



BioFrontiers Institute
Advanced Light Microscopy Core
UNIVERSITY OF COLORADO

Innovation without boundaries

Plateworks 6.10

Manual

Automation Pipeline for Opera Phenix

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advancedimaging.colorado.edu

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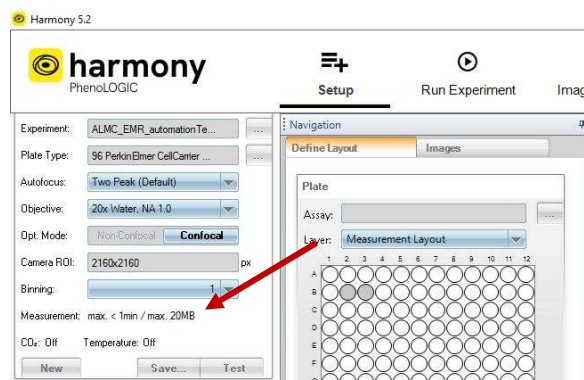
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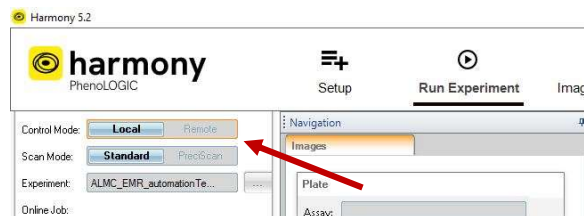
Loading a pre-made automation to image plate(s) with one experiment / same imaging settings

Set up Harmony experiment and load plate(s)

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plate(s):
 - a. For fixed plates: Insert plate(s) into the fixed plate rack, starting from the bottom. Position A1 in a well plate should be front-left of the plate rack or closest to you (next to the 1 sticker).
 - b. For incubated plates: Remove the rack by grabbing the handle on the top of the rack and rock it back and forth/pull toward you until it unclips from the holder. Insert plate(s) into the incubated plate rack, starting from the bottom. Position A1 in a well plate should be back-right of the plate rack when it is inserted into the holder (next to the 1 sticker).
2. In Harmony, in the *Setup* tab, load and set up your experiment, including the wells with your sample. Save it. **Write down the estimated time to run this experiment** (found near the Save button).



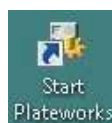
3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

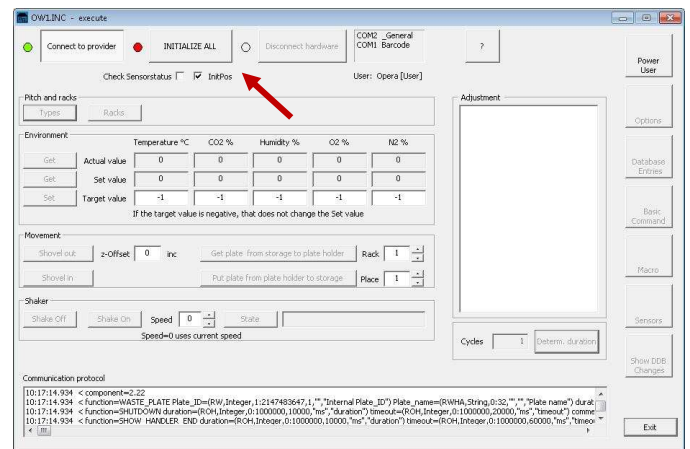
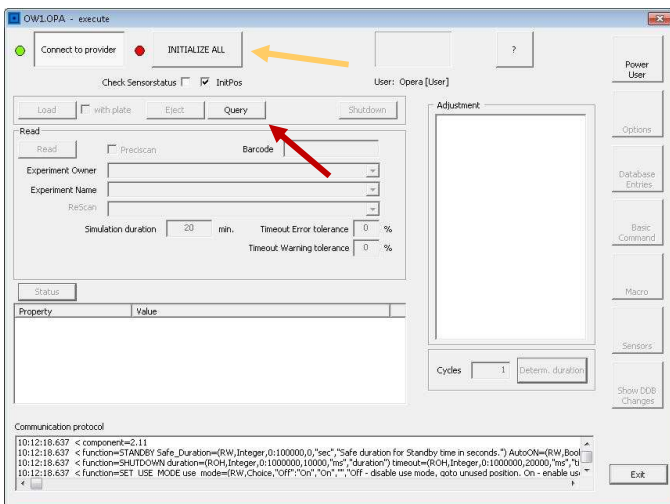


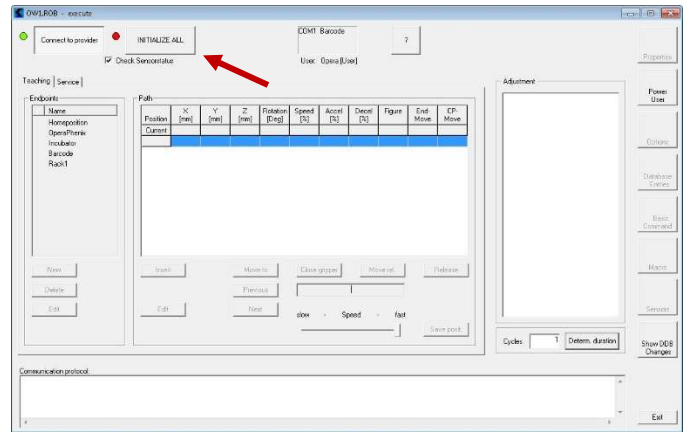
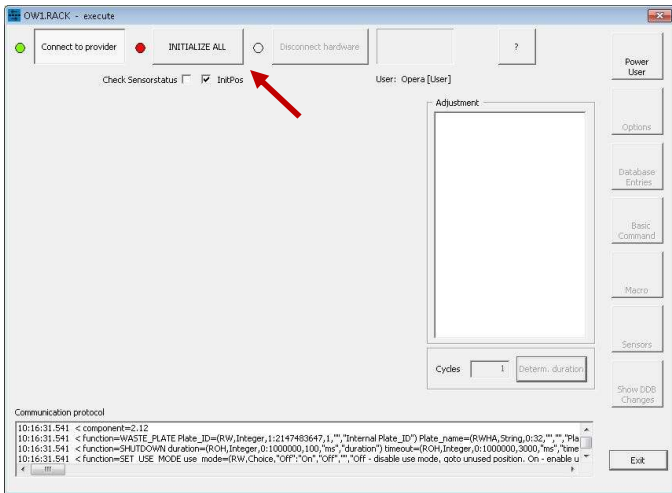
Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click **Service**.

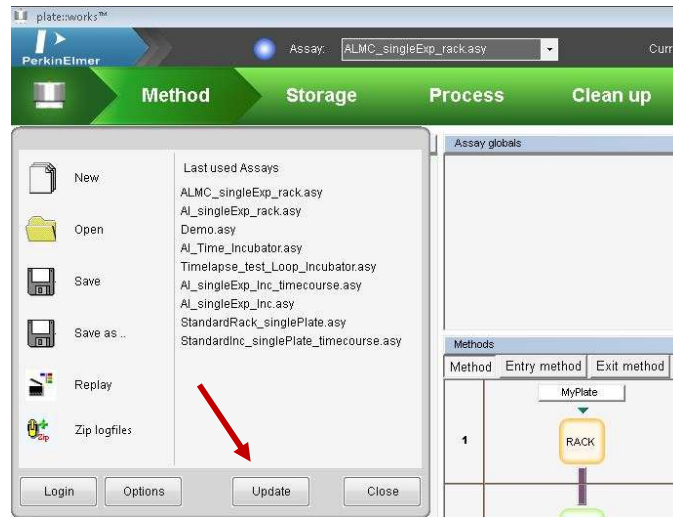


8. Right-click on **OPA** → **Direct Mode On**. In the pop-up window, click **Query** and then **Initialize All**. The loading door on the top of the microscope should open and close.
9. Right-click on **ROB** → **Direct Mode On**. In the pop-up window, click **Initialize All**. The robot hand should open and close.
10. Initialize the component where your plate(s) will be located/stored:
 - a. If using the incubator, right-click on **INC** → **Direct Mode On**. In the pop-up window, click **Initialize All**. The incubator should beep a few times.
 - b. If using the rack, right-click on **RACK** → **Direct Mode On**. In the pop-up window, click **Initialize All**.





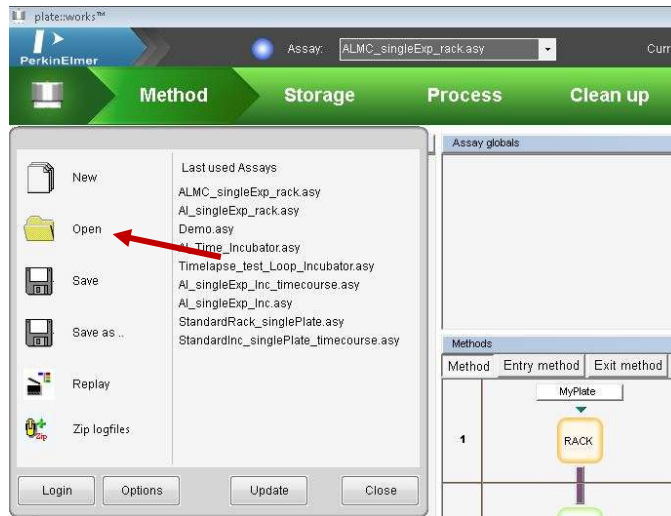
11. In the PlateWorks program, at the top-left of the screen, click the Phenix logo and then click *Update*. This will make sure that your new Harmony experiment is available to the automation software.



Load a pre-made automation

12. In the PlateWorks program, at the center-top of the screen, the Assay drop-down menu will load the most recent automations OR at the top-left corner of the screen, click the Phenix logo, and use *Open* to find your previous automation.





13. At the top left of the screen, click the *Method* chevron. Above the flow diagram, there should be a white rectangle with a plate name (e.g., *MyPlate*). Click this rectangle.
 - a. Adjust *PlateType* with the drop-down menu.



Method	Entry method	Exit method	Check
1	MyPlate RACK		MyPlate ● GET_NEW_PLATE
2	BCR		● READ_BARCODE
3	OPA		● Choose your experiment / imaging settings. ● Input the estimated time for the experiment (from the Harmony software). ● EXECUTE Owner: Palmer Lab Method: ALMC_BMR_automationTest\M EstDur: 1 min
4	RACK		● WASTE_PLATE



14. In the flow diagram, there should be an *OPA (component) | Execute (function)* step.
 - a. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: "Load Harmony experiment into PlateWorks".

- b. Adjust *Estimated duration* to reflect the estimated time written down from Harmony with a little extra time such that the measurement would not be aborted under normal circumstances (i.e., if the estimated time in Harmony was 20 minutes, enter 40 minutes; if the estimated time in Harmony was 60 minutes, enter 90 minutes). This value is for a timeout function to allow the automation to continue progressing if the plate fails to image correctly.
- c. *Timeout warning tolerance* = -1 (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
- d. *Timeout error tolerance* = -1 (default amount) or 110% (give error after experiment takes 10% more time than expected)

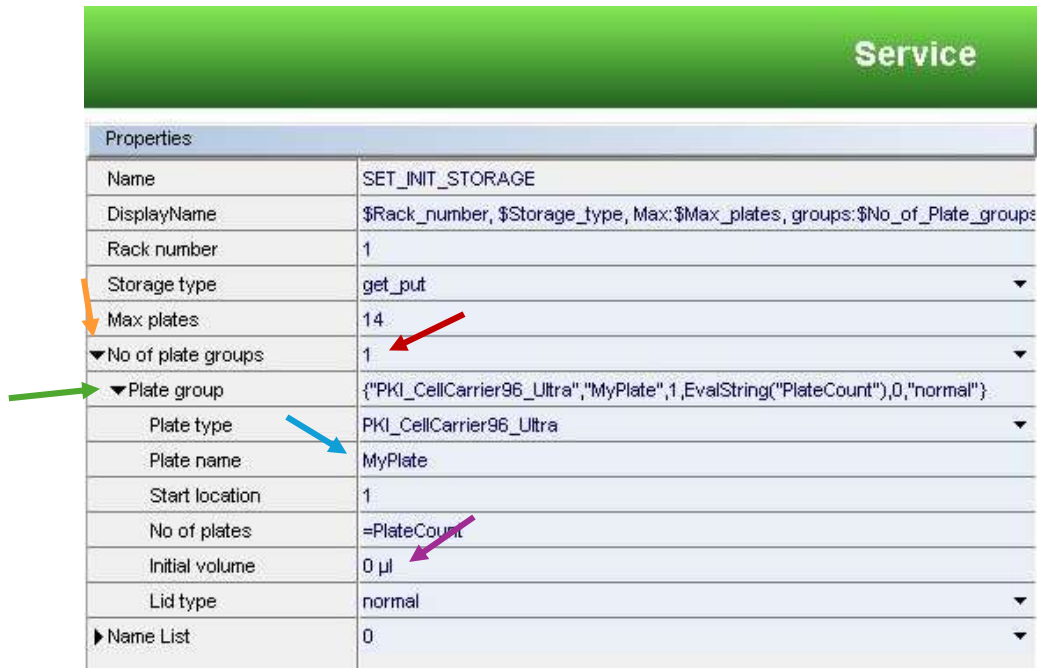
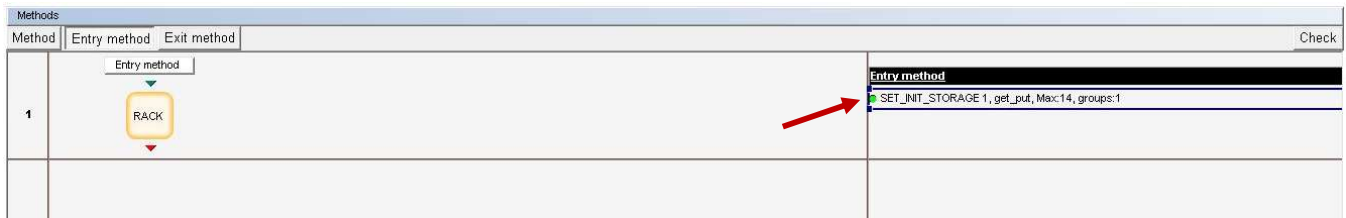
Methods			Check
Method	Entry method	Exit method	
1	MyPlate RACK		MyPlate ● GET_NEW_PLATE
2	BCR		● READ_BARCODE
3	OPA		● Choose your experiment / imaging settings. ● Input the estimated time for the experiment (from the Harmony software). ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTestVM EstDur: 1 min
4	RACK		● WASTE_PLATE

Service	
Properties	
Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_duration
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestVM
Remark	USE THE DIRECT MODE TOOL TO REFRESH THE LISTS!
Estimated duration	1 min
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

15. Just above the flow diagram, click the top *Entry method* tab.

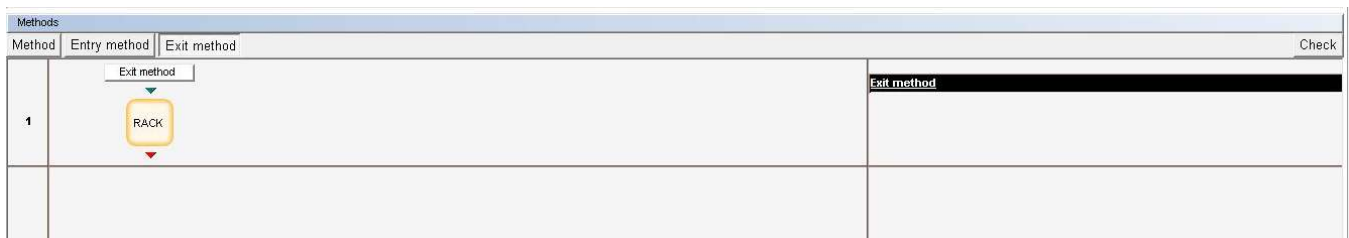
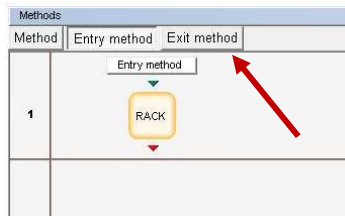
- a. Click the *SET_INIT_STORAGE* function.
 - i. *No of plate groups* = 1
 - ii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group*.
 - iii. Adjust *Plate type* with the drop-down menu.
 - iv. For fixed and mounted cells, *Initial volume* = 0. For fixed cells in PBS or for live cells in media, input the proper volume in μL .

Methods		
Method	Entry method	Exit method
1	MyPlate RACK	
2	BCR	
3	OPA	
4	RACK	



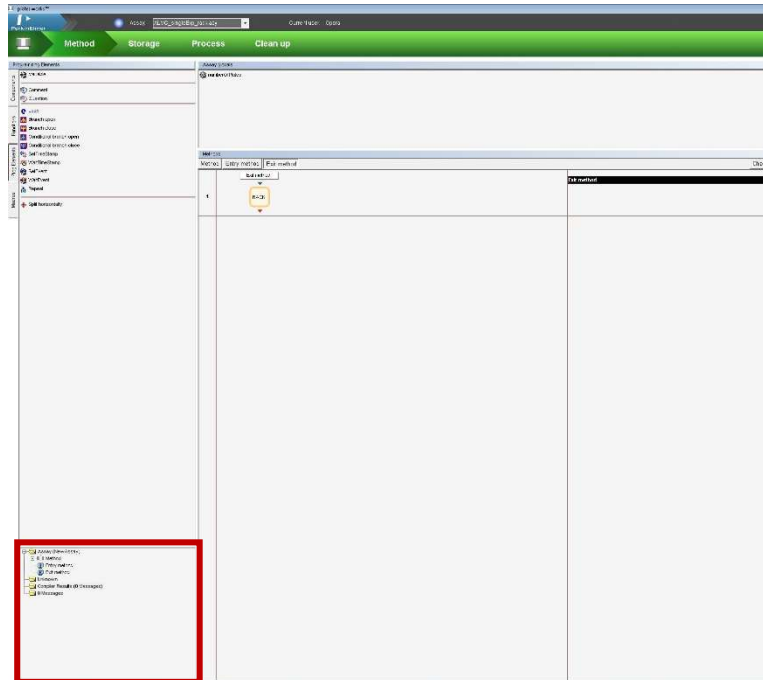
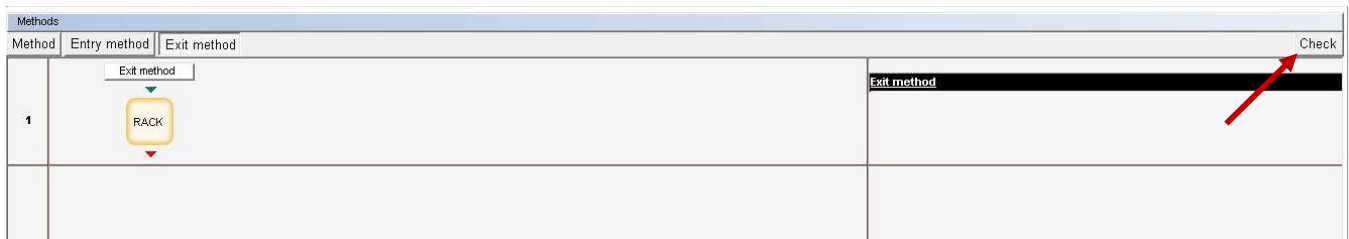
16. Click the *Exit method* tab, just above the flow diagram.

- a. Make sure the component matches where you put your plate and the final step of your flow diagram (RACK or INC). It should only be one step.



17. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.

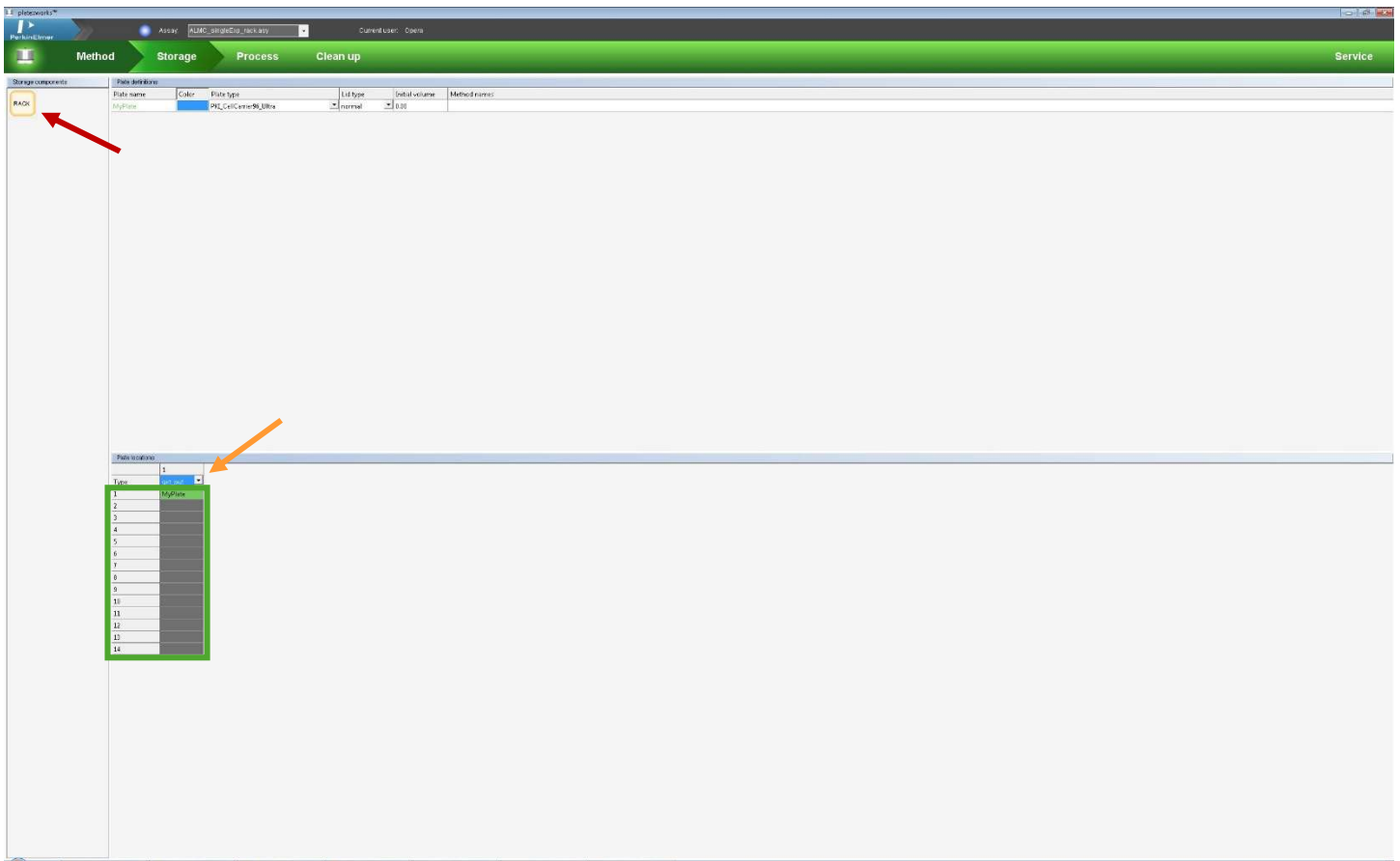
- a. Address any errors and repeat this step until no errors appear.



Run an automation

18. Click the *Storage* chevron at the top of the screen.
 - a. On the left side of the screen, click the location where your plate(s) will be located/stored.
 - b. Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack or the incubator.
 - c. Highlight the position(s) in the column where you will put your plate(s). The position(s) should turn blue.
 - d. Right-click → *Set places for '[plate name]'*. The position(s) should turn green.
 - e. Highlight the remaining position(s) in the column where there will not be plates. The position(s) should turn blue.
 - f. Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.
 - g. If using the incubator and if there are no plates in the second column, select *unused* from the drop-down menu at the top of the column.

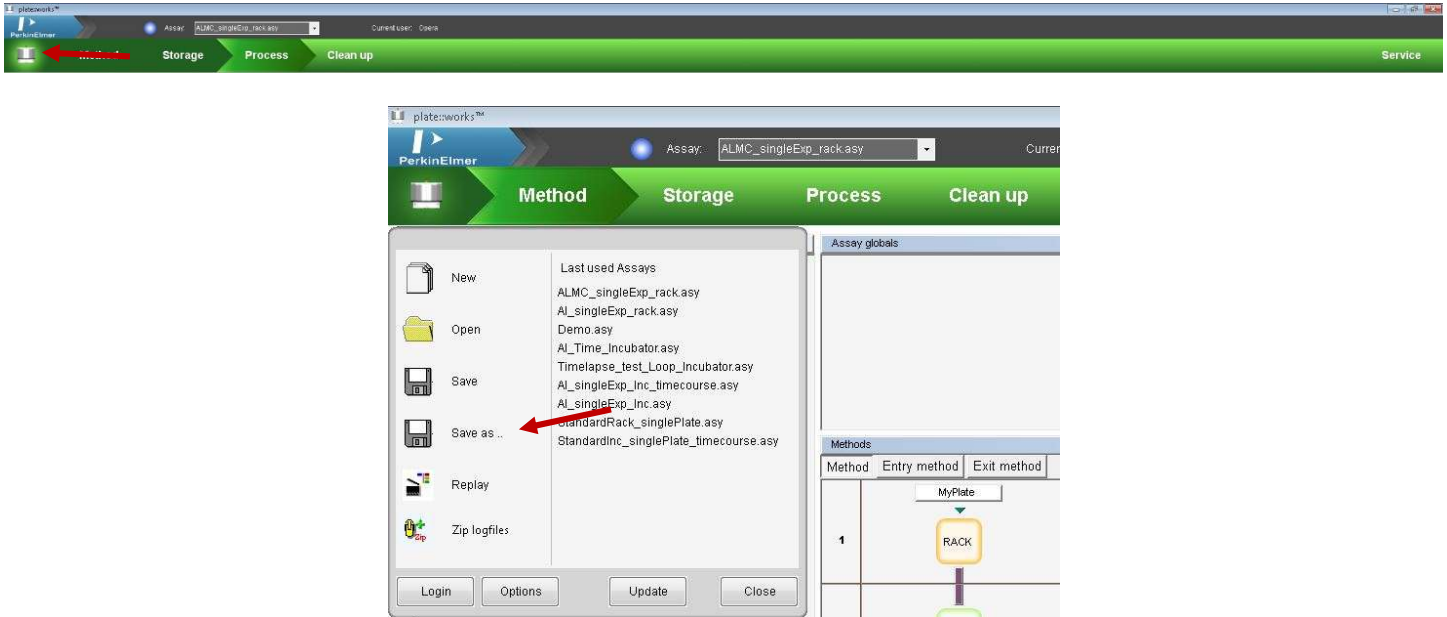




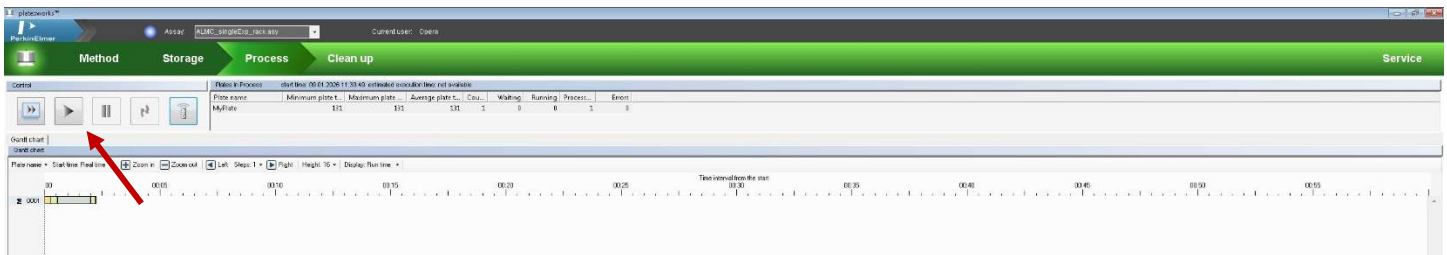
19. Click the *Process* chevron at the top of the screen.
 - a. Click the *Simulate assay* button (computer screen with double arrows).
 - b. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows, changing the *PlateCount* variable to match the number of plates that you want to run, and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.
 - c. If there are any errors, they will appear now and, sometimes, the software will ask you what to do.
 - d. If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
 - e. The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to. Clicking on the sections in the simulated time log will give you more details.



20. Fix any problems that arise and repeat the *Simulate* assay step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking Save or Save As...

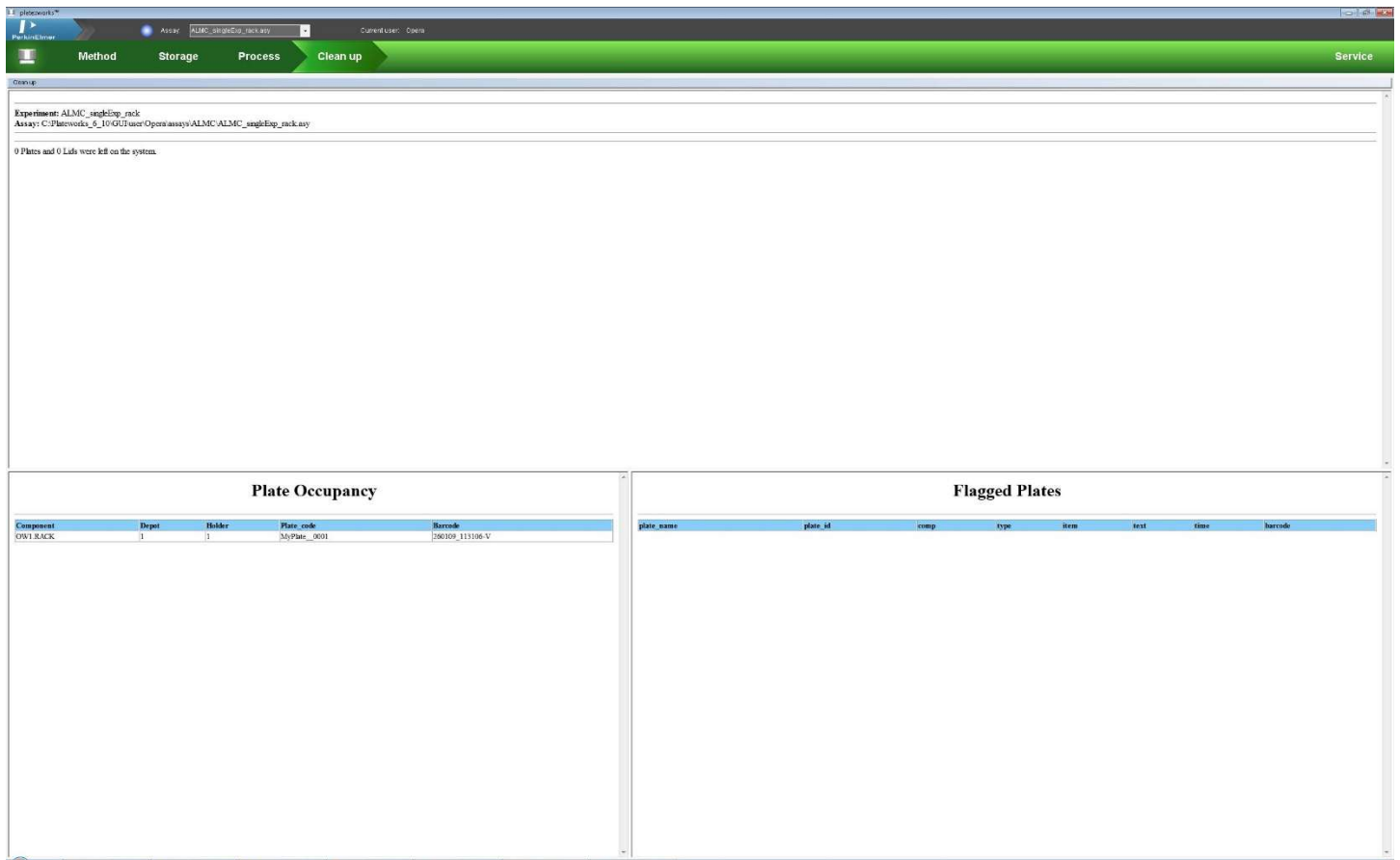


21. Click the *Run assay* button (single large play button) to start the automation.
 - a. In the *Assay Wizard* (blue pop-up window), click *Next* to move through the windows.
 - i. Change the value for the *PlateCount* variable = [number of plates].
 - ii. Change the *Experiment-ID* and the *Description* to fit your experiment.
 - iii. Click *Start*.
 - b. There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊
 - c. After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.



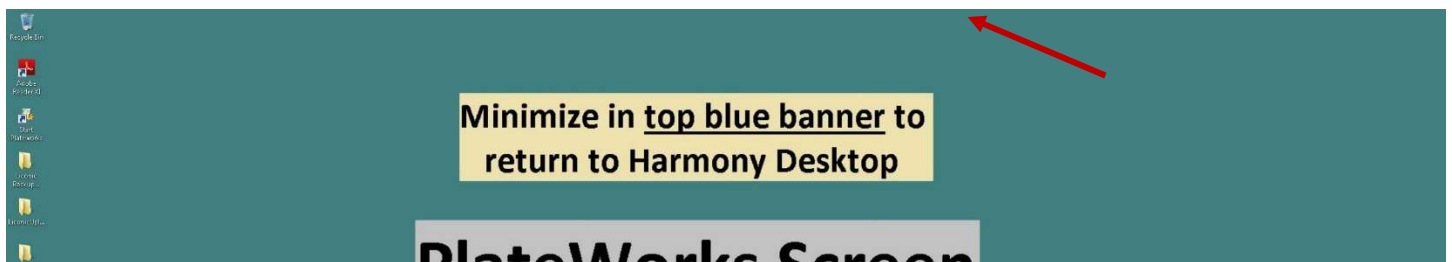
22. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plate(s) from the racks.



