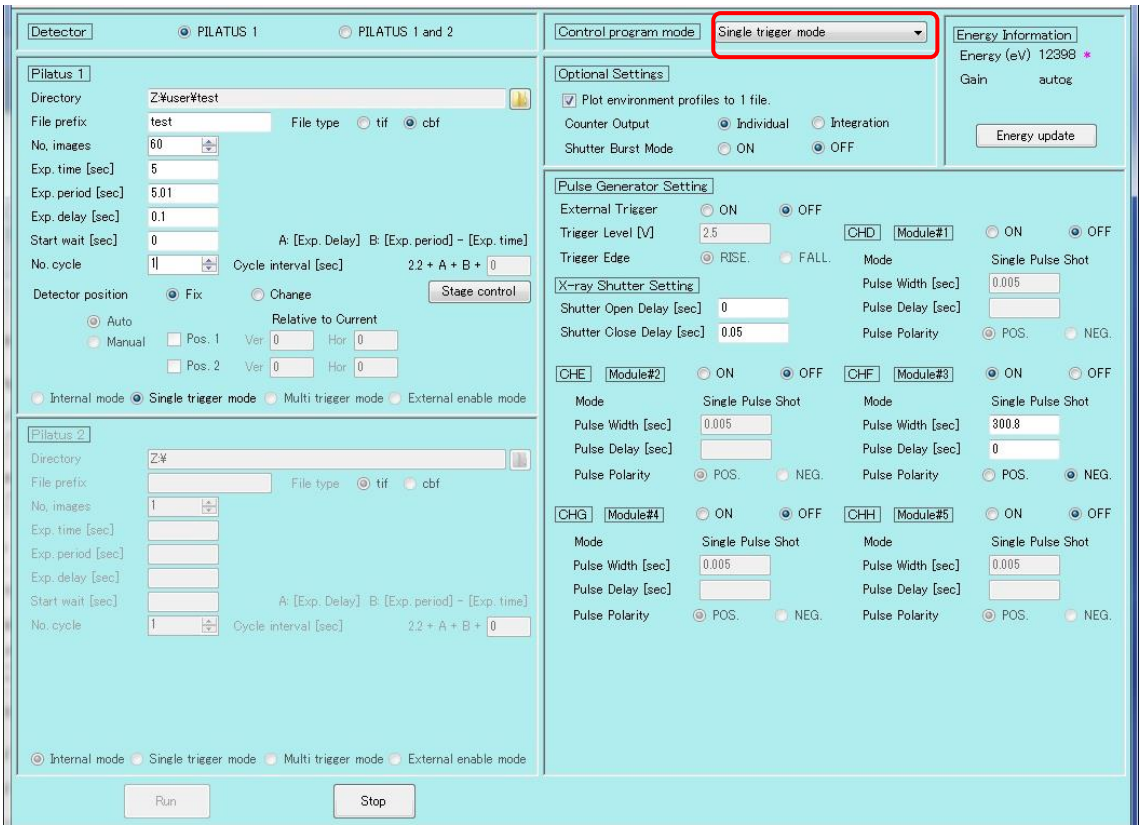
6.8. In the Control area of "Simple Flow Control" window, select "Ext. Start/Stop", and select "Falling Edge Start, Rising Edge Stop" from the pull-down menu and click "Run" button. The monitor area color changes to green, (Syringe pump is waiting for pulse signal.)

The measurements could be repeated several times until the Total Vol. reaches -450ul. (The syringe pump runs as long as the pulse signal is received.)

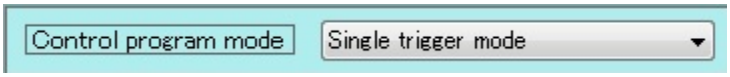




7. Setting up the PILATUS measuring software (on Measurement PC)



7.1. Set "Single trigger mode" in "Control program mode" of the PILATUS measurement software window.



7.2. Set parameters in PILATUS1 part of the PILATUS measurement software as follows.

Directory：The folder to store image files

File Prefix：The prefix of image file name

File type：Select either cbf of tif

No. images：60 Number of images

Exp. Time：5 Exposure time (sec)

Exp. period 5.01 Exposure period [enter (Exp. Time+0.01) or more.

