



## **TRIAx FLOW CELL**

### **MODEL**

**FC-1**

**FC-2**

**FC-3**

## **Quick start Manual v1.00**

**SOFTWARE VERSION 2.00+**

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1. SCAN MODE - Turn on the Fractionator/Gradient Station and put into SCAN mode before starting the software.



2. USER page

FlowCell software version 2.00E  
Triax PCB firmware version 0.62  
Fractionator firmware version 4.207

USB connection to the cUV established. Please enter your USER name, ROTOR, CORE USER if applicable, then press NEXT.

User Name:  ☐ CORE  
(avoids all but 6 main pages)

Rotor Manufacturer:  Rotor:

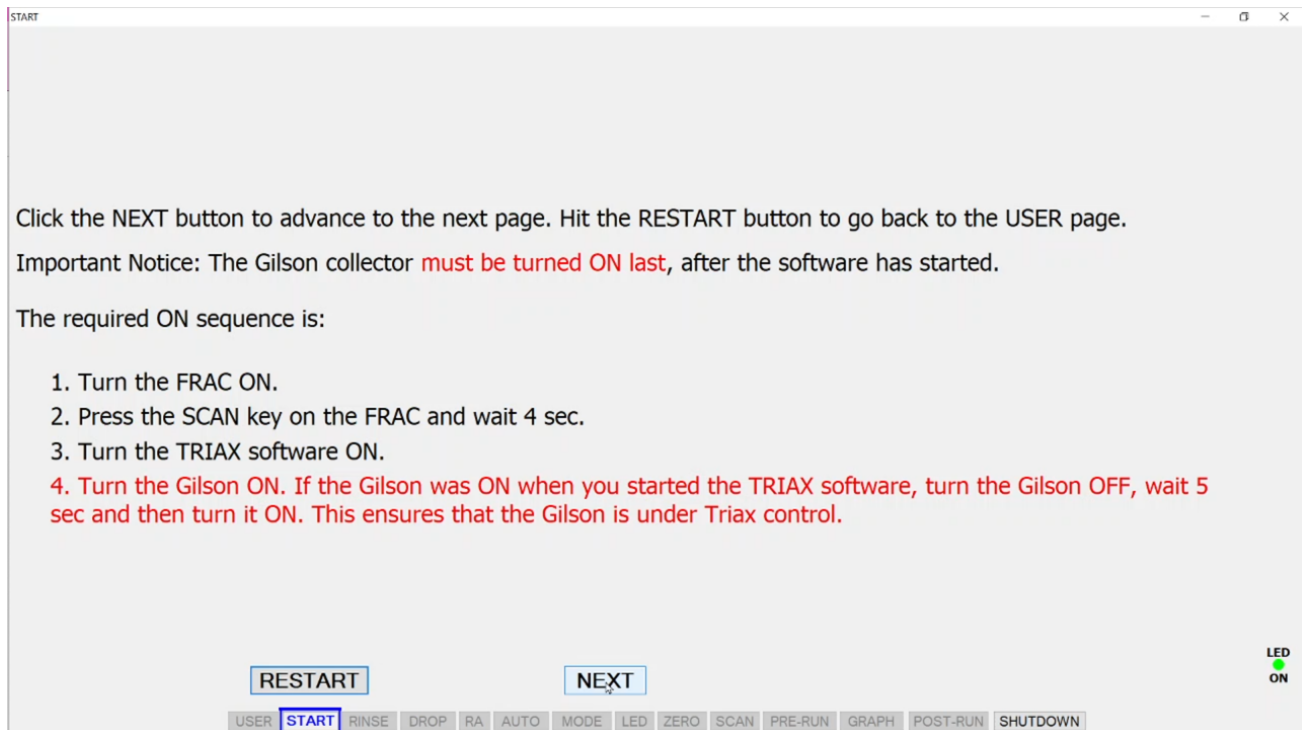
