This tutorial is an introduction to using the ARI Crystal Gryphon protocol. It is not intended to as an advanced text on use of the robot.

Guide to the Art Robbins Gryphon (and Phoenix) Crystallization Robot at UAB (CBSE)

The Art Robbins Phoenix Crystal Gryphon is a small, fast, efficient and affordable crystallization robot for setting up small drop volume screening trays. This document provides an introductory tutorial for use of the Crystal Gryphon in the UAB X-ray Crystallography core facility. A more in-depth manual of the operating procedures (including calibration, etc) can be found in the crystallization core. The personnel in charge of the core should be consulted before doing any extreme procedures to the robot or the computer.

The Crystal Gryphon is shown here:

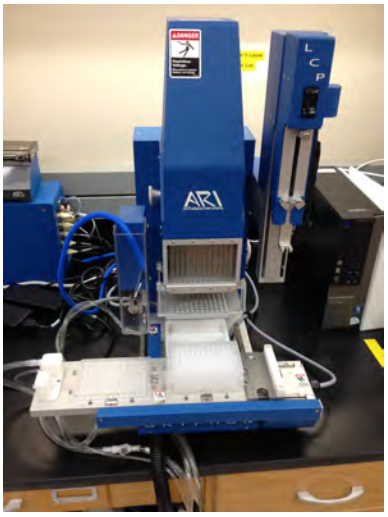


Figure 1 ARI Crystal Gryphon Robot AT CBSE

The Robot is modular in that there are external components that are necessary to make it function correctly. These include

- Computer (to the right, above) to control operations
- Vacuum/compressed air source to dispense solutions (blue box back left of the robot)
- Liquid pumps to dispense wash solutions (see below)

Figure 2 (left) Water sources and (right) waste carboy and pumps

With many moving precision parts, the collective unit is a high accuracy device that requires much care and attention to detail in order to prevent damaging the unit. For example there are two types of needles, a set of 96-dispensor needles for the 96-well block plates (~$100 per needle to replace, or ~$9,600 for the entire block) and the protein nano-needle (~$300). Each require free unobstructed movement and cleaning procedures to see that they remain un-clogged.

Safety

It is up to the operator to be the first line of defense in preventing an accident. The robot has many moving parts and collision with ill-placed body parts or inanimate objects can cause harm to the operator or the robot. Keep your hands clear of the machine whenever it is running a protocol (including the wash step) otherwise you risk bodily injury. This is the first concern. Second, damaging the robot and can be costly and result in downtime. Should you recognize an issue of danger, the protocol can be aborted with the STOP button or the robot could be powered down simply by turning the Tripp-Lite power strip to the off position. The latter is more timely.

Materials needed before running a typical protocol

- 96(1-3)-WELL SHALLOW FORM CRYSTALLIZATION PLATE
- HT CRYSTAL SCREEN
- PROTEIN
- NANO PURE WATER FOR WASHING 1 GALLON (MINIMUM 1 LITER/RUN)
- CRYSTAL CLEAR 3" TAPE (FOR SEALING THE CRYSTALLIZATION PLATE)
- 3” ALUMINUM TAPE (FOR SEALING DEEP WELL BLOCK)
- 0.2mL PCR-STYLE MICROFUGE TUBES (FOR PROTEIN)
- FRESHLY PREPARED 10% ZYMIT SOLUTION IN 0.2mL PCR-TYPE TUBE WITH NO LID
- FRESHLY PREPARED 0.02*M* EDTA SOLUTION IN 0.2 mL PCR-TYPE TUBE WITH NO LID

