



SIDNET Automation: A Primer

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Automation at SIDNET

Two key goals of laboratory automation are enabling assays which would require technicians to do tedious, repetitive work and ensuring uniform treatment of samples. The system installed at SIDNET has been designed to accomplish these two goals while being flexible enough to accommodate a range of assays. Listed below are the essential components of the system and their functions:

Thermo POLARA (control software) – the “brains” of the system. This is where the steps of an assay are laid out, timings of instrument operations and plate moves are adjusted, “calls” to instrument specific methods are made, and the overall assay sequenced. This is also the software that is used to run and monitor the assay in real-time.

Thermo Catalyst Express – the arm that transfers plates between instruments. This component also has a barcode reader, a plate rotation position, and attached storage locations for plates or lids.

BioTek ELX405 plate washer – a 96 channel plate washer. It can be used for 96 and 384 well microplates and can be set to wash reasonably adherent cells. It can also be used to dispense reagent as long as accuracy and precision are not too critical.

Perkin Elmer Envision plate reader – a spectrophotometer/luminometer/fluorometer for microplates. The data generator of the system.

Thermo Kendro Cytomat Incubator – a 42 microplate incubator with both temperature (~10°C – 50°C) and CO₂ control. Perkin Elmer Evolution P3 – a 96 channel pipettor. This type of device generally handles the key sample additions and other sensitive reagent handling.

Perkin Elmer PlateStak consists of 2 stacker modules, each with capacity for 50 standard -96,-384, or -1536 well microplates, 15 deepwell plates or 12 roborack tip boxes and the ability to sort and store plates for first-in, first-out processing.

Perkin Elmer Multiprobe – an 8 channel pipettor. This type of device can also handle key sample additions and other sensitive reagent handling. One very useful application for this instrument is cherry-picking or hit-picking wells of interest and compiling them to daughter plates.

POLARA Rationale

You use a POLARA lab system to automate laboratory protocols, such as methods and assays. You define the protocols in POLARA and then use POLARA's scheduler to generate the instructions the lab system follows to process the required number of samples through the protocols. The scheduler enables you to process samples through different protocols in parallel on the lab system, which boosts throughput and makes more efficient use of lab system resources and personnel.

The POLARA software instructs the lab system operator on what instruments to prepare to run a schedule, and how many containers to load. As the run progresses, POLARA tracks each sample as it moves through the protocol. If POLARA encounters any problems, such as a missing container, it alerts the operator. (If it is running in unattended mode, it will first attempt to deal with the problem on its own.) The operator can suspend or abort a run at any time. If there is an instrument failure, power outage, or some other reason for the operator to abort a run, the operator can save run recovery information. When the system is restarted, POLARA detects the saved information and asks whether the interrupted run should be recovered or abandoned. When the run is complete, the operator collects the sample data generated during the run, and the POLARA software readies the system to perform another run.

Typical Automated Assay Workflow

1. Develop automation amenable assay.

This seems obvious but not all assays that work on the bench can automatically be “ported” to automation. However, most assays can be modified to allow them to be run on an automated platform. The liaison between the automation specialist and the assay developer are very critical in order to ensure that development decisions are made with consideration for the capabilities and limitations of the available instruments.

2. Build and optimize automated protocol.

This involves identifying the integrated instruments that are needed for the assay and writing and testing necessary methods for them. Testing should be done with as many of the actual assay materials as possible; i.e. the same microplates, same buffers, temperatures etc. In addition, the overall assay method needs to be built in the “Master Control Program” (for the SIDNET system this means POLARA). Optimization involves dry runs of the both the instrument specific methods and the overall assay method. Critical adjustments include timing of the various steps, fine-tuning of liquid handling procedures, and optimization of the data acquisition.

3. Run small scale pilots.

This should be a bridge between the instrument specific method testing done above and running the assay with real samples. Run one or two plates through the assay and watch the whole process, especially if you intend to run the real assay without someone in attendance.

Safety

The integrated system at SIDNET has several key safety features that every user should be familiar with.

- Emergency Stop (E-Stop) buttons enable you to immediately halt container transport motion.
- The run-time Monitor window displays information during the progress of a run, including error messages and warnings.
- If POLARA detects that operator intervention is required during a run (for instance, if a non-standard container needs to be repositioned or removed), it suspends the run and alerts the operator.
- A three color run beacon provides a visual indication of system status.
- Safety guarding may either partially or completely enclose the system to prevent access during a run.
- An Uninterruptible Power Supply (UPS) can be configured to ensure that the system is halted in an orderly fashion if power is suddenly lost.
- An end-of-arm load limiter protects equipment from collision damage. The load limiter is a pneumatic safety-release device used with Thermo's more powerful CRS F3 robot. It is located between the gripper and the tool flange. If the load limiter detects a force greater than its pre-set threshold, it triggers an E-Stop and halts the arm.

Step-by-step Walkthrough of Real-time Assay

Starting POLARA

Start POLARA using one of the following methods:

- From the Start menu, select Programs>CRS Robotics>POLARA.
- Double-click on a "X.mdr" workspace file in Windows Explorer.

Opening a Workspace

A workspace contains profiles, methods and schedules (see illustrations and explanations below) created by the POLARA system admin. Before you can set up and start a run, you must select the workspace that contains the schedule for your run.