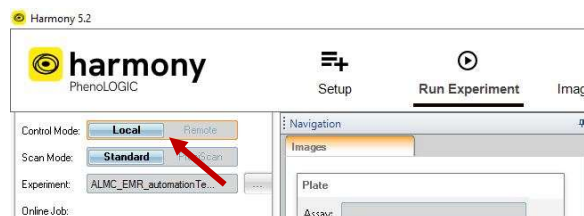


Look at and save your images

23. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



24. On the Harmony computer, re-open the *Harmony* application. In the *Run Experiment* tab, change the **Control to Local**. The images should be displayed in the *Run Experiment* tab.

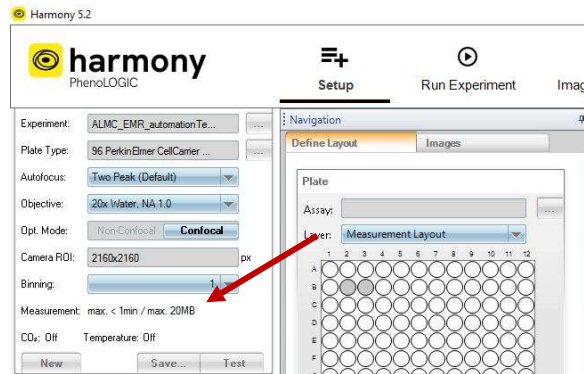


25. Save your images and their metadata using *Write Archive...* as normal.

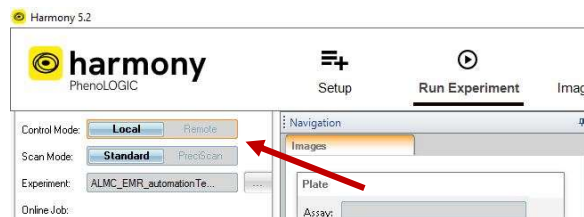
Loading a pre-made automation to image plates with two or more experiments / different imaging settings

Set up Harmony experiment and load plates

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plates:
 - a. For fixed plates: Insert plates into the fixed plate rack, starting from the bottom. Position A1 in a well plate should be front-left of the plate rack or closest to you (next to the **1** sticker).
 - b. For incubated plates: Remove the rack by grabbing the handle on the top of the rack and rock it back and forth/pull toward you until it unclips from the holder. Insert plates into the incubated plate rack, starting from the bottom. Position A1 in a well plate should be back-right of the plate rack when it is inserted into the holder (next to the **1** sticker).
2. In Harmony, in the *Setup* tab, load and set up all your experiments, including the wells with your sample. For each experiment, save it. **For each experiment, write down the estimated time to run the experiment** (found near the *Save* button).



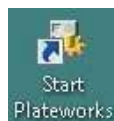
3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

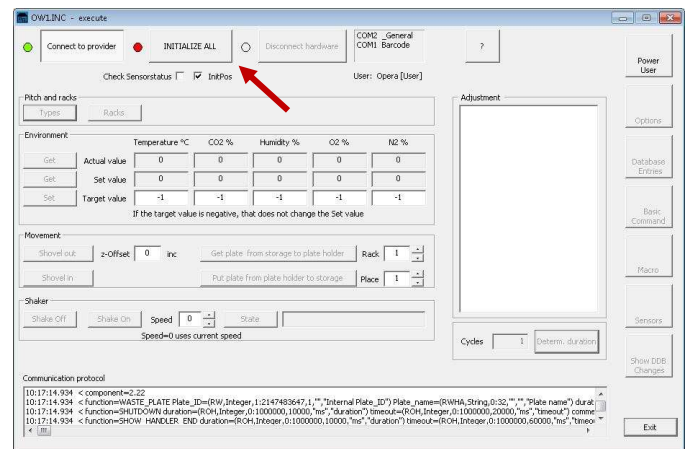
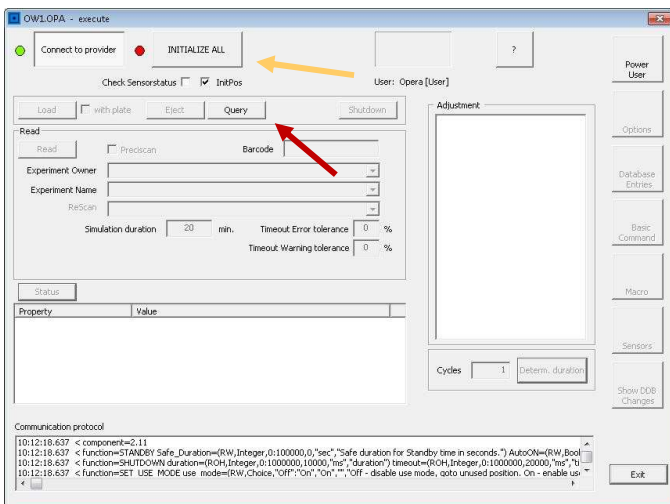


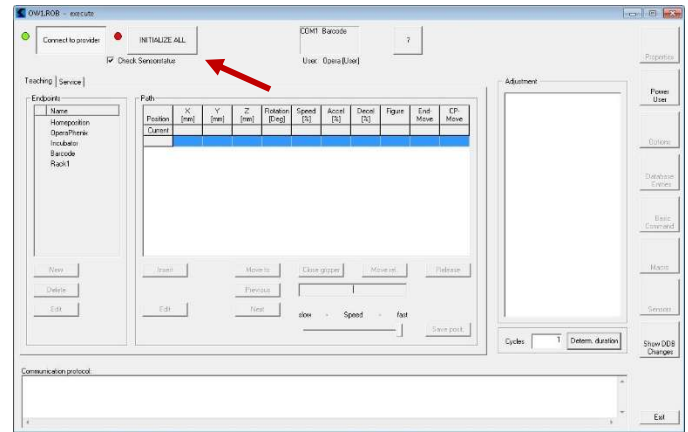
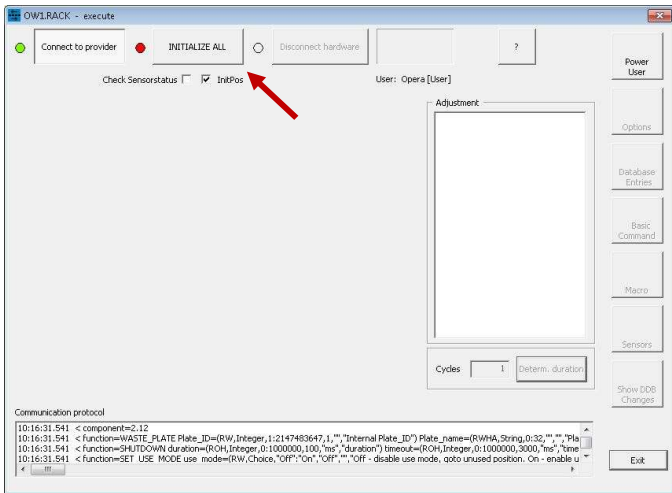
Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click **Service**.

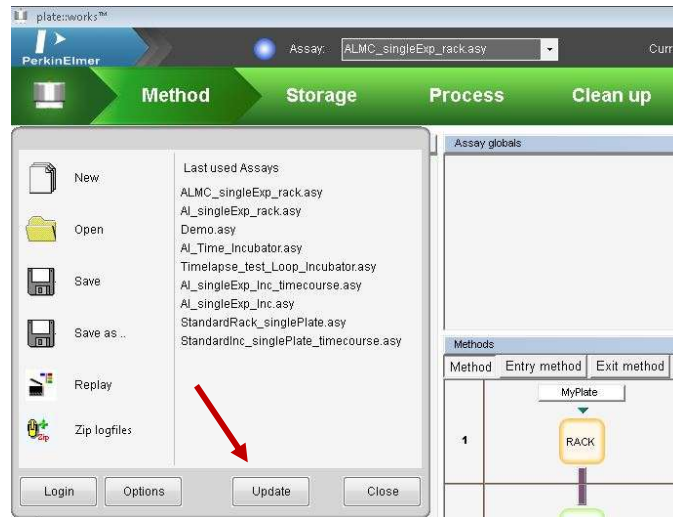


8. Right-click on **OPA** → **Direct Mode On**. In the pop-up window, click **Query** and then **Initialize All**. The loading door on the top of the microscope should open and close.
9. Right-click on **ROB** → **Direct Mode On**. In the pop-up window, click **Initialize All**. The robot hand should open and close.
10. Initialize the component where your plates will be located/stored:
 - a. If using the incubator, right-click on **INC** → **Direct Mode On**. In the pop-up window, click **Initialize All**. The incubator should beep a few times.
 - b. If using the rack, right-click on **RACK** → **Direct Mode On**. In the pop-up window, click **Initialize All**.





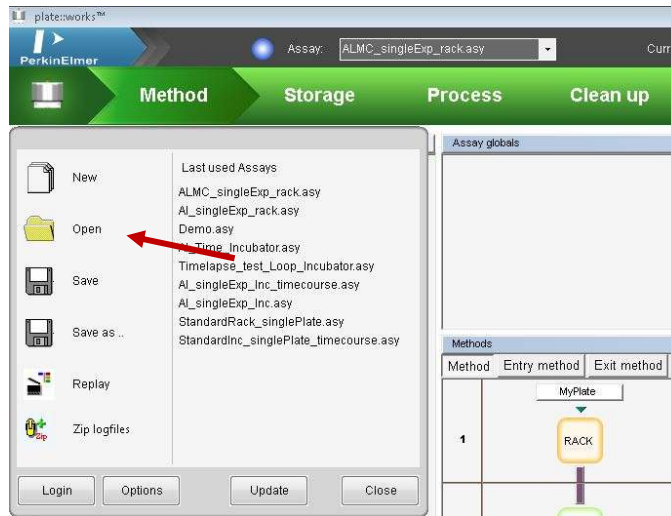
11. In the PlateWorks program, at the top-left of the screen, click the Phenix logo and then click *Update*. This will make sure that your new Harmony experiments are available to the automation software.



Load a pre-made automation

12. In the PlateWorks program, at the center-top of the screen, the Assay drop-down menu will load the most recent automations OR at the top-left corner of the screen, click the Phenix logo, and use *Open* to find your previous automation.





13. At the top left of the screen, click the *Method* chevron. There should be a *Method* tab for each experiment/imaging setting. In the *Method* tab, above the flow diagram, there should be a white rectangle with a plate name (e.g., *Plate1*). Click this rectangle.
 - a. Adjust *PlateType* with the drop-down menu.



Method 1	Method 2	Entry method	Exit method	Check
1	MyPlate1 RACK			MyPlate1 ● GET_NEW_PLATE
2	BCR			● READ_BARCODE
3	OPA			<ul style="list-style-type: none"> ● Choose your experiment / imaging settings for Experiment 1. ● Input the estimated time for the Experiment 1 (from the Harmony software). ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\M EstDur: 1 min
4	RACK			● WASTE_PLATE



14. In the flow diagram, there should be an *OPA (component) | Execute (function)* step.
 - a. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: "Load Harmony experiment into PlateWorks".
 - b. Adjust *Estimated duration* to reflect the estimated time written down from Harmony with a little extra time such that the measurement would not be aborted under normal circumstances (i.e., if the estimated time in Harmony was 20 minutes, enter 40 minutes; if the estimated time in

Harmony was 60 minutes, enter 90 minutes). This value is for a timeout function to allow the automation to continue progressing if the plate fails to image correctly.

- c. *Timeout warning tolerance* = -1 (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
- d. *Timeout error tolerance* = -1 (default amount) or 110% (give error after experiment takes 10% more time than expected)

The screenshot shows the 'Methods' window with a flow diagram on the left and a list of steps on the right. The flow diagram consists of four steps: 1. RACK, 2. BCR, 3. OPA, and 4. RACK. The right pane shows the following steps for 'MyPlate1':

- GET_NEW_PLATE
- READ_BARCODE
- Choose your experiment / imaging settings for Experiment 1.
- Input the estimated time for the Experiment 1 (from the Harmony software).
- EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\ EstDur: 1 min
- WASTE_PLATE

A red arrow points from the 'EXECUTE' step in the list to the 'OPA' step in the flow diagram.

The screenshot shows the 'Service' Properties window with the following fields:

Properties	
Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_duration
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTest\
Remark	USE THE DIRECT MODE TOOL TO REFRESH THE LISTS!
Estimated duration	1 min
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

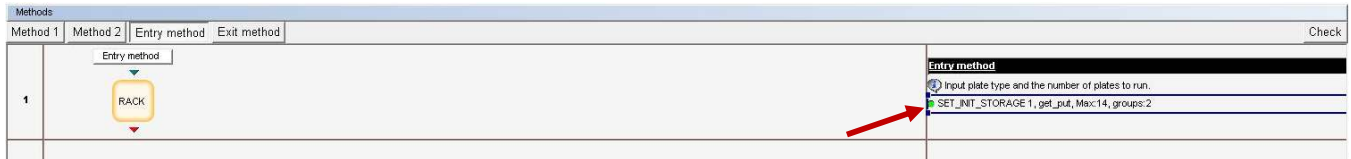
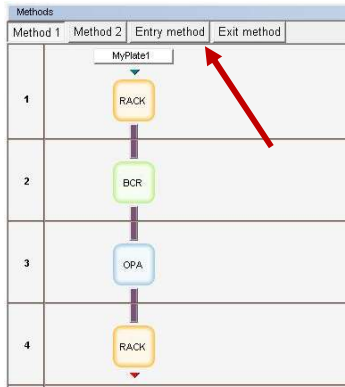
Red arrows point to the 'Experiment Name' and 'Remark' fields. An orange arrow points to the 'Estimated duration' field. A green arrow points to the 'Timeout error tolerance' field.

15. Repeat this step for each *Method* tab (e.g., *Method 1*) that holds a different experiment/imaging setting.

The screenshot shows the 'Methods' window with the flow diagram for 'MyPlate1' highlighted. The flow diagram consists of four steps: 1. RACK, 2. BCR, 3. OPA, and 4. RACK. A red arrow points to the 'MyPlate1' tab.

16. Just above the flow diagram, click the top *Entry method* tab.

- a. Click the *SET_INIT_STORAGE* function.
 - i. *No of plate groups* = [number of different experiments / imaging settings]
 - ii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group 1*. Adjust the *Plate type* in the drop-down menu.
 - iii. For fixed and mounted cells, *Initial volume* = 0. For fixed cells in PBS or for live cells in media, input the proper volume in μL .
 - iv. Repeat Steps 16aii – 16av for *Plate group 2*.

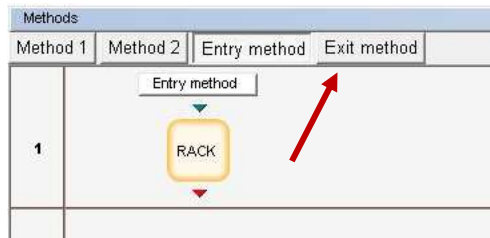


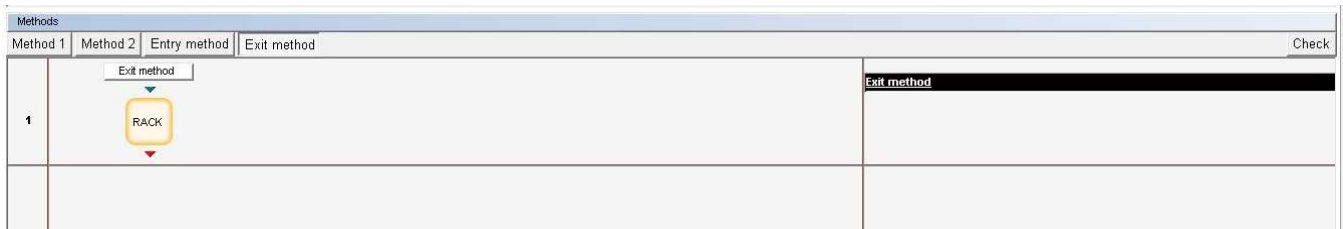
Service

Properties	
Name	SET_INIT_STORAGE
DisplayName	\$Rack_number, \$Storage_type, Max:\$Max_plates, groups:\$No_of_Plate_groups
Rack number	1
Storage type	get_put
Max. plates	14
No of plate groups	2
▼ Plate group 1	{"PK1_CellCarrier96_Ultra","MyPlate1",1,EvalString("PlateCountExp1"),0,"normal"}
Plate type	PK1_CellCarrier96_Ultra
Plate name	MyPlate1
Start location	1
No of plates	=PlateCountExp1
Initial volume	0 µl
Lid type	normal
▼ Plate group 2	{"PK1_CellCarrier96_Ultra","MyPlate2",2,EvalString("PlateCountExp2"),0,"none"}
Plate type	PK1_CellCarrier96_Ultra
Plate name	MyPlate2
Start location	2
No of plates	=PlateCountExp2
Initial volume	0 µl
Lid type	none
► Name List	0

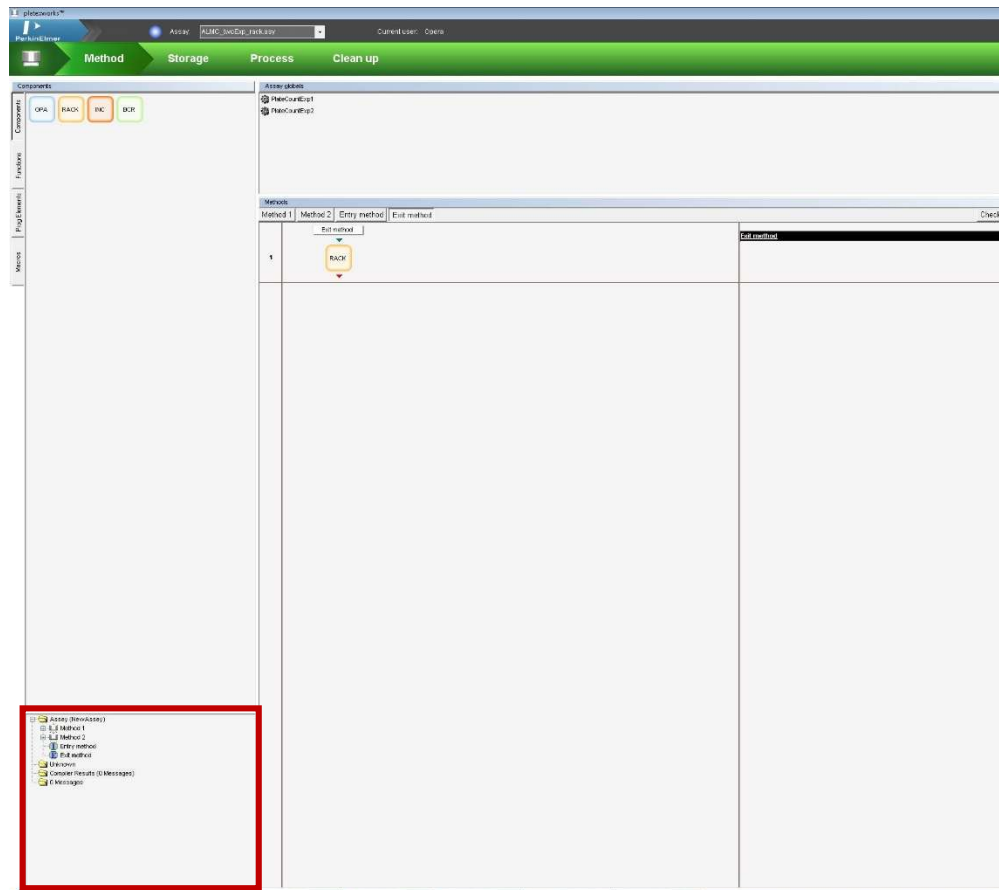
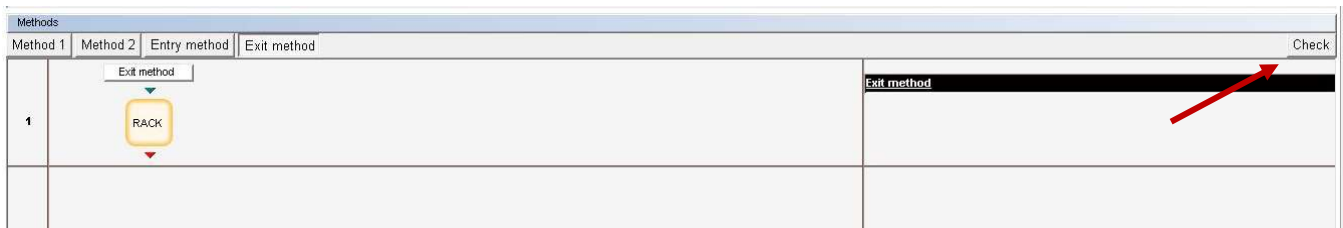
17. Click the *Exit method* tab, just above the flow diagram.

- a. Make sure the component matches where you put your plate and the final step of your flow diagram (RACK or INC). It should only be one step.





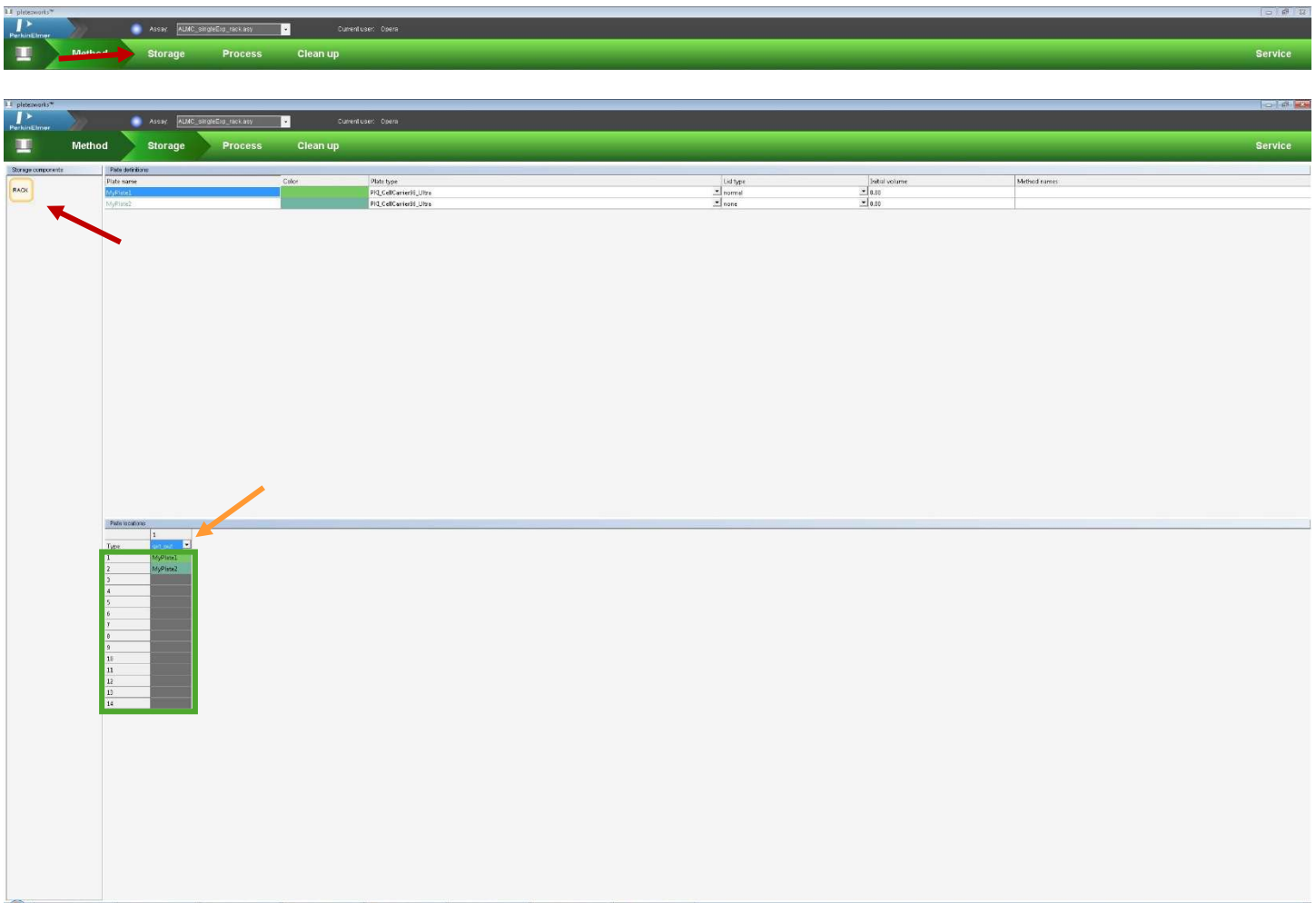
18. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.
- Address any errors and repeat this step until no errors appear.



Run an automation

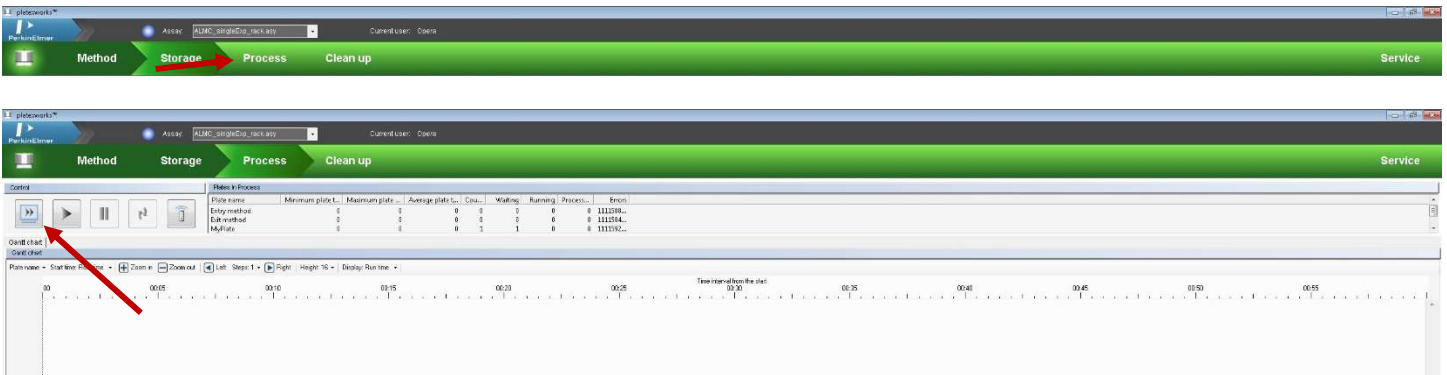
19. Click the *Storage* chevron at the top of the screen.
- On the left side of the screen, click the location where your plates will be located/stored.
 - Near the top of the screen, there is a table of the “plates” that are used in the automation.
 - There should be as many “plates” as there are different experiments/imaging settings. If there are not, see “Create or edit an automation for plates with two or more experiments / different imaging settings...”

- ii. Adjust the plate types.
- c. Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack or the incubator.
- d. Highlight the position(s) in the column where you will put your plates for Experiment 1. The position(s) should turn blue.
- e. Right-click → *Set places for '[Plate1]'*. The position(s) should turn green.
- f. Repeat Steps 19d – 19e for the other experiments and Plate types (e.g., *Plate2*).
- g. Highlight the remaining position(s) in the column where there will not be plates. The position(s) should turn blue.
- h. Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.
- i. If using the incubator and if there are no plates in the second column, select *unused* from the drop-down menu at the top of the column.



20. Click the *Process* chevron at the top of the screen.
 - a. Click the *Simulate assay* button (computer screen with double arrows).
 - b. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows, changing the *NumPlate* variables to match the number of plates that you want to run for each experiment, and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.
 - c. If there are any errors, they will appear now and, sometimes, the software will ask you what to do.

- d. If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
- e. The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to with the proper experiments / imaging settings. Clicking on the sections in the simulated time log will give you more details.



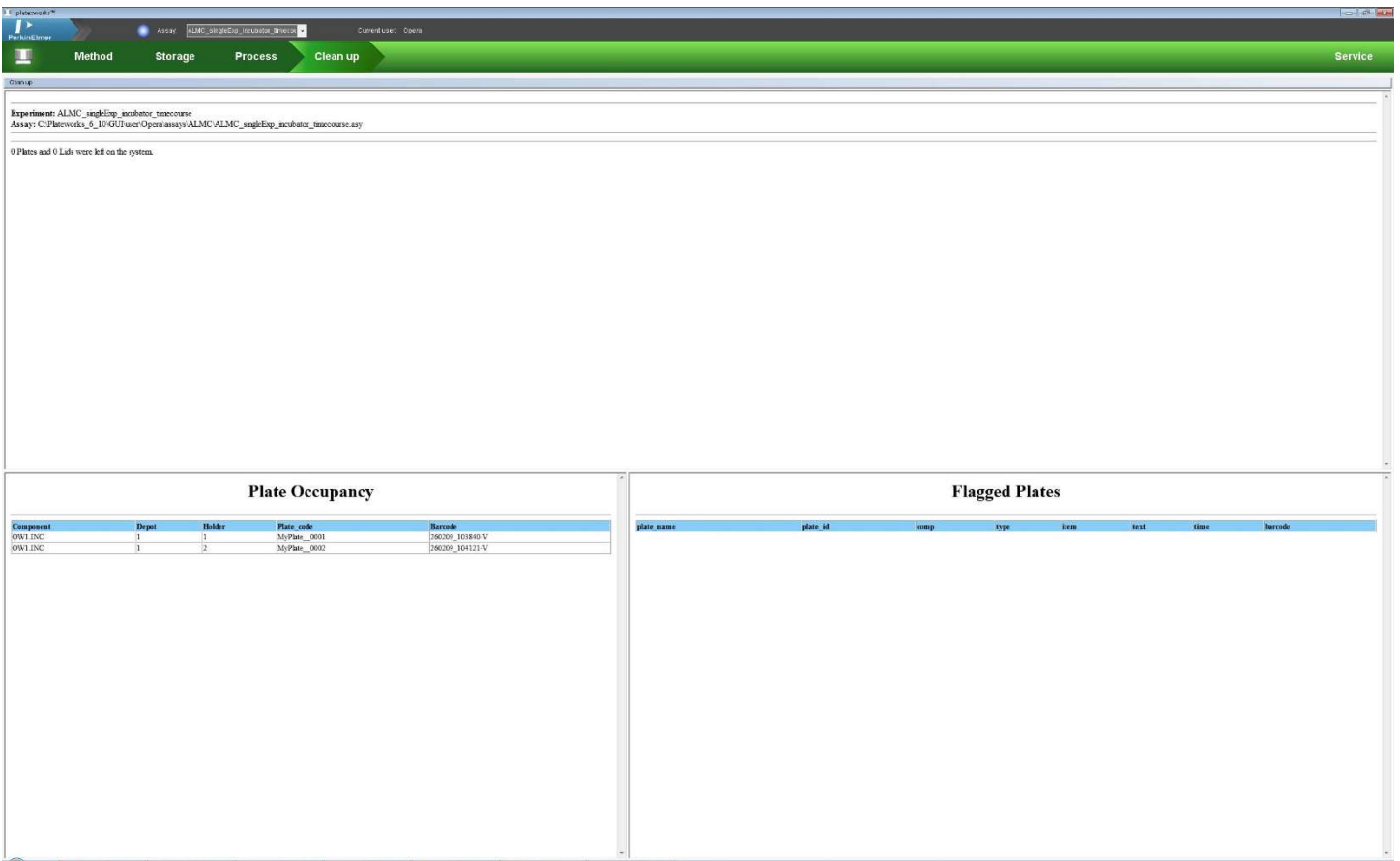
21. Fix any problems that arise and repeat the *Simulate* assay step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking *Save* or *Save As...*



22. Click the *Run* assay button (single large play button) to start the automation.
 - a. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows.
 - i. Change the value for the *PlateCount* variables = [number of plates for that experiment / imaging settings].
 - ii. Change the *Experiment-ID* and the *Description* to fit your experiment.
 - iii. Click *Start*.
 - b. There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊
 - c. After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.

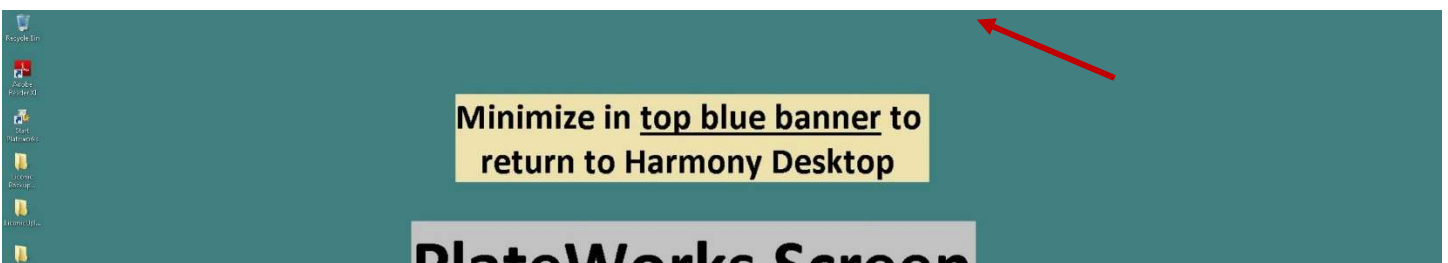


23. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plate(s) from the racks.