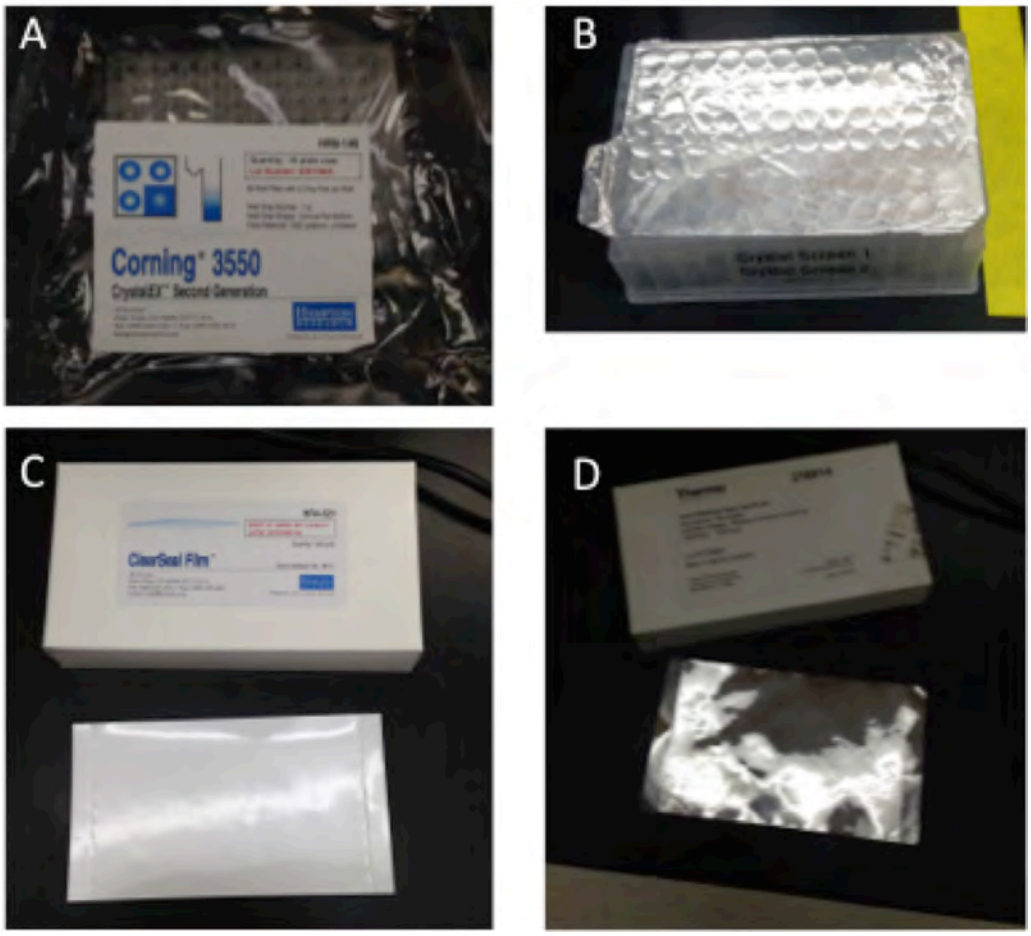


Fig #. Examples of (A) crystallization plate, (B) HT crystal screen in 96 well block plate, (C) Crystal Clear sealing tape and (D) aluminum sealing tape.

WATER

The robot works with external water sources so the user is required to maintain proper amounts of water in the clean water carboy and make sure that the waste water carboy has sufficient room for waste. As Shown in the figure #. The clean water carboy sits atop the bench with the waste on the floor next to the pumps.You should always confirm the following:

- There is **at least 1 gallon of clean water** in that carboy
- There is **at least 1 gallon empty space** in that carboy

POSITIONING PLATES ON THE DECK

The crystallization plate and the screening plate need to be properly positioned on the deck. Typically the crystallization tray is placed on the deck in position 1 and the plate containing the screening solutions in position 2. These are typical positions but should be verified with the protocols that you are using. Protocols are discussed later. It is critical for the robot that you pay extremely close attention to where you put the plates and that they are secured properly. Small errors in placement can cause thousands of dollars in damage and subsequent downtime. It is also critical that you place the crystallization plate type on the deck that corresponds with the protocol to be used. Crystallization plates are designed with different drop positions, etc. Using the wrong one can cause collisions to occur and subsequently damage and downtime. So to reiterate:

- Correctly identify the crystallization plate
- Correctly identify the screen buffer block
- Place the crystallization plate (in Position "1") and screen buffer (in Position "2") correctly.

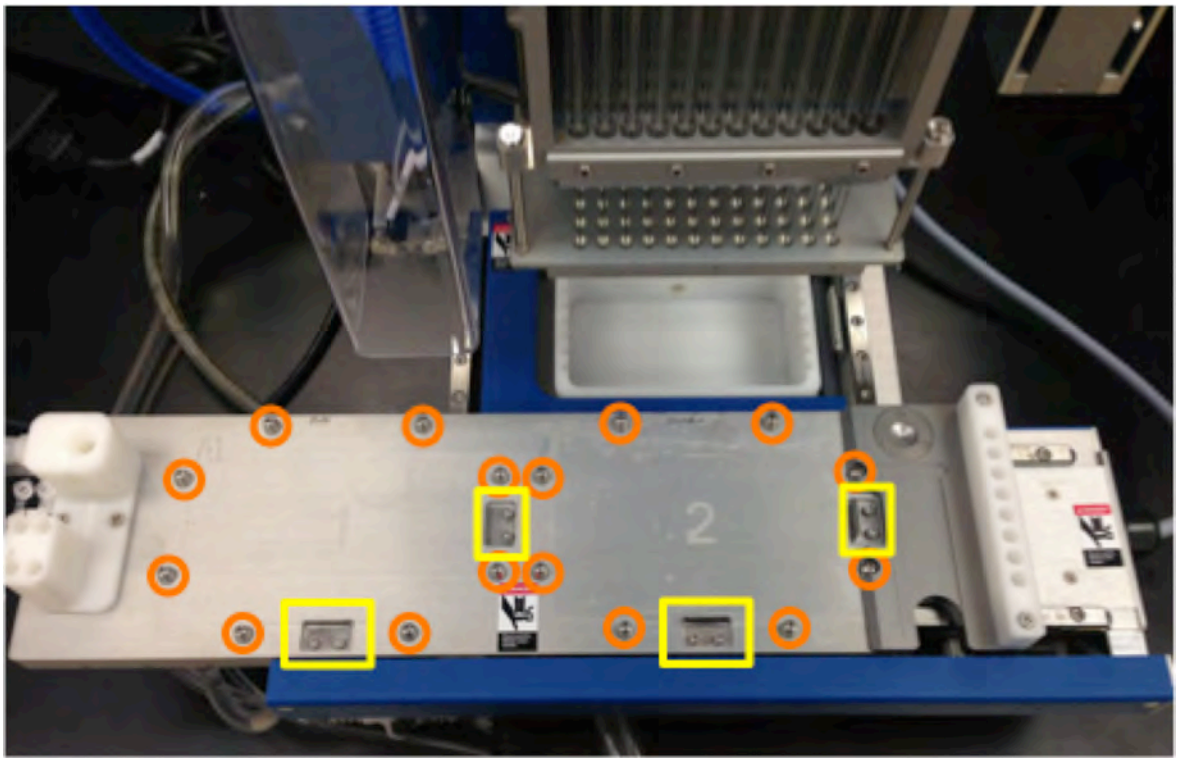


Figure #. Picture of the Deck. Plate positions are labeled 1 and 2. Positioning pins are circled in orange and tension clips in yellow. Typically the crystallization plate is placed in position 1 and the screen is in position 2.