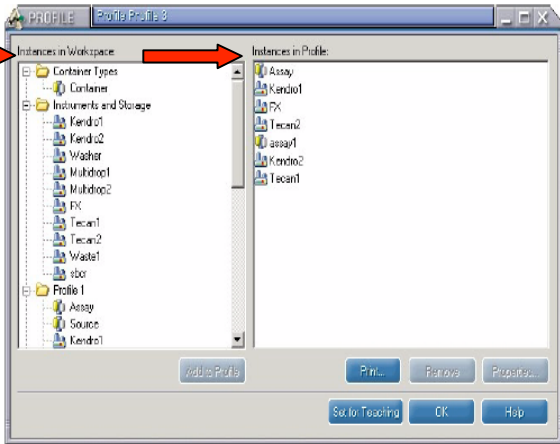
- From the File menu, select Open Workspace.
- On the toolbar, click Open Workspace.

Use the Open Workspace dialog box to locate and open the workspace. The name of the workspace and all schedules created for this workspace are listed in the left pane of the POLARA main window.

Profiles

A **profile** is the POLARA representation of the group of instruments and containers required to carry out a biological or chemical protocol.

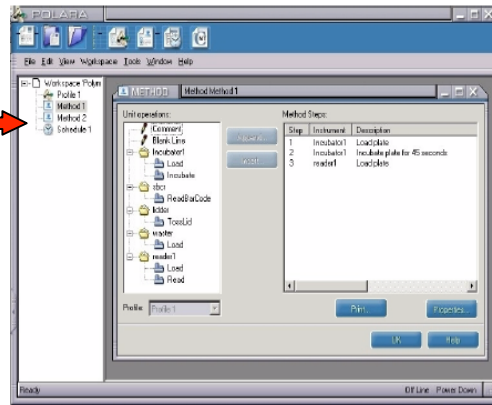
All the instances available for use in profiles are shown in the pane called **Instances in Workspace**. All of the container and instrument instances that have been added to the profile are shown under **Instances in Profile**. A container or instrument must be listed here for you to use it in a method



Methods

A **method** is a list of steps that defines the biological or chemical protocol that you want followed on an individual sample.

When you open a workspace, the methods associated with that workspace are shown in the left pane of the POLARA window.

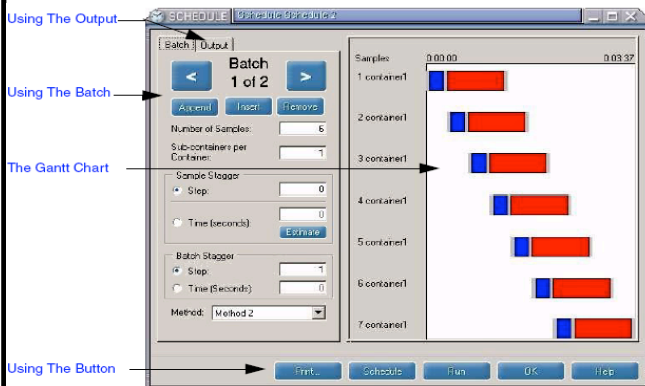


Some commonly used steps in POLARA methods include the following:

- **Load**: instructs POLARA where to pick up and place a container at the beginning of a method and end of the method
- **Incubate**: length of time a container should rest in the instrument. POLARA may be able to move other containers.
- **Transfer**, which moves material from one container to another
- **Read**, which tells POLARA to get data from an instrument such as a fluorescence or colorimeter reader.

Schedules

A **schedule** is the instructions the lab system follows to process the required number of samples through the selected method(s)



The POLARA schedule controls how multiple samples are processed by the system resources. A well-designed schedule yields increased throughput while optimizing sample uniformity.

Just as a method describes how a biological or chemical process is carried out on a single sample, a schedule defines how the process is carried out on multiple samples. By managing container movements and unit operations, the schedule makes the best possible use of available system resources.

Opening a Schedule

A schedule contains all the instrument and method information the system needs to perform a run.

To open the schedule for your run:

- Click the schedule in the left pane of the POLARA main window. The Schedule window opens at the Batch tab.

Generating the Schedule

Once you have opened a schedule, you can generate it.

To generate a schedule

1. On the Batch tab in the Schedule window, enter the number of samples for the first batch.
Note: Some highly optimized schedules will only run efficiently with a specific number of samples. If you encounter scheduling errors or are unsure if the schedule is satisfactory, check with the schedule developer.
2. If there is more than one batch in the schedule, scroll through the batches using the > and < buttons and enter the number of samples for each batch.
3. Click Schedule. POLARA generates the schedule, and displays information about the run in the Output tab of the Schedule window.

The Output tab provides the following information about the schedule:

- Running Time: displays the length of time that the run has proceed without interruption, in hours, minutes, and seconds
- Statistics: displays statistical information for each batch
- Gantt chart: displays a graphical representation of every unit operation performed on each sample

Setting up the Run

After opening and configuring a schedule, you are ready to set up the run.

To set up a run

1. In the Schedule window, click Run. POLARA displays the Setup for Run window
2. Carefully read and perform each step displayed in the Setup for Run window. Tip: Click Print to print a checklist of steps.
3. Click Site Parameters. Perform any steps displayed.
4. Click Start Run. The system initializes each instrument. During initialization the Monitor window appears and the following occurs:
 - The Initializing Status Indicator turns blue.

