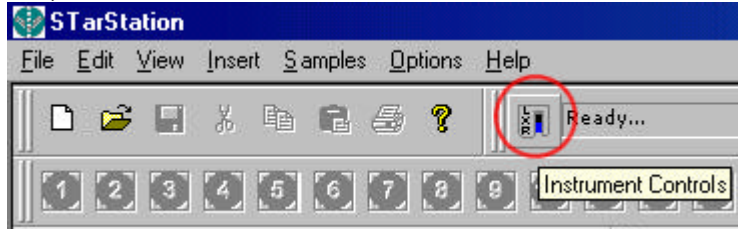



StarSystem Operation Guide (Sheet 1)

Switching on the Instruments

1. Turn on the STarSystem (Note that the XY Platform, Cytometer and Sheath Delivery Device all have individual power supplies).
 - Check for blue lights on the XY platform, Luminex 100 and Sheath Delivery Device (if present).
 - Verify that the compressor on the cytometer switches on within 1 minute of the unit being powered up (low rumbling noise).
2. Check that the waste bottle is empty and that the Sheath Bottle or SD System contains sufficient Luminex Sheath Buffer.
3. Start the STarStation Software and login.
4. Launch the STarStation Instrument Controls dialog by clicking the Instrument Controls icon or using the keyboard shortcut (Ctrl + I).

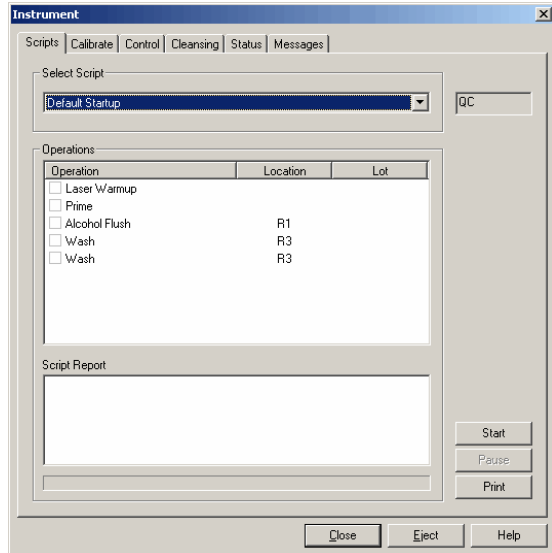


Warming up the optical system and cleansing the fluidic system

Note that IS version systems begin warming up immediately following power up. This can be confirmed by the Laser Status Indicator on the instrument Status Bar. During warm-up the laser status displays the laser warming icon . This will take 30 minutes (1800 seconds). A timer adjacent to the laser status indicator will countdown the remaining warm-up time.

If required remove and clean the sample probe to ensure that it is clean and free of any blockages.

5. Select the Scripts Tab and select the **default startup script**. The startup script will warm the detectors, prime the system to remove air bubbles from the sheath buffer inlet tubing and perform an alcohol wash cycle to remove air bubbles from the cuvette.



-**Eject** the plate holder and place a ACS utility plate on the tray ensuring that the commands outlined in the script are satisfied

R1 – Alcohol (require at least 3ml of 70% ethanol)

R3 – Fill with Sheath Buffer.

Note: if you have prepared your own startup script the above operations and well locations may differ.

-**Retract** the tray.
-Click the **Start** button on the scripts tab.

-Allow the system to warm up and for the optics to reach operational temperature.

On IS version STarSystems the wash, soak, backflush, prime, drain and Fill and sanitize are able to proceed during laser warm-up.

NOTE: If the system is left idle for 4 hr, the lasers will automatically turn off, when the detectors and lasers are powered down the Laser Status Icon on the STarStation status bar displays the laser sleeping icon




A 30 min warm-up period may again be required prior to reading an assay.

NOTE: If the waste is overfilled, the fluidics system may back up and the operation of the system may become less than optimal. The sheath reservoir contains enough fluid for approximately two 96-well plates. If the sheath fluid level falls below the "Sheath" output tubing on the bottle, the assay read cannot be completed.

6. Once the Warm-up, Prime and Wash steps have completed verify that the system pressure displayed on the

instrument status bar reads between 6 - 9 psi. Note: the system sheath and air pressures will fall following the completion of an instrument command when the compressor is disengaged. Confirm that the instrument

detectors are warm the Laser State icon should display 

Probe Height Adjustment


It is imperative, before reading your assay or attempting system calibration, that the height of the sample probe is altered so that it is optimal for the particular microtitre plate and sample volume combination used.