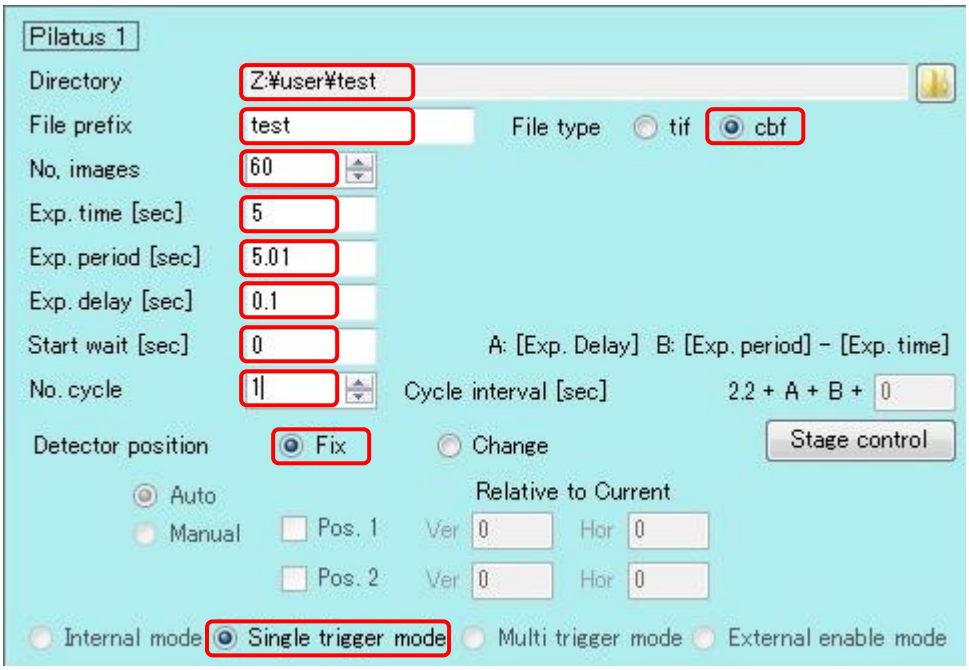
Exp. delay：0.1 [fixed value] interval from start signal pulse to start PILATUS actual exposure

Start wait : 0 [fixed value] waiting time until start signal pulse

No. cycle 1：Number of cycles

Detector position: Check “Fix”.

Check “Single trigger mode”.



7.3. Set parameters in “Pulse Generator Setting” part of the PILATUS measurement software as follows.

X-ray Shutter Setting

Shutter Open Delay：0

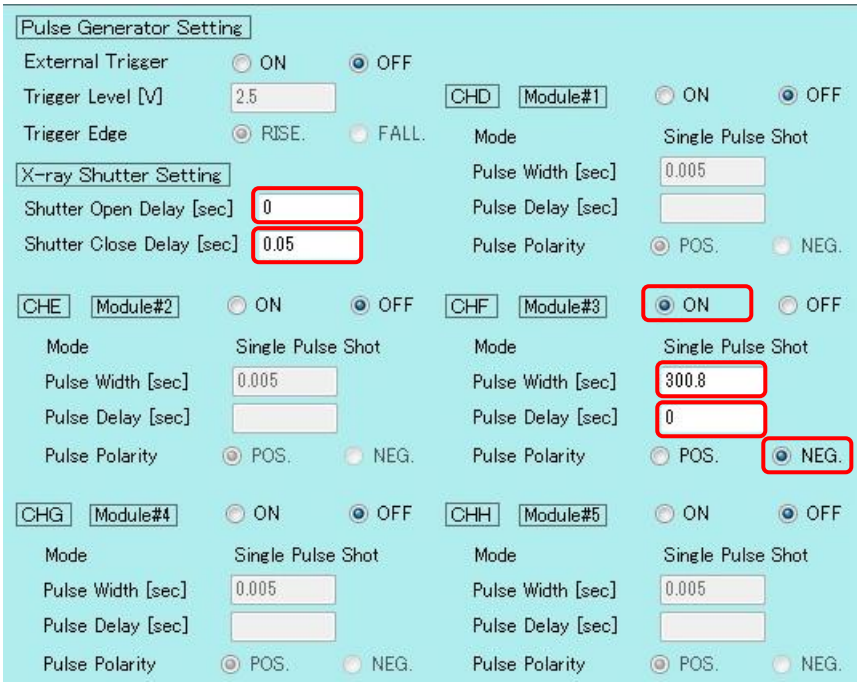
Shutter Close Delay：0.05

CHF：Check “ON”.

Pulse Width：300.8

Pulse Delay：0

Pulse Polarity：Check “NEG”.



8. Blank and test measurements are usually taken before the user starts their own experiment; if noted that by BL staff, proceed to 9.

8.1. The first run immediately after setting (or re-setting) the plunger to 0, the flow rate of the pump is unstable.
Therefore the blank run (without X-ray, cell, sample) is needed. Enter the parameters and click run on “PILATUS measurement software.