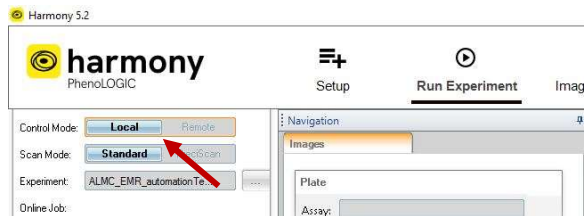


Look at and save your images

24. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



25. On the Harmony computer, re-open the *Harmony* application. **In the *Run Experiment* tab, change the *Control* to *Local*.** The images should be displayed in the *Run Experiment* tab.

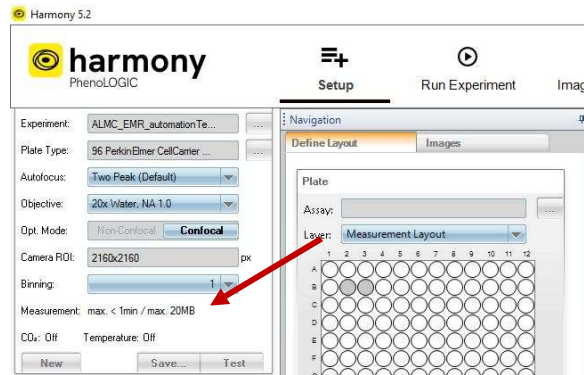


26. Save your images and their metadata using *Write Archive...* as normal.

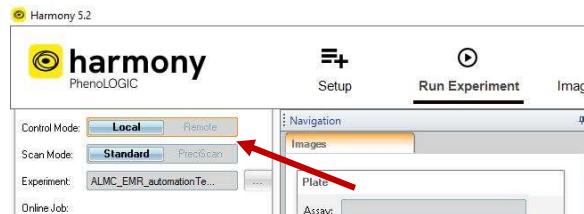
Create or edit an automation for plate(s) with a single experiment / same imaging settings from the fixed plate rack

Set up Harmony experiment and load plate(s)

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plate(s) into the fixed plate rack, starting from the bottom. Position A1 in a well plate should be front-left of the plate rack or closest to you (next to the 1 sticker).
2. In Harmony, in the *Setup* tab, load and set up your experiment, including the wells with your sample. Save it. **Write down the estimated time to run this experiment** (found near the *Save* button).



3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

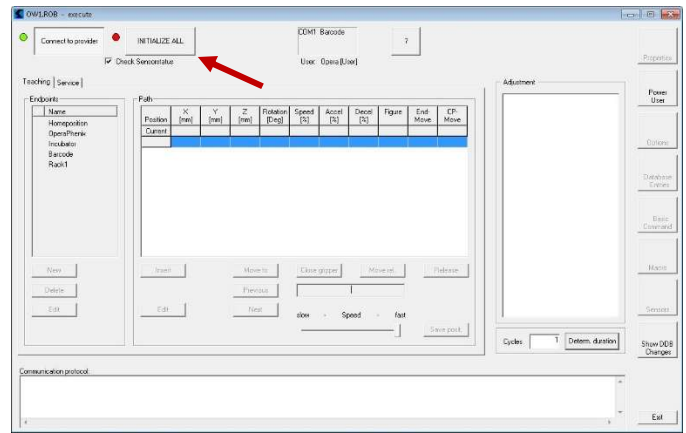
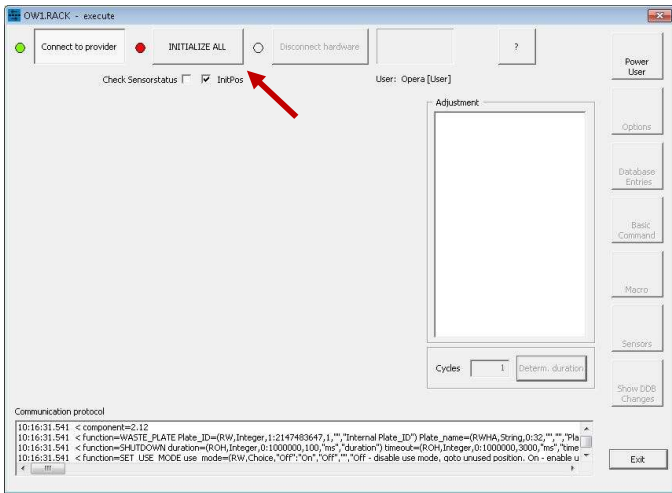
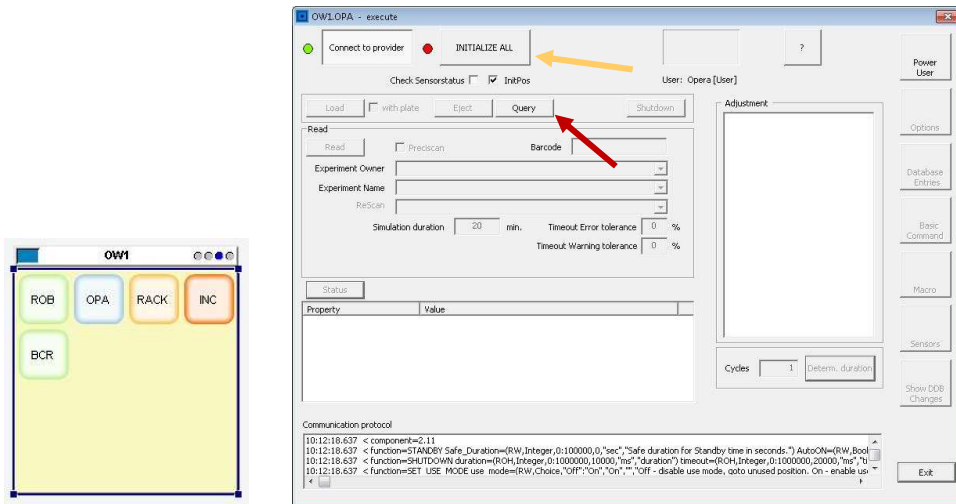


Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click *Service*.

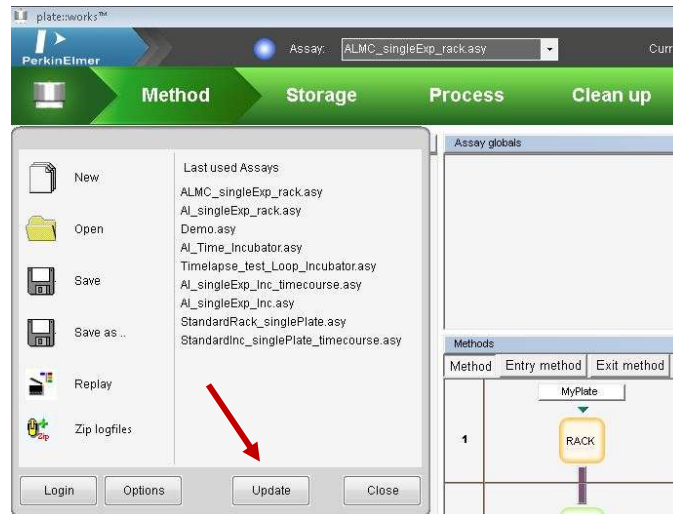


8. Right-click on *OPA* → *Direct Mode On*. In the pop-up window, click *Query* and then *Initialize All*. The loading door on the top of the microscope should open and close.
9. Right-click on *ROB* → *Direct Mode On*. In the pop-up window, click *Initialize All*. The robot hand should open and close.
10. Right-click on *RACK* → *Direct Mode On*. In the pop-up window, click *Initialize All*.



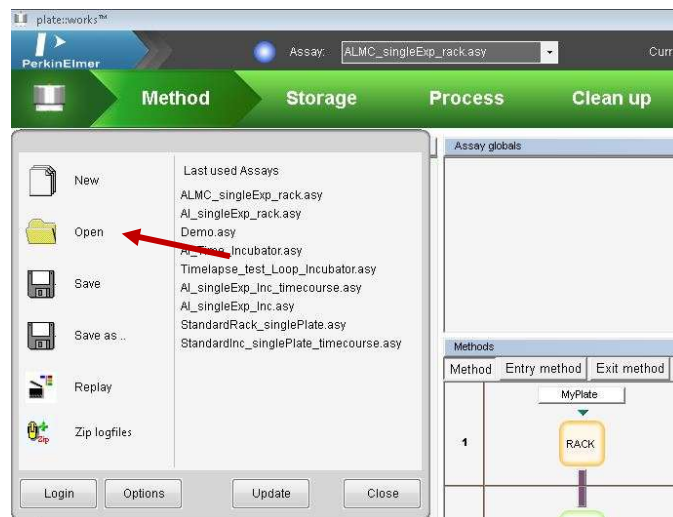
11. In the PlateWorks program, at the top-left of the screen, click the Phenix logo and then click *Update*. This will make sure that your new Harmony experiment is available to the automation software.





Load and edit a pre-made automation

12. Load a previous automation by clicking the Phenix logo and using *Open* to find the “ALMC_singleExp_rack” automation as a starting point.



13. The *Method* chevron shows the current automation with the thick black vertical lines indicating a movement by the robot arm.
 - a. If you need to change or add any components, click the left *Components* tab, and drag components to the flow diagram.
 RACK = fixed plate rack
 BCR = bar code reader
 OPA = Opera Phenix microscope



Methods			Check
Method	Entry method	Exit method	
1	MyPlate RACK		MyPlate ● GET_NEW_PLATE
2	BCR		● READ_BARCODE
3	OPA		<ul style="list-style-type: none"> Choose your experiment / imaging settings. Input the estimated time for the experiment (from the Harmony software). EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\EstDur: 1 min
4	RACK		● WASTE_PLATE

14. Above the flow diagram, there should be a white rectangle with a plate name (e.g., *MyPlate*). Click this rectangle.

- SourceCount* = *PlateCount* (variable)
- Adjust *PlateType* with the drop-down menu.
- Adjust *PlateName* if you'd like... but write down the new name, because it will be necessary later.

Methods			Check
Method	Entry method	Exit method	
1	MyPlate RACK		MyPlate ● GET_NEW_PLATE
2	BCR		● READ_BARCODE
3	OPA		<ul style="list-style-type: none"> Choose your experiment / imaging settings. Input the estimated time for the experiment (from the Harmony software). EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\EstDur: 1 min
4	RACK		● WASTE_PLATE

Service

Properties	
SourceCount	=PlateCount
PlateType	PK1_CellCarrier96_Ultra
Name	MyPlate
PlateLayout	
BatchSize	0
PlateTickRate	0 s
BatchGap	0 s

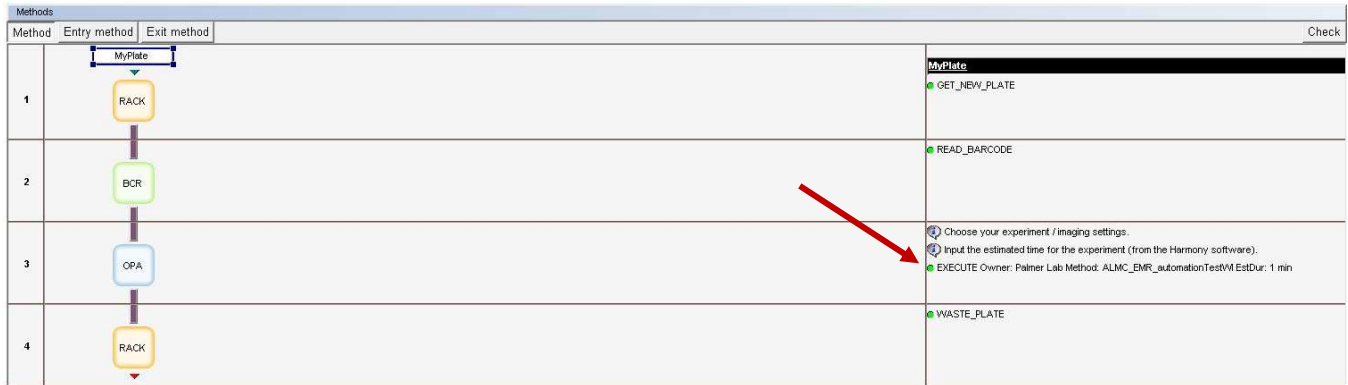
15. The first step should show *RACK* (component) | *GET_NEW_PLATE* (function). This will tell the automation to grab a plate starting from Position 1.

16. Keep the *BCR* (component) | *Read Barcode* (function) step as the second step in the flow even if you do not have barcodes on your plates. This will provide timestamps in the metadata.

17. The third step should be an *OPA* (component) | *Execute* (function) step.

- Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: "Load Harmony experiment into PlateWorks".

- b. Adjust *Estimated duration* to reflect the estimated time written down from Harmony with a little extra time such that the measurement would not be aborted under normal circumstances (i.e., if the estimated time in Harmony was 20 minutes, enter 40 minutes; if the estimated time in Harmony was 60 minutes, enter 90 minutes). This value is for a timeout function to allow the automation to continue progressing if the plate fails to image correctly.
- c. *Timeout warning tolerance* = -1 (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
- d. *Timeout error tolerance* = -1 (default amount) or 110% (give error after experiment takes 10% more time than expected)

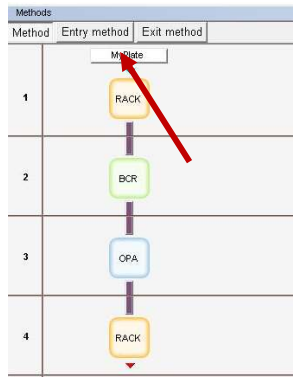



Service	
Properties	
Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_duration
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestVM
Remark	USE THE DIRECT MODE TOOL TO REFRESH THE LISTS!
Estimated duration	1 min
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

18. Finally, the fourth step should be a *RACK (component) | WASTE_PLATE (function)* step. This will tell the automation that after this flow, this plate will not be used again.

19. Just above the flow diagram, click the top *Entry method* tab.

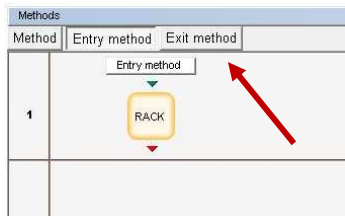
- a. Make sure the component matches where you put your plate and the first step of your flow diagram (RACK). It should only be one step.
- b. Click the *SET_INIT_STORAGE (function)*.
 - i. *Max plates* = 14
 - ii. *No of plate groups* = 1
 - iii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group*. Adjust the *Plate type* in the drop-down menu.
 - iv. Change the *Plate name* if you'd like to... (make sure it's the same plate name as before!)
 - v. *Start location* = 1
 - vi. *No of plates* = *PlateCount (variable)*
 - vii. For fixed and mounted cells, *Initial volume* = 0. For fixed cells in PBS or for live cells in media, input the proper volume in μL .
 - viii. Adjust *Lid type*.

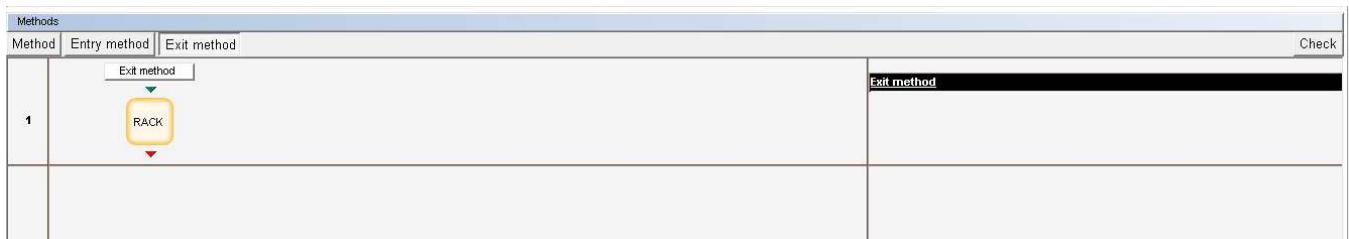


Methods			Check
Method	Entry method	Exit method	
1	<div style="border: 1px solid black; padding: 5px; text-align: center;"> Entry method  </div>		<div style="border: 1px solid black; padding: 5px;"> Entry method SET_INIT_STORAGE 1, get_put, Max:14, groups:1 </div>

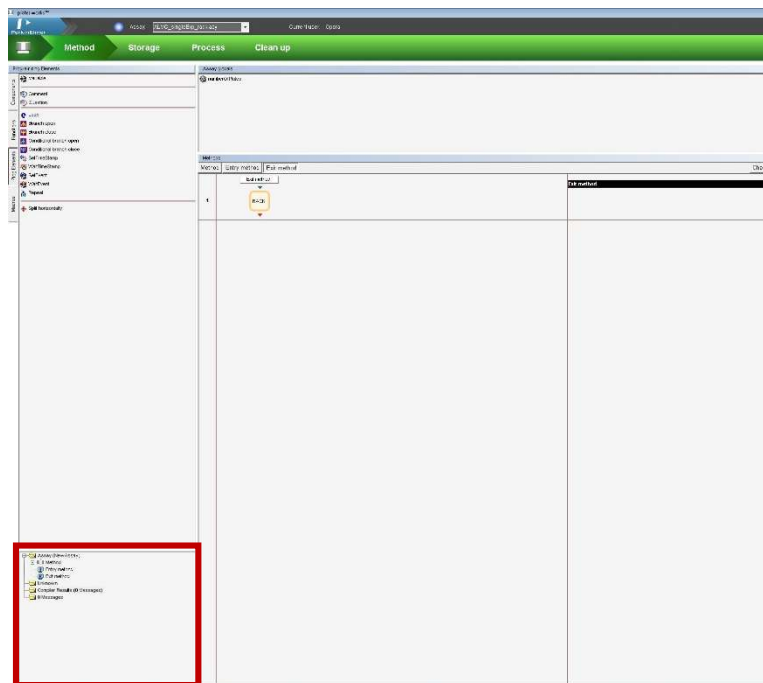
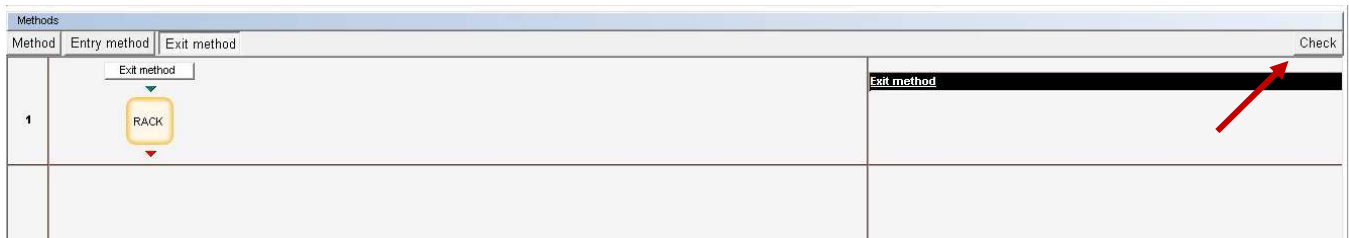
Properties	
Name	SET_INIT_STORAGE
DisplayName	\$Rack_number, \$Storage_type, Max:\$Max_plates, groups:\$No_of_Plate_groups
Rack number	1
Storage type	get_put
Max plates	14
No of plate groups	1
Plate group	{"PKI_CellCarrier96_Ultra", "MyPlate", 1, EvalString("PlateCount"), 0, "normal"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate
Start location	1
No of plates	=PlateCount
Initial volume	0 µl
Lid type	normal
Name List	0

20. Click the *Exit method* tab, just above the flow diagram.
 - a. Make sure the component matches where you put your plate and the final step of your flow diagram (RACK). It should only be one step.



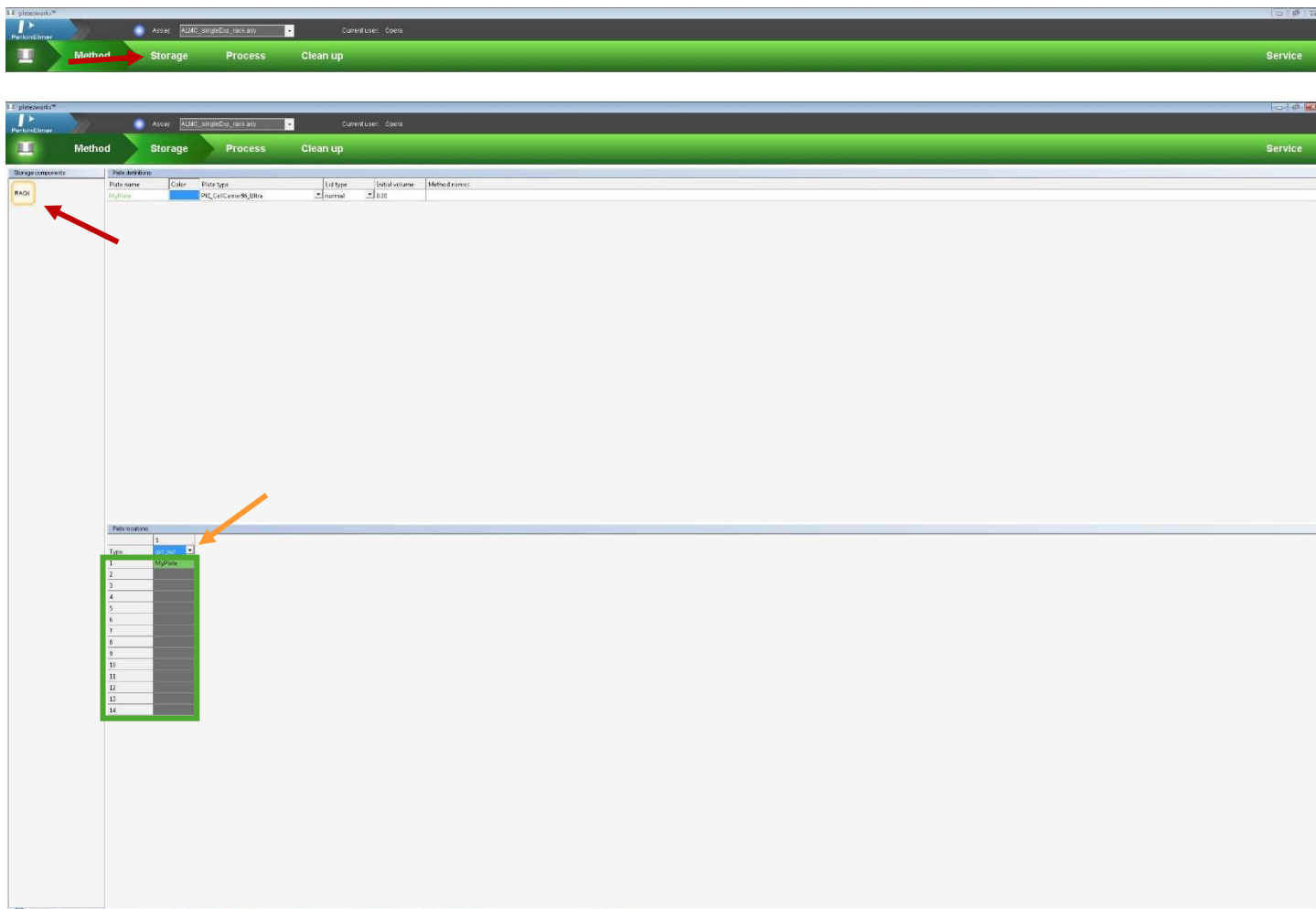


21. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.
- Address any errors and repeat this step until no errors appear.



Run an automation

22. Click the *Storage* chevron at the top of the screen.
- On the left side of the screen, click the *RACK* icon where your plate(s) will be located/stored.
 - Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack.
 - Highlight the position(s) in the column where you will put your plate(s). The position(s) should turn blue.
 - Right-click → *Set places for '[plate name]'*. The position(s) should turn green.
 - Highlight the remaining position(s) in the column where there will not be plates. The position(s) should turn blue.
 - Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.



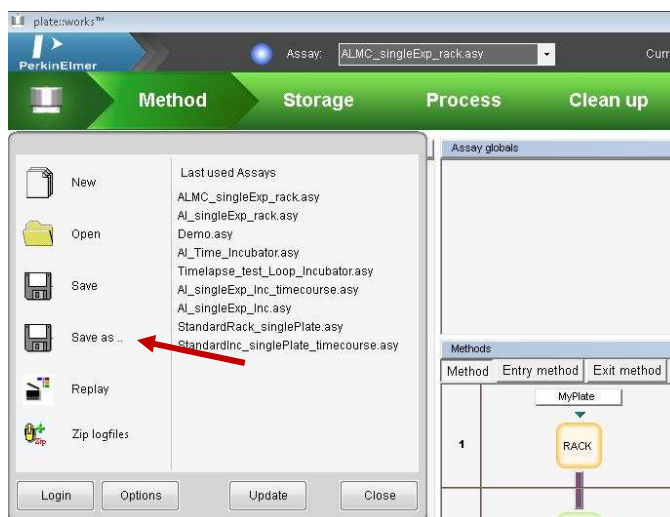
23. Click the *Process* chevron at the top of the screen.

- Click the *Simulate assay* button (computer screen with double arrows).
- In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows, changing the *PlateCount* variable to match the number of plates that you want to run, and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.
- If there are any errors, they will appear now and, sometimes, the software will ask you what to do.
- If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
- The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to. Clicking on the sections in the simulated time log will give you more details.



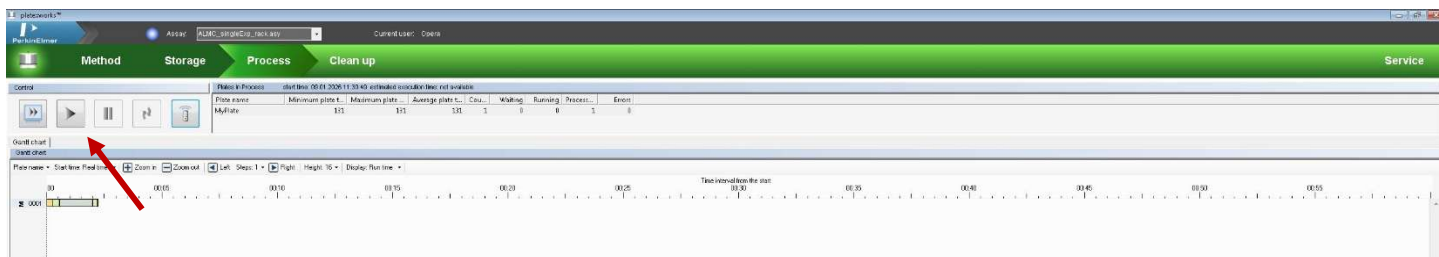


24. Fix any problems that arise and repeat the *Simulate* assay step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking Save As... and labeling it as [Lab]_[initials]_description. Please, do not save over the original ALMC automation!

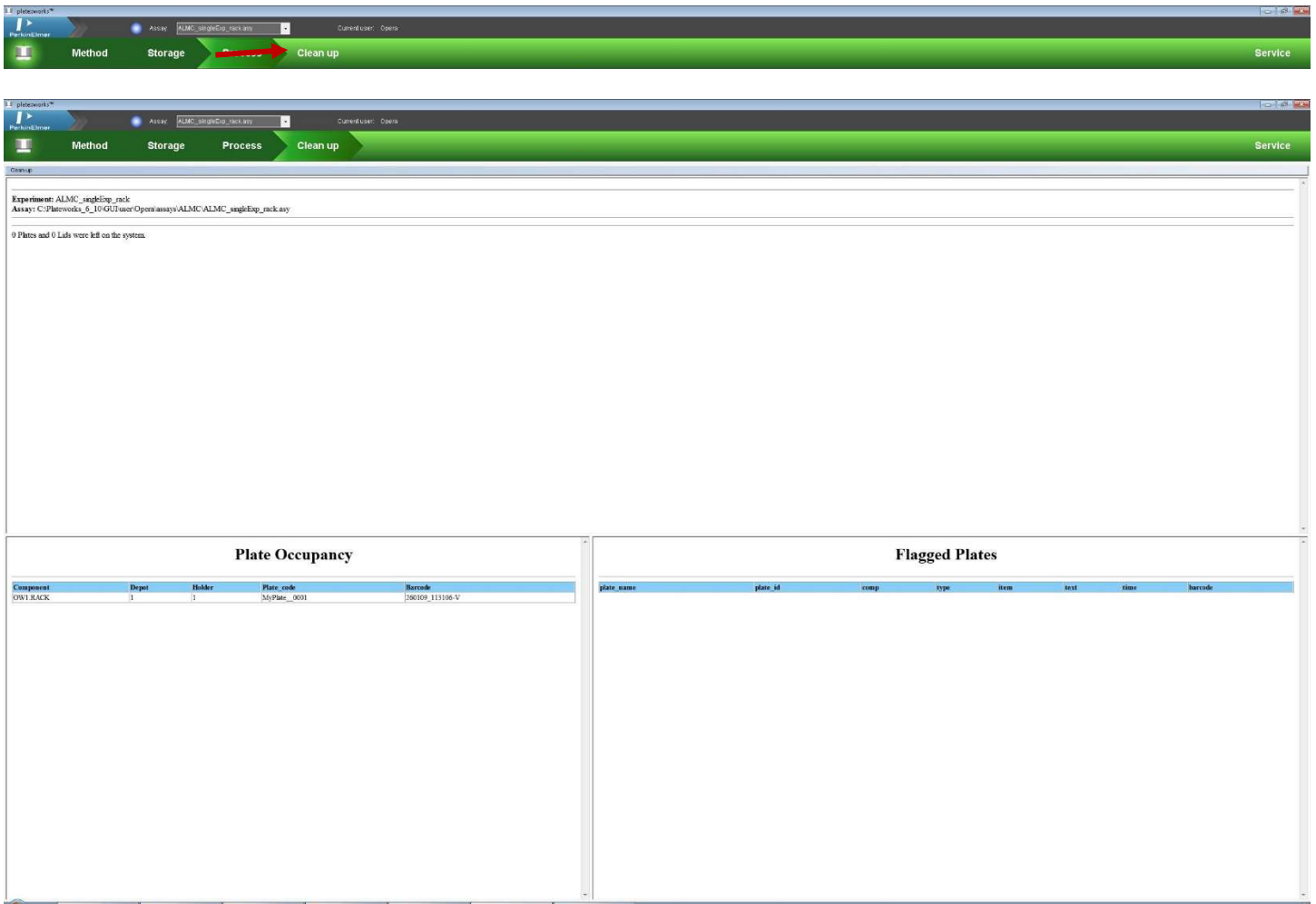


25. Click the *Run* assay button (single large play button) to start the automation.

- In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows.
 - Change the *PlateCount* variable = [number of plates].
 - Change the *Experiment-ID* and the *Description* to fit your experiment.
 - Click *Start*.
- There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊
- After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.

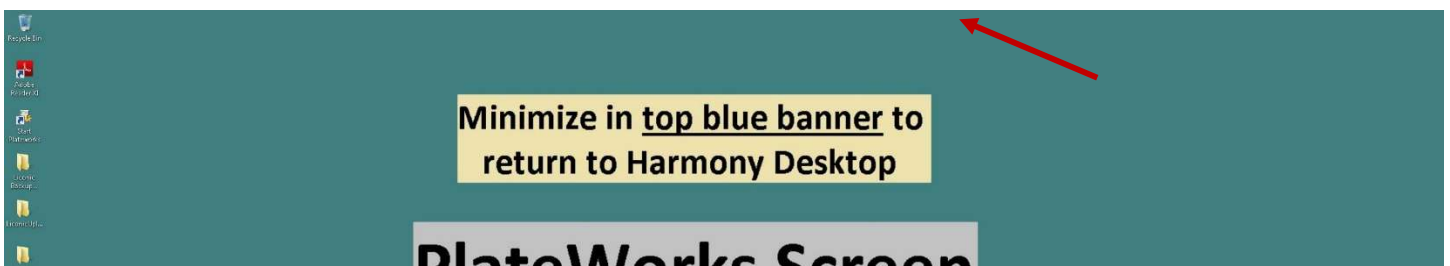


26. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plate(s) from the rack.

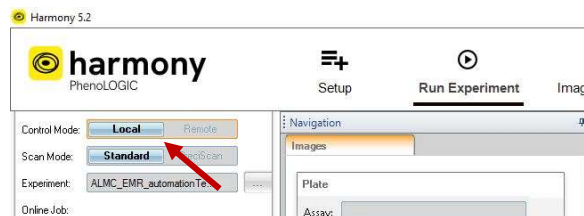


Look at and save your images

27. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



28. On the Harmony computer, re-open the *Harmony* application. In the *Run Experiment* tab, change the *Control* to *Local*. The images should be displayed in the *Run Experiment* tab.