ACS recommend that before adding your samples to a specific microtitre plate, that you adjust the probe height for that particular plate using 3 disks from the alignment kit. Also you should ensure that the final volume in each well is at least 30µl more than the sample volume that the instrument will take.

The probe height adjustment procedure is outlined in ACS Quick Reference document *-Probe Height Adjustment*.

System Calibration

It is recommend that the system be calibrated at least once a week and whenever the dCal Temperature displayed on the instrument status bar reads +/-3 °c.

The STarSystem calibration status is displayed on the instrument status bar  11 Dec 2004, the individual state of the previous reporter and classifier calibrations and the date on which they were performed is displayed.

Note: the dCal temperature should only used as criteria for deciding whether system calibration is required **after** the machine has been warmed up and cleansed. Since the ambient temperature of the instrument changes during system warm-up.


8. If you require a system calibration, ensure that the calibration bead bottles are vortexed prior to use.

- **Important:** Before vortexing, remove the calibration beads from 2–8°C storage and allow the beads to warm to room temperature.

Stage2:Preparing the Assay

Note: this step can be performed whilst the instrument is warming up.

Before you can start collecting sample data you must enter the details for the assay you wish to perform into the STarStation Assay Manager.

1. Ensure you are logged into STarStation under your account name (Assay information once added to the system is only available to the person that entered it.).
2. Start the Assay Manager by clicking the Assay Manager icon on the Managers Toolbar  use the shortcut key sequence **CTRL+M**, or select the Assay Manager option from the **Edit** Menu.
3. Click the **New** button.
4. Enter a name for the assay and the number of analytes that the assay detects. Set whether the assay requires the platform heater and the Press the **Next** button.
5. Enter the name of the analytes detected by the assay and the corresponding bead ID used to detect the analyte.
6. Enter the number of distinct standards and controls that you require for the assay.
7. Click Next.
8. Enter the standard concentration values for each analyte.
Note: Concentration values must be entered from low to high .i.e. standard 1 is the lowest concentration.
9. Enter the concentration values for any unknowns.
10. Review the assay details and then click the finish button to add the assay to your login account.

Please refer to the *STarSystem-Work Guide* and *Work Flow Presentation -Assay Manager* for more information on the Assay Manager.

STarStation System Operation Guide (Sheet 2)

Stage 3: Preparing the Plate/Worklist


Note: this step can be performed whilst the instrument is warming up.

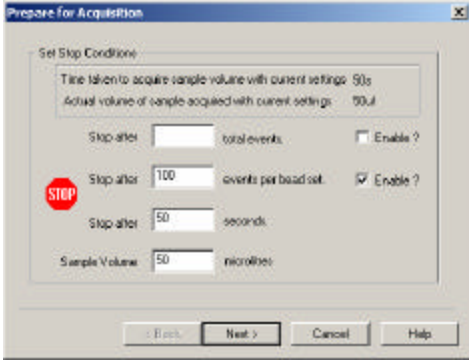
1. Ensure that you are logged-in to STarStation under your account.
2. Select the **Worklist** Tab.
3. From the Multiplex Assay drop-down list select the assay you wish to prepare a plate layout/ worklist for.
4. Add standards, controls and unknowns to the plate.
5. Select the direction of sample data collection.
6. Edit any dilution factors and sample id's as required.
7. Give the worklist a name and save it.
8. Select the **Close** option from the **File** menu.

Please refer to STarStation electronic help documentation and ACS Document *PD 035 712-Workflow Presentation - Worklist* for more information on Worklist creation.

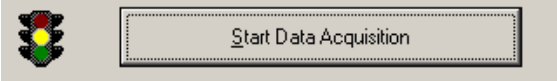
Stage 4: Reading the Plate

NOTE: Worklists must be created prior to reading a sample. The standard concentrations cannot be added / modified after the plate has been read. Therefore it is imperative that you ensure when creating and selecting Worklists that you assign time to check that the plate layout and assay described in the Worklist accurately reflect the physical setup of the microtitre plate containing your assay.