RESTART NEXT

USER START RINSE DROP RA AUTO MODE LED ZERO SCAN PRE-RUN GRAPH POST-RUN SHUTDOWN

LED ON

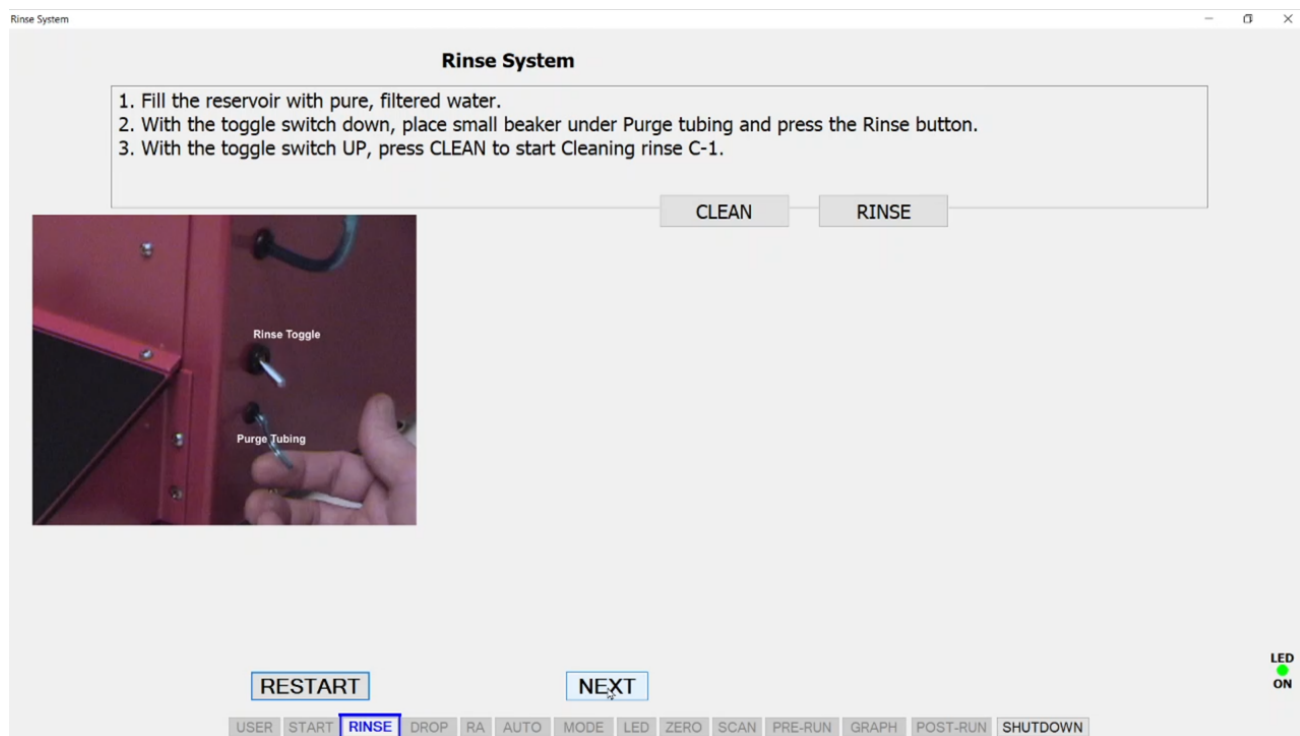
- a. User Name - Select an existing user name or enter a new one. A folder is created for each user's CSV files. The last settings user and rotor combination user are saved.
- b. Rotor - Be sure to select the correct rotor for proper functioning of the device.
- c. The "Core" check box can be selected once you are familiar with the software to skip the setup pages that are non essential.

### 3. START Page



- a. Key point - Turn on the Gilson Fraction Collector after the software has been opened.