- The CROSnt window opens briefly, showing the initialization process, then minimizes.
 - The results of the initialization are listed in the Summary tab of the Monitor window.
5. Check the Summary tab of the Monitor Window. You should see a list of instruments that have initialized successfully.

Using the Monitor Window

The Monitor window enables you to control a run, monitor the progress of samples through the system, check the status of instruments, and observe warning and error messages. The Monitor window has a toolbar, status indicator lamps, tabs, and a status bar.

Using the Monitor Toolbar

The toolbar buttons allow you to perform the following tasks:

Parameters: Opens the parameters dialog box, which allows you to view the location and name of the data log file, and the name of the sequence file.

- **data_dir:** The location of the POLARA data directory
- **log name:** The name of the log file that POLARA creates in the data directory for each run
- **sequence_name:** The name of the sequence file that controls the run

- **Attended:** Use this mode if an operator is overseeing the run.

When POLARA detects an error, it alerts operators who must clear the error before continuing the run.

- **Unattended:** Use this mode when an operator is not overseeing the run. POLARA will attempt to handle error conditions. If unable to do so in a safe manner, it suspends the system. You can change the mode of operation any time during the run. **Warning!** Do not change the following values without consulting your POLARA administrator:

Toolbar

Run status indicators

Status bar

Tabs

Reset Beacon: Resets the Run beacon and the status indicator lamps.

Start/Continue: Starts a run, and continue a run that has been Suspended.

Suspend: Suspends a run. The current unit operation will be completed before the robot stops moving.

Halt: Ends the run. A dialog box appears that prompts you to confirm that the run should be halted. When confirmed, the run ends.

Help: Displays online help for the current Monitor tab.

Understanding the Status Indicators

The status indicators display the state of the system.

Only one of the following status indicators will be lit at any time:

- Halted: Turns red when a run is halted.
- Initializing: Turns blue when the system is initializing.
- Running: Turns green when a run is underway.
- Suspended: Turns yellow when a run is paused.
- Complete: Turns blue when a run is complete.

The following status indicators may be lit individually or in combination:

- Error: Turns red when an error occurs.
- Warning: Turns yellow when a warning occurs.

Warning! The Suspend and Halt buttons do not stop robot motion immediately! In an emergency, press an E-Stop button.

Using the Monitor Tabs

Each tab displays selected information that helps you to monitor the progress of a run.

Summary Tab

The Summary tab displays the status of each sample and the status of each instrument in the schedule. Use this tab to check whether each instrument in the system has initialized properly. While an instrument is initializing, its status reads "Initializing". If initialization is successful, the status changes. If initialization is unsuccessful, the status remains "Initializing". In addition, the warning status indicator flashes yellow, and a message box appears that allows you to abort or retry initialization. The example below shows seven instruments that have successfully initialized.

Samples Tab

The Samples tab displays the status of a specific sample. Use this tab to view the containers and method steps used by this sample.

Instruments Tab

The Instruments tab displays the status of a specific instrument. Use this tab to check whether the instrument is operational and to view details about each nest in the instrument.

Sequence Tab

The Sequence tab displays the progress of the unit operations in the schedule. Use this tab to monitor whether a unit operation is underway, or complete. Unit operations that are underway are indicated by a green arrow, while completed operations are indicated by a red checkmark.

The Sequence tab displays only one segment of the currently running schedule. To select the segment displayed, use the segment display control on the right edge of the form.

The segment display control represents all the segments of the schedule as a column of rectangles: the red rectangle indicates the currently displayed segment, while the green rectangle indicates the segment containing the currently executing unit operation.

To select the sequence segment to display

- Click the rectangle representing the segment.

Tip: If you hold the mouse pointer over a rectangle, the segment display control shows the range of method steps in that segment.

To select the segment display mode

- Click the button to switch between display modes: either scrolling, which keeps the currently executing unit operation displayed, or static.

To view the unit operations in the segment

- Use the scroll bar at the right of the list to scroll through the unit operations in the schedule.

Note: You can use the Sequence tab to set automatic pause points in the schedule, as described in “Inserting Pause Points into a Run” below.

Messages Tab

The Messages tab displays all warning and error messages issued by the system. Keep this tab selected during a run to view the messages as they occur.

Status Bar

The Status bar displays the following information about the system:

- Status: the status of the system. This will be either Running, Suspended, or Halted.
- Time: the time that the system has been running since the run was started. This does not include time while the run was Suspended.
- Slip: the time the system was Suspended during a run
- Heart: A beating heart indicates communication between the POLARA system computer and the lab system.

Starting a Run

After setting up the run, you are ready to start the run.

Note: The following procedure assumes you have powered up your system as described in the system manual.

To start a run

1. Take the following steps:
 - If you are restarting a recovered run, ensure that the containers that were in process when the run was aborted are where POLARA expects them to be, based on the handling selected for the interrupted operations. For more information about handling options, see “Handling Interrupted Operations” below.
 - Ensure that all laboratory personnel are clear of the system, and that there are no objects on the table that may obstruct robot motion.
 - If the lab system uses an articulated robot, ensure robot arm power is on.
2. In the Monitor window click Start/Continue. On AR systems, if the robot is not currently within the safe start zone, the Robot Start Position dialog box appears. Respond to the dialog as follows:
 - a Move the robot to a safe location.
 - b Click Start Run. The run begins processing.See “Setting the AR Start Position” below for details on setting and saving the start position. When a run completes, the “Complete” status indicator lamp on the toolbar turns blue, and the beacon turns red.