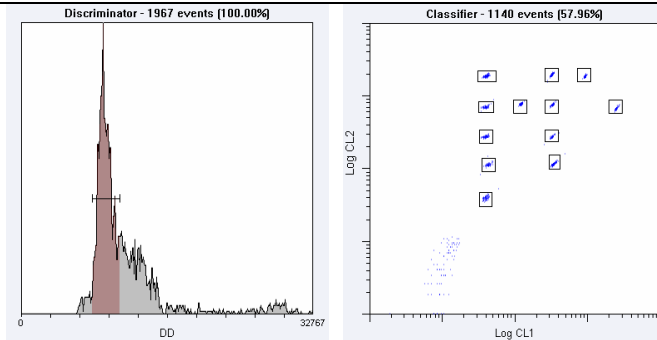
1. Check that the filter plate is flat. While pressing on one end of the plate, observe the distance that the opposite end of the plate is raised off a flat surface. If the distance is greater than 1 mm, transfer all contents to a flat-bottom 96-well plate or another pre-wetted filter plate and proceed with reading the assay (having ensured optimal probe height).
2. Visually inspect the plate and ensure that corresponding assay wells are filled with buffer prior to placing the plate on the XY platform.
3. Shake the assay plate at 1,100 rpm for 30 sec immediately before starting the run. Failure to do so will result in an increased read time due to settling of the beads. Remove the sealing tape and any plate cover before placing the plate on the XY platform.
4. Select the Acquisition Tab in the STarStation software.
5. From the file menu select the **Open** option or click the **File Open** Icon on the file toolbar.
6. Select the Worklist which corresponds with your sample plate from the Worklists directory.
7. After STarStation opens the template Worklist you should verify that the correct Worklist has been selected via inspecting that the number of analytes the Worklist has been setup to detect corresponds with either the number of analyte tabs displayed in the data grid or the number of bead regions displayed on the classifier plot. If there is a discrepancy in the Worklist setup, return to the Worklist tab to review or edit the Worklist.
8. Press the Start Acquisition button 
9. The first page of the acquisition wizard is displayed,
 - Enable events per bead set (100).
 - Enter a sample volume.
 - Set the stop time equal to the sample volume (assuming that the sample injection rate is set at 1µl/s).



10. Press the **Next** Button and set the Platform Heater temperature if necessary.
11. Press the **Next** button and Eject the plate holder and load the assay microtiter plate.
12. Proceed to the final page of the acquisition wizard, Press the **Start Data Acquisition** Button



13. Once data collection begins use the DD plot and Classifier plot to verify that adequate bead events are detected and that the distinct color coded beads are classified appropriately. When satisfied that sufficient beads are being counted we recommend leaving the system to collect data (modifications to gating or classification can be performed via the list mode data during analysis).

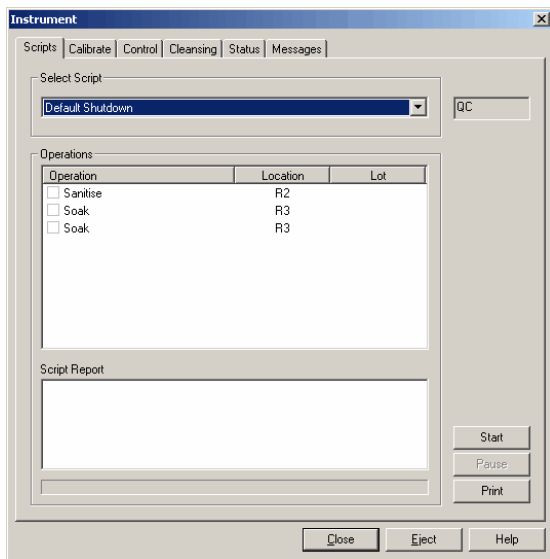


NOTE: It is possible to skip specific wells or analyze a well (sample) a second time using the pause acquisition function of the STarStation software.

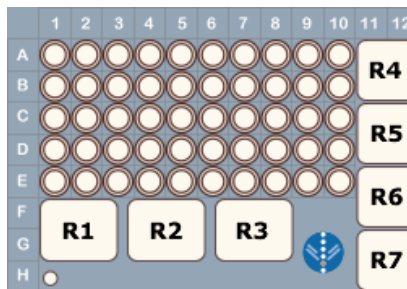
Note: If reading more than 1 plate, empty the waste and refill the sheath containers after each plate is run. We also recommend performing an alcohol flush followed by two washes with sheath fluid between each plate and warming the laser.

Stage 5: Preparing STarSystem for Shutdown

1. When the assay reading is complete, select the **Scripts** tab from the STarStation instrument controls and select the **default shutdown script**.
2. The shutdown script will decontaminate the system and wash the salt-containing sheath buffer from the sample needle and sample tubing reducing the potential for salt precipitation.
3. **Eject** the plate holder and place a ACS utility plate on the tray ensuring that the commands outlined in the script are satisfied



- R2 - 3ml of 10% household bleach or hypochlorite solution.
- R3 - 2ml Distilled Water



4. Click the **Start** button to launch the script.
5. When the Shutdown script completes the STarSystem can be powered off.

Note: It is recommended that you wash with Distilled water (Soak) at least three times before shutting down the instruments. This step is crucial in preventing the precipitation of salts from the sheath buffer in both the sample tubing and sample probe.