9. Setting up cell and sample loading

9.1. Solution sample cell（standard solution sample cell for users are available.）
Using standard solution sample cell with default length of HIBIKI tube,
40ul sample volume could be loaded.
The cell volume H: 2.5 x W: 6 x T: 1.25 =18.75 ul -> 40 - 19 = 21ul. So 21ul sample solution can be flowed during the measurement.

Standard solution sample cell (40 ul red solution loaded)

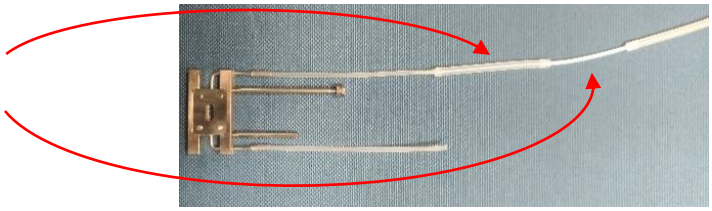
9.2. To increase sample volume for measurement, change longer HIBIKI tube.（The volume of HIBIKI tube is about 5 ul/cm）

It is recommended to set same length HIBIKI tube at the inlet and outlet of solution sample cell. Because moving sample solution stays within another side of HIBIKI tube of sample solution cell after measurement.

9.3. Connect solution sample cell and peristaltic pump with short silicone tube and short HIBIKI tube (2~3 cm) for connection.

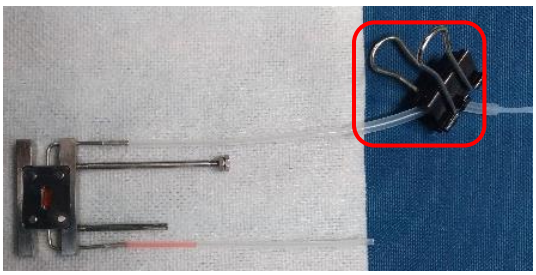
Silicone tube

HIBIKI tube



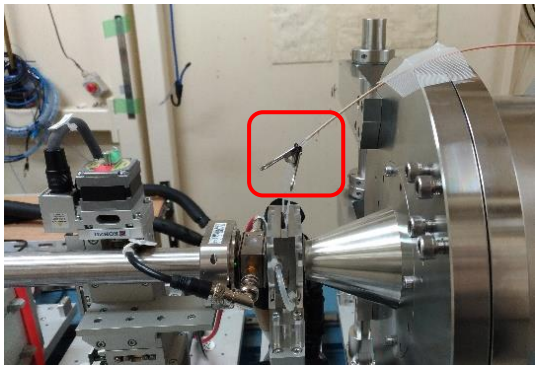
9.4. Aspirate the sample with a peristaltic pump at slow aspiration rate (about 2~3) and stop as soon as the cell window is filled with solution. If too much solution is drawn, adjust by pumping in the opposite direction.（Marking the liquid level on HIBIKI tube as a guide.）

9.5. Close short silicone tube with the binder clip and remove it from the peristaltic pump, leaving the connecting silicone tube on the solution sample cell.

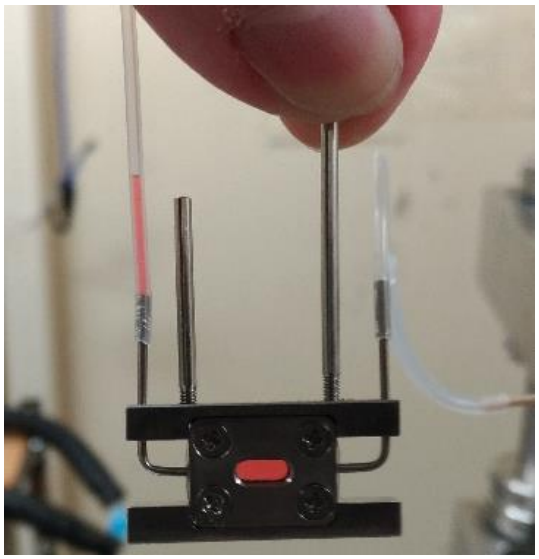


10. Setting of Solution sample cell and measurement

10.1. Set the solution sample cell in the cell holder and connect the PEEK tube of syringe pump with the silicone tube of the solution sample cell.
(When the HIBIKI tube length of the cell is changed or at first measurement.)
Fix the PEEK tube with adhesive tape to prevent the slippage of the cell position due to the tension of the tubes.



10.2. Remove the binder clip.
Remove the solution sample cell once from the cell holder.
Check that there are no bubbles in the cell and that the liquid level has not moved significantly from the initial level.
Set the solution sample cell again.