Setting the AR Start Position

On AR systems, POLARA needs to know that the robot is in a safe position before it starts a run. To do this, it checks the current robot position against a defined safe start position at the beginning of each run. The safe start position is a set of XYZ coordinates, with +/- tolerances, defining a three-dimensional zone within the robot's range of motion. Any position within this zone is considered a safe position from which to start a run.

You specify the safe start position using the Robot Start Position dialog box: The Robot Start Position dialog box is opened in two ways:

- It opens automatically when you click Start/Continue on the Monitor window, if the robot is not currently within the safe start position zone.
 - It opens when you select Robot Start Position from the View menu.
- Note: The buttons on the dialog box differ depending on how it was opened.

To set and save the robot start position

1. If the robot is not in a safe position to start a run, move it to a safe position.
Tip: The Actual Robot Position fields show the XYZ coordinates for the current position of the robot.
2. When the robot is positioned, click Use This Position as Start. The current coordinates are copied into the Robot Starting Position fields.
3. If necessary, modify the Plus Tolerance and Minus Tolerance settings to define the zone around the start position, within which the robot is safe to start a run.
4. Make sure that the checkbox above the buttons is enabled so that the system always checks the position of the robot before starting a run.
5. Save the settings by doing one of the following:
 - If the dialog opened when you clicked Start/Continue on the Monitor window, click Start Run to save the settings and start the run.
 - If you opened the dialog by selecting Robot Start Position from the View menu, click OK to save the settings.

Monitoring a Run

As the run progresses, you monitor the run for warnings or errors. When POLARA encounters a condition that warrants a warning or error, it alerts the operator using the Run beacon (if installed), issues a message in the Messages tab of the Monitor window, and may display an error message box with several options.

Understanding the Run Beacon

The Run beacon is an optional external device that displays the status of the run. As you perform a run, you can look at the Run beacon to quickly check the status of the run. The beacon uses three colored lights that are lit individually or in combination as follows:

Green

The green beacon light indicates the system is running, as follows:

- Solid green: The run is proceeding normally and no warnings have been issued.
- Solid green, solid yellow: The system is running, but warnings have been issued. Depending on the type of warning, the run may become suspended if corrective action is not taken.
- Solid green, flashing yellow: The system is running, but an error occurred that has not been cleared.

Yellow

The yellow beacon light indicates something is wrong, as follows:

- Solid yellow: A warning has occurred.
- Flashing yellow: An error has occurred.

Red

The red beacon light indicates the system is not running, as follows:

- Solid red: The run has completed.
- Flashing red: Initialization has completed without fault. The system is in a suspended state.
- Flashing red, flashing yellow: An error has occurred and the run was suspended. Once corrective action is performed, the run can be continued.

Understanding Warnings and Error Messages

POLARA issues warnings and error messages when it encounters a condition that should be noted or corrected.

Warnings

POLARA issues a warning when it encounters a condition that should be noted and reported but is not serious enough to warrant suspending the run. It alerts operators to a warning in the following ways:

- A warning appears in the Messages tab of the Monitor window.
- The beacon turns yellow.
- The warning status indicator turns yellow.

Error Messages

POLARA suspends the run when it encounters an error that requires intervention. It alerts operators in the following ways:

- Depending on the type of error, a message box may appear on the screen. The message box displays the specific error POLARA encountered and offers optional steps you can take to resolve the issue.
- An error message appears in the Messages tab of the Monitor window.
- The Run beacon flashes yellow.
- The error status indicator turns red.

Suspending a Run

POLARA suspends a run when it encounters an error that requires operator intervention. You may suspend a run to attend to a warning issued by POLARA, to adjust a container, or to refill reagents.

When you or POLARA suspends a run, the following occurs:

- The system completes any unit operations in progress but does not begin new unit operations.

- The suspended status indicator lamp turns yellow, and remains yellow until you click either Start/Continue or Halt.
- The run time stops accumulating and slip time (the amount of time that the run slips from the scheduled time) accumulates until the run is continued.
- The beacon turns yellow.

To suspend a run

- Click Suspend in the Monitor toolbar.

Continuing a Suspended Run

When a run has been suspended, you must ensure it can safely continue before resuming the run in POLARA.

To resume a suspended run

1. Ensure that all laboratory personnel are clear of the system, and that there are no objects on the table that may obstruct the motion of the robot or other instruments.
2. Ensure the robot is in a safe position.
3. Click Start/Continue. The run continues with the next unit operation in the schedule. When the run restarts, the running status indicator turns green.

Warning! Suspending the run does not immediately stop the motion of the robot and other instruments. Nor does it remove power. Always suspend a run and press an E-Stop before reaching into the system.

Inserting Pause Points into a Run

Besides allowing you to pause a run manually, POLARA automatically suspends a run when it encounters a pause point.

You use pause points to force the system to suspend in such cases as:

- When operators must refill reagents
- When operators must add containers to the system at the last possible moment (such as containers that must be kept refrigerated as long as possible before being used in a run)

Note: You can insert pause points anywhere except on Convey operations.