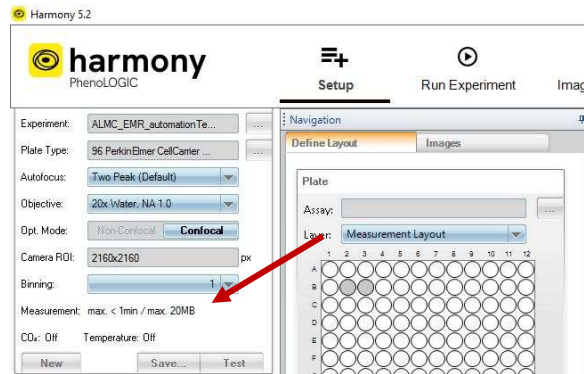


29. Save your images and their metadata using *Write Archive...* as normal.

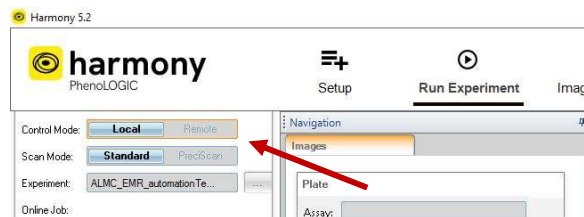
Create or edit an automation for plates with two or more experiments / different imaging settings from the fixed plate rack

Set up Harmony experiment and load plates

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plates into the fixed plate rack, starting from the bottom. Position A1 in a well plate should be front-left of the plate rack or closest to you (next to the 1 sticker).
2. In Harmony, in the *Setup* tab, load and set up all your experiments, including the wells with your sample. For each experiment, save it. **For each experiment, write down the estimated time to run the experiment** (found near the *Save* button).



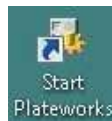
3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

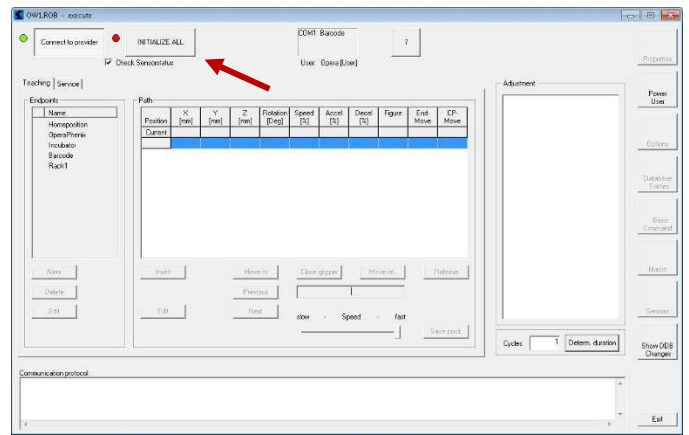
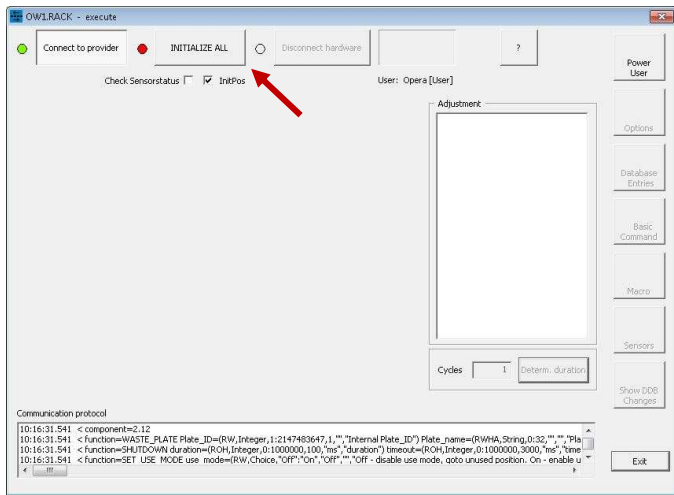
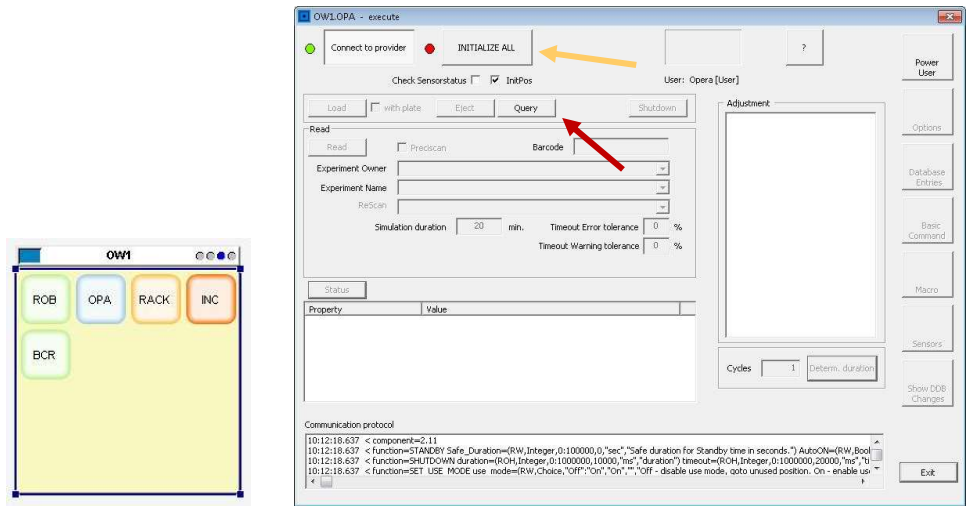


Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click *Service*.

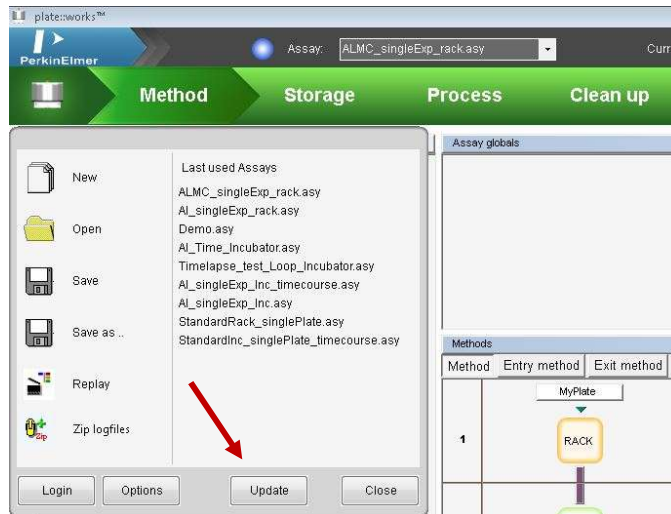


8. Right-click on OPA → *Direct Mode On*. In the pop-up window, click *Query* and then *Initialize All*. The loading door on the top of the microscope should open and close.
9. Right-click on ROB → *Direct Mode On*. In the pop-up window, click *Initialize All*. The robot hand should open and close.
10. Right-click on RACK → *Direct Mode On*. In the pop-up window, click *Initialize All*.



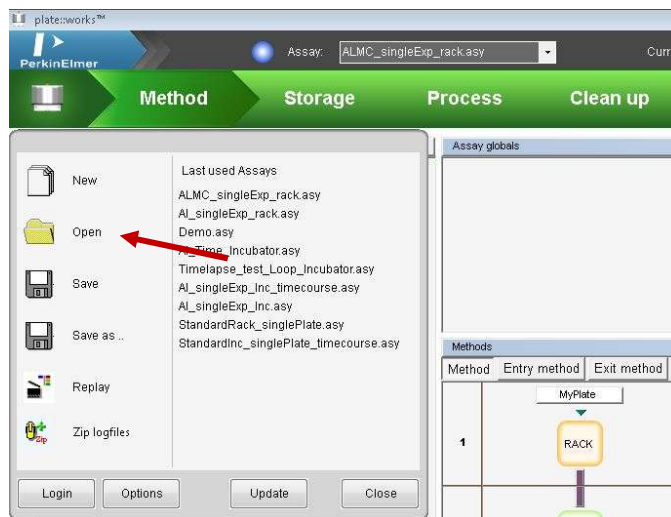
11. In the PlateWorks program, at the top-left of the screen, click the Phenix logo and then click *Update*. This will make sure that your new Harmony experiments are available to the automation software.





Load and edit a pre-made automation

12. Load a previous automation by clicking the Phenix logo and using *Open* to find the “ALMC_twoExp_rack” automation as a starting point.



13. The *Method* chevron shows the current automation with the thick black vertical lines indicating a movement by the robot arm.
 - a. If you need to change or add any components, click the left *Components* tab, and drag components to the flow diagram.
 RACK = fixed plate rack
 BCR = bar code reader
 OPA = Opera Phenix microscope





13. There should be a *Method* tab for each experiment/imaging setting. In the *Method* tab, above the flow diagram, there should be a white rectangle with a plate name (e.g., *MyPlate1*). Click this rectangle.
 - a. *SourceCount* = *PlateCountExp1* (variable) or *PlateCountExp2* (variable)
 - b. Adjust *PlateType* with the drop-down menu.
 - d. Change *PlateName* if you'd like... but write down the new name, because it will be necessary later.
 - c. If there is not a *Method* tab for each experiment/imaging setting, right-click on the *Method* tab → *Add method* to create a new *Method* tab. Build the same flow diagram in this new *Method 1* tab by dragging components, functions, and elements into the flow diagram.



15. The first step should show *RACK* (component) | *GET_NEW_PLATE* (function). This will tell the automation to grab a plate starting from Position 1.
16. Keep the *BCR* (component) | *Read Barcode* (function) step as the second step in the flow even if you do not have barcodes on your plates. This will provide timestamps in the metadata.
17. The third step should be an *OPA* (component) | *Execute* (function).
 - a. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: "Load Harmony experiment into PlateWorks".
 - b. Adjust *Estimated duration* to reflect the estimated time written down from Harmony with a little extra time such that the measurement would not be aborted under normal circumstances (i.e., if

the estimated time in Harmony was 20 minutes, enter 40 minutes; if the estimated time in Harmony was 60 minutes, enter 90 minutes). This value is for a timeout function to allow the automation to continue progressing if the plate fails to image correctly.

- c. *Timeout warning tolerance* = -1 (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
- d. *Timeout error tolerance* = -1 (default amount) or 110% (give error after experiment takes 10% more time than expected)

The screenshot shows the 'Methods' window with a tab for 'Method 1'. On the left, a flow diagram for 'MyPlate1' shows a sequence of steps: RACK (orange), BCR (green), OPA (blue), and RACK (orange). On the right, the 'Plate1' section lists the following steps:

- GET_NEW_PLATE
- READ_BARCODE
- Choose your experiment / imaging settings for Experiment 1.
- Input the estimated time for the Experiment 1 (from the Harmony software).
- EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTestVM EstDur: 1 min
- WASTE_PLATE

A red arrow points from the 'EXECUTE' step in the list to the 'OPA' step in the flow diagram.

The screenshot shows the 'Service' window with a 'Properties' section. The properties are as follows:

Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_duration
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestVM
Remark	USE THE DIRECT MODE TOOL TO REFRESH THE LISTS!
Estimated duration	1 min
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

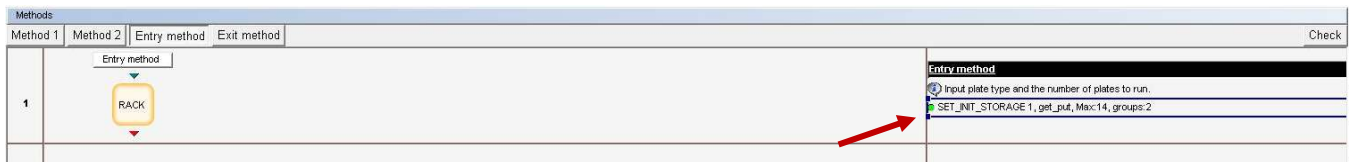
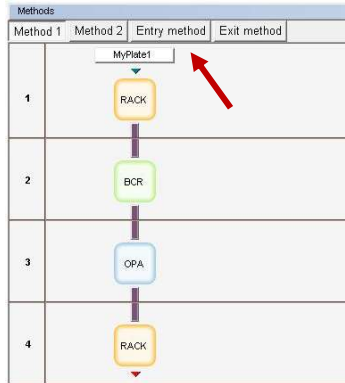
Red and orange arrows point to the 'Experiment Owner' and 'Experiment Name' dropdown menus, respectively. A green arrow points to the 'Estimated duration' field.

- 18. Finally, the fifth step should be a *RACK (component) | WASTE_PLATE (function)* step. This will tell the automation that after this flow, this plate will not be used again, but that it should return to the fixed plate rack.
- 19. Repeat this step for each *Method* tab (e.g., *Method 1*) that holds a different experiment/imaging setting.

The screenshot shows the 'Methods' window with a tab for 'Method 1'. The flow diagram for 'MyPlate1' is shown, with a red arrow pointing to the first 'RACK' step (orange box).

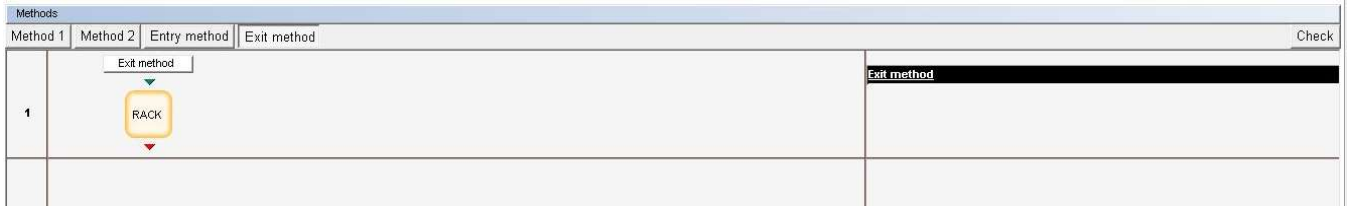
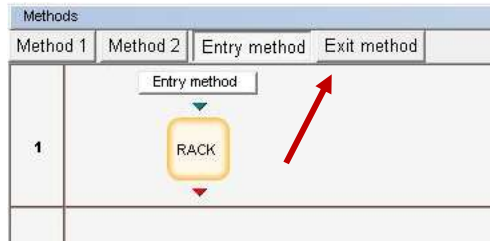
- 20. Just above the flow diagram, click the top *Entry method* tab.
 - a. Make sure the component matches where you put your plates and the first step of your flow diagram (RACK). It should only be one step.

- b. Click the SET_INIT_STORAGE (function).
 - i. *Max plates* = 22
 - ii. *No of plate groups* = [number of different experiments/imaging settings]
 - iii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group 1*. Adjust the *Plate type* in the drop-down menu.
 - iv. Change the *Plate name* if you'd like to... (make sure it's the same plate name as before!)
 - v. *Start location* = 1
 - vi. *No of plates* = *PlateCountExp1* (variable)
 - vii. For fixed and mounted cells, *Initial volume* = 0. For fixed cells in PBS or for live cells in media, input the proper volume in μL .
 - viii. Adjust *Lid type*.
 - ix. Repeat Steps 20biii – 20bvii for *Plate group 2*.

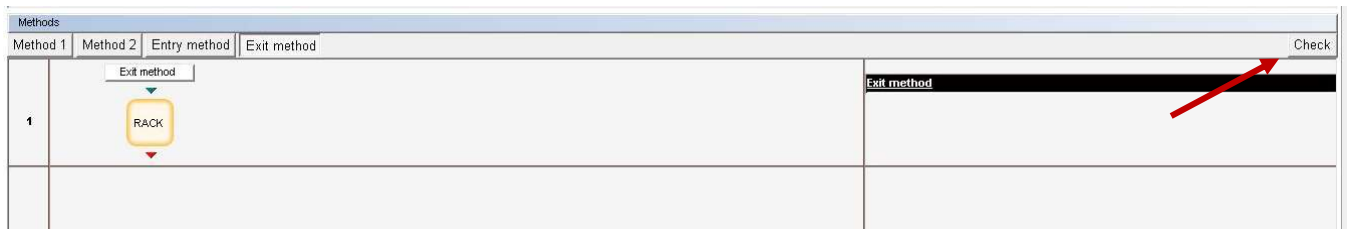


Properties	
Name	SET_INIT_STORAGE
DisplayName	\$Rack_number, \$Storage_type, Max:\$Max_plates, groups:\$No_of_Plate_groups
Rack number	1
Storage type	get_put
Max plates	14
No of plate groups	2
▼ Plate group 1	{("PKI_CellCarrier96_Ultra","MyPlate1",1,EvalString("PlateCountExp1"),0,"normal"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate1
Start location	1
No of plates	=PlateCountExp1
Initial volume	0 μl
Lid type	normal
▼ Plate group 2	{("PKI_CellCarrier96_Ultra","MyPlate2",2,EvalString("PlateCountExp2"),0,"none"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate2
Start location	2
No of plates	=PlateCountExp2
Initial volume	0 μl
Lid type	none
► Name List	0

21. Click the *Exit method* tab, just above the flow diagram.
 - a. Make sure the component matches where you put your plate and the final step of your flow diagram (RACK).

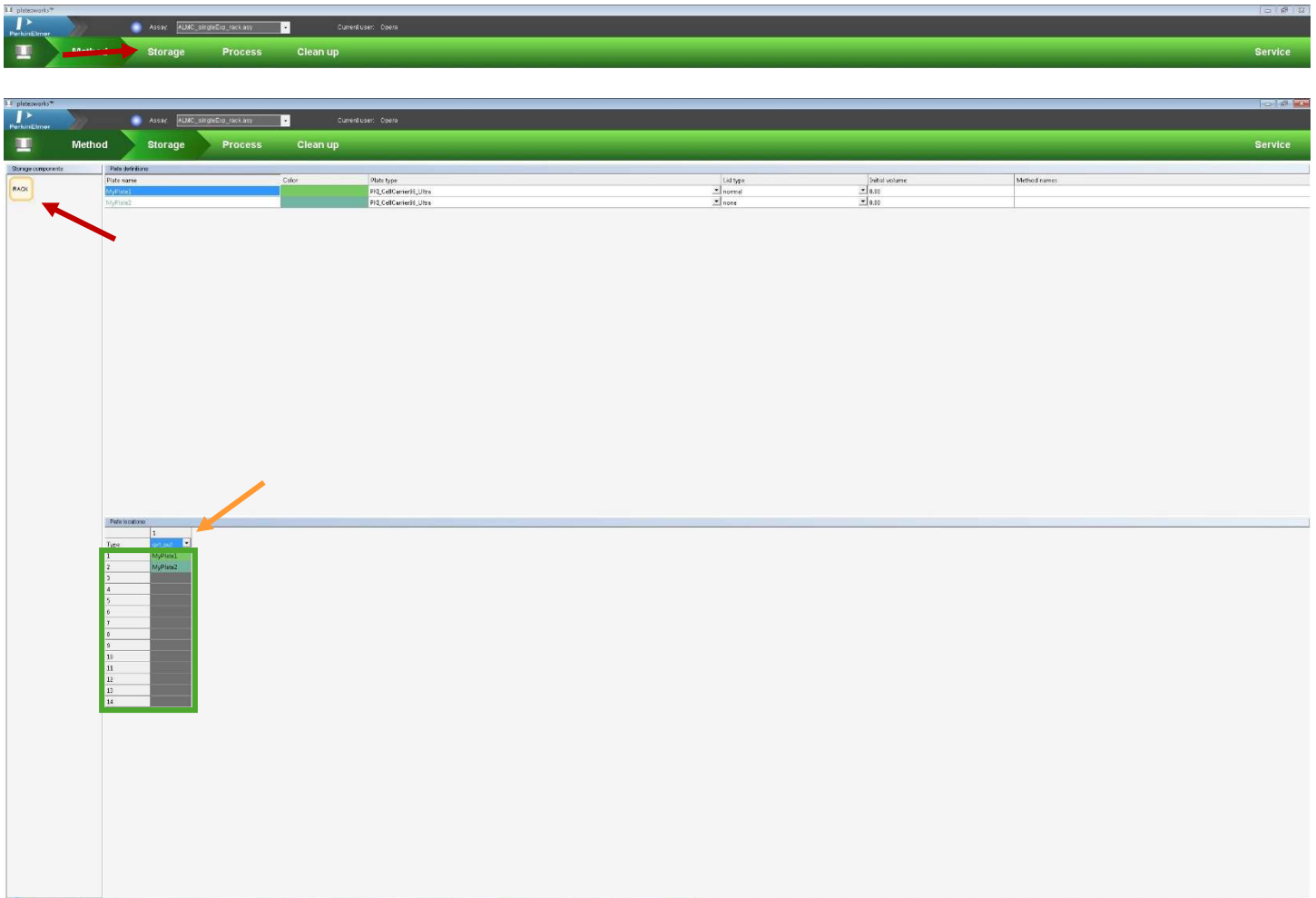


22. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.
- a. Address any errors and repeat this step until no errors appear.



Run an automation

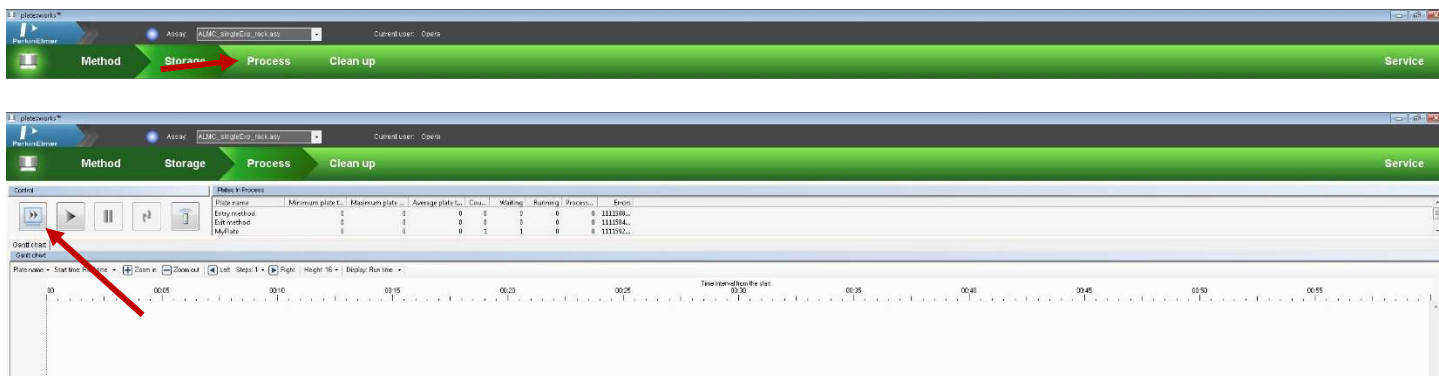
24. Click the *Storage* chevron at the top of the screen.
 - a. On the left side of the screen, click the *RACK* icon where your plates will be located/stored.
 - b. Near the top of the screen, there is a table of the “plates” that are used in the automation.
 - i. There should be as many “plates” as there are different experiments/imaging settings.
 - ii. If you need to add a “plate”, right-click → *Add Plate*.
 - iii. Adjust the plate types.
 - c. Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack.
 - d. Highlight the position(s) in the first column where you will put your plates for Experiment 1. The position(s) should turn blue.
 - e. Right-click → *Set places for [MyPlate1]*. The position(s) should turn green.
 - f. Repeat Steps 24d – 24e for the other experiments and Plate types (e.g., *MyPlate2*).
 - g. Highlight the remaining position(s) in the column where there will not be plates. The position(s) should turn blue.
 - h. Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.



25. Click the *Process* chevron at the top of the screen.
 - a. Click the *Simulate assay* button (computer screen with double arrows).
 - b. In the *Assay Wizard* (green pop-up window click *Next* to move through the windows, changing the *PlateCount* variables to match the number of plates that you want to run in each experiment,

and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.

- If there are any errors, they will appear now and, sometimes, the software will ask you what to do.
- If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
- The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to with the proper experiments / imaging settings. Clicking on the sections in the simulated time log will give you more details.

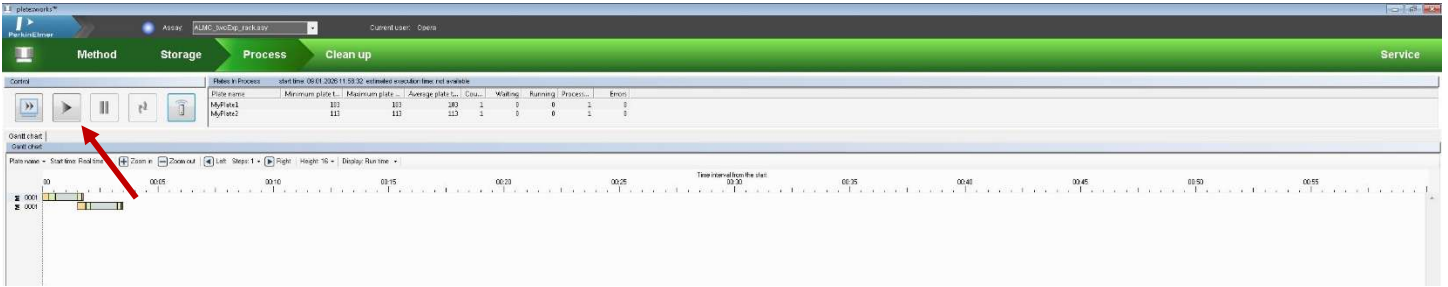


26. Fix any problems that arise and repeat the *Simulate assay* step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking *Save As...* and labeling it as *[Lab]_[initials]_description*. **Please, do not save over the original ALMC automation!**

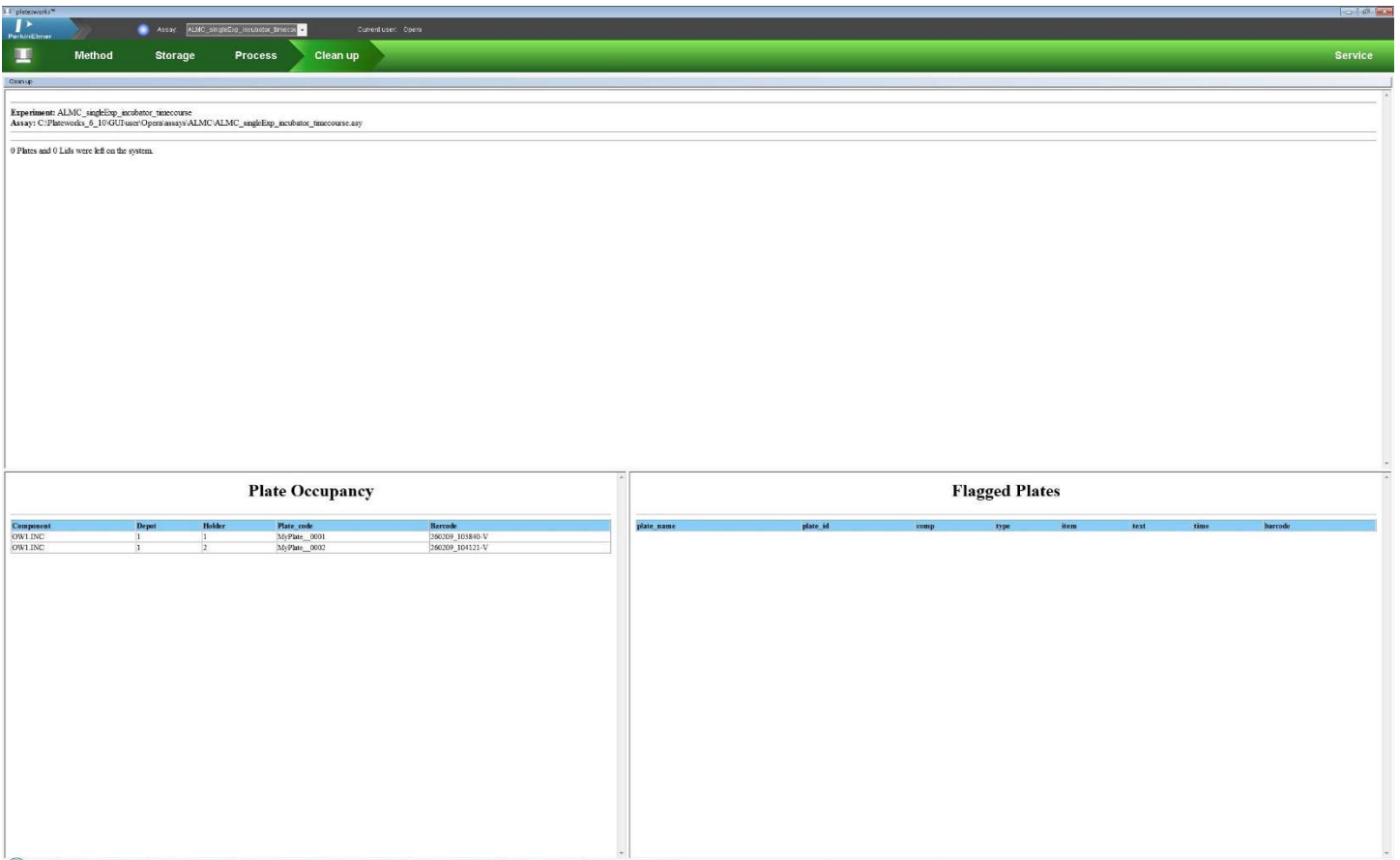


- Click the *Run assay* button (single large play button) to start the automation.
 - In the *Assay Wizard* (blue pop-up window), click *Next* to move through the windows.
 - Change the value for the *PlateCountExp1* variable = [number of plates in Experiment 1].
 - Change the value for the *PlateCountExp2* variable = [number of plates in Experiment 2].
 - Change the *Experiment-ID* and the *Description* to fit your experiment.
 - Click *Start*.
 - There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊

- c. After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.



28. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plates from the incubator.

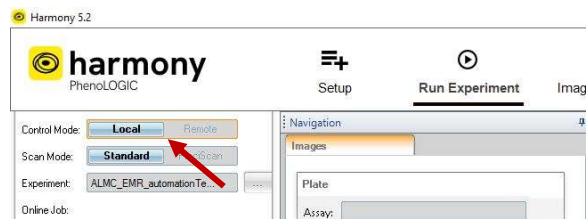


Look at and save your images

29. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



30. On the Harmony computer, re-open the *Harmony* application. In the *Run Experiment* tab, change the **Control to Local**. The images should be displayed in the *Run Experiment* tab.

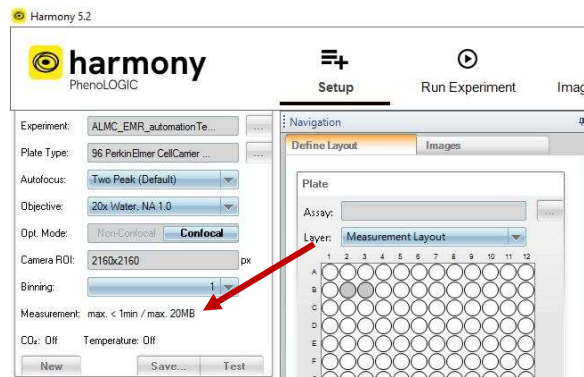


31. Save your images and their metadata using *Write Archive...* as normal.

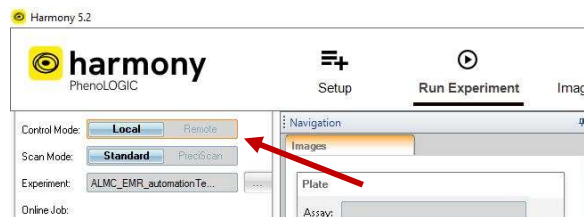
Create or edit an automation for plate(s) with a single experiment / same imaging settings from the incubator

Set up Harmony experiment and load plate(s)

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plate(s) into the incubator. Remove the rack by grabbing the handle on the top of the rack and rock it back and forth/pull toward you until it unclips from the holder. Insert plate(s) into the incubated plate rack, starting from the bottom. Position A1 in a well plate should be back-right of the plate rack when it is inserted into the holder (next to the **1** sticker).
2. In Harmony, in the *Setup* tab, load and set up your experiment, including the wells with your sample. Save it. **Write down the estimated time to run this experiment** (found near the *Save* button).