#### 4. RINSE page



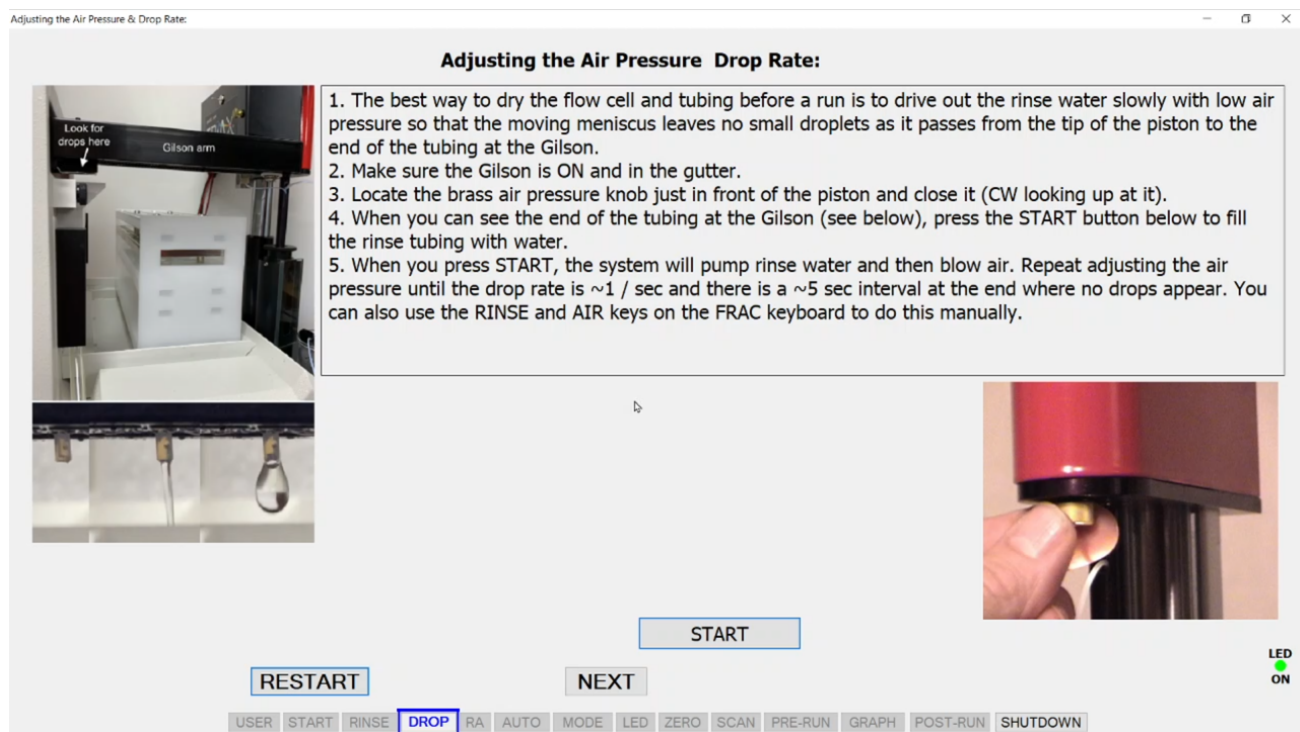
- a. This is the reservoir being referenced. Follow the instructions on the page.



## 5. DROP page

Adjusting the Air Pressure & Drop Rate:

**Adjusting the Air Pressure Drop Rate:**



1. The best way to dry the flow cell and tubing before a run is to drive out the rinse water slowly with low air pressure so that the moving meniscus leaves no small droplets as it passes from the tip of the piston to the end of the tubing at the Gilson.
2. Make sure the Gilson is ON and in the gutter.
3. Locate the brass air pressure knob just in front of the piston and close it (CW looking up at it).
4. When you can see the end of the tubing at the Gilson (see below), press the START button below to fill the rinse tubing with water.
5. When you press START, the system will pump rinse water and then blow air. Repeat adjusting the air pressure until the drop rate is  $\sim 1$  / sec and there is a  $\sim 5$  sec interval at the end where no drops appear. You can also use the RINSE and AIR keys on the FRAC keyboard to do this manually.