To insert a pause point

1. With the system running, select the Sequence tab in the Monitor window.
2. If necessary, use the segment display control to find the unit operation before which you want the system to pause.
3. Right-click on the line for that unit operation and choose Insert pause from the pop-up menu. POLARA displays the information for the operation in red.

To re-start the system after a pause point

1. Ensure that all laboratory personnel are clear of the system, and that there are no objects on the table that may obstruct the motion of the robot or other instruments.
2. Ensure the robot is in a safe position.
3. Click Start/Continue. The run continues with the unit operation at which you inserted the pause.

To search for pause points

1. Select the Sequence tab in the Monitor window.
2. Right-click on any unit operation and choose Display next pause or Display previous pause. POLARA finds the next or previous pause in the schedule and displays the subset of operations that contain the pause.

To remove a pause point

1. 1 Select the Sequence tab in the Monitor window.
2. 2 Use the Subset Selector or the Display next/previous pause feature to find the unit operation that is marked for the pause you wish to remove.
3. Right-click on the unit operation and choose Remove pause. POLARA displays the information for the operation in black.

Adjusting Containers or Reagents During a Run

During a run, you may need to adjust containers or refill reagents inside the system.

To adjust containers or refill reagents

1. Click Suspend in the Monitor window.
2. Wait until the robot stops moving and the Run beacon light flashes yellow, indicating the run is suspended.
3. Press an E-Stop button. You may now enter the system workspace.
4. Adjust the containers or reagents on the table as necessary.
5. Recover the system.

Warning! Always suspend the run and press an E-Stop button before working with the system. This removes arm power to the robot and prevents it from moving.

Warning! Pressing the E-Stop only removes power from the robot. Be sure you understand what instruments are still operational before you reach into the system. If unsure, consult your POLARA administrator

Adding Samples to a Run

POLARA 2.1 and higher enables you to add samples to a run in progress. To add samples to a run, you schedule them as a new batch in the schedule that controls the run. POLARA then replaces the unexecuted part of the run's schedule with the updated schedule.

To add samples to a run

1. Open the schedule controlling the run by clicking on its name in the left pane of the POLARA main window.
2. Click Append to add a new batch to the schedule. The schedule editor displays a blank batch form.
3. Take the following steps:
 - Select a Method to be used with the new samples.
 - Enter the Number of Samples to be processed.
 - If necessary, enter sample and batch staggers required to optimize the run.

Note: The researcher or administrator who normally creates schedules should provide the operator with appropriate sample and batch staggers for the schedule.
4. Click Schedule. The scheduler generates a new Gantt chart for the schedule.
5. Click Commit to replace the unexecuted portion of the running schedule with the new schedule. POLARA displays in the Monitor's Message tab the number of the sequence step at which the switch-over to the new schedule will occur. POLARA then displays the Setup for Run window, which lists the containers needed for the added samples and the nests to place them in.
6. Wait until the mover serving the instrument to which the containers must be added has finished getting or putting a container.
7. Add the containers to the instrument, performing any instrument-specific steps that might be necessary. For example, you might have to power off the instrument before you add the containers, and bring it back online afterwards. For details, refer to the system manual.
8. Click Start Run on the Setup for Run window.

Note: It is not necessary to suspend the run to add containers. In fact, suspending the run might add so much slip time that POLARA will not be able to switch over to the new schedule. If this occurs, you have to re-schedule the added samples.

Halting a Run

To halt a run

1. Click Halt. A dialog box appears that prompts you to confirm that the run should be halted.
2. Click Yes to halt the run. Robot motion stops and the run ends. The halted status indicator lamp turns red, and the Run beacon turns red. The Shutdown Options dialog box appears.
3. Choose the appropriate Shutdown Option:
 - If you have no intention of attempting to recover the run, click Abort immediately and DO NOT SAVE.

- If you wish to wait until all motion is completed, and intend to recover the run later, click Abort once no plates are in motion (at sequence step n) and save.
4. Click OK. Container transport motion stops according to the specified Shutdown Option and the run ends. The halted status indicator turns red, and the beacon turns red.
Warning! The Halt button does not immediately stop the motion of the robot and other instruments. Nor does it remove power. In an emergency use the E-Stop button.
Caution! Do not use the Halt button unless you want to end a run. Use the Suspend button to pause the system briefly.

Recovering an Aborted Run

If you saved run information when aborting a run, you can recover the run.

Note: Until you recover an interrupted run, or explicitly command POLARA to abandon the saved run information, POLARA displays the run recovery options every time you start it or open the workspace containing the schedule that controlled the interrupted run.

Whether you choose to recover or abandon the run depends on factors that include the following:

- The duration of the interruption. If the contents of your samples are sensitive to delays in processing, you must consider whether the duration of the interruption would invalidate the data from the samples that have not completed processing.
- The number of samples remaining. If only a small percentage of the total number of samples remains to be processed, it might be simpler to schedule them into a new run.

Understanding Run Recovery

When POLARA saves run information, it stores the operations that were still in progress when the run was aborted. Before you can resume a run, you must tell POLARA how to handle the interrupted operations, and then ensure that the containers affected by these operations are where POLARA expects them to be when the run resumes.

