

### **Continuing An Assay Reading Following a System Error.**

This document outlines a procedure to minimise the time required for system startup should a software or hardware problem occur during an assay reading which necessitates that the STarSystem cytometer is restarted

STarStation has been designed so that all of the fluorescent data detected by the STarSystem cytometer during sample acquisition are saved. All of the fluorescent signal data detected by the Luminex 100's four detectors (CL1, CL2, DD and RP1) are stored in List-Mode Data (LMD) files. The creation of separate LMD data files for each microplate well allows STarStation users to reanalyze assay data (recalculate raw median fluorescence, modify the singlet gate and move/resize microsphere classifier regions) after the assay data has been collected.

It may be necessary to cycle the power to the STarSystem instruments and/or restart the STarStation software If one of the following scenarios occurs:

1) STarSystem has become unresponsive for prolonged periods of time during a sample reading and you are unable to regain control via the **Clear Queue** function on the Control tab of the Instrument

Controls dialog and the **Abort Acquisition**  function has no affect it may be necessary to restart both the software and the instrument.


Note: you should confirm via visual inspection of the sheath buffer reservoir and waste bottle that the interruption to data collection has not been caused by an overfull waste bottle or an inadequate volume of sheath buffer in the SD system or sheath bottle.

Also confirm that the system has not aborted acquisition, when acquisition has been terminated the status of the Luminex Controller and of STarStation are "Ready". If the states of the Luminex Controller and or STarStation software display Busy or Acquiring for prolonged periods without any discernible activity from the system and the time displayed on the instrument status toolbar does not increment the system has attained an unresponsive state.

Note: Before restarting the system users should *rule out* a common cause of interruption to assay readings which is a pressurization timeout. When acquiring assay data the STarSystem pressurizes so that the sheath and air pressure are within a defined pressure range (usually between 6 and psi units). If during the data acquisition process the system is unable to attain pressure in this range the Luminex Controller Status will display the state "Pressurizing" and if this state persists for 5 minutes (because an insufficient supply of sheath buffer makes it impossible to pressurize the system or the cap of the sheath buffer bottle is loose) the system will generate a command failure state and the assay reading will be terminated. Pressurization issues can be diagnosed via the sheath and air pressure status indicators in the Instrument Status bar at the bottom right corner of the STarStation software interface.

2) An interruption to the power supply prematurely terminates an assay reading.

If either of the two scenarios above occurs and the power to the STarSystem cytometer is interrupted, when the LX100 restarts a laser warm-up operation is required, even if the cytometer has been previously warmed up.

Note: IS-version cytometers automatically begin warm-up  of the fluorescent detectors upon start-up.

3) Next you will need to select the worklist which was being acquired when the interruption occurred.

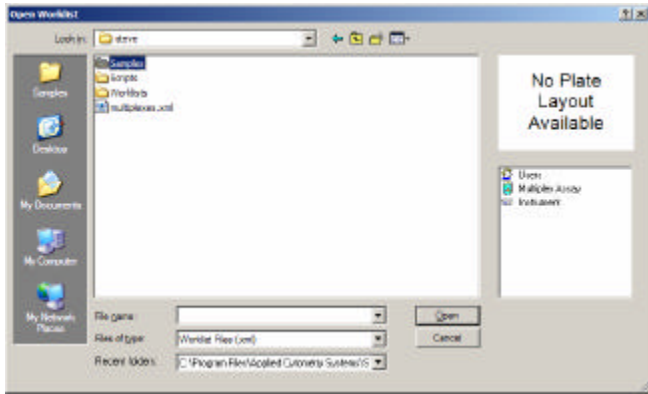
a) Activate the Acquisition View by clicking the Acquisition Tab.

b) Select the **Open** option from the file menu or press the open icon on the file toolbar.