RESTART START NEXT

USER START RINSE **DROP** RA AUTO MODE LED ZERO SCAN PRE-RUN GRAPH POST-RUN SHUTDOWN

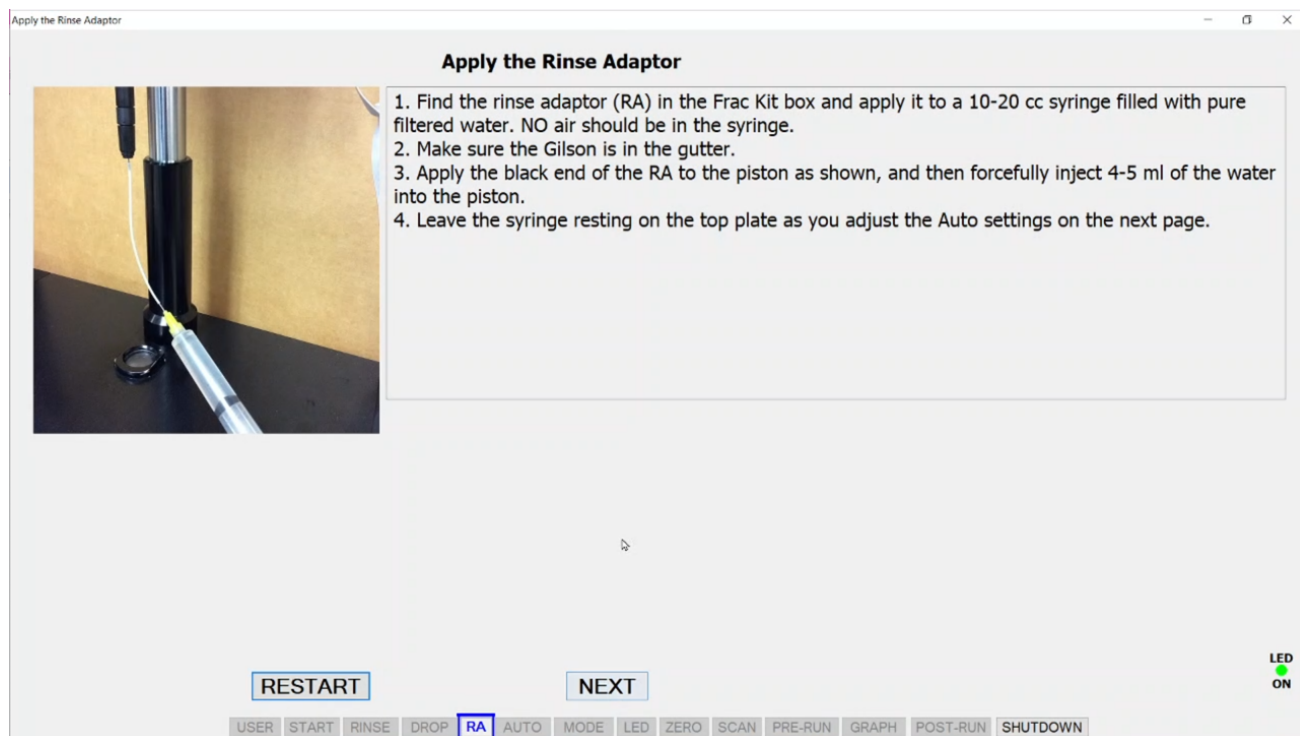
LED ON

- a. This adjustment should not have to be made regularly. This optimizes the system's ability to dry itself. If the air blows too hard droplets may be left in the tubing which can be problematic if you choose to start your data collection by “meniscus sensing” on the SCAN page.

## 6. Rinse Adaptor page

Apply the Rinse Adaptor

**Apply the Rinse Adaptor**



1. Find the rinse adaptor (RA) in the Frac Kit box and apply it to a 10-20 cc syringe filled with pure filtered water. NO air should be in the syringe.
2. Make sure the Gilson is in the gutter.
3. Apply the black end of the RA to the piston as shown, and then forcefully inject 4-5 ml of the water into the piston.
4. Leave the syringe resting on the top plate as you adjust the Auto settings on the next page.

RESTART NEXT

USER START RINSE DROP **RA** AUTO MODE LED ZERO SCAN PRE-RUN GRAPH POST-RUN SHUTDOWN

LED ON

- a. Follow the instructions. Injecting pure water into the system using the rinse adaptor is necessary for adjusting the ontime on the LED page and for Zeroing the flow cell. Water pumped through the system with the rinse pump contains microbubbles that give false absorbance readings.

## 7. AUTO page

AUTOMATIC

Select	Name	Description	RINSE / AIR
<input checked="" type="checkbox"/>	A-1	Dry	A15 ▼
<input checked="" type="checkbox"/>	A-2	Adjusting the air pressure	R1 A10 ▼
<input checked="" type="checkbox"/>	A-3	Air during descent +	3 sec after stop
<input checked="" type="checkbox"/>	G-1	Gilson to gutter = END on Gilson	
<input checked="" type="checkbox"/>	C-1	Pre / Post run cleaning	R3 A2 R3 A2 R3 A2 R4 A15 ▼

Click on any box to delete it, insert another box or change the Air or Rinse on time. Add an Air step with the letter 'A' followed by a number in seconds. Add a Rinse step with the letter 'R' followed by a number in seconds.

☒ Show air / rinse dialog on screen

RESTART NEXT

USER START RINSE DROP RA **AUTO** MODE LED ZERO SCAN PRE-RUN GRAPH POST-RUN SHUTDOWN

LED ON

- a. You can adjust the various automatic cleaning cycles the system performs here. Generally speaking the default settings are good and no adjustments are necessary.