POLARA provides the following options for handling interrupted operations:

- Resume the operation. POLARA takes into account the time that has elapsed since the run was aborted, and adjusts the operation's times accordingly. For example, if a container had incubated for 10 minutes of a scheduled 30 minute period when the run was aborted, and if the run recovery was started 10 minutes later,

POLARA sets the time remaining in the incubation operation to 10 minutes ($30 - (10 + 10) = 10$). POLARA assumes the container is in the same place it was when the operation was interrupted.

- Restart the operation. POLARA ignores the elapsed operation time and re-issues the command to perform the operation. POLARA assumes the container is where it should be when the operation starts.

Caution! If you resume a run without ensuring that the containers in process are where POLARA expects them to be, you may lose run data, damage equipment, or spoil samples.

- Skip the operation. POLARA assumes the operation is complete. When the run resumes, POLARA will issue the command to perform the next operation on the container as originally scheduled. POLARA assumes the container is where it should be when the operation has completed.

Note: When a run is aborted and saved, POLARA may not have detailed knowledge of the status of each interrupted operation. For example, if an interrupted operation was a Run Program operation for a liquid handler, POLARA may not know what operations the program was performing on the container. Therefore, you must consider carefully which of the three options for handling interrupted operations will result in the most safe and effective way to resume the run.

Starting Run Recovery

When POLARA detects saved run information, it displays a Saved Run Detected dialog box. The options available depend on whether POLARA detected the run information when it started or when you opened the workspace containing the schedule for the interrupted run:

The Saved Run Detected forms enable you to start run recovery, postpone it, or abandon the saved run information.

To start run recovery

1. Select Open the workspace and commence run recovery or Enable run recovery facilities.
2. Click OK. POLARA displays the Run Recovery - Containers In Progress form.

To postpone run recovery

1. To postpone recovering the run, select Handle run recovery later. At the start of the next run, POLARA asks you whether to overwrite the

- information it saved on the previous aborted run. If you choose not to overwrite the information, you cannot save information on the new run.
2. Click OK.

To abandon saved run information

1. If you do not wish to recover the run at any time, select Abandon saved run information. POLARA will delete the saved run information.
2. Click OK.

Handling Interrupted Operations

When you choose to recover an interrupted run, POLARA displays the Run Recovery - Containers in Process form. This form displays a table showing the operations that were in progress when the run was aborted.

It consists of the following columns:

- Step, which is the step in the schedule where the interrupted operation was taking place
- Instrument, which is the instrument the container was in when the run was aborted
- Sample, which is the number of the sample to which the container belongs
- Container, which is the container on which the operation was being performed
- Pending Operation, which is the operation that was interrupted when the run was aborted
- Start Time, which is the time (in seconds) at which the interrupted operation started
- Duration, which is the duration (in seconds) defined for the operation
- Action, a drop-down list from which you select how POLARA should handle the interrupted operation

To handle interrupted operations

1. Click the Action cell in the Containers In Instruments table and select one of the following ways to reschedule the operation in the drop-down list:
 - Restart Operation. When you restart the run, POLARA assumes the container is in the source nest for the operation.
 - Resume Operation. When you restart the run, POLARA assumes the container is where it was when the operation was interrupted. It

shortens the duration of the operation in the schedule's Gantt chart to account for the time the container has spent in the instrument before and during the interruption.

- Skip Operation. POLARA marks the operation as completed. When you restart the run, POLARA assumes the container is in the destination nest for the operation.

2. Take one of the following steps:

- Click OK to continue run recovery.
- Click Cancel to close the Run Recovery form and postpone run recovery. To resume run recovery, choose Run Recover in the Workspace menu.

Restarting the Run

When you click OK in the Run Recovery form, POLARA prepares to restart the run. But before you click Start/Continue in the Monitor, you must make sure that each container that was in process when the run was aborted is where POLARA expects it to be, based on the options you selected in the Run Recover form. For information on how instrument servers handle interrupted operations, refer to the instruments' interface guides.

To restart the run

1. Ensure that the containers that were in process when the run was aborted are where POLARA expects them to be, based on the handling you selected for the interrupted operations.
2. Click Start/Continue to resume the run.
Caution! If you resume a run without ensuring that the containers in process are where POLARA expects them to be, you may lose data, damage equipment, or spoil samples.

Finishing Up After a Run

After a run completes, the red light on the Run beacon will be lit, and the complete status indicator turns blue. Once you have confirmed the run has ended, remove containers and reagents from the system as necessary.

Tips and Tricks for Instruments in the Thermo integrated system

Cytomat

- If controlled CO2 level is necessary make sure to follow start-up process posted on the side of the unit
- Leave user access doors open when the unit is not being used for an extended time. This prevents an internal switch from failing due to moisture build-up inside the Cytomat.
- If the Cytomat is going to be used at a temperature around or below room temperature then the circulating chiller should be utilized. The temperature set-point on the chiller is automatically controlled by the Cytomat. Ensure that adequate coolant is in the chiller.

Inheco Thermoshake

- Controlled by WinPrep for Multiprobe:
(C:\Packard\MultiPROBE\bin\InhecoComm_Teleshake.MPT)
- Leaving the Thermoshake on the Evolution deck can be hazardous due to the height of the unit, especially when testing methods in WinPrep
- You cannot pick and place a microplate onto the Thermoshake with tips still on the head, there is not adequate travel in the Z drive

Multiprobe

- This instrument is not integrated with POLARA, but methods/scripts can be called up from WinPrep for the Evolution
- If the Multiprobe will be used in an assay coordinated by POLARA, it should be powered on and initialized before starting the assay as initialization takes about 5 minutes.