Plates should be placed inside of the positioning pins and tension clips with the A1 position of the plate to the top left, as in he picture here:

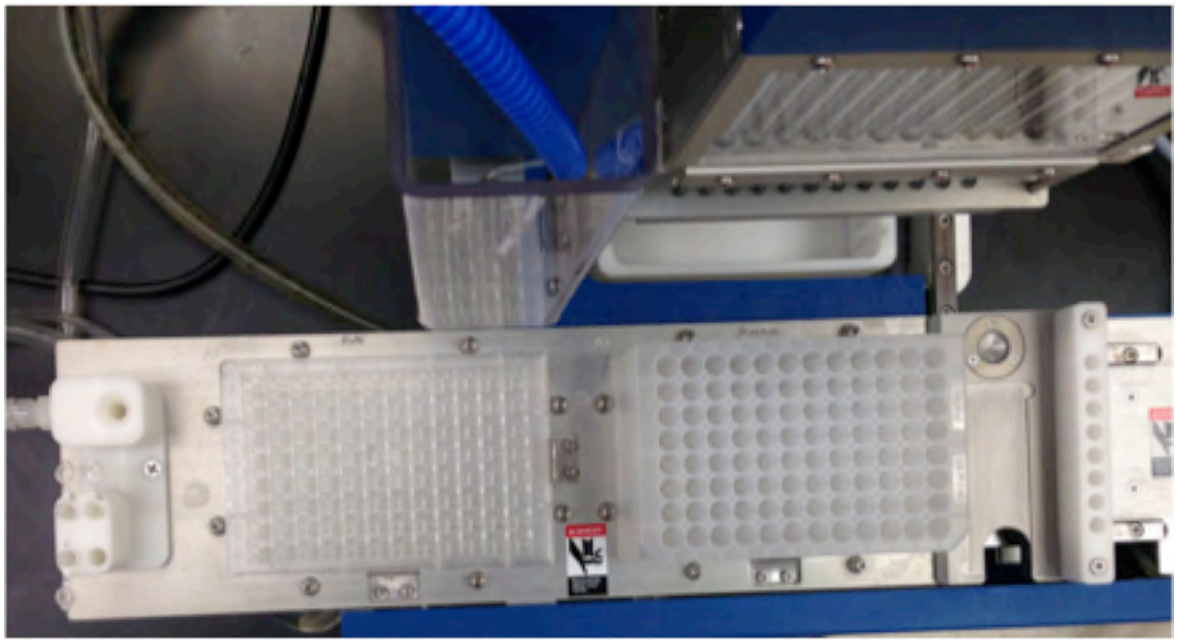


Figure #. Deck with plates positioned correctly

It can't be understated that placement of the plates is the biggest source of error and one of the most repetitive and costly.

Positioning protein on the deck

You can have place up to 200 µL each of four different protein solutions on the deck. Proteins are placed in blocks 10 and 11 (positions "10 A1, 10A2" and "11A1, 11A2"). Proteins are placed in 200 µL PCR tube with lid cut-off. The lid is removed to eliminate any

Figure #. There are four slots for protein on the deck. Three positions are occupied in this picture. The used are 10 A1, 10A2 and 11A1. 11A2 not occupied. The schematic is shown to the right. Nano need wash staion is on top.

DOUBLE CHECKING PLACEMENT OF PLATES AND PROTEINS

Now that you have everything placed and are ready to proceed to the computer-aided part of the experiment, take a second and double check that everything is in the right position. You may be glad that you did!

TURN ON THE ROBOT

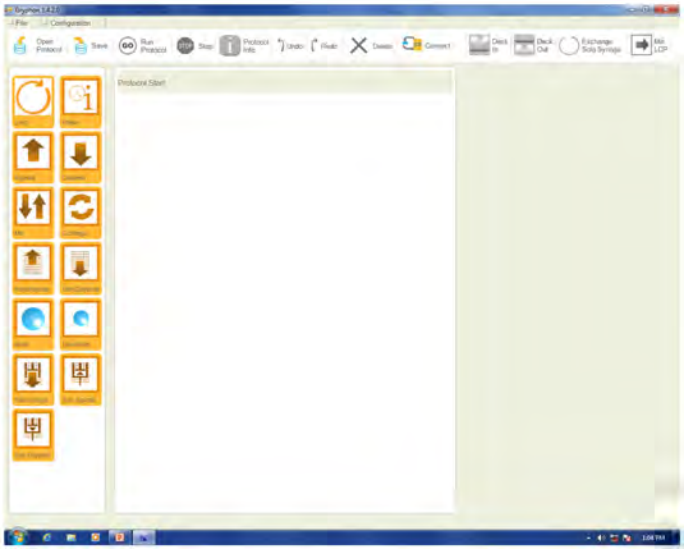
Locate the 4-outlet TRIPP-LITE power strip and turn the switch to "ON" position on the power-strip. This power-strip energizes all the components that are needed for Gryphon to function except for the computer. The user may hear the sound of a motor running which is normal. A green light on the left-side of the Gryphon head assembly will illuminate indicating all the components are energized.

Gryphon Power Strip

USING THE COMPUTER TO CONTROL THE CRYSTAL GRYPHON

The computer to the right of the robot controls the Crystal Gryphon.