## 8. MODE page

- Mode select - Choose what channels to use by checking/unchecking the boxes.
- These are only relevant to a user who is swapping in a new filter kit for a fluor or changing between 260nm and 280nm in a given channel. Changing a channel from 260nm to 280 nm UV requires careful calibration of the photodiodes, please consider contacting BioComp for assistance if you choose to do so.
- The Gradients Box- All of these fields will be recorded with the user's data into the CSV file and are purely informational. Their purpose is to give context to the data for reference in the future.
- Sample Volume - the volume of sample/lysate that is layered on top of the gradient after it has been formed.
- Gradient - The solute the gradient was made from, its range of concentrations and mode of measurement when mixing the gradient solutions.
- Number of tubes - the number of tubes in the rotor during the centrifuge run.
- Speed - The centrifugation speed. We recommend running at the maximum speed the rotor is capable of, to keep the centrifugation time as short as possible and prevent diffusion of the peaks.
- Time - The time of centrifugation

- i.  $w2t$  - Known as Omega squared  $t$ , this is the total centrifugal force applied during a run. If this value is entered, the centrifuge will stop the run when the value has been reached, regardless of the time and speed entered.
- j. Temperature - The temperature of centrifugation. The default value is 4 deg C.

## 9. LED page

**DUAL UV, SINGLE FLUORESCENT CONNECTIONS**

LED3 (VIS LED 569 nm) LED2 (UV2 LED 280 nm) LED1 (UV1 LED 260 nm) PD6 (VIS SAMPLE PD 569 nm) PD5 (VIS SAMPLE PD 569 nm) PD4 (UV2 SOURCE PD 280 nm) PD3 (UV1 SOURCE PD 260 nm) PD2 (UV2 SAMPLE PD 280 nm) PD1 (UV1 SAMPLE PD 260 nm)

**a** Data points/mm: 10.0

**b** Piston Speed: 0.20 mm/sec

**c** # of samples to average: 1

Time to scan 78.3 mm gradient: 6.53 minutes

Data points per 78.3 mm gradient: 783

☒ USE 2 PDs FOR FLUORESCENCE

Integration time: 500 ms

Lowering data pts/mm or piston speed increases integration time

Increasing integration time reduces noise

Apply Rinse Adapter and inject water before adjusting ON time.

LED On Time (ms): 10

LED1(260 nm)	LED2(280 nm)	LED3(mKate2 - 569 nm)
Sample: 76144	Sample: 274024 800-900k	Sample: 456
Source: 33711	Source: 34429 >100k, <= sample	

