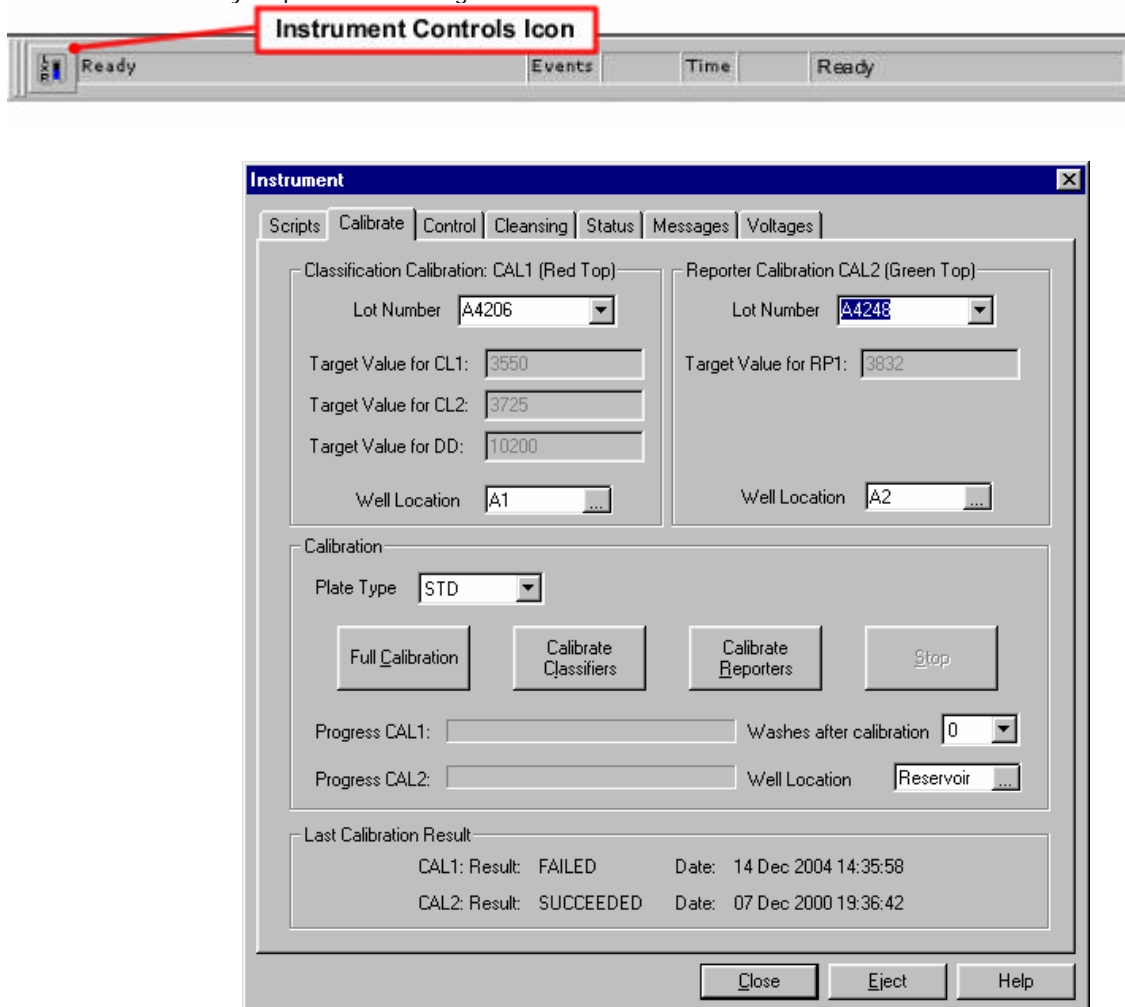



# STarSystem Calibration

**Step 1** Display the STarStation Instrument Controls by selecting the Instrument Controls option from the **View** Menu, using the CTRL + I shortcut key sequence or clicking the Instrument Controls icon.



**Note:** before attempting calibration ensure that the laser status icon indicates that the system has been warmed up  and that reservoir or programmed wash location contains sufficient sheath fluid to allow the post calibration wash of the instrument described in Step 10. The Sample Probe height and cleanliness should be verified before calibration.