In order to sync the computer with the robot, double-click on Gryphon 1.4.2.0 icon on the computer Desktop .
This launches the Gryphon program, and the Gryphon GUI will pop-up:

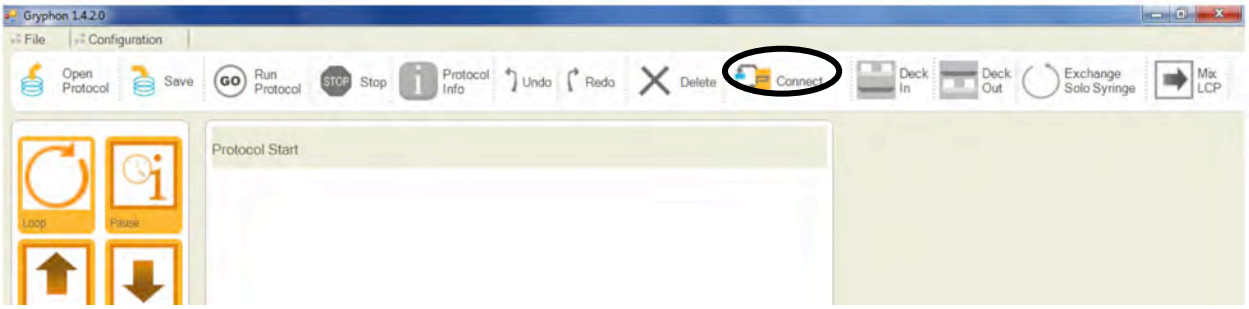


In the GUI, click the "Connect" Icon. This connects the hardware with the computer. Motors will be initialized. So don't be alarmed with the noise.

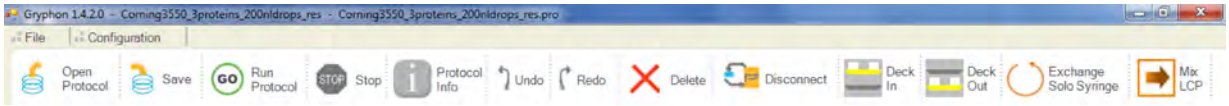
The Deck and the Nano Needle will move so make sure that the area around the deck is clear. Also, during this time, Connect will change to Starting.

After initializing, "Starting" will change to "Disconnect". At this point, the computer and the Crystal Gryphon are synced and the computer has control of the robot.

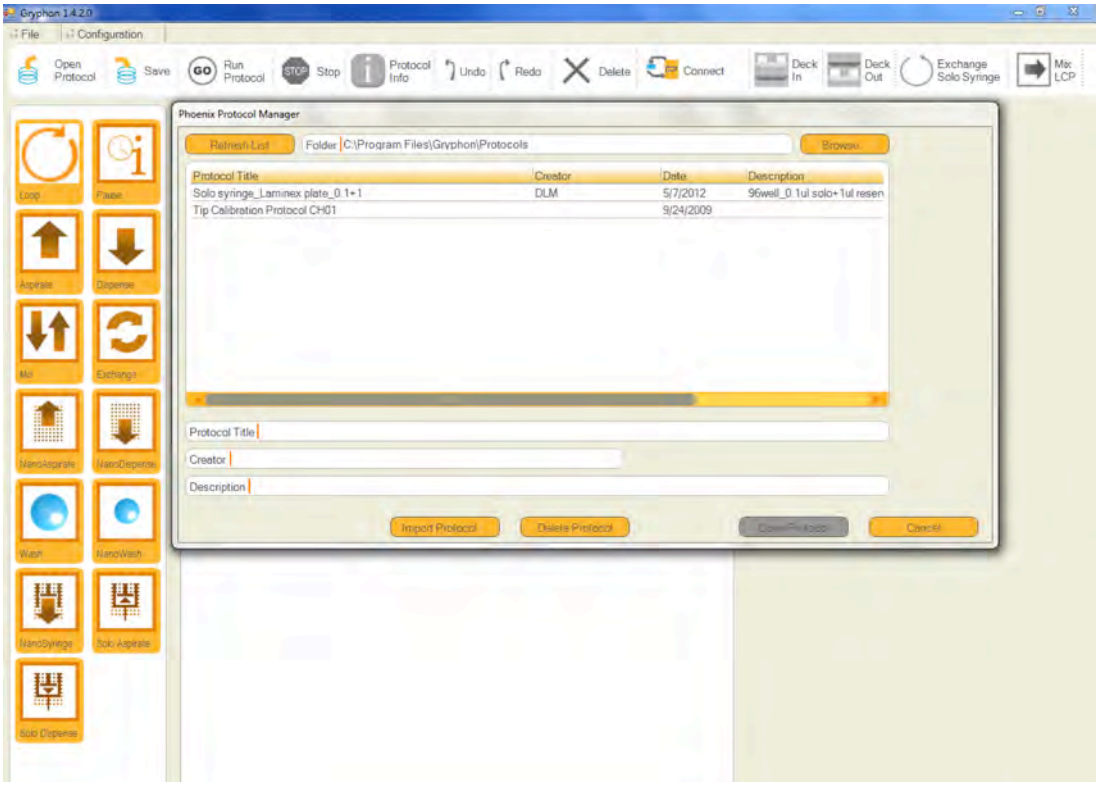
Click Connect:



Once they are synced the radio button changes to "Disconnect" :