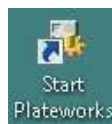
3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

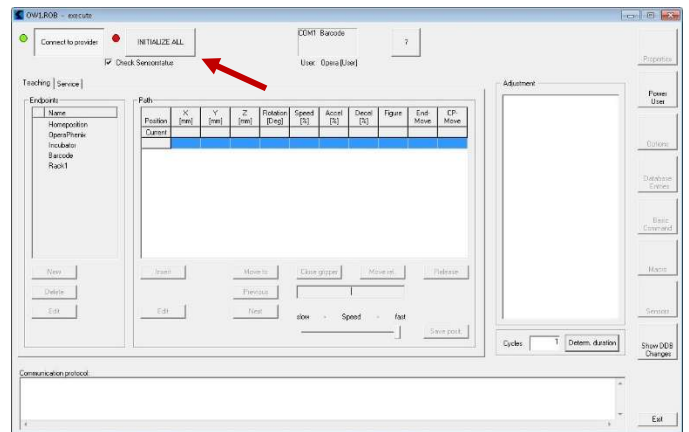
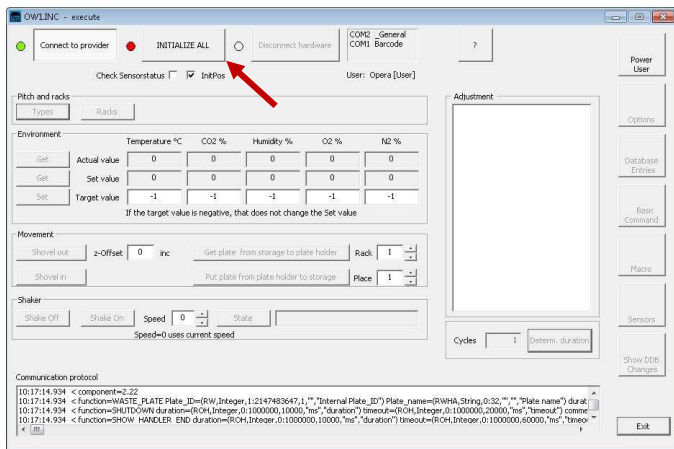
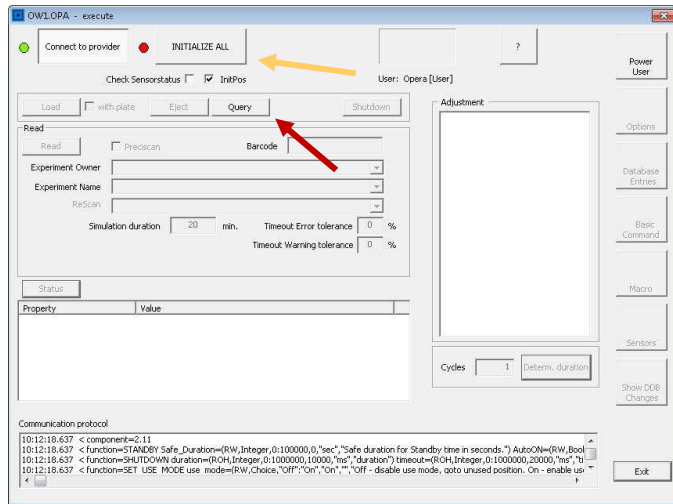


Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click *Service*.

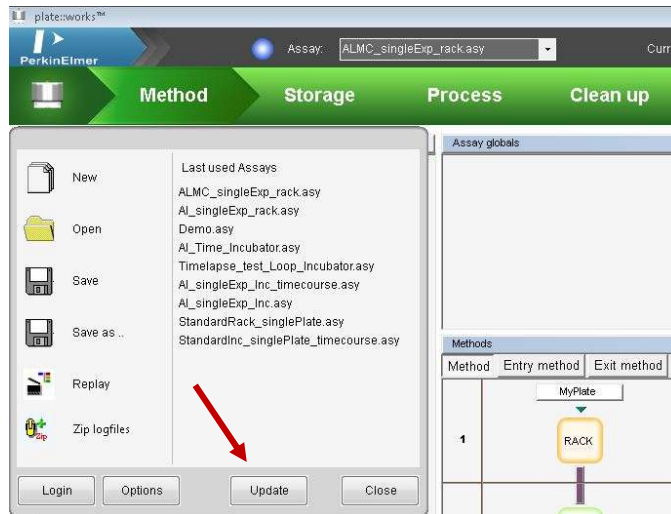


8. Right-click on OPA → *Direct Mode On*. In the pop-up window, click *Query* and then *Initialize All*. The loading door on the top of the microscope should open and close.
9. Right-click on ROB → *Direct Mode On*. In the pop-up window, click *Initialize All*. The robot hand should open and close.
10. Right-click on INC → *Direct Mode On*. In the pop-up window, click *Initialize All*. The incubator should beep a few times.



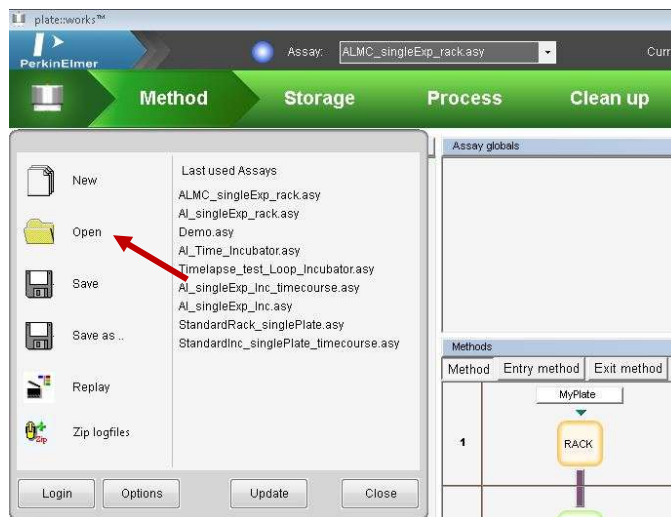
11. In the PlateWorks program, at the top-left of the screen, click the Phenix logo and then click *Update*. This will make sure that your new Harmony experiment is available to the automation software.





Load and edit a pre-made automation

12. Load a previous automation by clicking the Phenix logo and using *Open* to find the “ALMC_singleExp_incubator” automation as a starting point.



13. The *Method* chevron shows the current automation with the thick black vertical lines indicating a movement by the robot arm.

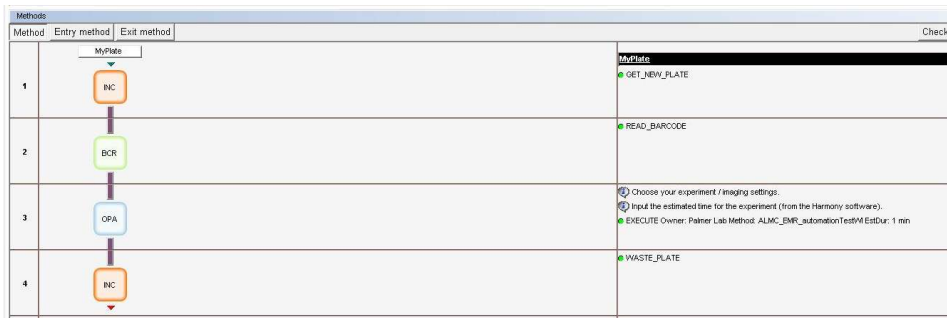
a. If you need to change or add any components, click the left *Components* tab, and drag components to the flow diagram.

INC = incubated plate rack

BCR = bar code reader

OPA = Opera Phenix microscope





14. Above the flow diagram, there should be a white rectangle with a plate name (e.g., *MyPlate*). Click this rectangle.

- a. *SourceCount* = *PlateCount*
- b. Adjust *PlateType* with the drop-down menu.
- c. Adjust *PlateName* if you'd like... but write down the new name, because it will be necessary later.



Service

Properties	
SourceCount	=PlateCount
PlateType	PK1_CellCarrier96_Ultra
Name	MyPlate
PlateLayout	
BatchSize	0
PlateTickRate	0 s
BatchGap	0 s

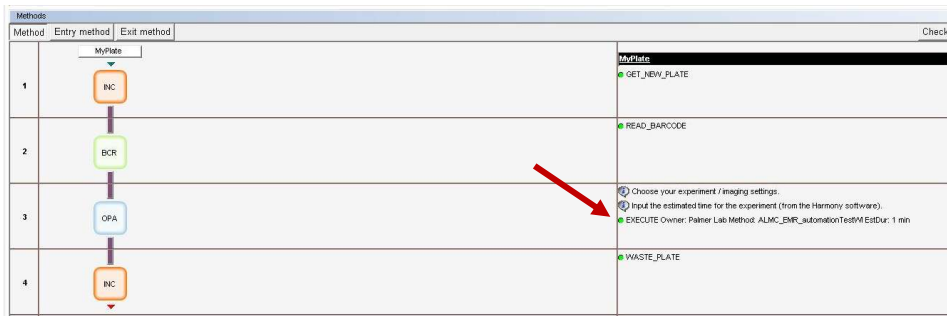
15. The first step should show *INC (component) | GET_NEW_PLATE (function)*. This will tell the automation to grab a plate starting from Position 1.

16. Keep the *BCR (component) | Read Barcode (function)* step as the second step in the flow even if you do not have barcodes on your plates. This will provide timestamps in the metadata.

17. The third step should be an *OPA (component) | Execute (function)* step.

- a. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: "Load Harmony experiment into PlateWorks".
- b. Adjust *Estimated duration* to reflect the estimated time written down from Harmony with a little extra time such that the measurement would not be aborted under normal circumstances (i.e., if the estimated time in Harmony was 20 minutes, enter 40 minutes; if the estimated time in Harmony was 60 minutes, enter 90 minutes). This value is for a timeout function to allow the automation to continue progressing if the plate fails to image correctly.

- c. *Timeout warning tolerance* = -1 (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
- d. *Timeout error tolerance* = -1 (default amount) or 110% (give error after experiment takes 10% more time than expected)

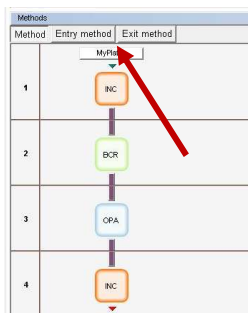


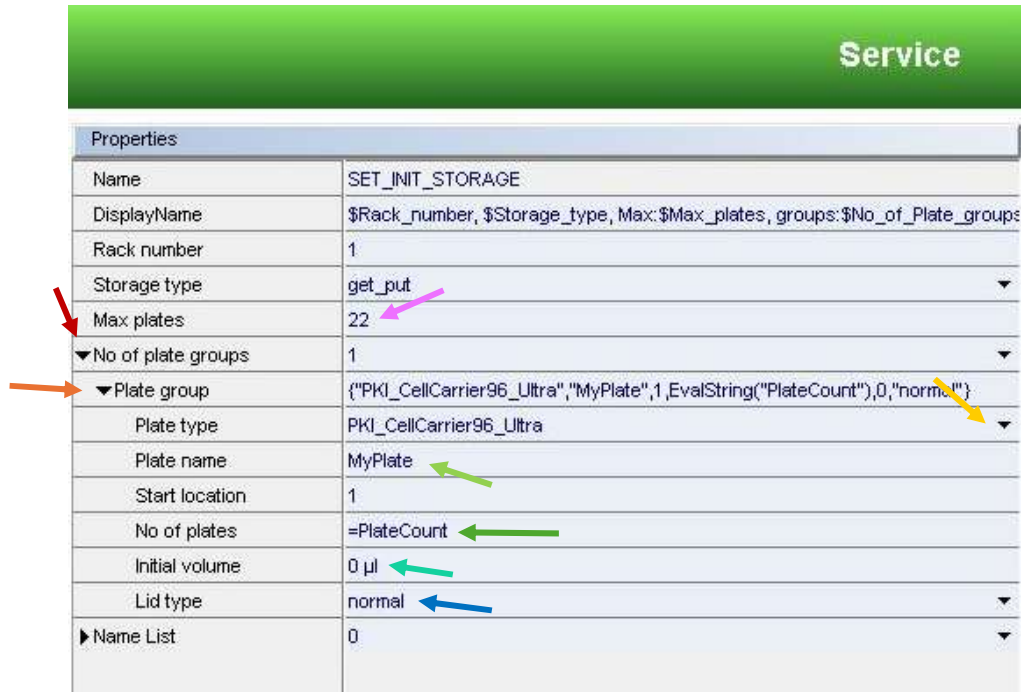
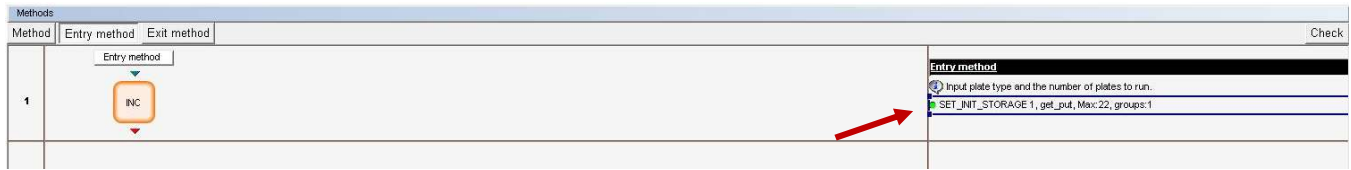
The screenshot shows the 'Service' window with a 'Properties' table for the EXECUTE function. The table has the following data:

Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_duration
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestM
Remark	USE THE DIRECT MODE TOOL TO REFRESH THE LISTS!
Estimated duration	1 min
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

A green arrow points to the 'Timeout error tolerance' field, and two orange arrows point to the 'Experiment Owner' and 'Experiment Name' fields.

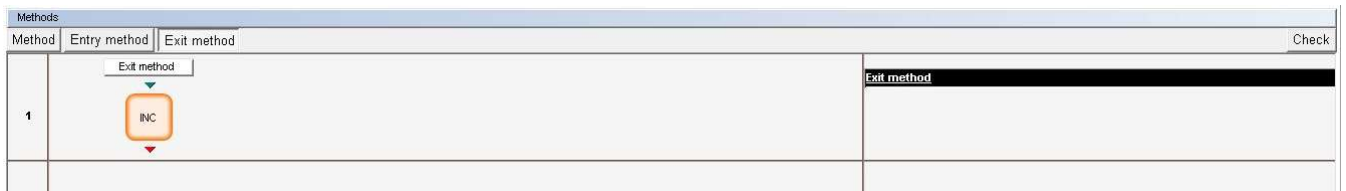
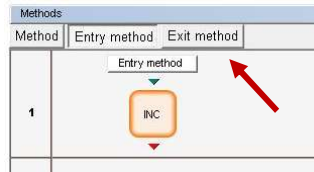
- 18. Finally, the fourth step should be an *INC (component) | WASTE_PLATE (function)* step. This will tell the automation that after this flow, this plate will not be used again, but that it should return to the incubator.
- 19. Just above the flow diagram, click the top *Entry method* tab.
 - a. Make sure the component matches where you put your plate and the first step of your flow diagram (INC). It should only be one step.
 - b. Click the *SET_INIT_STORAGE (function)*.
 - i. *Max plates* = 22
 - ii. *No of plate groups* = 1
 - iii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group*. Adjust the *Plate type* in the drop-down menu.
 - iv. Change the *Plate name* if you'd like to... (make sure it's the same plate name as before!)
 - v. *Start location* = 1
 - vi. *No of plates* = *PlateCount* (variable)
 - vii. For live cells in media, input the proper volume in μL .
 - viii. Adjust *Lid type*.





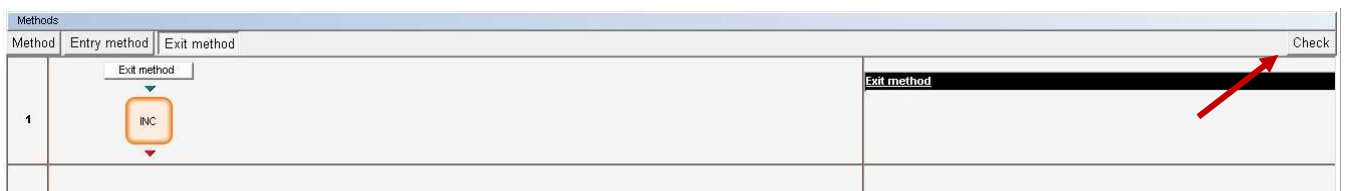
20. Click the *Exit method* tab, just above the flow diagram.

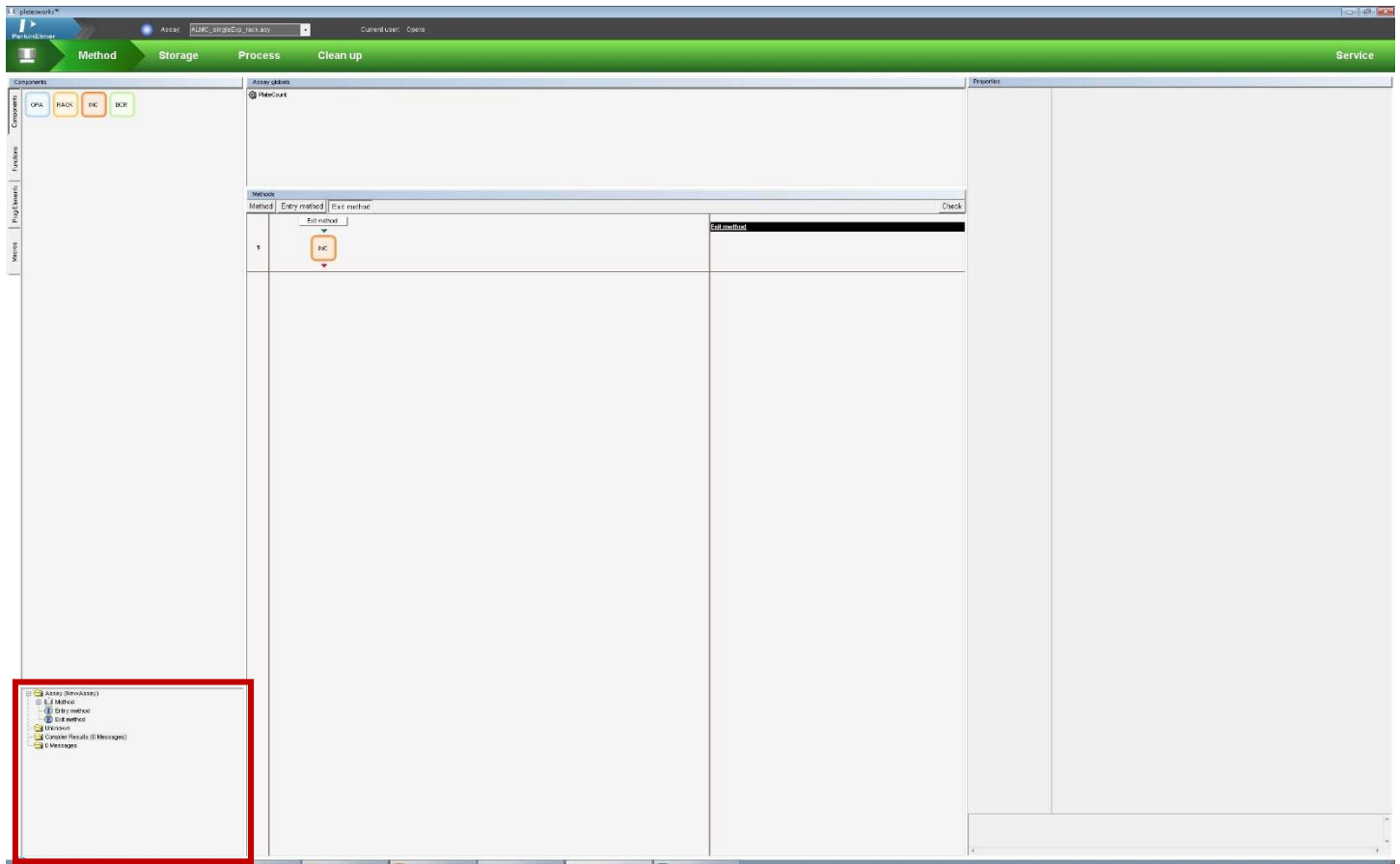
- a. Make sure the component matches where you put your plate and the final step of your flow diagram (INC). It should only be one step.



21. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.

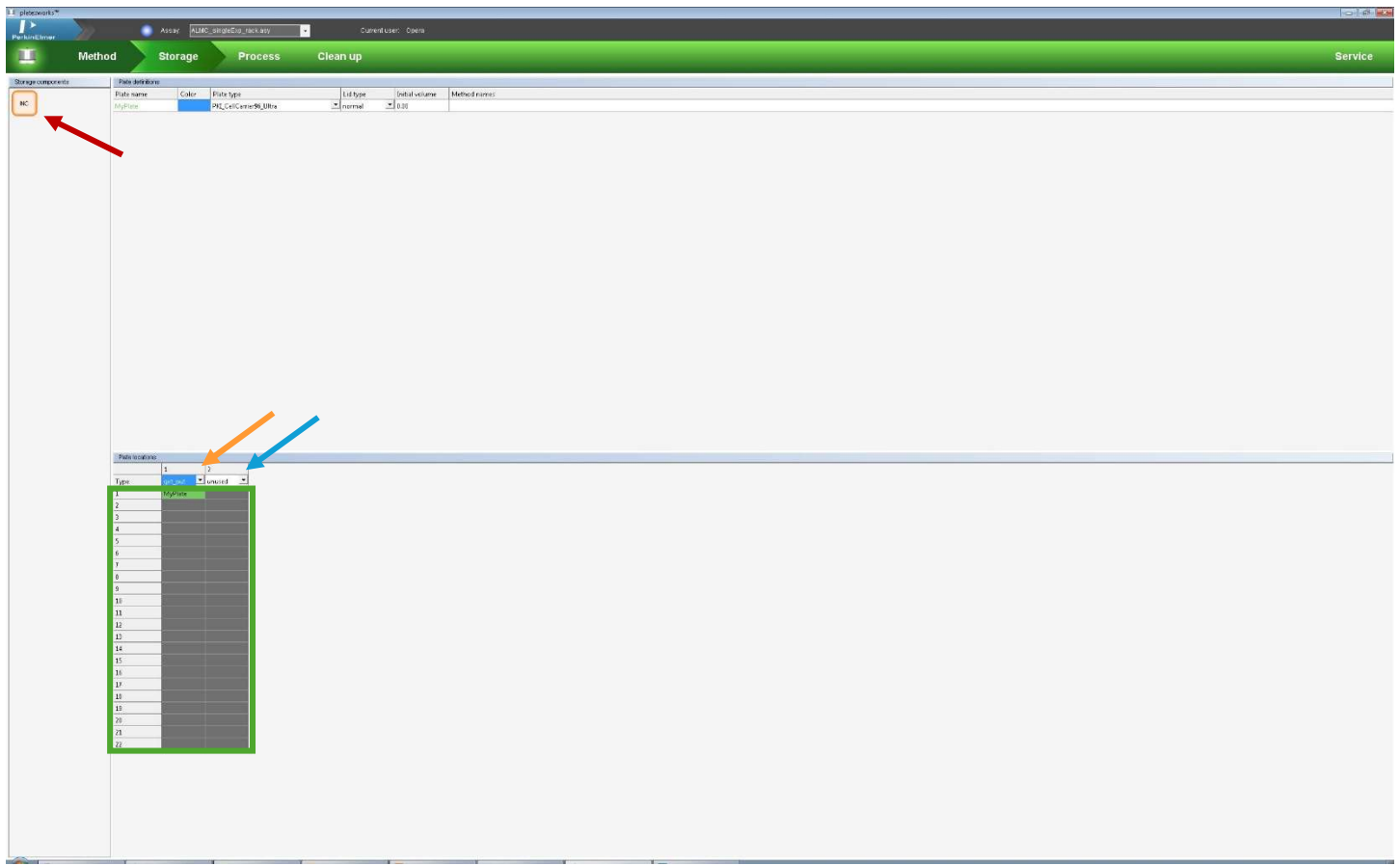
- a. Address any errors and repeat this step until no errors appear.



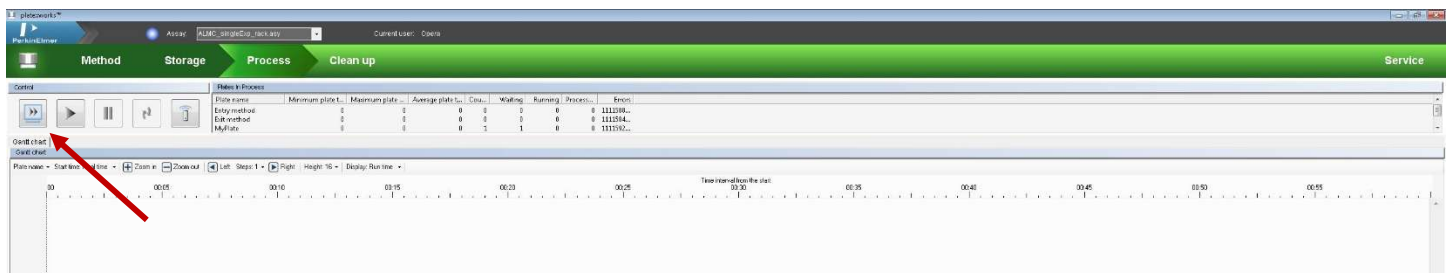


Run an automation

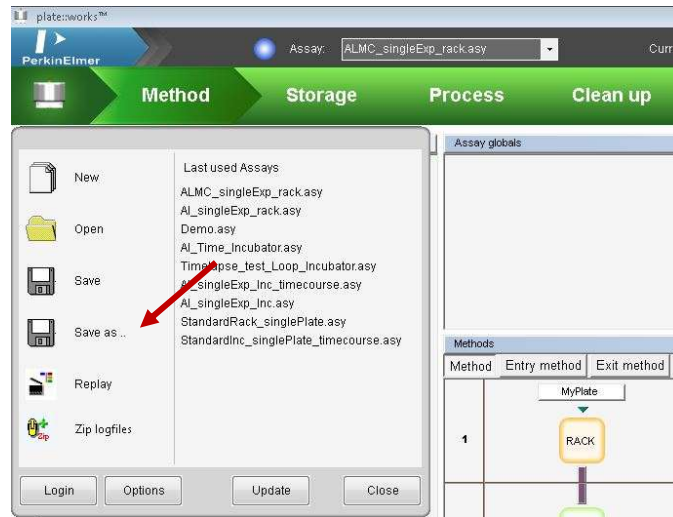
22. Click the *Storage* chevron at the top of the screen.
 - a. On the left side of the screen, click the *INC* icon where your plate(s) will be located/stored.
 - b. Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack.
 - c. Highlight the position(s) in the column where you will put your plate(s). The position(s) should turn blue.
 - d. Right-click → *Set places for '[plate name]'*. The position(s) should turn green.
 - e. Highlight the remaining position(s) in the column where there will not be plates. The position(s) should turn blue.
 - f. Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.
 - g. If there are no plates in the second column, select *unused* from the drop-down menu at the top of the column.



23. Click the *Process* chevron at the top of the screen.
 - a. Click the *Simulate assay* button (computer screen with double arrows).
 - b. In the *Assay Wizard* (blue pop-up window), click *Next* to move through the windows, changing the *PlateCount* variable to match the number of plates that you want to run, and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.
 - c. If there are any errors, they will appear now and, sometimes, the software will ask you what to do.
 - d. If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
 - e. The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to. Clicking on the sections in the simulated time log will give you more details.



24. Fix any problems that arise and repeat the *Simulate* assay step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking *Save As...* and labeling it as *[Lab]_[initials]_description*. Please, do not save over the original ALMC automation!

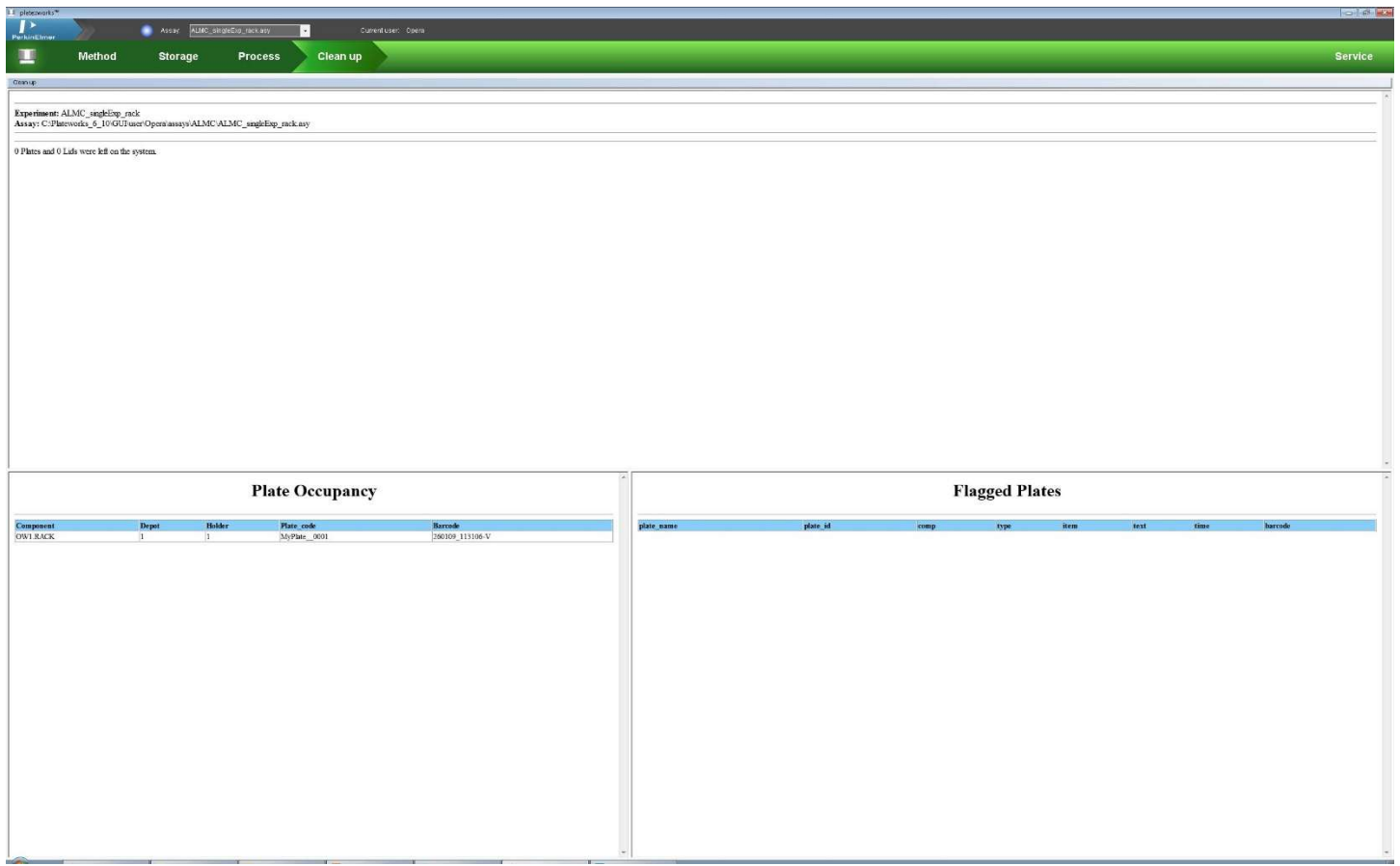


25. Click the *Run assay* button (single large play button) to start the automation.
 - a. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows.
 - i. Change the *PlateCount* variable = [number of plates].
 - ii. Change the *Experiment-ID* and the *Description* to fit your experiment.
 - iii. Click *Start*.
 - b. There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊
 - c. After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.



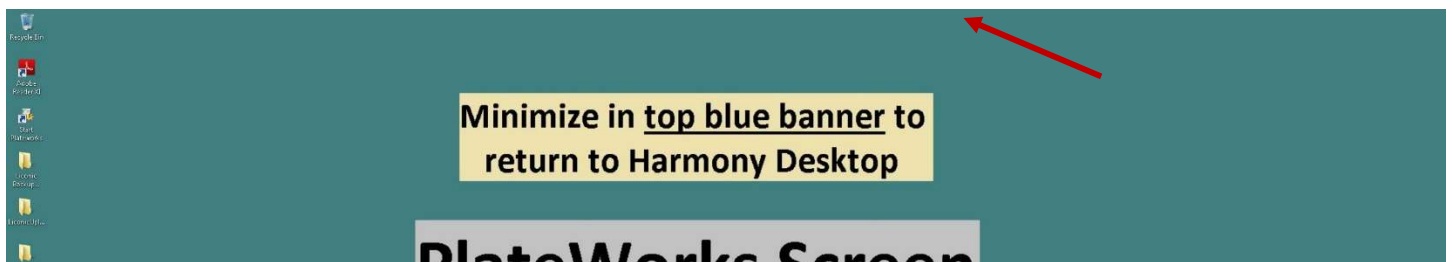
26. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plate(s) from the incubator.



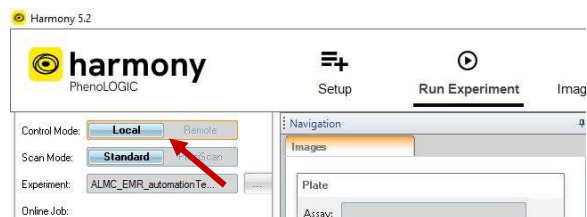


Look at and save your images

27. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



28. On the Harmony computer, re-open the *Harmony* application. In the **Run Experiment** tab, change the **Control to Local**. The images should be displayed in the *Run Experiment* tab.

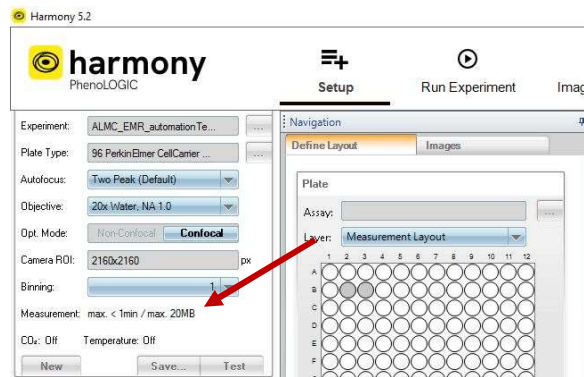


29. Save your images and their metadata using *Write Archive...* as normal.

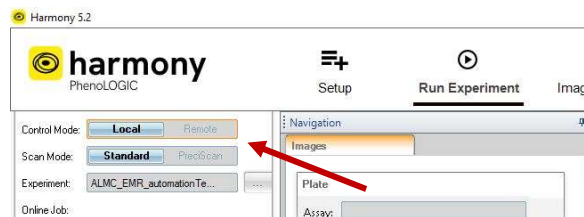
Create or edit an automation for plates with a single experiment / same imaging settings from the incubator with timelapse imaging

Set up Harmony experiment and load plates

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plate(s) into the incubator. Remove the rack by grabbing the handle on the top of the rack and rock it back and forth/pull toward you until it unclips from the holder. Insert plate(s) into the incubated plate rack, starting from the bottom. Position A1 in a well plate should be back-right of the plate rack when it is inserted into the holder (next to the **1** sticker).
2. In Harmony, in the *Setup* tab, load and set up your experiment, including the wells with your sample. Save it. **Write down the estimated time to run this experiment** (found near the *Save* button).