Note: 1044175 = saturation

RESTART NEXT

USER START RINSE DROP RA AUTO MODE **LED** ZERO SCAN PRE-RUN GRAPH POST-RUN SHUTDOWN

LED ON

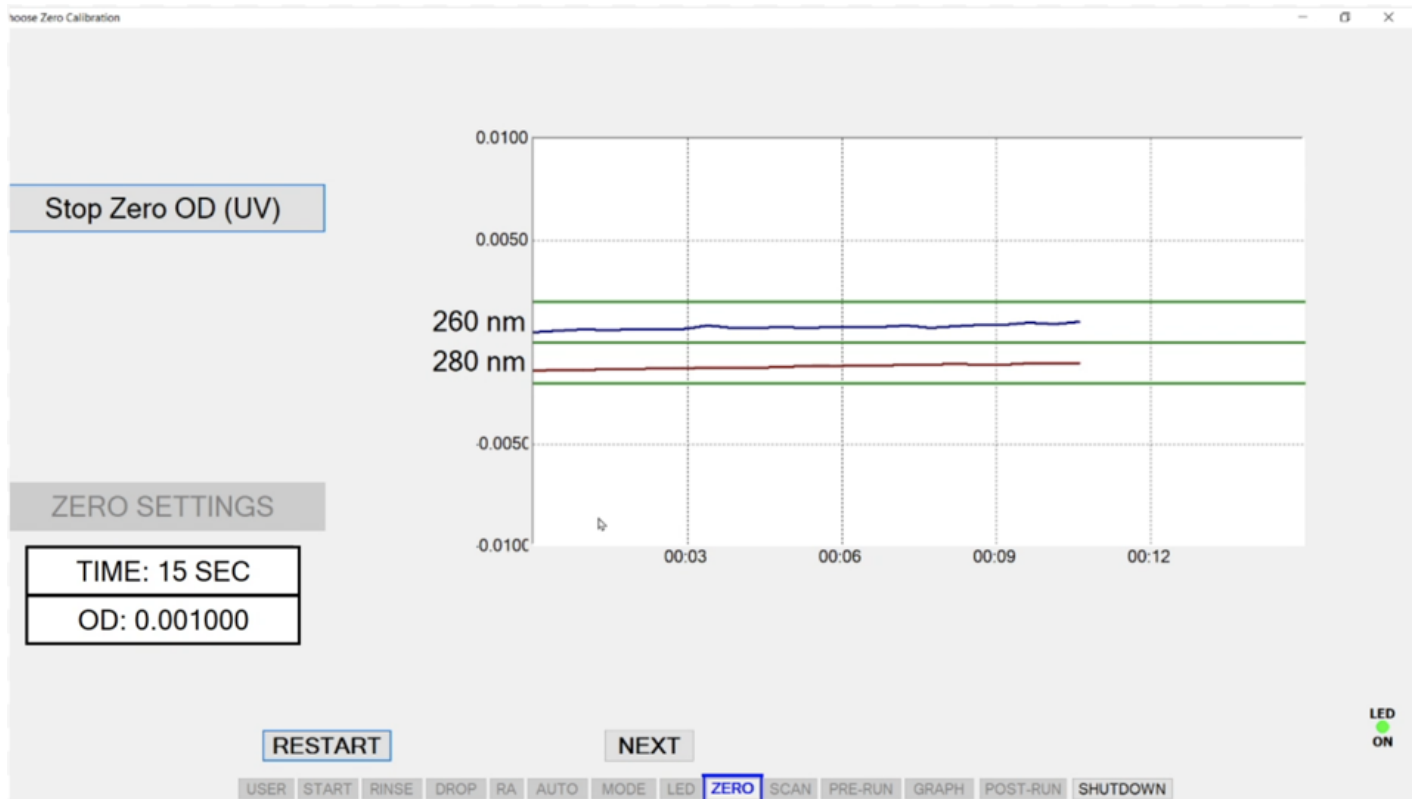
- a. Data points/mm. - The number of data points per mm of the tube. 10 is a good setting for smooth peaks and good resolution. Higher values may increase the resolution of the system but will also increase the relative amplitude of the noise in the system cancelling some or all of the gains made. It is very rare for a significant peak to be less than 0.1mm in height.
- b. Piston Speed. - The speed at which your gradient will be fractionated by the downward motion of the piston. There is no strict rule but generally speaking large tubes and more concentrated gradients demand slower speeds. 0.2 or 0.3mm/sec are good starting points.
- c. # of Samples to Average - If dealing with a very small sample that makes peaks with values below 0.05OD, the profile may look noisy. Increasing this value may help suppress the appearance of noise but normally should not be necessary.
- d. LED sample and source values
  - i. This is the raw data from the photodiodes.

- ii. The sample value is the data from the photodiode that is detecting the light that has passed through/been emitted by your sample and is the main source of the OD reading or fluorescence emittance
- iii. The source value is from a photodiode pointed directly at a UV led. This data is used to subtract variation in the emitted light from the sample value. There is no source PD in Fluorescent channels
- iv. All sample and source channels have a maximum value of 1044175 units. Above this value, the photodiodes are saturated and can not record meaningful data.

e. LED On-time Slider

- i. Having used the rinse adaptor to push Milli-q into the flow cell, use this slider to keep the sample value of the UV LED between 800 000 and 900 000 units. In systems with 2 UV channels, set the value of the most powerful UV LED in this range. Normally this will be the 280nm LED. This setting will maximize the sensitivity of the flow cell while avoiding accidental flatlining of data.

## 10. ZERO page



- a. ZERO once per day.

- b. Make sure to push filtered water into the flow cell with the rinse adaptor before zeroing.

## 11. SCAN page

**SW41Ti**

Mode: Dual OD With Single Fluorescence

LED1 Wavelength: 260 nm

LED2 Wavelength: 280 nm

Samples/mm: 10.0

Start recording data when: Meniscus Sensing.