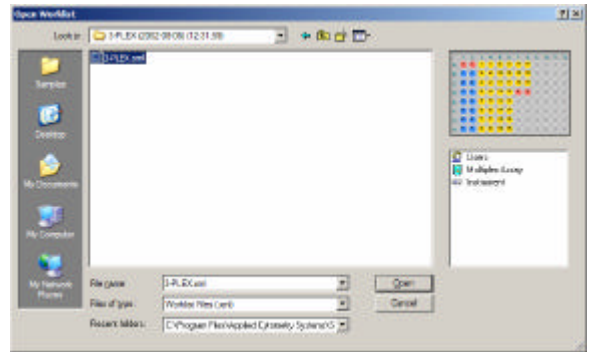
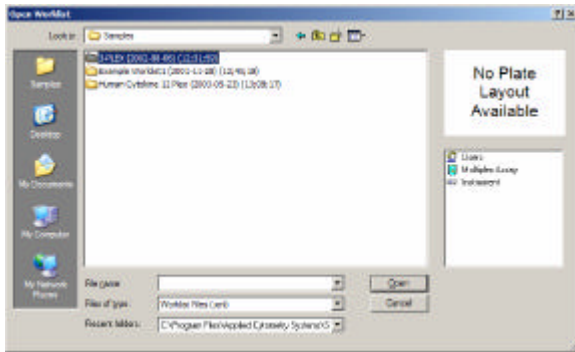
c) The **Open Worklist** Dialog is displayed.

d) Navigate to the **Samples** folder and select the directory that corresponds with the worklist which was interrupted (see the sequence of diagrams below).

Note: When the Open worklist command is selected in the Acquisition view the open file dialog starts in the logged in users worklist folder where the template worklists are located. When acquisition of a template worklist begins, STarStation creates a folder in the logged-in user's samples directory with the name of the worklist and a timestamp corresponding with the date and time acquisition began. The LMD files for each of the acquired wells and the worklist file are stored in this folder. The worklist file is the file with the ".xml" extension.




e) Next select the worklist file from within the selected folder.



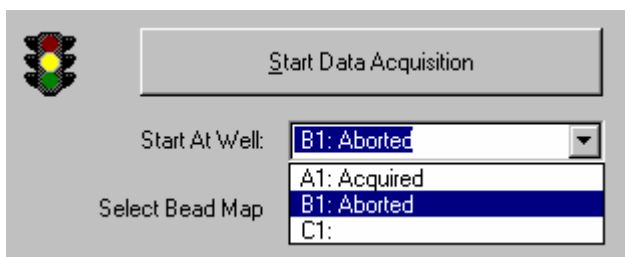
f) When the worklist is opened in the acquisition view, the LMD column in the data grid should contain LMD file references and that the sample column has progress indicators indicating that wells on the plate have been previously acquired.

Sample	Location	Multiplex	Events
✓	A1	Development	300
✓	B1	Development	300
✓	C1	Development	300
✓	D1	Development	300
✓	E1	Development	300
✓	F1	Development	300
✓	G1	Development	300
✓	H1	Development	300
✓	A2	Development	300
✓	B2	Development	300
✓	C2	Development	300
✓	D2	Development	300
✓	E2	Development	300
✓	F2	Development	300
✓	G2	Development	300
⊗	H2	Development	76

g) Press the **Start Acquisition** button  or press the **F9** key to start the Acquisition Wizard.

h) Modify, if required, acquisition parameters on the first page of the Acquisition Wizard.  
 Note: Any wells which have already been acquired will have received 162µl of sheath buffer from the post acquisition wash, the concentration of microspheres in pre-acquired wells may be less than the concentration prior to data collection.

i) On the final page of the Acquisition Wizard the **Start at Well** control permits the selection of a well on the worklist from which the restarted acquisition can commence.



The items in the **Start At Well** list will give you an indication as to how the wells have been processed. Whether they were aborted or acquired or whether they timed out during data collection. If the well has not been sampled it will have no comment after the well location. Select from this list were you would like to restart data collection (this by default will be the first well in the sequence for

which there is no LMD file).

j) Once the restart location has been selected, press the Start Data Acquisition button to begin collecting data.