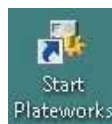
3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

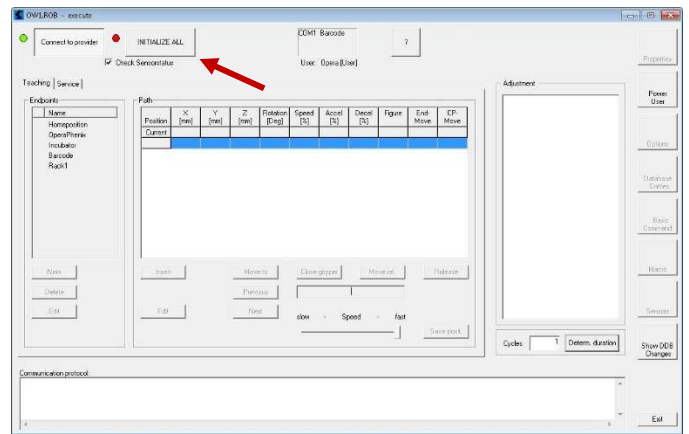
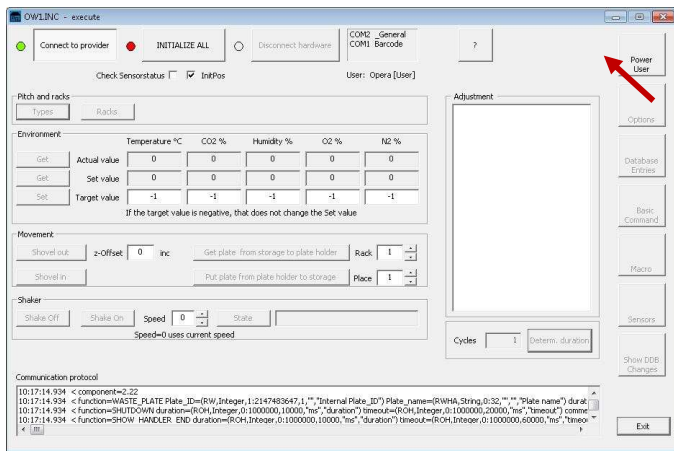
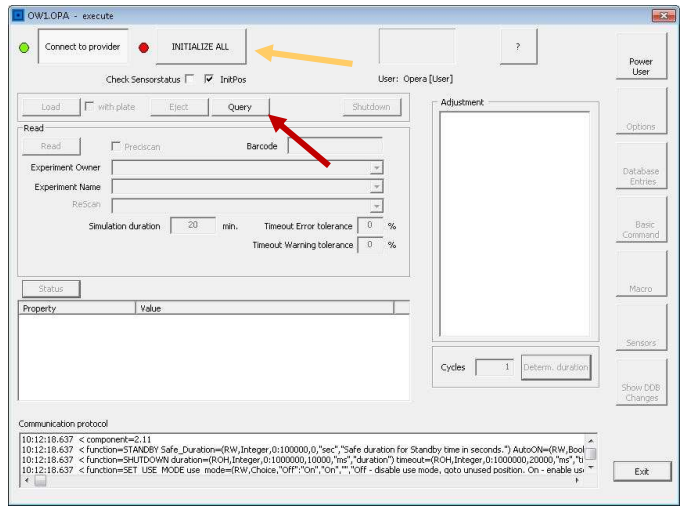


Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click *Service*.

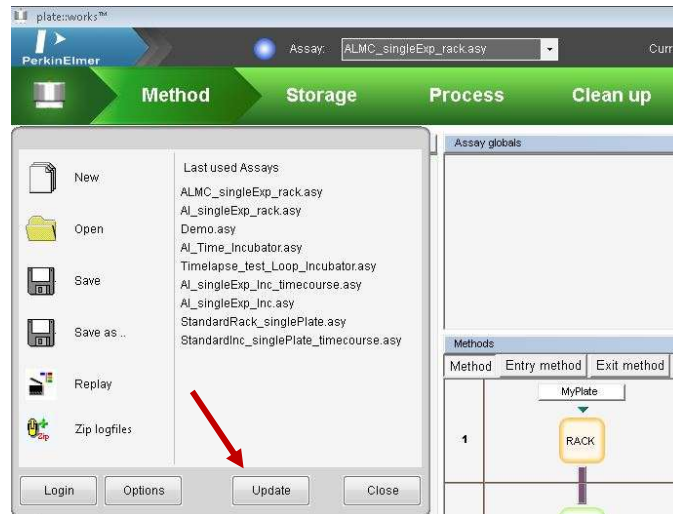


8. Right-click on *OPA* → *Direct Mode On*. In the pop-up window, click *Query* and then *Initialize All*. The loading door on the top of the microscope should open and close.
9. Right-click on *ROB* → *Direct Mode On*. In the pop-up window, click *Initialize All*. The robot hand should open and close.
10. Right-click on *INC* → *Direct Mode On*. In the pop-up window, click *Initialize All*. The incubator should beep a few times.



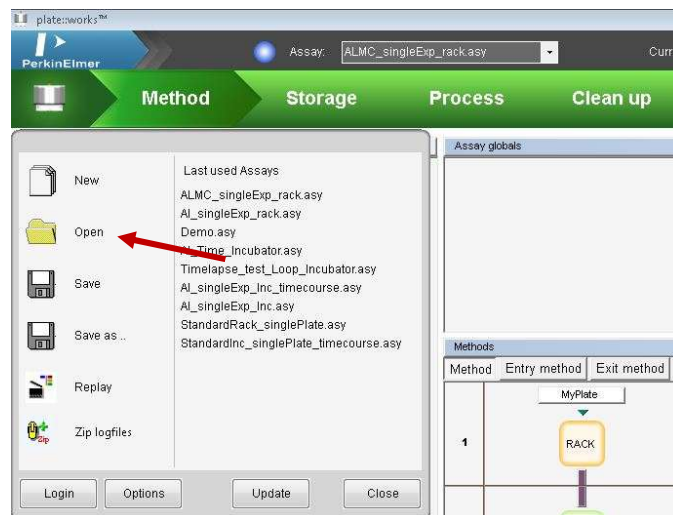
11. In the PlateWorks program, at the top-left of the screen, click the Phenix logo and then click *Update*. This will make sure that your new Harmony experiment is available to the automation software.





Load and edit a pre-made automation

12. Load a previous automation by clicking the Phenix logo and using *Open* to find the “ALMC_singleExp_incubator_timecourse” automation as a starting point.



13. In *Method* chevron, the *Method* tab shows the current automation with the thick black vertical lines indicating a movement by the robot arm and the thin blue vertical arrows indicating a loop.
 - a. If you need to change or add any components, click the left *Components* tab, and drag components to the flow diagram.
 - INC = incubated plate rack
 - BCR = bar code reader
 - OPA = Opera Phenix microscope
 - b. If you need to add loops, click the left *ProgElements* tab and drag the *Loop (element)* to a component on the flow diagram. Loops can then be dragged to the final component of the loop to complete the loop.

Methods			Check
Method	Entry method	Exit method	
1	MyPlate INC		MyPlate ● GET_NEW_PLATE
2	BCR		● READ_BARCODE
3	OPA		⚙ SetTimeStamp 🔗 Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMF_automationTest\M EstDur: =Estimated_Read
4	INC		⌚ WaitTimeStamp =60*(Interval - Plate_Transit_Time), {}
5	OPA		⌚ WaitTimeStamp =60*Interval, {} ⚙ SetTimeStamp 🔗 Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMF_automationTest\M EstDur: =Estimated_Read
6	INC		● WASTE_PLATE

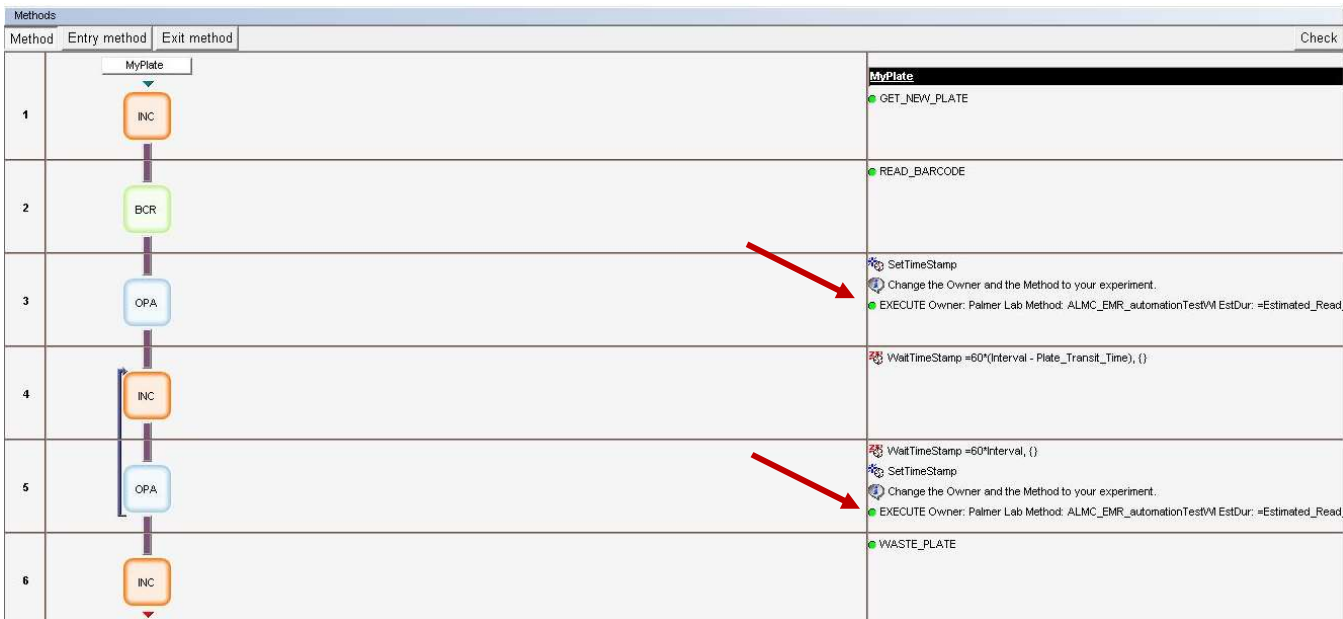
14. Above the flow diagram, there should be a white rectangle with a plate name (e.g., *MyPlate*). Click this rectangle.

- $SourceCount = PlateCount$ (variable)
- Adjust *PlateType* with the drop-down menu.
- Change *PlateName* if you'd like... but write down the new name, because it will be necessary later.
- $BatchSize = CalculatedBatchSize$ (variable)
- $BatchGap = ((CalculatedBatchSize * Estimated_Read_Time) + ((Number_of_Timepoints - 1) * Interval)) * 60$

Methods			Check
Method	Entry method	Exit method	
1	MyPlate INC		MyPlate ● GET_NEW_PLATE
2	BCR		● READ_BARCODE
3	OPA		⚙ SetTimeStamp 🔗 Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMF_automationTest\M EstDur: =Estimated_Read
4	INC		⌚ WaitTimeStamp =60*(Interval - Plate_Transit_Time), {}
5	OPA		⌚ WaitTimeStamp =60*Interval, {} ⚙ SetTimeStamp 🔗 Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMF_automationTest\M EstDur: =Estimated_Read
6	INC		● WASTE_PLATE

Service	
Properties	
SourceCount	=PlateCount
PlateType	PKI_CellCarrier96_Ultra
Name	MyPlate
PlateLayout	
BatchSize	=CalculatedBatchSize
PlateTickRate	0 s
BatchGap	$=((\text{CalculatedBatchSize} * \text{Estimated_Read_Time}) + ((\text{Number_of_Timepoints} - 1)$

15. The first step should show *INC (component) | GET_NEW_PLATE (function)*. This will tell the automation to grab a plate starting from Position 1.
16. Keep the *BCR (component) | Read Barcode (function)* step as the second step in the flow even if you do not have barcodes on your plates. This will provide timestamps in the metadata.
17. The third step should be an *OPA (component) | SetTimeStamp (element), Execute (function)* step to start a timer and to image the plate for the first time.
 - a. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: "Load Harmony experiment into PlateWorks".
 - b. *Estimated duration = Estimated_Read_Time (variable)*
 - c. *Timeout warning tolerance = -1 (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)*
 - d. *Timeout error tolerance = -1 (default amount) or 110% (give error after experiment takes 10% more time than expected)*



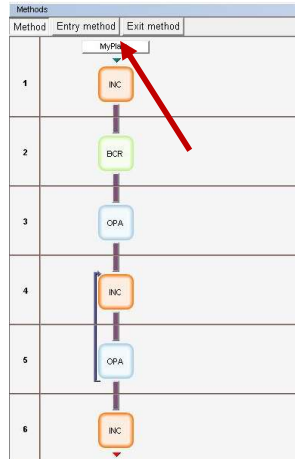
Service	
Properties	
Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_du
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestWJ_4Ch
Remark	USE THE DIRECT MODE TOOL TO REFRESH THE LISTS!
Estimated duration	=Estimated_Read_Time
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

18. The fourth step should show an *INC (component) | WaitTimeStamp (element)* step to incubate the plate for the desired period of time in the incubator before moving the plate to the Phenix for imaging.
 - a. $WaitTimeStamp = 60 * (Interval - Plate_Transit_Time), \{ \}$
19. The fifth step should be an *OPA (component) | WaitTimeStamp (element), SetTimeStamp (element), Execute (function)* step to incubate the plate for any remaining time of the desired period of time before imaging it again.
 - a. $WaitTimeStamp = 60 * Interval, \{ \}$
 - b. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: “Load Harmony experiment into PlateWorks”.
 - c. $Estimated\ duration = Estimated_Read_Time$ (variable)
 - d. $Timeout\ warning\ tolerance = -1$ (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
 - e. $Timeout\ error\ tolerance = -1$ (default amount) or 110% (give error after experiment takes 10% more time than expected)

Service	
Properties	
Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_du
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestWJ_4Ch
Remark	USE THE DIRECT MODE TOOL TO REFRESH THE LISTS!
Estimated duration	=Estimated_Read_Time
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

20. There should be a *Loop* that connects from Step 3 (*OPA | StartTimeStamp, Execute*) to Step 5 (*OPA | WaitTimeStamp, StartTimeStamp, Execute*).
 - a. $Loops = Number_of_Timepoints$ (variable)
21. Finally, the sixth step should be an *INC (component) | WASTE_PLATE (function)* step. This will tell the automation that after this flow, this plate will not be used again, but that it should return to the incubator.
22. Just above the flow diagram, click the top *Entry method* tab.
 - a. Make sure the component matches where you put your plate and the first step of your flow diagram (*INC*). It should be two steps.
 - b. Click the *SET_INIT_STORAGE* (function).

- i. *Max plates* = 22
- ii. *No of plate groups* = 1
- iii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group*. Adjust the *Plate type* in the drop-down menu.
- iv. Change the *Plate name* if you'd like to... (make sure it's the same plate name as before!)
- v. *Start location* = 1
- vi. *No of plates* = *PlateCount* (variable)
- vii. For live cells in media, input the proper volume in μL .
- viii. Adjust *Lid type*.







Methods			Check
Method	Entry method	Exit method	
1	INC		<p>Entry method</p> <ul style="list-style-type: none"> Input the number of plates: SET_INT_STORAGE 1, get_put, Max:22, groups:1
2	INC		

Service



Properties	
Name	SET_INIT_STORAGE
DisplayName	\$Rack_number, \$Storage_type, Max:\$Max_plates, groups:\$No_of_Plate_groups
Rack number	1
Storage type	get_put
Max plates	22
▼ No of plate groups	1
▼ Plate group	{"PKI_CellCarrier96_Ultra", "MyPlate", 1, EvalString("PlateCount"), 100, "normal"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate
Start location	1
No of plates	=PlateCount
Initial volume	100 µl
Lid type	normal
▶ Name List	0

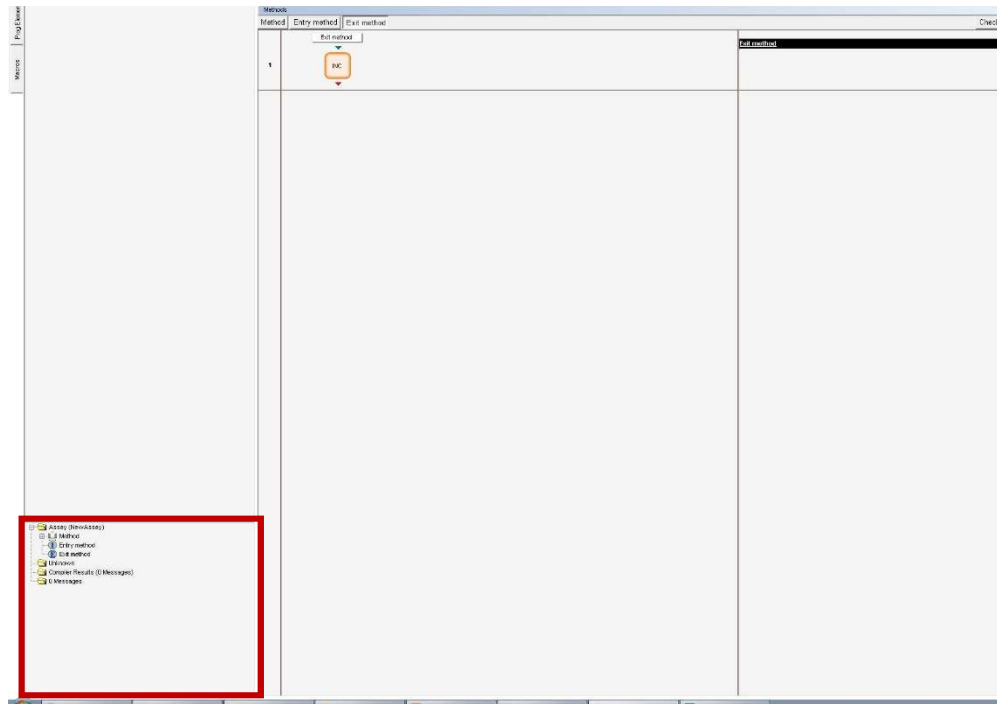
23. Click the *Exit method* tab, just above the flow diagram.
- Make sure the component matches where you put your plate and the final step of your flow diagram (INC).

Methods	
Method	Entry method Exit method
1	<div style="text-align: center;"> Entry method  </div>
2	<div style="text-align: center;">  </div>

Methods		
Method	Entry method Exit method	Check
1	<div style="text-align: center;"> Exit method  </div>	<div style="text-align: center;"> Exit method  </div>

24. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.
- Address any errors and repeat this step until no errors appear.

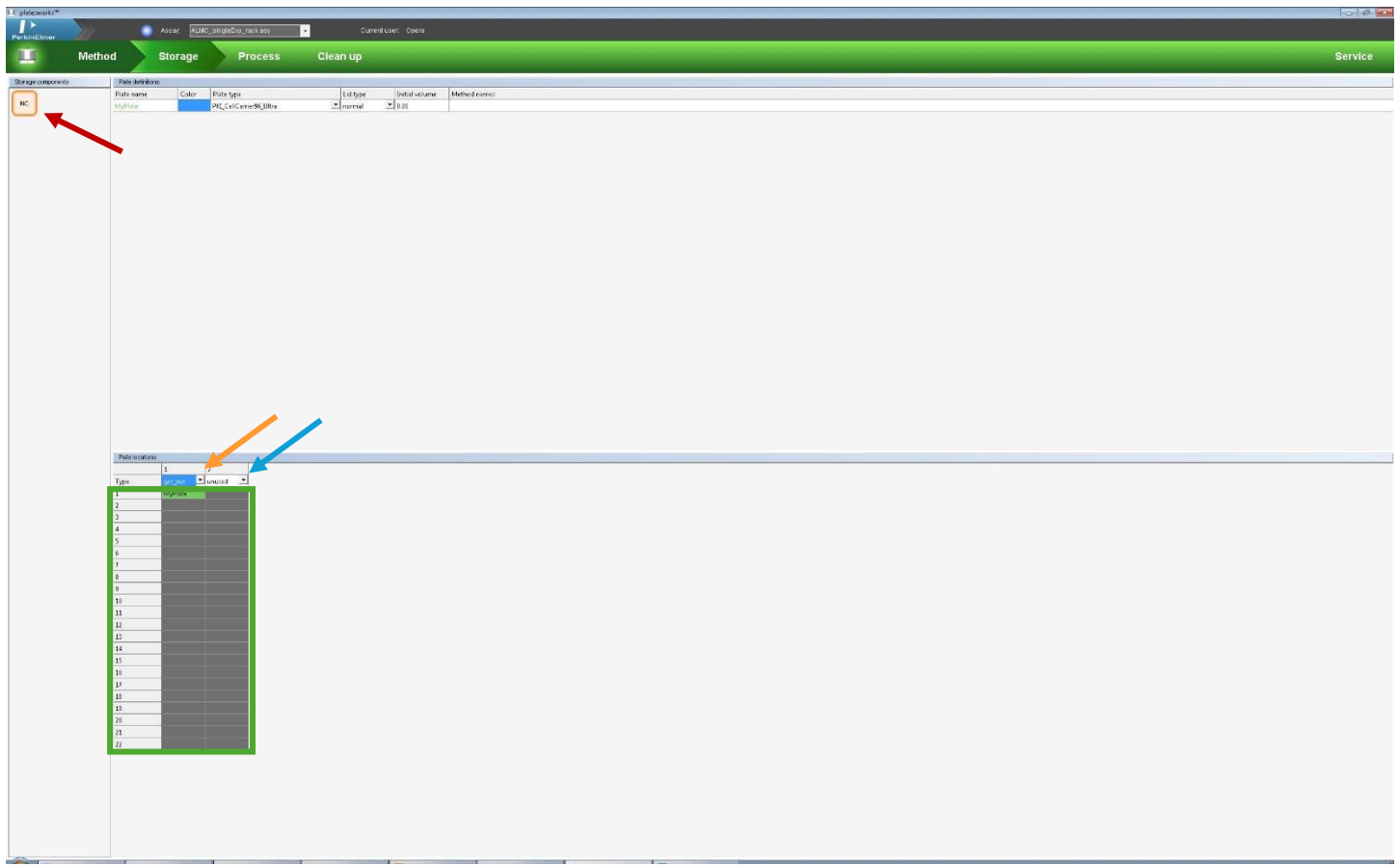
Methods		
Method	Entry method Exit method	Check
1	<div style="text-align: center;"> Exit method  </div>	<div style="text-align: center;"> Exit method  </div>



Run an automation

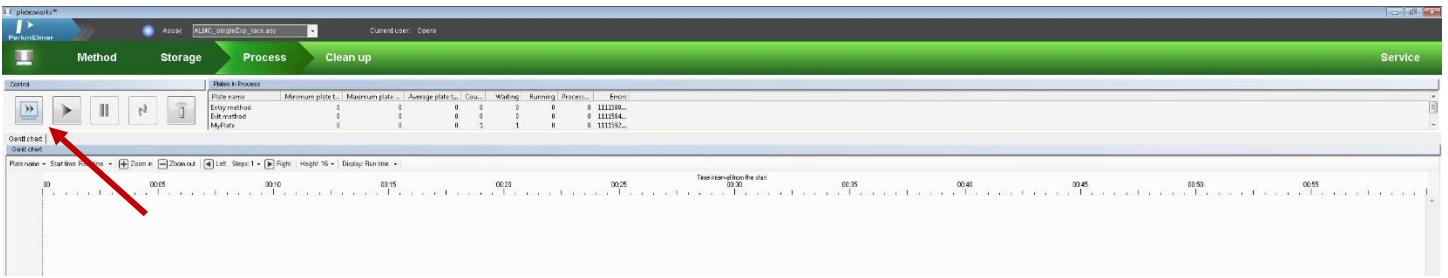
25. Click the *Storage* chevron at the top of the screen.
 - a. On the left side of the screen, click the *INC* icon where your plates will be located/stored.
 - b. Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack.
 - c. Highlight the position(s) in the column where you will put your plates. The position(s) should turn blue.
 - d. Right-click → *Set places for '[plate name]'*. The position(s) should turn green.
 - e. Highlight the remaining position(s) in the column where there will not be plates. The position(s) should turn blue.
 - f. Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.
 - g. If there are no plates in the second column, select *unused* from the drop-down menu at the top of the column.





26. Click the *Process* chevron at the top of the screen.
 - a. Click the *Simulate assay* button (computer screen with double arrows).
 - b. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows, changing the *PlateCount* variable to match the number of plates that you want to run, changing the *Number_of_Timepoints* variable to match the number of timepoints that you want, changing the *Estimated_Read_Time* variable and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.
 - i. Adjust *Estimated_Read_Time* to reflect the estimated time written down from Harmony with a little extra time such that the measurement would not be aborted under normal circumstances (i.e., if the estimated time in Harmony was 20 minutes, enter 40 minutes; if the estimated time in Harmony was 60 minutes, enter 90 minutes). This value is for a timeout function to allow the automation to continue progressing if the plate fails to image correctly.
 - c. If there are any errors, they will appear now and, sometimes, the software will ask you what to do.
 - d. If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
 - e. The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to and that the timecourse looks correct. Clicking on the sections in the simulated time log will give you more details.





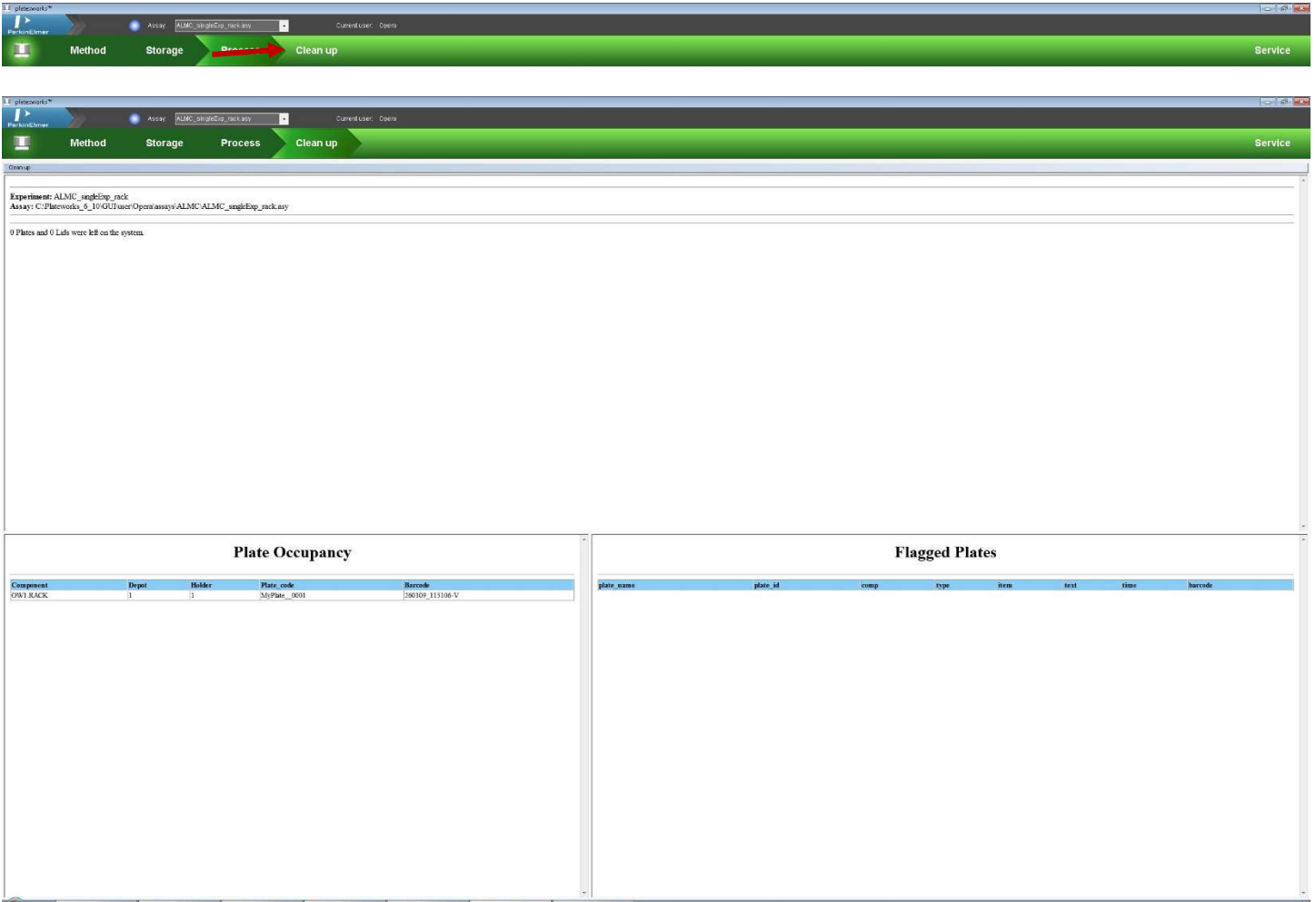
27. Fix any problems that arise and repeat the *Simulate assay* step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking *Save As...* and labeling it as *[Lab]_[initials]_description*. Please, do not save over the original ALMC automation!



28. Click the *Run assay* button (single large play button) to start the automation.
- In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows.
 - Change the *PlateCount* variable = [number of plates].
 - Change the *Number_of_Timepoints* variable = [number of timepoints].
 - Change the *Estimated_Read_Time* variable = [estimated time to image your plate once]
 - Change the *Experiment-ID* and the *Description* to fit your experiment.
 - Click *Start*.
 - There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊
 - After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.



29. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plates from the incubator.

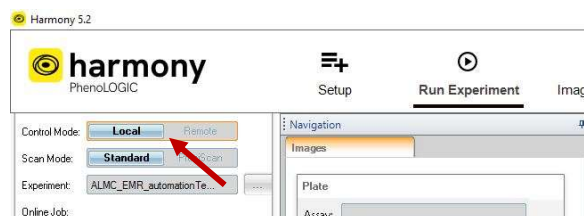


Look at and save your images

30. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



31. On the Harmony computer, re-open the *Harmony* application. In the *Run Experiment* tab, change the **Control to Local**. The images should be displayed in the *Run Experiment* tab.

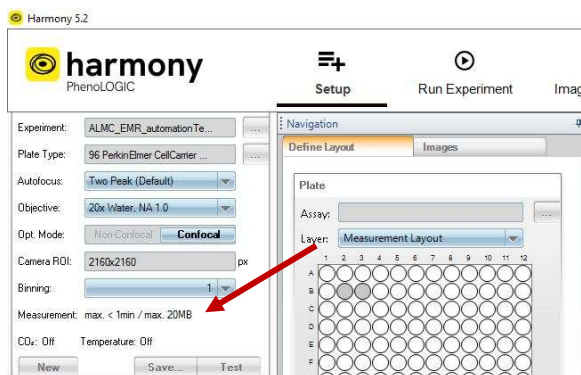


32. Save your images and their metadata using *Write Archive...* as normal.

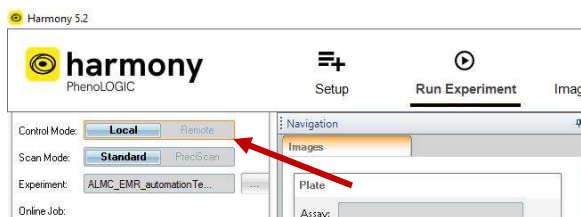
Create or edit an automation for plates with two or more experiments / different imaging settings from the incubator

Set up Harmony experiment and load plates

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plates into the incubator. Remove the rack by grabbing the handle on the top of the rack and rock it back and forth/pull toward you until it unclips from the holder. Insert plate(s) into the incubated plate rack, starting from the bottom. Position A1 in a well plate should be back-right of the plate rack when it is inserted into the holder (next to the 1 sticker).
2. In Harmony, in the *Setup* tab, load and set up all your experiments, including the wells with your sample. For each experiment, save it. **For each experiment, write down the estimated time to run the experiment** (found near the *Save* button).