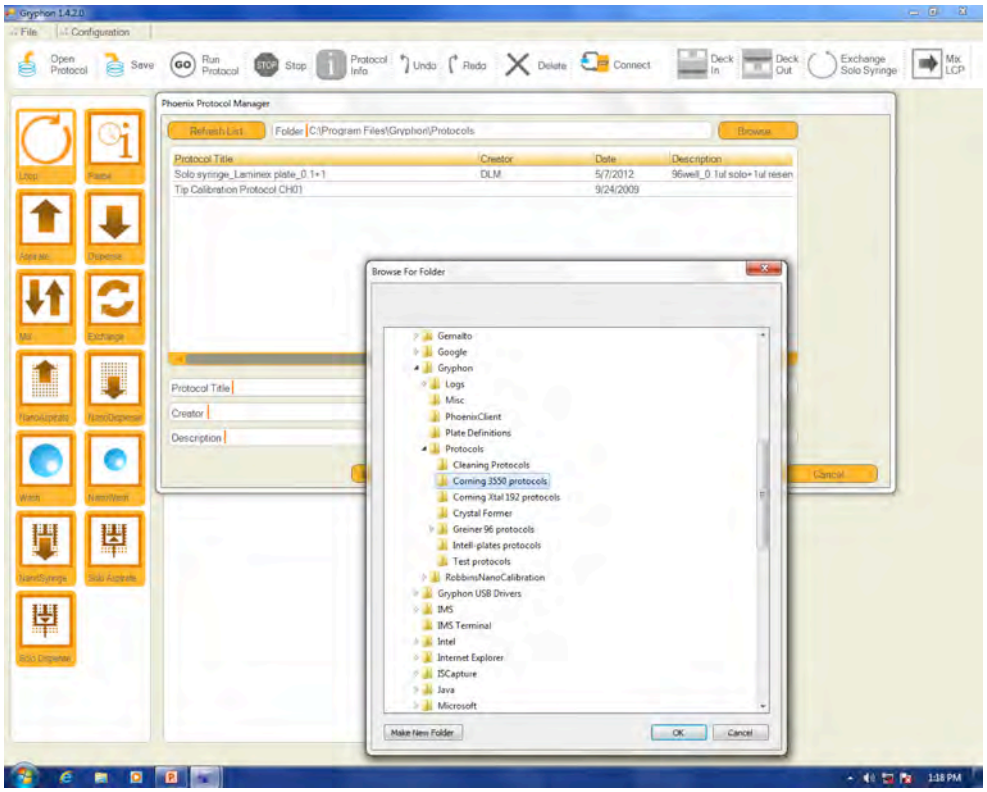


Once the computer and Gryphon are synced, you can proceed to open a protocol. To do this click on the “Open Protocol”

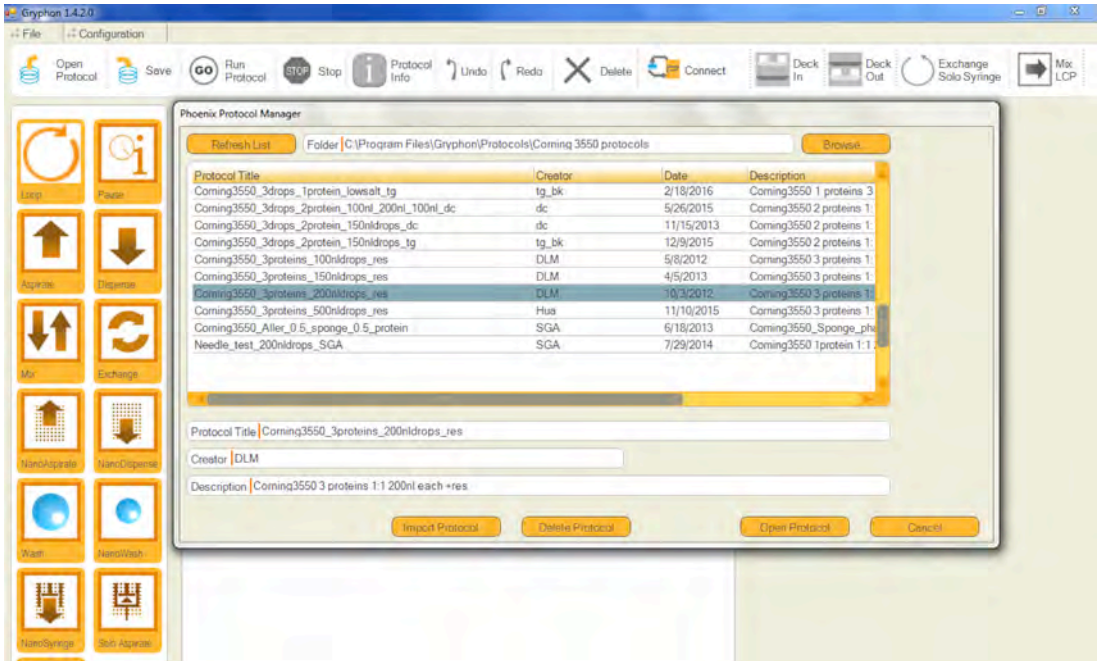


button or select it from the File dropdown menu. The following menu will pop-up:

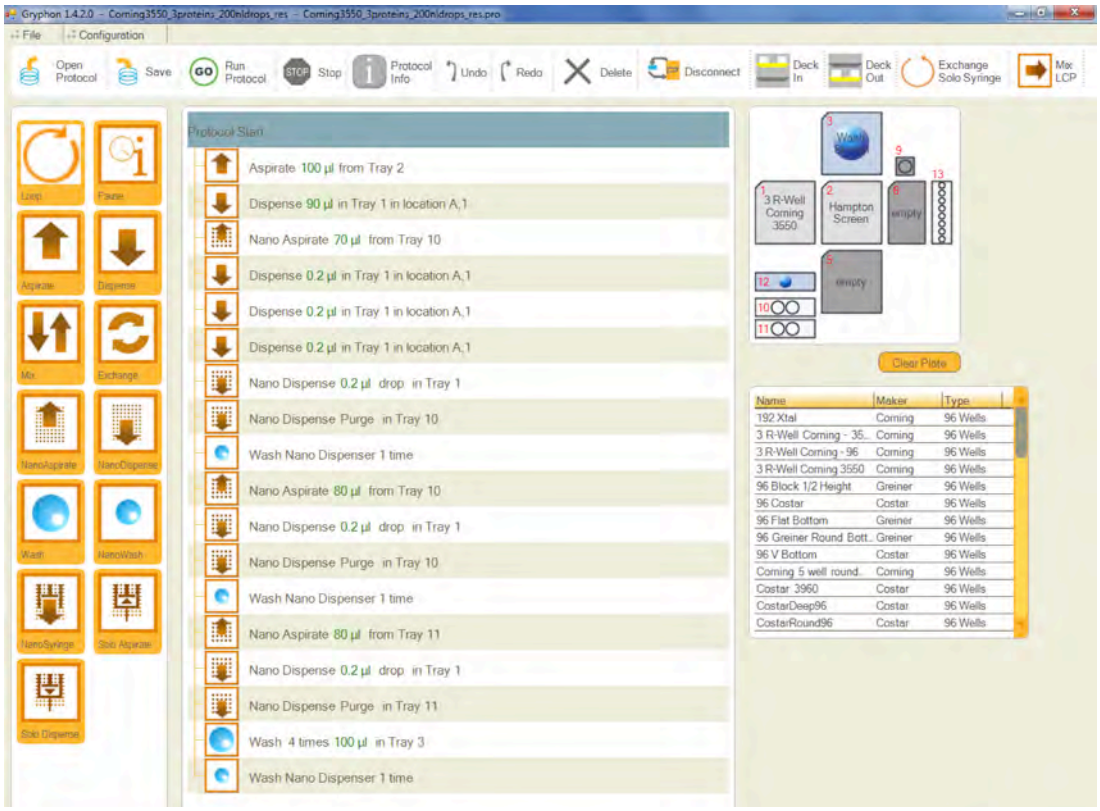
You can begin to navigate through the protocols by then clicking “Browse” in the new window. The protocols are stored within subdirectories found in C:\Program Files\Gryphon\Protocols . The subdirectories are separated according to crystallization plate type in addition to special cleaning protocols. A clean instrument is a must in order to maintain the needles in good working condition.



Navigate to this this directory and then into the Corning 3550 protocols directory. It should look like this:



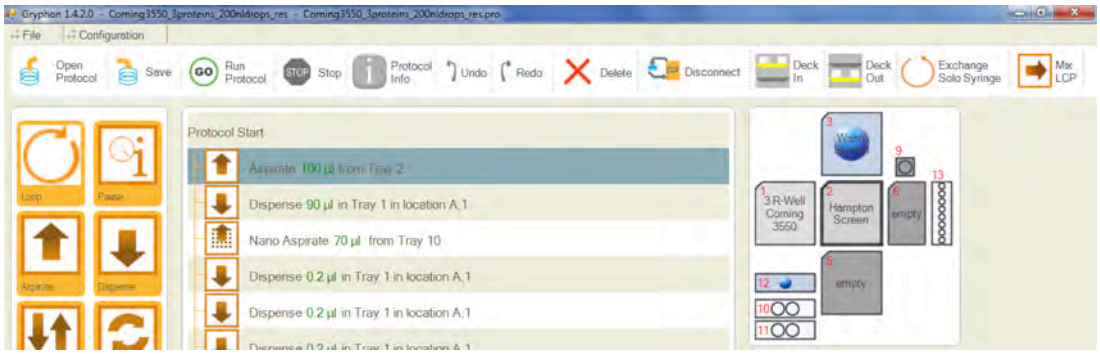
Select the protocol named “Corning3550_3proteins_200nldrops_res”, followed by clicking on the “Open Protocol” radio button. This will load the protocol into the GUI Window which will look like this:



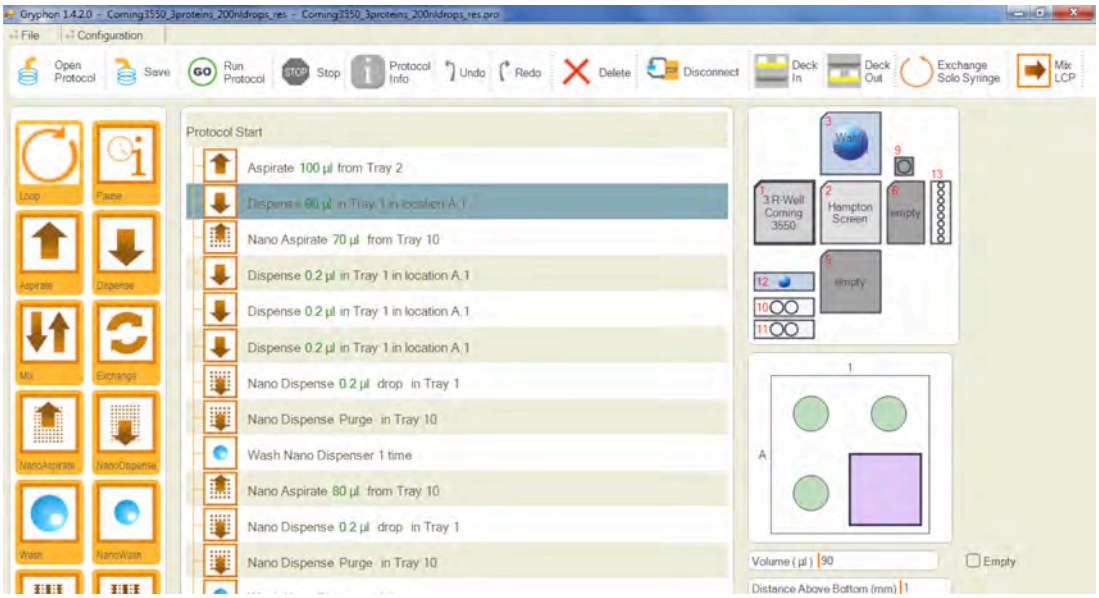
The protocol is list of lines that are called out to the robot. Each line consists of an operation, a component, a movement and is programmed with care so be careful to maintain the procedure as it was when you started it. If you have questions about a particular procedure, just ask the staff in the CBSE. Notice in the upper right quadrant, there is a schematic of the robot and it lists in the positions of the 3550 crystallization plate and the screen.