Stak

- In order to communicate with this instrument via POLARA you must have the serial port switch set to THERMO. To communicate with it via WinPrep for EP3 you must have the switch set to PE SYSTEM.
- This unit can be used to read barcodes, implementation is not complete. It only supports the narrower of the two barcode labels that SIDNET has.

Evolution

- Using the MPD manual control window to pick and place a plate will most likely fail; it seems that the XY coordinates for positions on the deck are not setup properly. However using the pick and place function within a method works fine
- To use a custom VB script create a new Function by right-clicking on the Start of Test line of the method, select the Runtime Parameters tab and click the Functions... button. Click Add in the new window, then Edit. You can give the script an appropriate name in the new window, provide a description of what it does and select the Type of script. The User Sub/Function type can be called as a step in the method. The Pre\Post Callback type can be called within a step to either precede or follow it.
- If you want to load less than 96 tips on the head then make sure to set the number in the tip load step.
- The Plate Replication button is somewhat like a wizard which guides you through setting up a process which involves pipetting from a source to one or more plates. However it is generally safer to build the method yourself in a Custom Procedure subroutine.
- If a method is to be called by POLARA then ensure that Pre Initialize and Post Test Processing are unchecked in the Config/Initialize Options window. Having Pre Initialize checked will cause the Evolution to go through the initialize process every time the method is called by POLARA and this includes attempting to initialize the PlateStak which can only be controlled by POLARA. Having Post Test Processing checked will cause the head to return to the home position after the method completes and this position could result in a collision with the CatEx arm.
-

CatEx

- Standard Shelf 1 has the top 7 positions reserved for delidding, the remaining 8 can be used for plate storage
- Ensure that the safety door is firmly closed otherwise arm power cannot be enabled on the CatEx
- When teaching instrument positions for the CatEx be sure to press the Update V3 File button before exiting the EasyTeach window
- This is the only unit in the system that has an on-board CPU with files that should be backed up. Here's how to perform the backup:
 1. Turn on CatEx main power, wait for startup to complete
 2. Prepare a backup folder using Windows Explorer
 3. Open ActiveRobot Explorer (Start/All Programs/CRS ActiveRobot/ActiveRobot Explorer)
 4. Select the conf folder, you should see ~11 files
 5. Press CTRL+A to select all of them

6. Click and drag the files to the backup folder you created in Windows Explorer. The cursor may pause at the edge of the ActiveRobot window, this is normal.

ELx405

- This unit has three fluid-related sensors: a liquid sensor, a liquid flow sensor and a vacuum sensor. The liquid and liquid flow sensors have been turned off for convenience but should be turned on if needed for a real assay. This will prevent issues from inadequate priming or miscalculation of the volume of buffer required for the assay.
- It is very important before running a full-scale assay to check that all nozzles on both the dispense and aspirate manifolds are not blocked or restricted. The best way to test this is to have the unit dispense >100ul of buffer into a plate and visually check that all wells contain the buffer and appear to have equal volumes. Then run an aspiration, preferably one that should remove all buffer from the wells. Again, visually inspect the wells to confirm that all nozzles are clear.

EnVision

- The plate handling forks are set to never retract. This prevents them from ever retracting when the CatEx is delivering a plate to the EnVision.
- POLARA's data handler will transfer reads from the EnVision data folder to the data folder on the POLARA pc as long as the Assay Name used in the Runtime Parameters dialog is less than 26 characters long.

Component Startup Checklist

1. Catalyst Express
 - Turn on power
 - Press Arm Power button
 - Ensure arm is in park position
2. Cytomat

(For full temp and CO₂ controlled operation this unit requires 5-8 hours lead time to complete the auto-start routine)

 - Turn key to power on
 - If testing system only, press i/alarm button when buzzer sounds and close doors, skip remaining steps
 - If necessary, fill connected refrigerator unit with brine, level should be 5-10 mm from upper edge
 - Open main valve on CO₂ tank, do not adjust regulator valves at this point
 - Power on refrigerator unit
 - Leave Cytomat doors open
 - If humidity is desired fill bottom of incubator with clean distilled water up to the indicator line
 - On the Cytomat, set the desired temperature by holding the oC button
 - Set the desired CO₂ level by holding the % CO₂ button
 - Hold the auto-start button for 5 seconds
 - Close both doors
 - Don't open the unit until the display shows the desired temperature and %CO₂ values otherwise the startup routine will start over.
 - Adjust valves on CO₂ regulator to adjust outlet pressure to ~ 15 psi
 - See Cytomat 2 Operating Instructions P. 47-50 for more details.
3. Evolution/PlateStak
 - Power on both units
 - Make sure data switch under Multiprobe is turned to Thermo position, this allows Polara to communicate with the PlateStak, the PlateStak requires no further attention other than loading of microplates
 - Make sure tipload station, tipwash station, bird-feeder reservoir and all plate positions have the right holders and labware in place
 - If using tipwash station make sure source and waste reservoirs and filled/emptied as appropriate; power on peristaltic pump and ensure pump speed matches that used during testing. Also, this pump can sometimes fail to prime on the waste line which causes the tipwash reservoir to overflow, a test run is highly recommended unless the reservoir has recently been used successfully
 - If using bird-feeder reservoir make sure it is clean and filled with necessary reagent
 - Load clean tips
 - Scripts to be used must be in the folder C:\EvolutionScripts