**Step 2** Select the **Calibrate** tab.

**Step 3** Select the appropriate CAL1 and CAL2 reagents using the lot Number drop-down lists. Lot Number and Target Values for the CAL1 Classification Calibrator (CL1/CL2/DD) and the CAL2 Reporter Calibrator (RP1) are entered via the STarStation Reagent Manager. Target values can be found in the Certificate of Analysis documentation for the calibrators.

**Step 4** When the reagents have been selected the target values for the beads will be displayed. **Confirm that the correct calibration Microsphere Lot and target values are displayed.**

**Step 5** Select the Well location for CAL1 and CAL2 Calibrators, ensuring that the locations are appropriate for the type of microtiter plate used (Standard 96 well plate or ACS Utility/QC plate).

**Step 6** Ensure that the Calibration reagents are at room temperature. Vortex Calibrators and place 5 drops of CAL1 (Red topped bottle) into the selected Well e.g. A1. Place 5 drops of CAL2 (Green topped bottle) into the selected Well e.g. A2.

**Step 7** Click the **Eject** button and place the Microtitre plate onto the XY platform. Check that the Reservoir is filled with Sheath Fluid. Retract the XY Platform tray by clicking the **Retract** button.

**Step 8** Click the **Full Calibration** button to begin a full calibration, STarSystem will begin Calibration of CAL1 followed by CAL2. Note: The **Calibrate** button performs both CAL1 and CAL2 calibration operations whereas **Calibrate Classifiers** and **Calibrate Reporters** only perform the respective single operations.

**Step 9** The Instrument Status Toolbar at the top of the Main Window will display the progress of Calibration and report on the success or failure. View the Progress Bars on the Calibrate tab to check the progress of the calibration of CAL1 and or

CAL2 as appropriate.  
 The *Last Calibration Result* window on the Instrument Controls Calibrate tab will indicate the success or failure of system calibration.

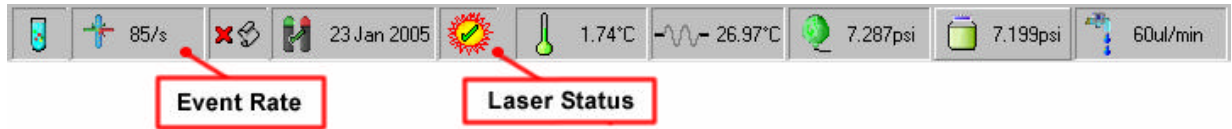
**Step 10**

After the Calibration operations have completed, a number of wash cycles (specified in the **wash after calibration** field) is performed.

Note: It is also possible to calibrate STarSystem using a calibration script, refer to Applied Cytometry Systems document *Quick Reference: STarSystem Calibration Using a Script*.

**Troubleshooting**

The event rate during calibration can be observed via the Instrument Status bar. The event rate during calibration should be in the range 100 to 300 events/sec.



A low event rate indicates sub optimal probe height adjustment or a blockage in the sample probe/sample tubing.

If a low event rate is displayed verify that the sample probe is clean, by removing the probe and flushing it with distilled water using a narrow gauge syringe. The probe height should also be verified.

### Classification Calibration (CAL1) - Phase 1: Doublet Discriminator Calibration

Progress CAL1:

When CAL1 calibration starts the instrument status will display "Calibrating CAL1" and the Luminex controller status "Acquiring".

Calibrating CAL1
Events
Time
Busy

Progress CAL1:

When Phase 1 completes the progress bar appears as shown on the left. If phase 1 fails\* (phase 1 times out after approx. 180 seconds) this indicates that the probe height may be incorrect, the sample probe blocked or that there are no CAL1 Microspheres in the selected well.

### Classification Calibration - Phase 2: Classifier (CL1 and CL2) Calibration

Calibrating CAL1
Events
Time
Busy

Progress CAL1:

When Phase 2 of CAL1 calibration completes the progress bar will appear as shown on the left. If phase 2 fails check that the microspheres are within date and that the correct target values have been entered into the Reagent Manager and the correct bead lot has been placed in the well.

### Reporter Detector (RP1) Calibration (CAL2)

Progress CAL2:

When CAL1 calibration starts the instrument status will display "Calibrating CAL2" and the Luminex controller status "Acquiring".

Calibrating CAL2
Events
Time
Busy

Progress CAL2:

When RP1 calibration completes the progress bar will appear as shown on the left. If RP1 calibration fails confirm that the beads are within expiration date and that the correct lot and target information is present in the calibration script/calibrate tab.

Calibration Troubleshooting