Max. vol./fraction: 1.3

Length of Tubing: 290 mm

Piston Speed: 0.20 mm/sec

Fractionate by: Select one of the three modes

Number of fractions: 14

Distance/fraction: 5.59 mm

Volume/fraction: 0.80 ml

☒ Recover the dead volume in an additional fraction.

Less Than Full Stroke Run

Start: 0.00 mm (0.0 mm Slow start)

Stop: 78.30 mm (78.3 mm Bottom)

RESET

RESTART

NEXT

USER START RINSE DROP RA AUTO MODE LED ZERO **SCAN** PRE-RUN GRAPH POST-RUN SHUTDOWN

LED ON

- a. The “Start recording data when” dropdown:
  - i. Meniscus sensing - the flow cell looks for the change of OD that represents the meniscus of the top of the gradient before it begins to record data. This helps to align peaks from similar runs with each other.
  - ii. Slow Start Point - Data is recorded from a set point near the top of the tube regardless of the exact height of the gradient in the tube.
- b. Max. vol./fraction
  - i. This is a fail-safe setting to prevent the user from collecting fractions too big for the vessels they are collecting into. If collecting into 0.5mL eppies this value should be changed to 0.4mL. If collecting into a 96 well rack set this 0.1mL smaller than the volume of the wells.
- c. Length of tubing.
  - i. This should match the length of the tubing that connects the Triax flow cell and the fraction collector and is used to calculate the dead volume between the point of data collection and deposition of the gradient into a fraction. This allows the software to accurately display the fraction marks relative to the peaks in the data.

d. Number of fractions - Distance/fraction -Volume/Fraction

- i. Changing one value changes the others. You may define the size of your fractions using any of the three values. The volume per fraction may not exceed the max volume per fraction covered earlier.

e. Recover the dead volume in an additional fraction

- i. During the fractionation, the system is displacing the gradient out of the tube, and so can not recover what is in the dead volume of the piston tip and flow cell when the piston tip reaches the bottom of its stroke. If you want the system to automatically recover this dead volume into an additional fraction, check this box put an extra tube in the rack.

f. Less Than Full Stroke Run

- i. If your particles of interest end up in a relatively narrow and predictable sector of a given gradient, you may use these settings to collect fractions of only that sector, saving time and materials.
- ii. To narrow the range either type in the Starting distance and Stopping distance or click and drag with your cursor.

12. PRE-RUN page:

PRE-RUN TASKS

1. Using last zero calibration.
2. Remove Rinse Adaptor
3. Blow the system  (A-1) or  (C-1) if not clean.
4. Blot the valve and apply a clean, dry piston tip.
5. Apply the cap to the gradient, insert it in the holder and mount the holder under the piston, facing front.
6. Name the run
7. Press NEXT to go to the GRAPH

USER START RINSE DROP RA AUTO MODE LED ZERO SCAN **PRE-RUN** GRAPH POST-RUN SHUTDOWN

LED ON

- a. Follow the instructions and give your data a name.

13. GRAPH page

- a. Start a scan by hitting the START SCAN button
- b. Data Display Checkboxes
  - i. The sample and source values are raw data and are only of interest for diagnostic purposes.
  - ii. You can turn the Absorbance and Fluorescence data on or off as desired. It impacts the data displayed on the graph but not the data recorded.
- c. Y Auto Scale - This checkbox scales the graph to your data, Occasionally debris with very high OD at the top of the gradient will drown out the peaks you wish to see. You can uncheck this box and then adjust the scale of the Y-axis during a run to remedy this. Again, this does not impact the data being recorded, only how it is displayed on this page.

#### 14. POST-RUN page

POST-RUN

1. Save Run

YES NO

2. Was this the last run?

YES NO

3. Another identical run?

YES NO

RESTART NEXT

USER START RINSE DROP RA AUTO MODE LED ZERO SCAN PRE-RUN GRAPH POST-RUN SHUTDOWN

LED ON

- a. Save Run
  - i. allows the user to save their data as a CSV file. The default location is in the C:\FlowCell\Users\\*\*run name\*\*.
- b. Was this the last run?
  - i. If you select yes you will be prompted to do the final cleaning of the machine
- c. Another identical run?

- i. If NO is selected you will return to the User page of the software and you can then move through all the pages and adjust your settings as required.

#### 15. SHUT DOWN page

- a. Follow the instructions to do a final cleaning of the instrument
- b. Sterilize
  - i. These steps should be followed weekly if the instrument is in regular use and before the instrument will be left unused for sometime.