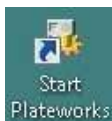
3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

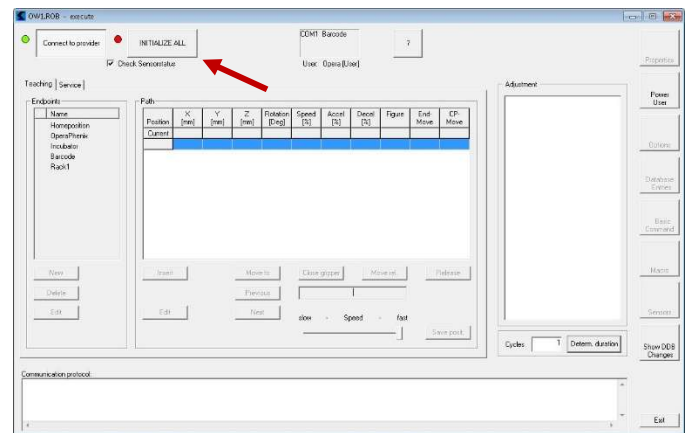
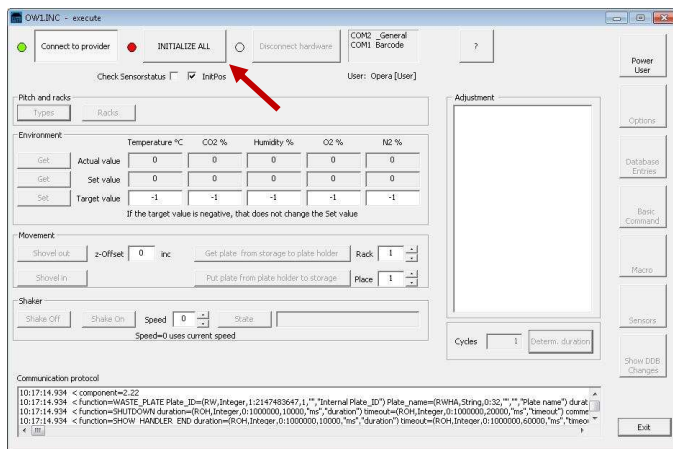
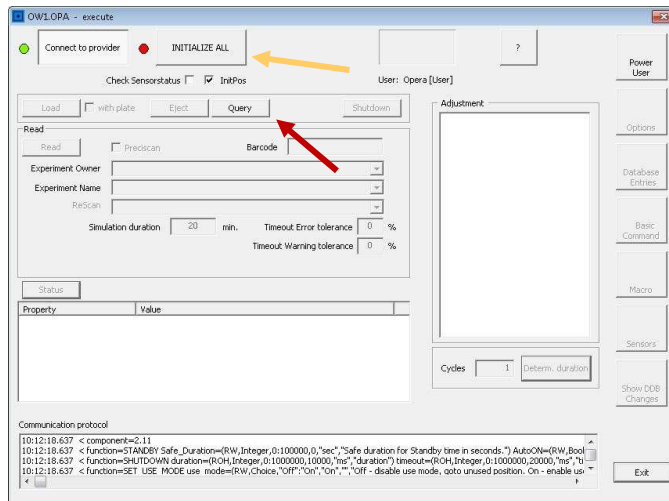


Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click **Service**.

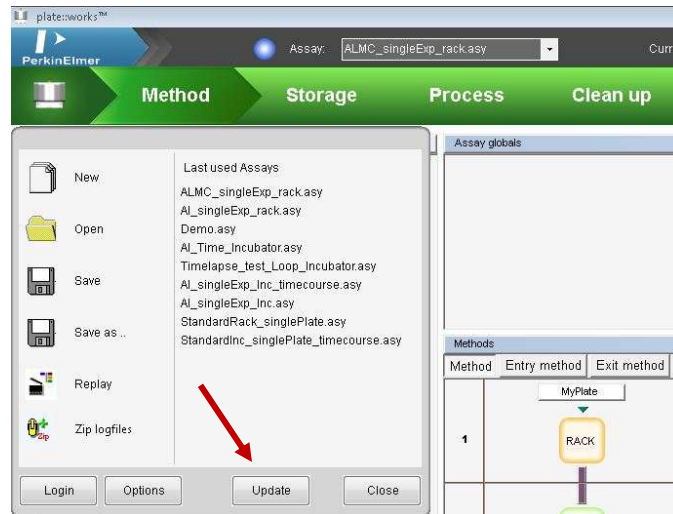


8. Right-click on **OPA** → **Direct Mode On**. In the pop-up window, click **Query** and then **Initialize All**. The loading door on the top of the microscope should open and close.
9. Right-click on **ROB** → **Direct Mode On**. In the pop-up window, click **Initialize All**. The robot hand should open and close.
10. Right-click on **INC** → **Direct Mode On**. In the pop-up window, click **Initialize All**. The incubator should beep a few times.



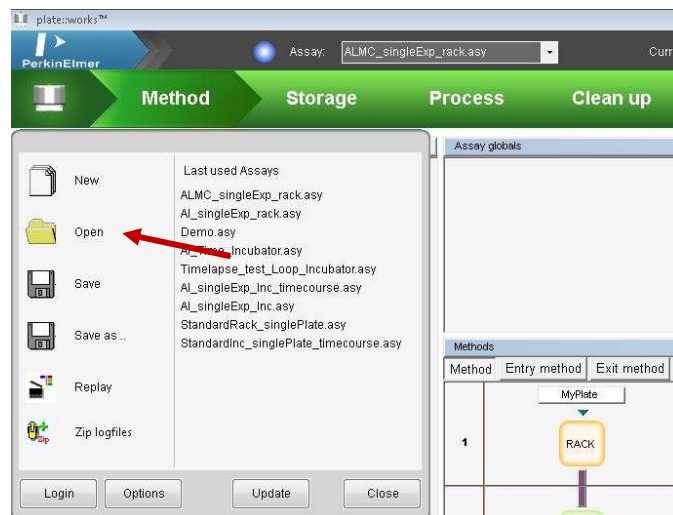
11. In the PlateWorks program, at the top-left of the screen, click the **Phenix** logo and then click **Update**. This will make sure that your new Harmony experiments are available to the automation software.





Load and edit a pre-made automation

12. Load a previous automation by clicking the Phenix logo and using *Open* to find the “ALMC_twoExp_incubator” automation as a starting point.



13. In the *Method* chevron, the *Method* tab shows the current automation with the thick black vertical lines indicating a movement by the robot arm.
 - a. If you need to change or add any components, click the left *Components* tab, and drag components to the flow diagram.
 INC = incubated plate rack
 BCR = bar code reader
 OPA = Opera Phenix microscope





14. There should be a *Method* tab for each experiment/imaging setting. In the *Method* tab, above the flow diagram, there should be a white rectangle with a plate name (e.g., *MyPlate1*). Click this rectangle.
- SourceCount* = *PlateCountExp1*
 - Adjust *Plate type* with the drop-down menu.
 - Change *PlateName* if you'd like... but write down the new name, because it will be necessary later.
 - If there is not a *Method* tab for each experiment/imaging setting, right-click on the *Method* tab → *Add method* to create a new *Method 1* tab. Build the same flow diagram in this new *Method 1* tab by dragging components, functions, and elements into the flow diagram.

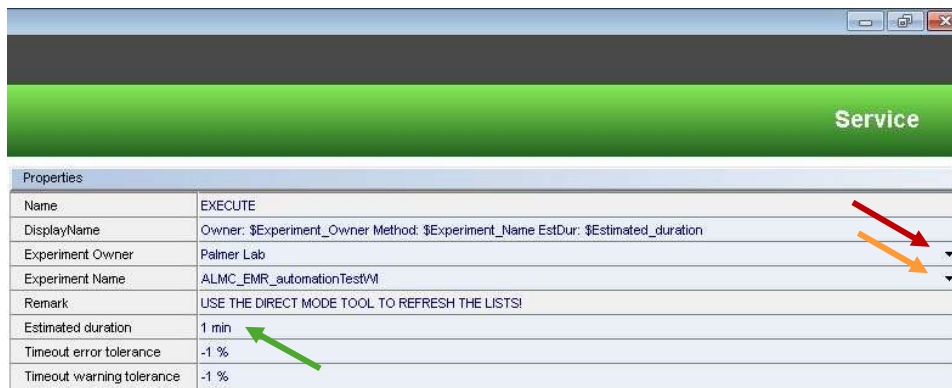
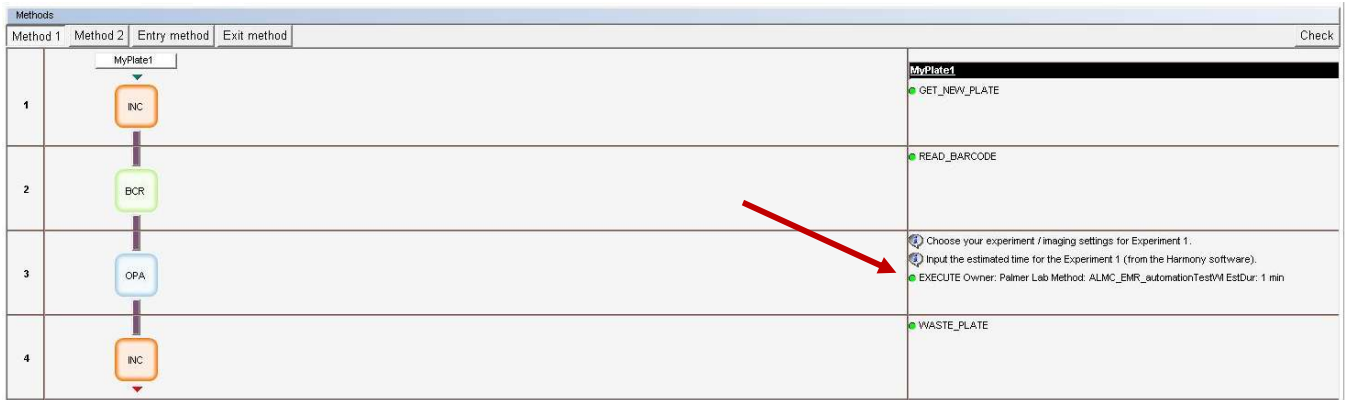


Service

Properties	
SourceCount	=PlateCountExp1
PlateType	PKI_CellCarrier96_Ultra
Name	MyPlate1
PlateLayout	
BatchSize	0
PlateTickRate	0 s
BatchGap	0 s

- The first step should show *INC (component)* | *GET_NEW_PLATE (function)*. This will tell the automation to grab a plate starting from Position 1.
- Keep the *BCR (component)* | *Read Barcode (function)* step as the second step in the flow even if you do not have barcodes on your plates. This will provide timestamps in the metadata.
- The third step should be an *OPA (component)* | *Execute (function)*, *Start time stamp (element)* step.

- Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: “Load Harmony experiment into PlateWorks”.
- Adjust *Estimated duration* to reflect the estimated time written down from Harmony with a little extra time such that the measurement would not be aborted under normal circumstances (i.e., if the estimated time in Harmony was 20 minutes, enter 40 minutes; if the estimated time in Harmony was 60 minutes, enter 90 minutes). This value is for a timeout function to allow the automation to continue progressing if the plate fails to image correctly.
- Timeout warning tolerance* = -1 (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
- Timeout error tolerance* = -1 (default amount) or 110% (give error after experiment takes 10% more time than expected)



- Finally, the fourth step should be an *INC (component) | WASTE_PLATE (function)* step. This will tell the automation that after this flow, this plate will not be used again, but that it should return to the incubator.
- Repeat Steps 14–18 for each *Method* tab (e.g., *Method 1*) that holds a different experiment/imaging setting.

Methods			
Method 1	Method 2	Entry method	Exit method
1	MyPlate1	INC	MyPlate1 ● GET_NEW_PLATE
2		BCR	● READ_BARCODE
3		OPA	④ Choose your experiment / imaging settings for Experiment 1. ④ Input the estimated time for the Experiment 1 (from the Harmony software). ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTestW EstDur: 1 min
4		INC	● WASTE_PLATE

20. Just above the flow diagram, click the top *Entry method* tab.

- a. Make sure the component matches where you put your plates and the first step of your flow diagram (INC). It should only be one step.
- b. Click the SET_INIT_STORAGE (function).
 - i. *Max plates* = 22
 - ii. *No of plate groups* = [number of different experiments/imaging settings]
 - iii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group 1*. Adjust the *Plate type* in the drop-down menu.
 - iv. Change the *Plate name* if you'd like to... (make sure it's the same plate name as before!)
 - v. *Start location* = 1
 - vi. *No of plates* = *PlateCountExp1*
 - vii. For fixed and mounted cells, *Initial volume* = 0. For fixed cells in PBS or for live cells in media, input the proper volume in μL .
 - viii. Adjust *Lid type*.
 - ix. Repeat Steps 20biii – 20bvii for *Plate group 2*.

Methods			
Method 1	Method 2	Entry method	Exit method
1	MyPlate1	INC	MyPlate1 ● GET_NEW_PLATE
2		BCR	● READ_BARCODE
3		OPA	④ Choose your experiment / imaging settings for Experiment 1. ④ Input the estimated time for the Experiment 1 (from the Harmony software). ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTestW EstDur: 1 min
4		INC	● WASTE_PLATE

Methods			
Method 1	Method 2	Entry method	Exit method
1	Entry method	INC	Entry method ④ Input plate type and the number of plates to run. ● SET_INIT_STORAGE 1, get_put, Max:22, groups:2

Service	
Properties	
Name	SET_INIT_STORAGE
DisplayName	\$Rack_number, \$Storage_type, Max:\$Max_plates, groups:\$No_of_Plate_groups
Rack number	1
Storage type	get_put
Max plates	22
▼ No of plate groups	2
▼ Plate group 1	{"PKI_CellCarrier96_Ultra","MyPlate1",1,EvalString("PlateCountExp1"),0,"normal"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate1
Start location	1
No of plates	=PlateCountExp1
Initial volume	0 µl
Lid type	normal
▼ Plate group 2	{"PKI_CellCarrier96_Ultra","MyPlate2",2,EvalString("PlateCountExp2"),0,"none"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate2
Start location	2
No of plates	=PlateCountExp2
Initial volume	0 µl
Lid type	none
► Name List	0

21. Click the *Exit method* tab, just above the flow diagram.

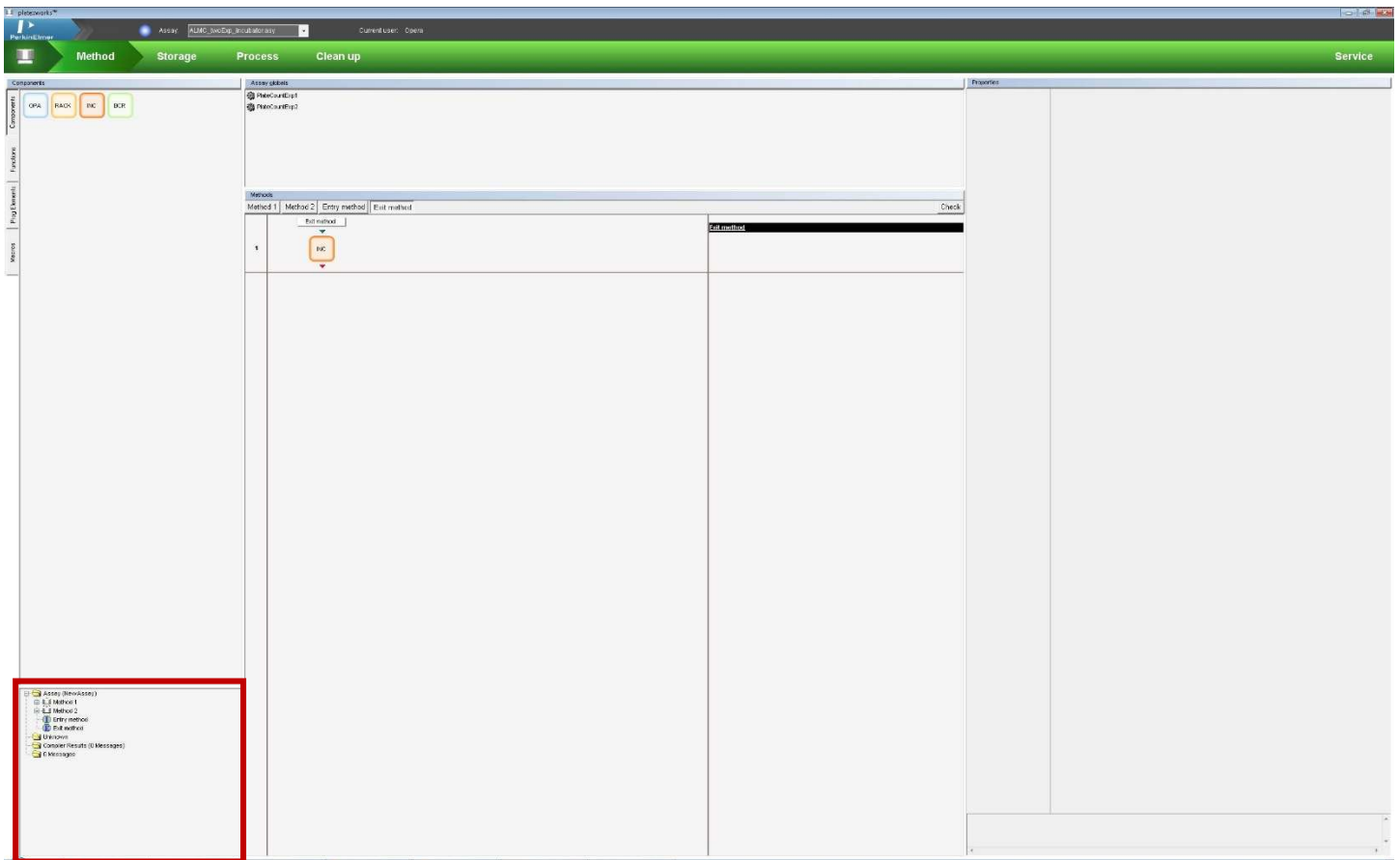
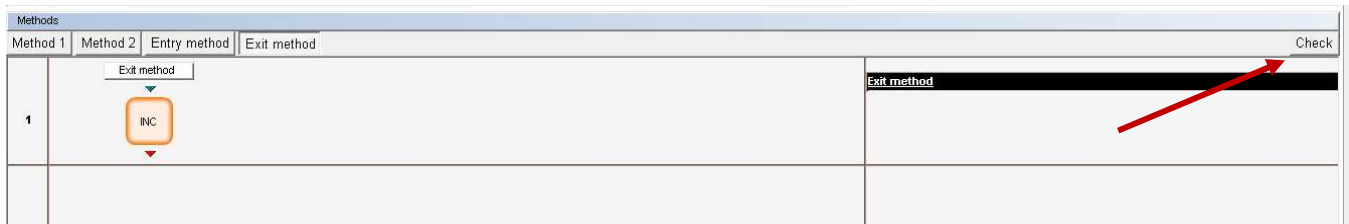
- a. Make sure the component matches where you put your plate and the final step of your flow diagram (INC).

The screenshot shows the 'Methods' configuration window. The 'Exit method' tab is selected, and the 'INC' component is highlighted in the flow diagram. The 'Check' button is visible in the top right corner.

The screenshot shows the 'Methods' configuration window. The 'Exit method' tab is selected, and the 'INC' component is highlighted in the flow diagram. The 'Check' button is visible in the top right corner.

22. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.

- a. Address any errors and repeat this step until no errors appear.

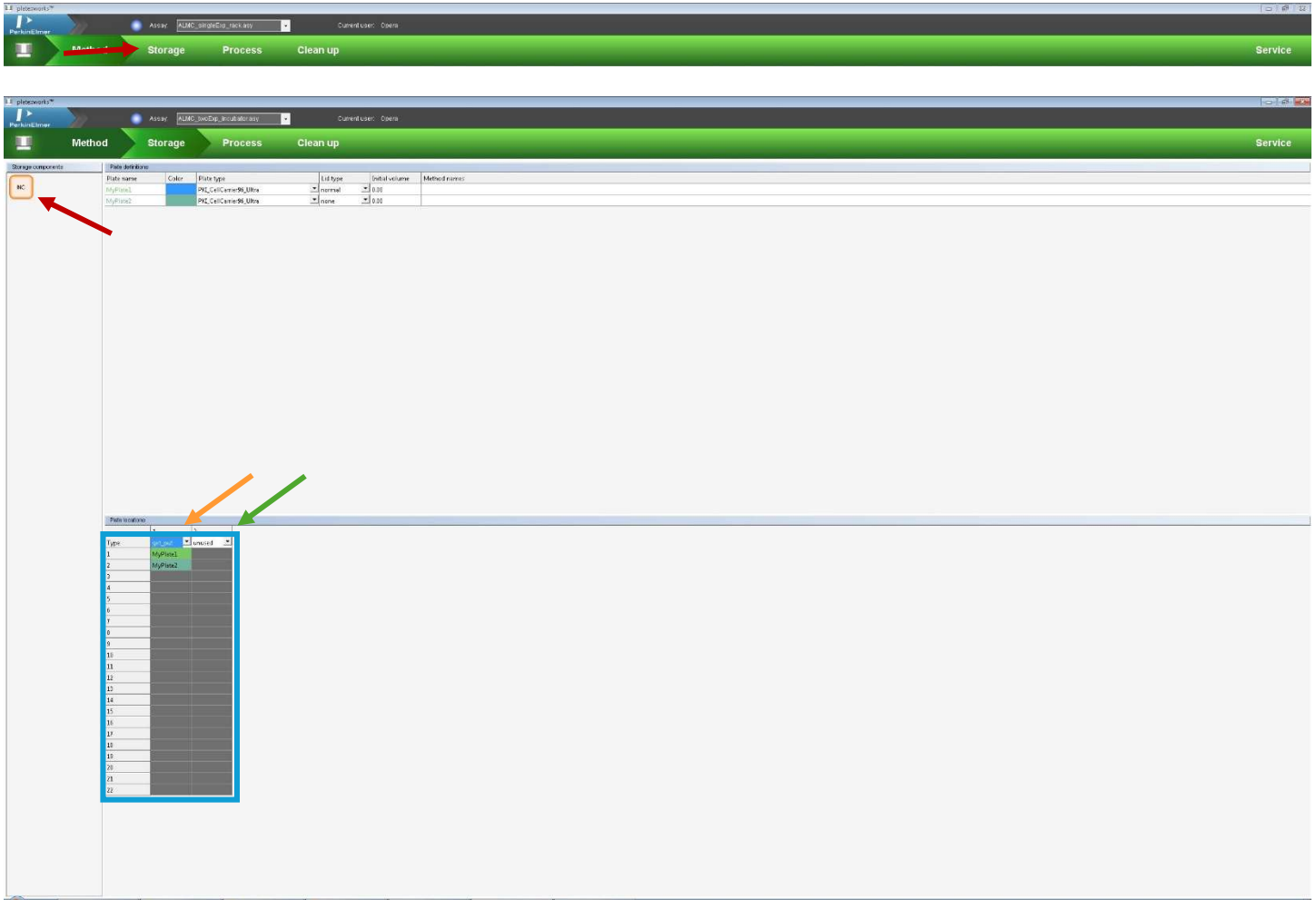


Run an automation

23. Click the *Storage* chevron at the top of the screen.

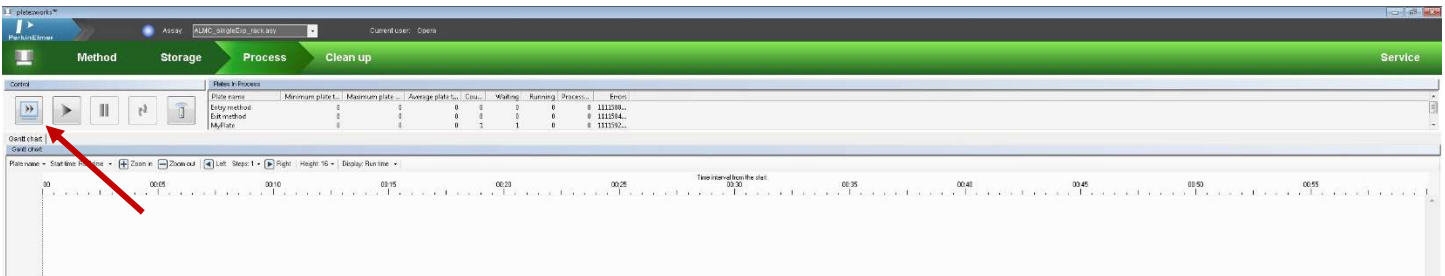
- a. On the left side of the screen, click the *INC* icon where your plates will be located/stored.
- b. Near the top of the screen, there is a table of the “plates” that are used in the automation.
 - i. There should be as many “plates” as there are different experiments/imaging settings.
 - ii. If you need to add a “plate”, right-click → *Add Plate*.
 - iii. Adjust the plate types.
- c. Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the first column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack.
- d. Highlight the position(s) in the first column where you will put your plates for Experiment 1. The position(s) should turn blue.
- e. Right-click → *Set places for '[Plate1]'*. The position(s) should turn green.
- f. Repeat Steps 23d – 23e for the other experiments and Plate types (e.g., *Plate2*).
- g. Highlight the remaining position(s) in the column where there will not be plates. The position(s) should turn blue.
- h. Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.

- i. If there are no plates in the second column, select *unused* from the drop-down menu at the top of the column.

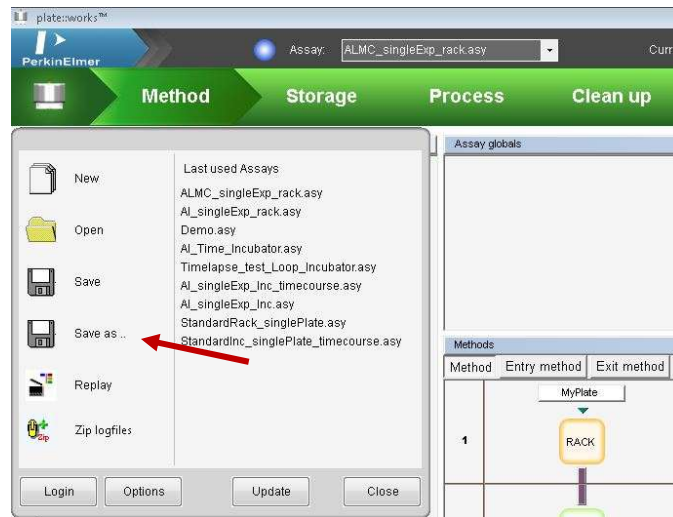


24. Click the *Process* chevron at the top of the screen.
 - a. Click the *Simulate assay* button (computer screen with double arrows).
 - b. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows, changing the *PlateCount* variables to match the number of plates that you want to run for each experiment, and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.
 - c. If there are any errors, they will appear now and, sometimes, the software will ask you what to do.
 - d. If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
 - e. The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to with the proper experiments / imaging settings and that the timecourse looks correct. Clicking on the sections in the simulated time log will give you more details.

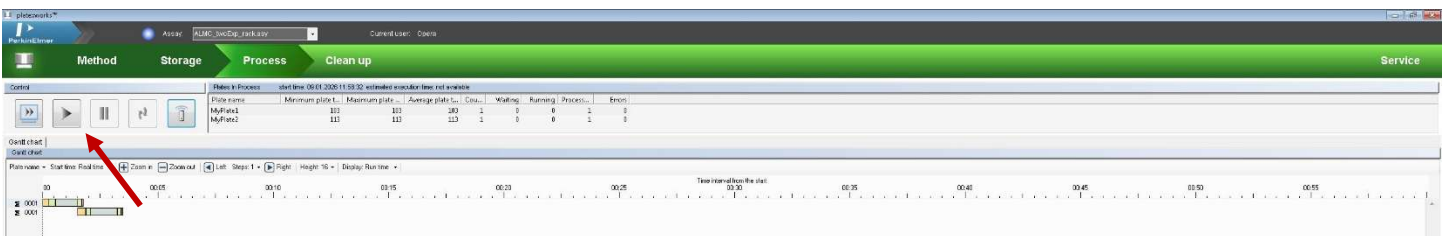




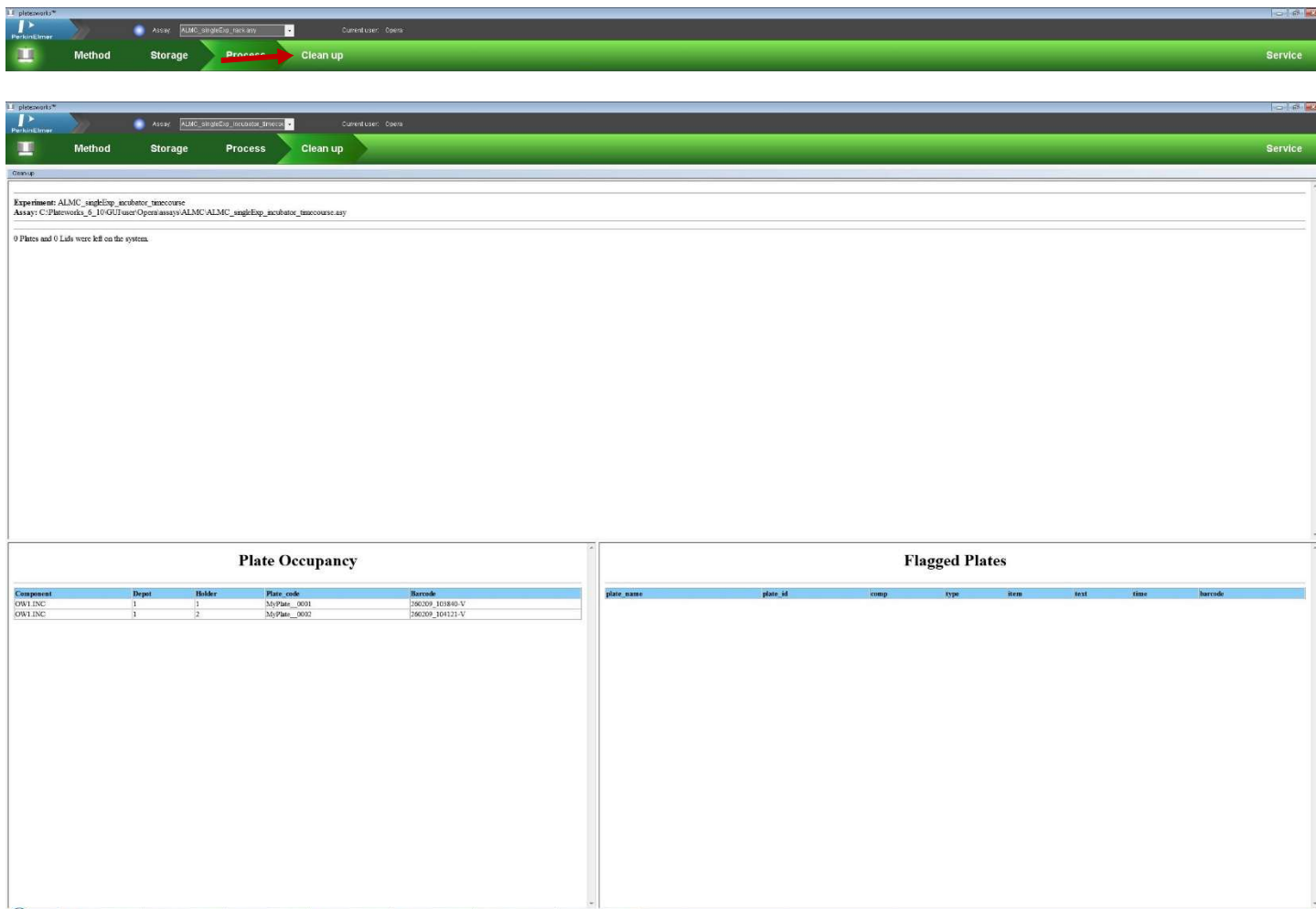
25. Fix any problems that arise and repeat the *Simulate* assay step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking Save As... and labeling it as [Lab]_[initials]_description. Please, do not save over the original ALMC automation!



26. Click the *Run* assay button (single large play button) to start the automation.
 - a. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows.
 - i. Change the *PlateCountExp1* = [number of plates in Experiment 1].
 - ii. Change the *PlateCountExp2* = [number of plates in Experiment 2].
 - iii. Change the *Experiment-ID* and the *Description* to fit your experiment.
 - iv. Click *Start*.
 - b. There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊
 - c. After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.



27. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plates from the incubator.

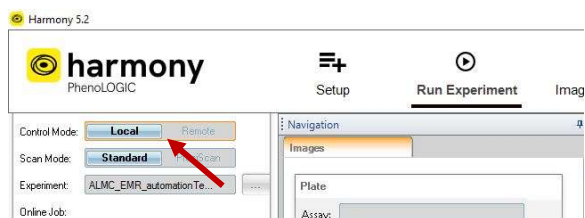


Look at and save your images

28. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



29. On the Harmony computer, re-open the *Harmony* application. In the **Run Experiment** tab, change the **Control to Local**. The images should be displayed in the *Run Experiment* tab.