- On the Evolution PC, startup the Evolution pipe server, clicking OK at the first window
4. ELx405
- Power on unit
 - Use on-board routines or software interface to make sure all dispense and aspirate needles are functioning, use stylus, rinse/soak routines, and/or sonicating self clean as necessary
 - Fill/empty reservoirs, make sure the right buffer is in the reservoir called in the wash method (A/B/C/D), also make sure that a pre-wash prime is included if a different buffer is selected mid-run
5. Envision
- Power on unit
 - Make sure that the read protocol is in the Protocols/Users/Polara folder in the Envision software
 - The save path for the protocol's data output must be C:\EnvData\ - On the Envision PC, startup the Envision pipe server, clicking OK at the first window

Labware Preparation Checklist

- Make sure labware is automation friendly or preferably already been tested on the units that will perform assay operations
- Ensure that labware is loaded in correct orientation; generally labware will be loaded into the Cytomat and/or the PlateStak which are both labeled to indicate orientation. If labware is to be loaded directly on the Evolution deck then make sure it is in the same orientation as the plates that will come from the Cytomat and/or PlateStak
- Barcodes should be on the top long side of the plate, i.e. if plate is held with A1 to the top and left then barcode should be on the “north” edge of the plate

Component Shutdown Checklist

1. Catalyst Express
 - Ensure arm is in park position (Use the Teach tool to park arm if necessary)
 - Before turning off the power, shutdown the Catalyst Express' operating system by holding the Home button and pressing/releasing the Pause/Continue button, wait for the "System Halted" message on the display before continuing
 - Turn off power
2. Cytomat
 - Turn off refrigerator unit if it was used
 - Close main valve on CO2 tank
 - Turn off Cytomat and leave doors open (letting the interior dry out seems to prolong the life of an internal sensor of the Cytomat)
3. Evolution/PlateStak
 - Close pipe server on Evolution PC
 - Turn off PlateStak
 - Turn off peristaltic pump
 - Turn off Evolution
 - Empty and clean bird feeder reservoir if it was used
 - Place a cover on the tipwasher reservoir to keep it clean
 - Remove and discard used tips (keep the box and lid)
 - Empty tipwash waste reservoir, fill source reservoir with distilled water
4. ELx405
 - Turn off washer
 - Empty waste reservoir
5. Envision
 - Close pipe server on Envision PC
 - Turn off reader

Tips on Method Generation

- Basic requirements are a series of “unit operations”, ordered in time sequence.
- If you want “containers” to return to the same position in the instrument that they came from then check “Reserve Nest”. Note that you must check it for every instance of that instrument in your method otherwise the order will get mixed up or the instrument may attempt to place a container where one already exists.
- Timings for load/unload and step duration should be as accurate as possible, with extra time allowed if in doubt
- When timing between 2 steps is critical set a 1 second buffer time in the first step, this forces the scheduler to start moving the container to the next step within 1 second. Leaving it at 0 seconds means it is not critical and the scheduler may leave whatever time works best for the overall sequence. Note that setting a buffer time can make scheduling many samples difficult due to the inflexibility.
- Schedule the method using 1 sample and run it. A log file will be generated which can be used to tighten up the timings. This is a good idea to avoid having the CatEx waiting for a unit that has already completed its operation. Also, when estimating the total run time for a screen having these times accurate improves the quotation.
- Once the timings have been tightened for 1 sample, the method can be scheduled for an increasing number of samples, say 2, 5, 10, 15, 25, to whatever level is expected to be required. If the schedule of 2 samples contains white gaps or downtime, all the gaps can be added together and the time in seconds used as the sample stagger, rescheduling should give a sequence with no white gaps. If not, iterations of this process should make the timings per sample as uniform as possible.

Teaching Positions

1. Power on arm
2. Load Polara
3. When CatEx is ready, press Arm Power button
4. In Polara open LOR772 workspace (or applicable workspace)
5. Select Workspace/Change Physical Settings
6. Press Yes
7. Press Teach
8. Press Unpark Robot, press Yes, wait for arm to complete initialization
9. In new window, select instrument to teach and set the instrument type in the box on the left, press Launch Teach
10. Select position to be re-taught and select the Nest position in the Teachable Locations area of the window, press Move To Location
11. Gripper should be open, if not press Gripper Open button
12. Place teach tool in position with corner dot up and towards the back of the gripper.
13. Press Gripper Close, the tool should be dripped in the upper holes, in landscape orientation when facing the grippers head on
14. Use the Plate Motion buttons to move the gripper into the desired position, note that you have to hold the CTRL keyboard button to enable actual movement by the arm
15. When the gripper is in the perfect position (ha!) press the Re-Teach button, and press Yes to write the coordinates permanently to file
16. Test the position repeatedly to confirm and also test moving the tool from another position to the one being taught. Even better is to move it from a different instrument. Try to teach the position so that the arm doesn't force the plate down further than physically possible or end up too high and slightly drop the plate.
17. When done, press OK, and press Yes to close the Teach application
18. Before pressing Exit in the EasyTeach window press Park Robot. Press Yes to confirm if the arm is in a safe position to move to park.
19. Press Exit to close the window
20. Press Close to exit the Physical Settings window