- **Position 1**: Corning 3550 plate
- **Position 2**: Hampton Screen in Deep Well Block
- **Position 3**: Wash Tray
- **Position 10 A1, A2**: At least 50 µL of protein each
- **Position 11 A1**: At least50 µL of protein
- **Position 12**: Nano wash station

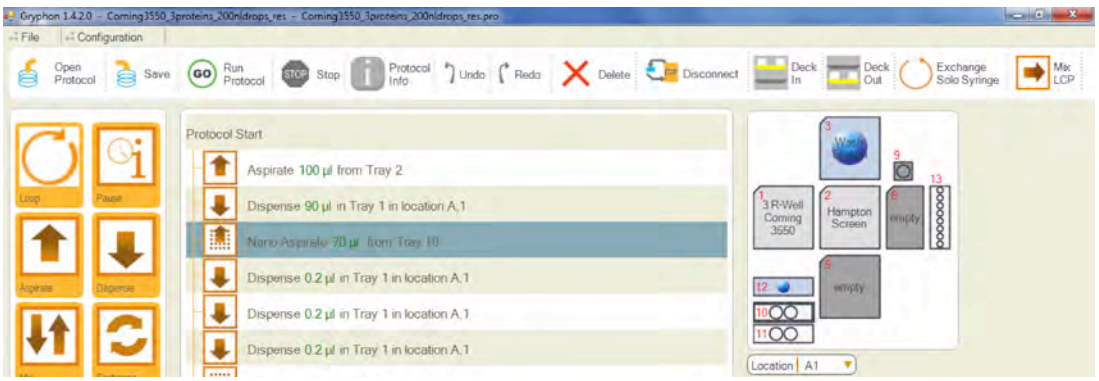
In the protocol, you will see procedures labeled: aspirate, dispense, wash and nano aspirate, nano dispense, nano wash. The first three refer to procedures with the 96 block of needles that handle the screen, the last three are commands for the protein needle. The first line here “Aspirate 100 µl from Tray 2” tell the robot to take 100 µl in the 96 needle block from tray 2 (the screening plate):



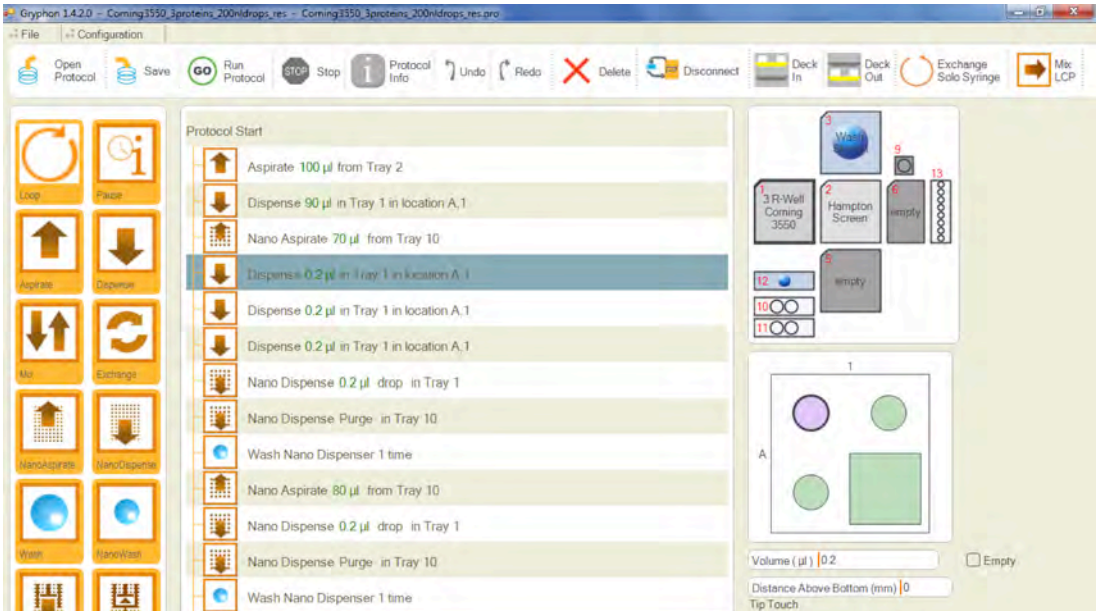
Line 2 tells the robot to dispense 90 µl into the reservoir of the crystallization plate. Here, the crystallization plate in position 1 is highlighted, as is the purple box. The three circles represent the three crystallization drop positions in the Corning 3550 plate. The box is the reservoir:



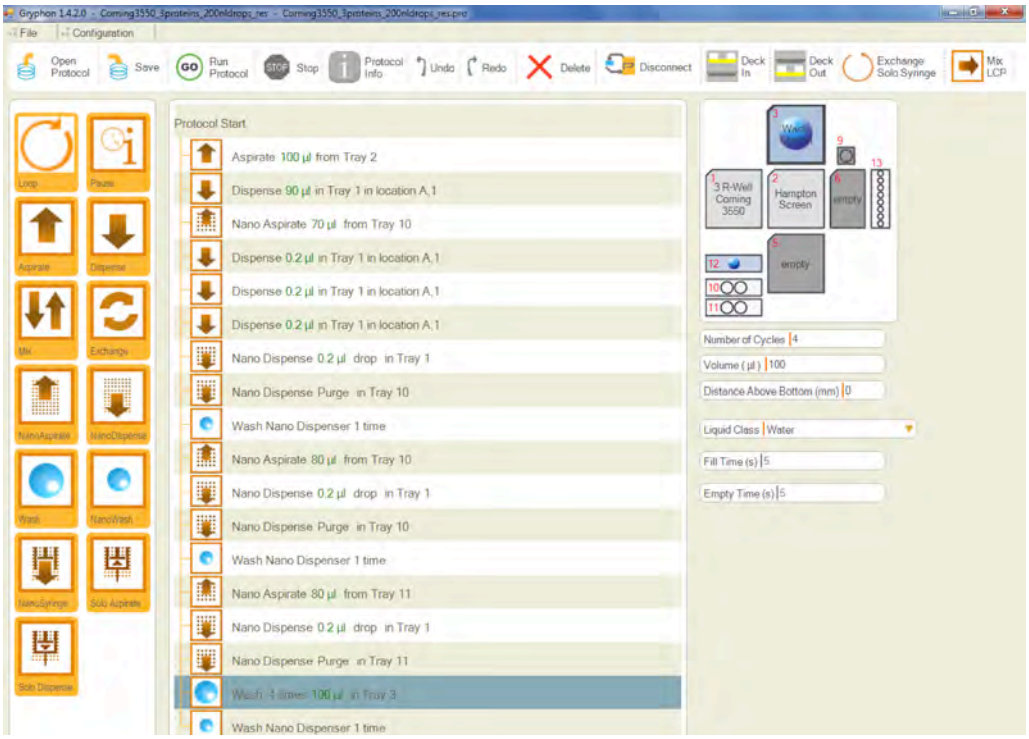
This is followed by the first action with the protein needle. In the example below the protein needle will take 70 ml from the protein in the left position of block 10 (notice that block 10 is highlighted). We know it is the left position because below that the location is designated “A1”:



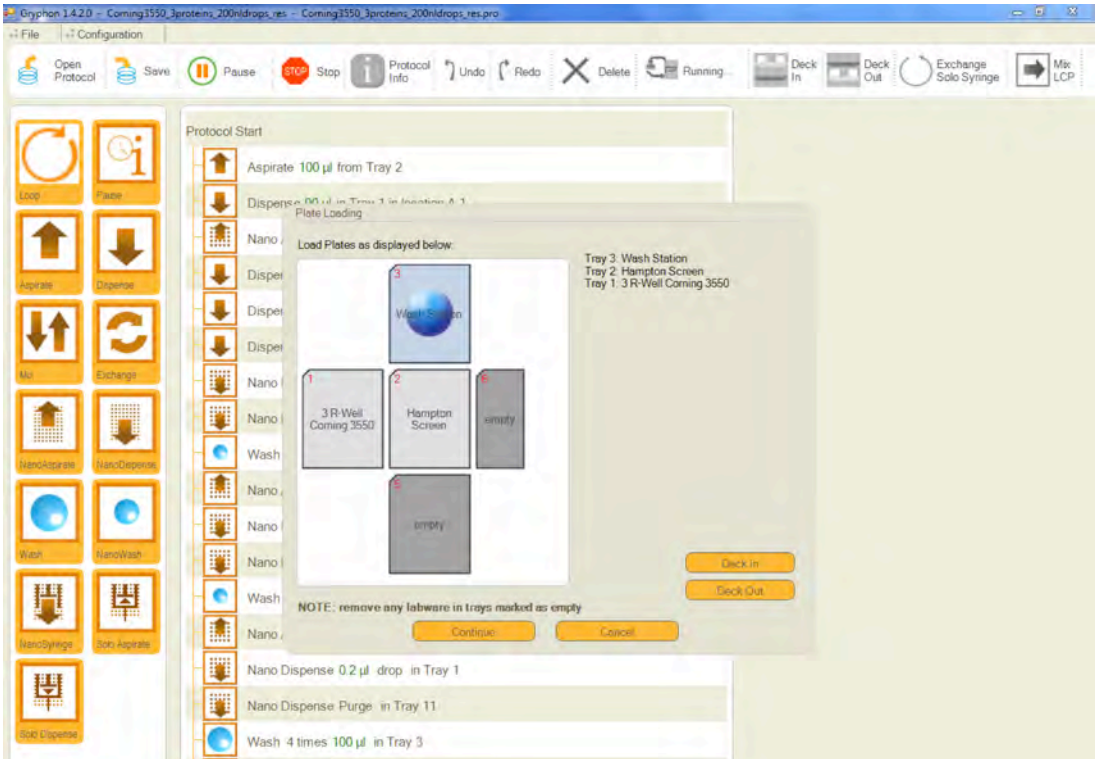
You can then see that the 96-needle block then dispenses into each of the three drop positions. In the highlighted step here, notice that the crystallization plate (block 1) is highlighted and the drop position 1 is highlighted and shaded in purple. In this step reservoir solution is dispensed in that position. The next two lines tell the robot to dispense to drops 2 and 3 (top right and bottom left), respectively:



The protocol will continue until protein has been dispensed from each position block 10 A11 to drop 2 and the block 11 A1 to drop 3. At the conclusion of the run the protocol dictates that all of the needles are washed thoroughly, then will conclude:



After you have checked once again that the experiment is appropriately set-up and the robot is unobstructed. You can begin the run by clicked on the GO- Run Protocol radio button. This will prompt the following window:



If you are ready, you can hit continue and the protocol will commence. Good Luck!!!!!

If you perceive a danger to you or the instrument, you can abort by hitting STOP. Turning the power off is another option.

This is a simple tutorial. If you need further assistance, please see the CBSE staff.