10.3. Close the experimental hatch and start the measurement.
During the measurement, check that the system is working properly as described below.

1) The color of monitor area in "Simple Flow Control" window turns from green to pink.

2) The "Total Volume" value in “Simple Flow Control“ window is changing as planned.
3) The measured images does not contain scattering from air bubbles and/or air interface.

11. After the measurement, Cleaning and Drying of solution sample cell

11.1. After the measurement, disconnect the PEEK tube of syringe pump and the silicone tube of solution sample cell.
And then take the solution sample cell out.

11.2. Cleaning and drying (sample collection) can be carried out in the same way as for conventional experiments. As long as the Solution Flow System is used properly, the connecting short silicon tube does not contact with the solution and can be removed and used again without washing. (If short silicon tube is wet, it needs to be washed and dried).
In case the HIBIKI tube is long, it may take some time to dry. After 1~2 minutes of drying, connect another side HIBIKI tube and the air pump to speed up drying.

12. Repeated measurements

12.1. Repeat steps 7, 9, 10 and 11 to carry out the measurement. "Simple Flow Control" software is set to move the syringe only while the pulse is receiving, up to the set "volume" value.

13. re-setting the plunger

After the several times measurements, the plunger position must be returned when approaching the set "volume" value.

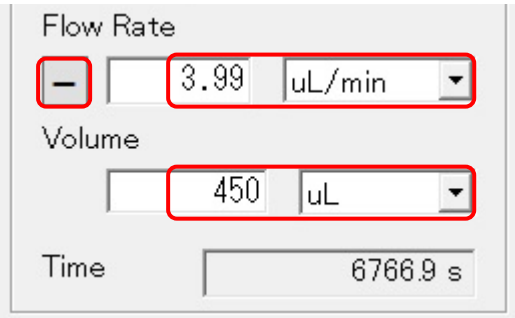
13.1. After the measurement, disconnect the PEEK tube of syringe pump and the silicone tube of solution sample cell.
And then take the solution sample cell out.

13.2. Click "Stop" button in the Control area of “Simple Flow Control” window to end the pulse waiting state.

13.3. Set the flow rate in "Simple Flow Control" window to +100 ul/min and set “Volume" value to the absolute value of the present "Total Volume" value in the Monitor area.

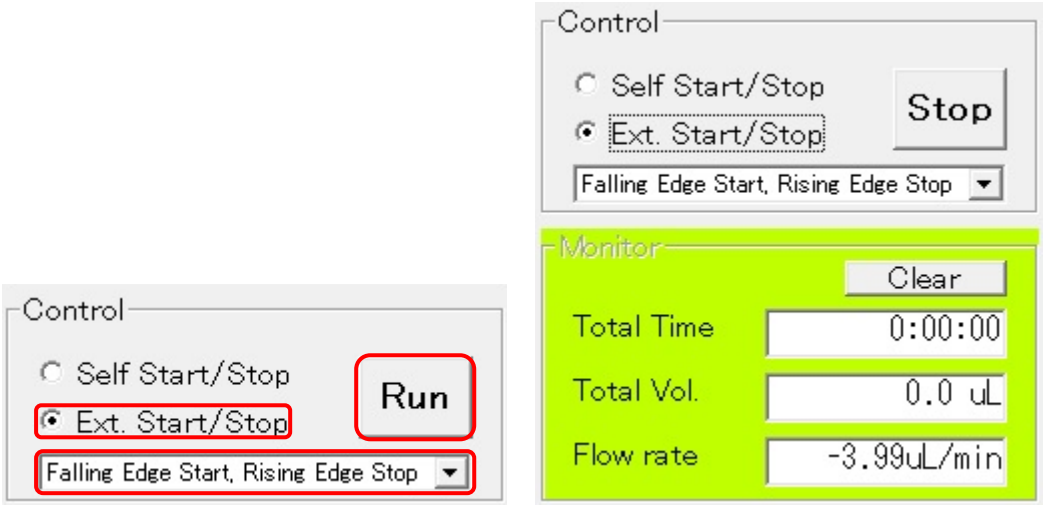
13.4. Check “Self Start/Stop" on the Control area of "Simple Flow Control" window. And click Run button.
The plunger of the syringe pump is returned to its initial position.

13.5. Restore "Flow rate” and "Volume" values which are used at latest measurement.
（Flow rate is NEGATIVE value.）



13.6. In the Control area of "Simple Flow Control" window, select "Ext. Start/Stop", and select "Falling Edge Start, Rising Edge Stop" from the pull-down menu and click "Run" button.

The monitor area color changes to green, (Syringe pump is waiting for pulse signal.)



13.7. Carry out a blank run, and the continue experiments.