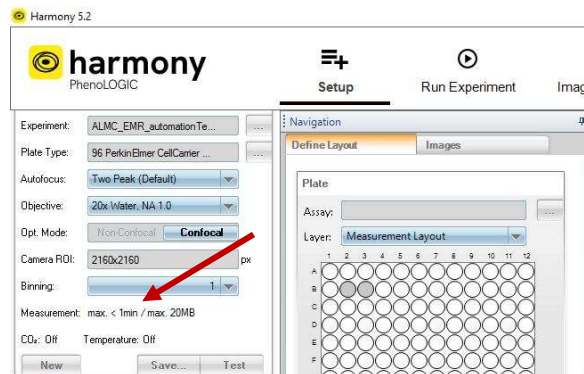


30. Save your images and their metadata using *Write Archive...* as normal.

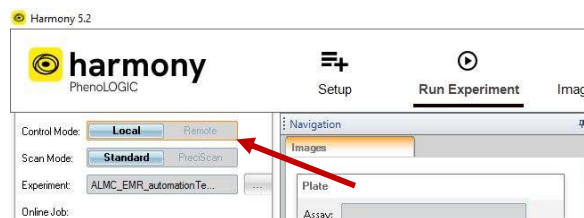
Create or edit an automation for plates with two or more experiments / different imaging settings from the incubator with timelapse imaging

Set up Harmony experiment and load plates

0. If you need to test imaging settings for this type of sample, insert your plate directly into the Phenix and set up the imaging settings like normal.
1. Load plates into the incubator. Remove the rack by grabbing the handle on the top of the rack and rock it back and forth/pull toward you until it unclips from the holder. Insert plate(s) into the incubated plate rack, starting from the bottom. Position A1 in a well plate should be back-right of the plate rack when it is inserted into the holder (next to the 1 sticker).
2. In Harmony, in the *Setup* tab, load and set up all your experiments, including the wells with your sample. For each experiment, save it. **For each experiment, write down the estimated time to run the experiment** (found near the Save button).



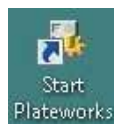
3. In Harmony, in the *Run Experiment* tab, in the upper-left corner, change the *Control* to *Remote*.



4. Minimize Harmony.
5. On the desktop, in the middle of the screen, double click the *PlateWorks* app. This will open a remote session to the PlateWorks computer.



6. In the remote session, open the *Start Plateworks* app.

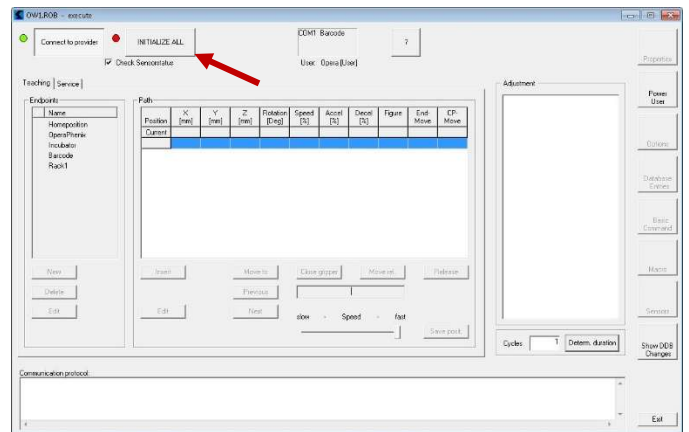
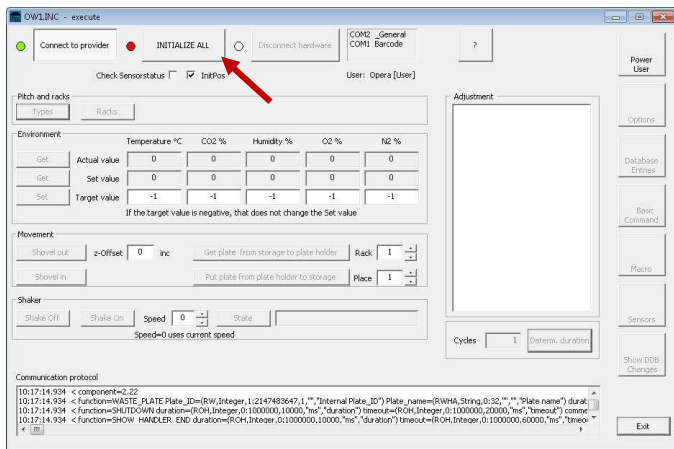
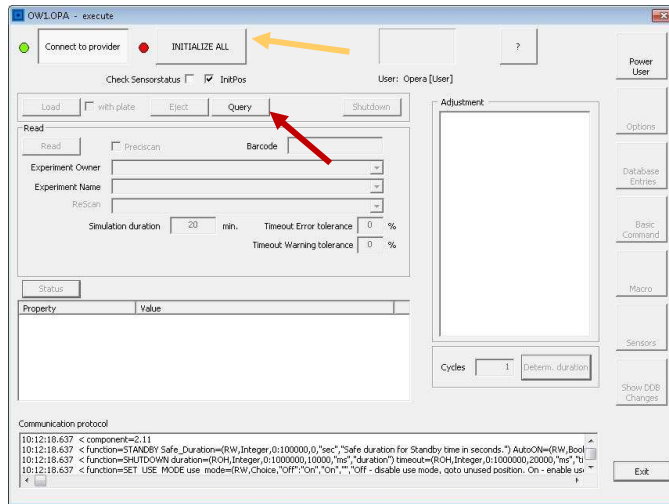


Load Harmony experiment into PlateWorks

7. In the PlateWorks program, at the top-right of the screen, click *Service*.

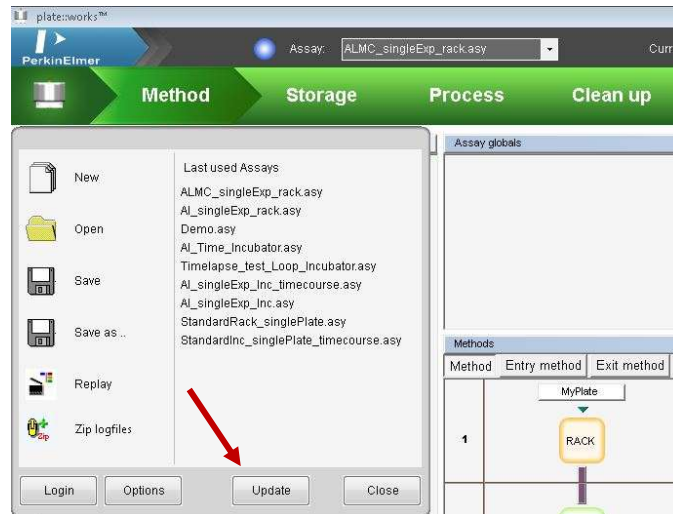


8. Right-click on OPA → *Direct Mode On*. In the pop-up window, click *Query* and then *Initialize All*. The loading door on the top of the microscope should open and close.
9. Right-click on ROB → *Direct Mode On*. In the pop-up window, click *Initialize All*. The robot hand should open and close.
10. Right-click on INC → *Direct Mode On*. In the pop-up window, click *Initialize All*. The incubator should beep a few times.



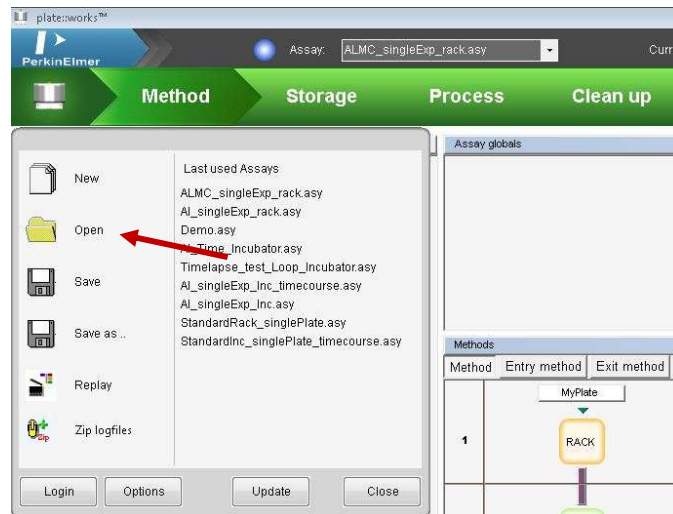
11. In the PlateWorks program, at the top-left of the screen, click the Phenix logo and then click *Update*. This will make sure that your new Harmony experiments are available to the automation software.





Load and edit a pre-made automation

12. Load a previous automation by clicking the Phenix logo and using *Open* to find the “ALMC_twoExp_incubator_timecourse” automation as a starting point.



13. In the *Method* chevron, the *Method* tab shows the current automation with the thick black vertical lines indicating a movement by the robot arm and the thin blue vertical arrows indicating a loop.
 - a. If you need to change or add any components, click the left *Components* tab, and drag components to the flow diagram.
 - INC = incubated plate rack
 - BCR = bar code reader
 - OPA = Opera Phenix microscope
 - b. If you need to add loops, click the left *ProgElements* tab and drag the *Loop (element)* to a component on the flow diagram. Loops can then be dragged to the final component of the loop to complete the loop.

Methods				Check
Method 1	Method 2	Entry method	Exit method	
	MyPlate1			MyPlate1
1	INC			● GET_NEW_PLATE
2	BCR			● READ_BARCODE
3	OPA			⚙️ SetTimeStamp ⚙️ Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\M EstDur: =Estimated_Read
4	INC			⚙️ WaitTimeStamp =60*(Interval - Plate_Transit_Time), {}
5	OPA			⚙️ WaitTimeStamp =60*Interval, {} ⚙️ SetTimeStamp ⚙️ Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\M EstDur: =Estimated_Read
6	INC			● WASTE_PLATE

14. There should be a *Method* tab for each experiment/imaging setting. In the *Method* tab, above the flow diagram, there should be a white rectangle with a plate name (e.g., *Plate1*). Click this rectangle.
- SourceCount = PlateCount (variable)
 - Adjust PlateType with the drop-down menu.
 - Change PlateName if you'd like... but write down the new name, because it will be necessary later.
 - BatchSize = CalculatedBatchSize_Exp1 (variable)
 - BatchGap = $((\text{CalculatedBatchSize_Exp1} * \text{Estimated_Read_Time_Exp1}) + ((\text{Number_of_Timepoints} - 1) * \text{Interval})) * 60$
 - If there is not a *Method* tab for each experiment/imaging setting, right-click on the *Method* tab → *Add method* to create a new *Method* tab. Build the same flow diagram in this new *Method* tab by dragging components, functions, and elements into the flow diagram.

Methods				Check
Method 1	Method 2	Entry method	Exit method	
	MyPlate1			MyPlate1
1	INC			● GET_NEW_PLATE
2	BCR			● READ_BARCODE
3	OPA			⚙️ SetTimeStamp ⚙️ Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\M EstDur: =Estimated_Read
4	INC			⚙️ WaitTimeStamp =60*(Interval - Plate_Transit_Time), {}
5	OPA			⚙️ WaitTimeStamp =60*Interval, {} ⚙️ SetTimeStamp ⚙️ Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\M EstDur: =Estimated_Read
6	INC			● WASTE_PLATE

Service	
Properties	
SourceCount	=PlateCountExp1
PlateType	PKI_CellCarrier96_Ultra
Name	MyPlate1
PlateLayout	
BatchSize	=CalculatedBatchSize_Exp1
PlateTickRate	0 s
BatchGap	=((CalculatedBatchSize_Exp1 * Estimated_Read_Time_Exp1) + ((Number_of_Tir

15. The first step should show *INC (component) | GET_NEW_PLATE (function)*. This will tell the automation to grab a plate starting from Position 1.
16. Keep the *BCR (component) | Read Barcode (function)* step as the second step in the flow even if you do not have barcodes on your plates. This will provide timestamps in the metadata.
17. The third step should be an *OPA (component) | SetTimeStamp (element), Execute (function)* step to start a timer and to image the plate for the first time.
 - a. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: “Load Harmony experiment into PlateWorks”.
 - b. *Estimated duration = Estimated_Read_Time_Exp1* (variable)
 - c. *Timeout warning tolerance = -1* (ignore warnings) or *110%* (give warning after experiment takes 10% more time than expected)
 - d. *Timeout error tolerance = -1* (default amount) or *110%* (give error after experiment takes 10% more time than expected)

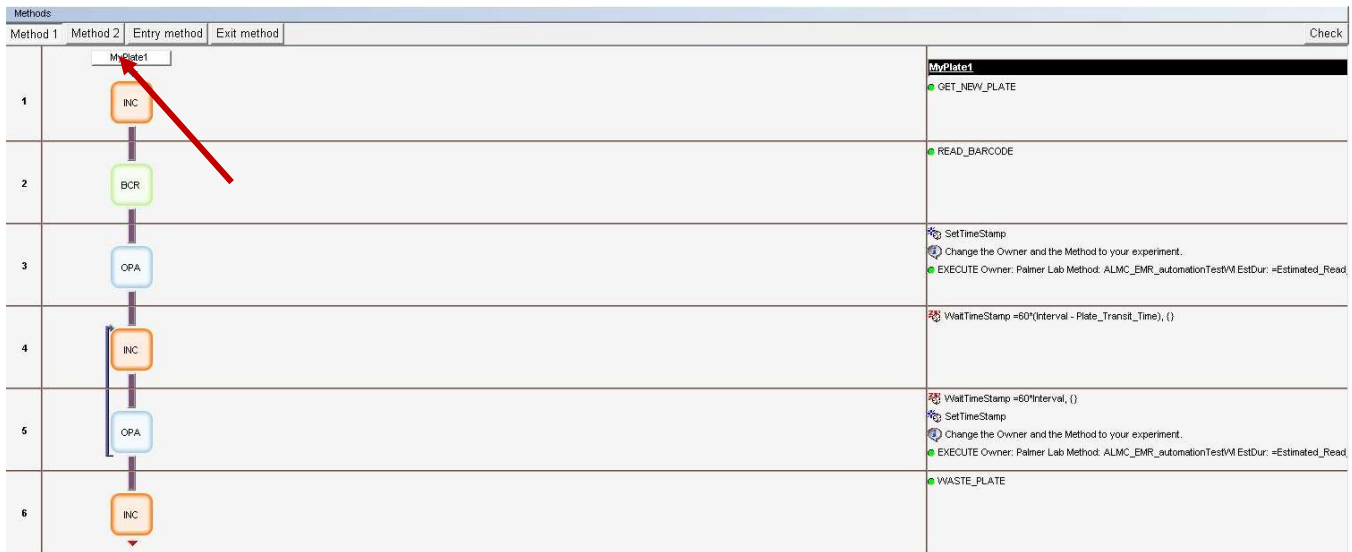
Methods		Check
Method 1	Method 2	Entry method
1	MyPlate1 INC	MyPlate1 ● GET_NEW_PLATE
2	BCR	● READ_BARCODE
3	OPA	⌚ SetTimeStamp ⚙ Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\M EstDur: =Estimated_Read
4	INC	⌚ WaitTimeStamp =60*(Interval - Plate_Transit_Time), {}
5	OPA	⌚ WaitTimeStamp =60*(Interval, {} ⌚ SetTimeStamp ⚙ Change the Owner and the Method to your experiment. ● EXECUTE Owner: Palmer Lab Method: ALMC_EMR_automationTest\M EstDur: =Estimated_Read
6	INC	● WASTE_PLATE

Service	
Properties	
Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_du
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestW
Remark	USE THE DIRECT MODE TO REFRESH THE LISTS!
Estimated duration	=Estimated_Read_Time_Exp1
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

18. The fourth step should show an *INC (component) | WaitTimeStamp (element), SetTimeStamp (element)* step to incubate the plate for the desired period of time in the incubator before moving the plate to the Phenix for imaging.
 - a. $WaitTimeStamp = 60 * (Interval - Plate_Transit_Time), \{ \}$
19. The fifth step should be an *OPA (component) | WaitTimeStamp (element), SetTimeStamp (element), Execute (function)* step to incubate the plate for any remaining time of the desired period of time before imaging it again.
 - a. $WaitTimeStamp = 60 * Interval, \{ \}$
 - b. Click on the *Execute* function and select the *Experiment Owner* and *Experiment Name* with the drop-down menu. If your experiment is not there, repeat the steps from the prior section: "Load Harmony experiment into PlateWorks".
 - c. $Estimated\ duration = Estimated_Read_Time_Exp1$ (variable)
 - d. $Timeout\ warning\ tolerance = -1$ (ignore warnings) or 110% (give warning after experiment takes 10% more time than expected)
 - e. $Timeout\ error\ tolerance = -1$ (default amount) or 110% (give error after experiment takes 10% more time than expected)

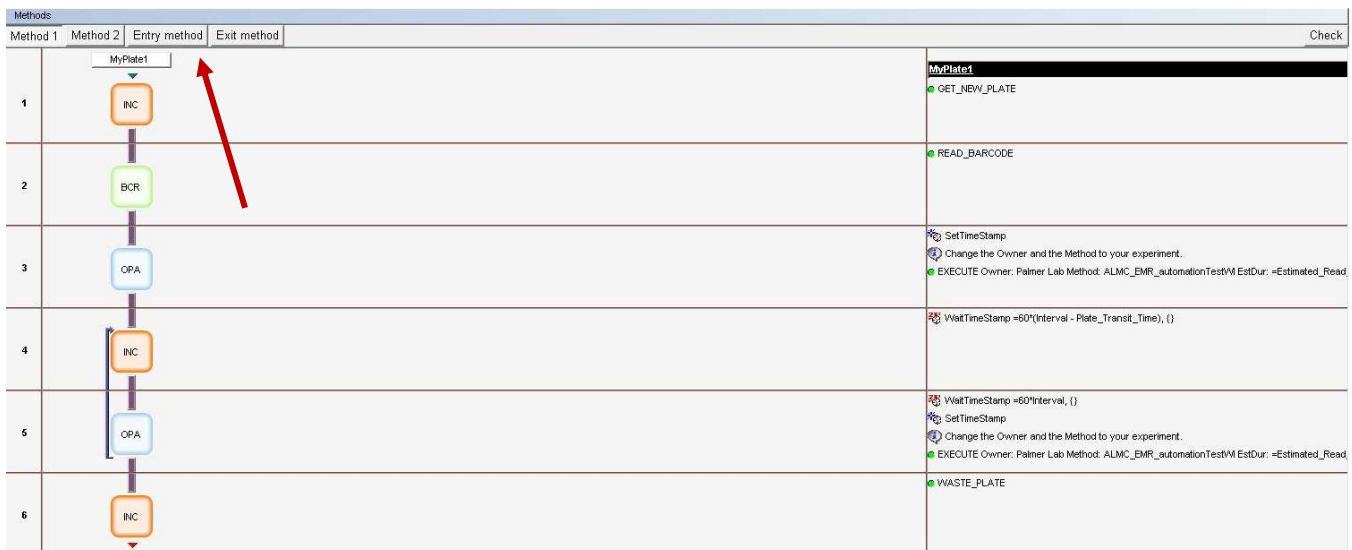
Service	
Properties	
Name	EXECUTE
DisplayName	Owner: \$Experiment_Owner Method: \$Experiment_Name EstDur: \$Estimated_du
Experiment Owner	Palmer Lab
Experiment Name	ALMC_EMR_automationTestW
Remark	USE THE DIRECT MODE TO REFRESH THE LISTS!
Estimated duration	=Estimated_Read_Time_Exp1
Timeout error tolerance	-1 %
Timeout warning tolerance	-1 %

20. There should be a *Loop* that connects from Step 3 (*OPA | StartTimeStamp, Execute*) to Step 5 (*OPA | WaitTimeStamp, StartTimeStamp, Execute*).
 - a. $Loops = Number_of_Timepoints$
21. Finally, the sixth step should be an *INC (component) | WASTE_PLATE (function)* step. This will tell the automation that after this flow, this plate will not be used again, but that it should return to the incubator.
22. Repeat this step for each *Method* tab that holds a different experiment/imaging setting.



23. Just above the flow diagram, click the top *Entry method* tab.

- a. Make sure the component matches where you put your plates and the first step of your flow diagram (INC). It should be two steps.
- b. Click the SET_INIT_STORAGE (function).
 - i. *Max plates* = 22
 - ii. *No of plate groups* = [number of different experiments / imaging settings]
 - iii. Click the down arrow next to *No of plate groups*. Then click the down arrow next to *Plate group 1*. Adjust the *Plate type* in the drop-down menu.
 - iv. Change the *Plate name* if you'd like to... (make sure it's the same plate name as before!)
 - v. *Start location* = 1
 - vi. *No of plates* = *PlateCountExp1* (variable)
 - vii. For live cells in media, input the proper volume in μL .
 - viii. Adjust *Lid type*.
 - ix. Repeat Steps 23iii – 23vii for *Plate group 2*.



Methods

Method 1 | Method 2 | Entry method | Exit method | Check

1	<p>Entry method</p>	<p>Entry method</p> <ul style="list-style-type: none"> Input the number of plates. SET_INIT_STORAGE 1, get_put, Max:22, groups:2
2		

Service

Properties

Name	SET_INIT_STORAGE
DisplayName	\$Rack_number, \$Storage_type, Max:\$Max_plates, groups:\$No_of_Plate_groups
Rack number	1
Storage type	get_put
Max plates	22
▼ No of plate groups	2
▼ Plate group 1	{"PKI_CellCarrier96_Ultra","MyPlate1",1,EvalString("PlateCountExp1"),0,"normal"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate1
Start location	1
No of plates	=PlateCountExp1
Initial volume	0 µl
Lid type	normal
▼ Plate group 2	{"PKI_CellCarrier96_Ultra","MyPlate2",2,EvalString("PlateCountExp2"),0,"none"}
Plate type	PKI_CellCarrier96_Ultra
Plate name	MyPlate2
Start location	2
No of plates	=PlateCountExp2
Initial volume	0 µl
Lid type	none
▶ Name List	0

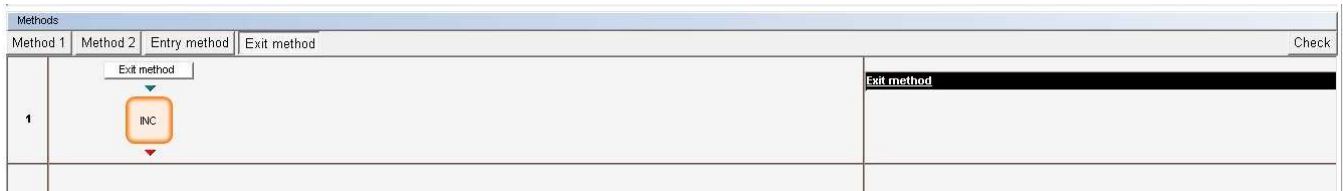
24. Click the *Exit method* tab, just above the flow diagram.

- a. Make sure the component matches where you put your plate and the final step of your flow diagram (INC).

Methods

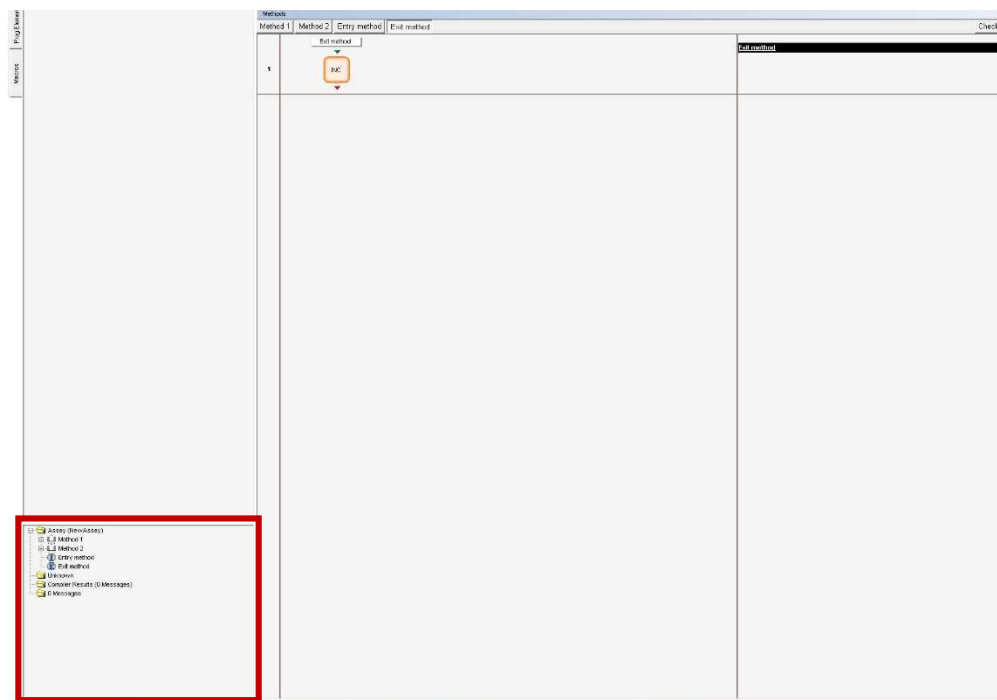
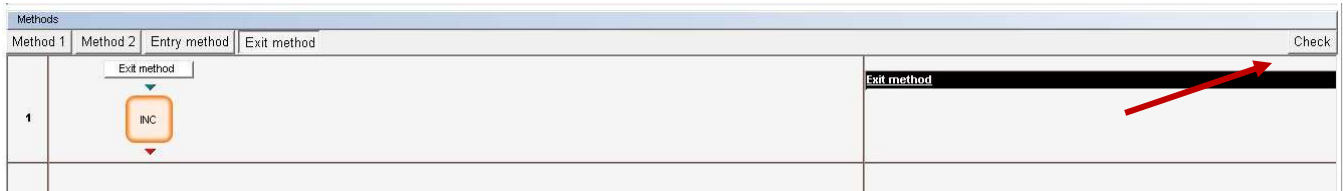
Method 1 | Method 2 | Entry method | Exit method | Check

1	<p>Entry method</p>	<p>Exit method</p> <ul style="list-style-type: none"> Input the number of plates. SET_INIT_STORAGE 1, get_put, Max:22, groups:2
2		



25. Near the top-right of the flow diagram, click the *Check* button to check for errors. Errors will appear in the bottom-left corner of the screen.

a. Address any errors and repeat this step until no errors appear.

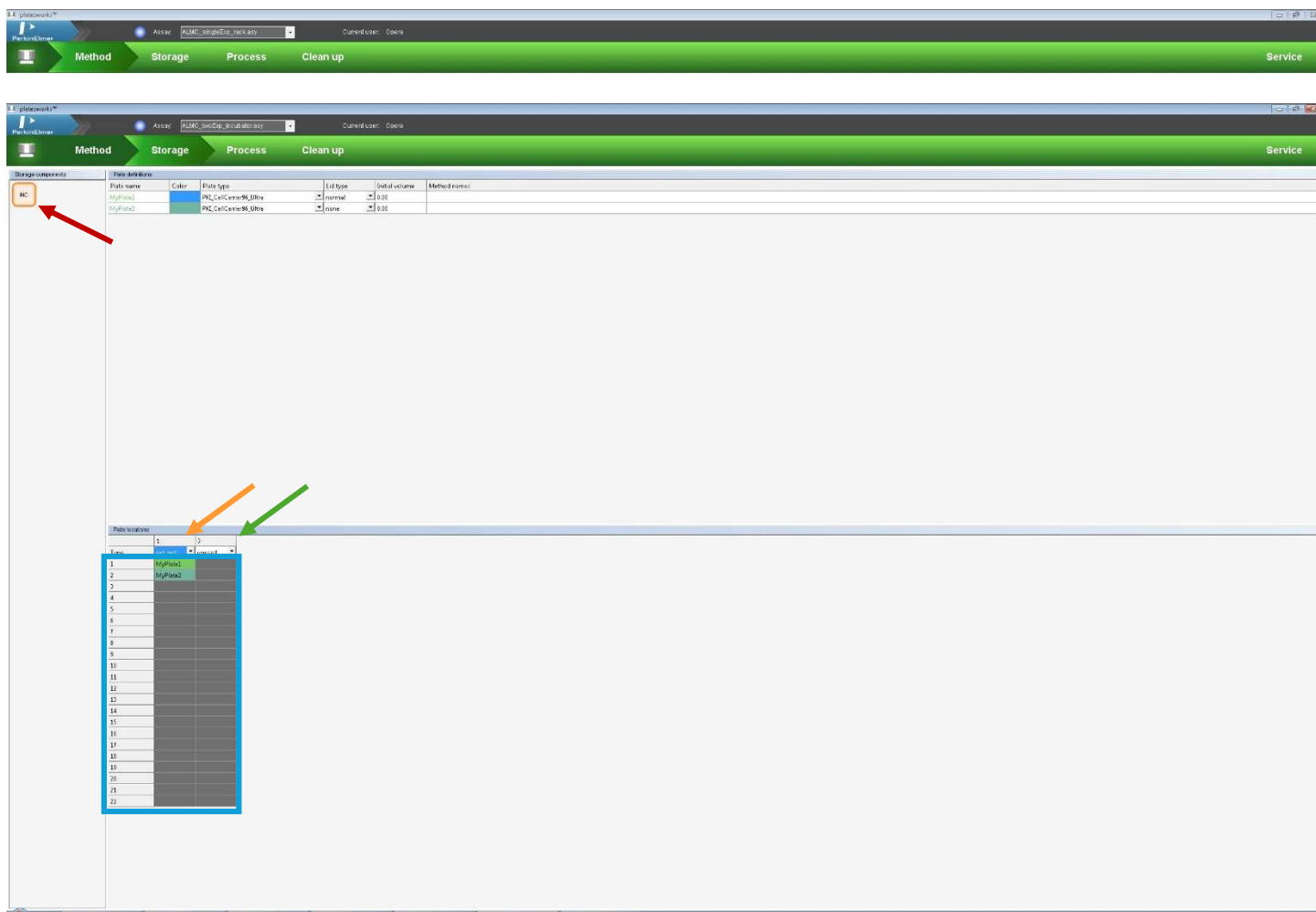


Run an automation

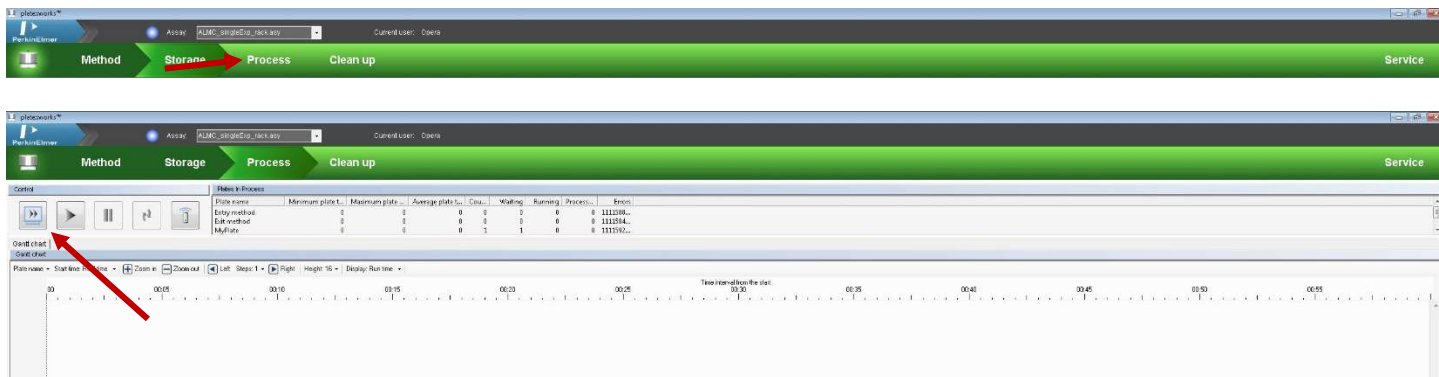
26. Click the *Storage* chevron at the top of the screen.

- a. On the left side of the screen, click the *INC* icon where your plates will be located/stored.
- b. Near the top of the screen, there is a table of the “plates” that are used in the automation.
 - i. There should be as many “plates” as there are different experiments/imaging settings.
 - ii. If you need to add a “plate”, right-click → *Add Plate*.
 - iii. Adjust the plate types.
- c. Near the bottom of the screen, there is a diagram of *Plate locations*. At the top of the first column, select *get_put* from the drop-down menu. This will allow the robot to get plates and put plates in this column of the rack.
- d. Highlight the position(s) in the first column where you will put your plate(s) for Experiment 1. The position(s) should turn blue.
- e. Right-click → *Set places for [MyPlate1]*. The position(s) should turn green.
- f. Repeat Steps 26d – 26e for the other experiments and Plate types (e.g., *MyPlate2*).

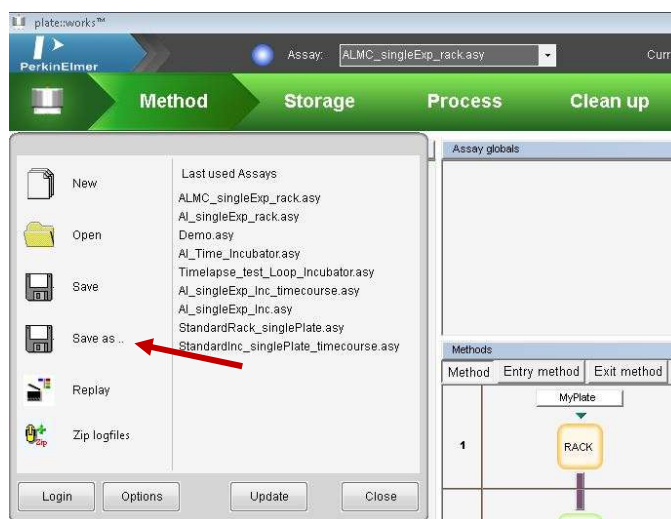
- g. Highlight the remaining position(s) in the column where there will not be plate(s). The position(s) should turn blue.
- h. Right-click → *Set places as 'unused'*. The position(s) should turn grey. Setting these position(s) as unused ensures that the robot will not put the plate back in a different position.
- i. If there are no plates in the second column, select *unused* from the drop-down menu at the top of the column.



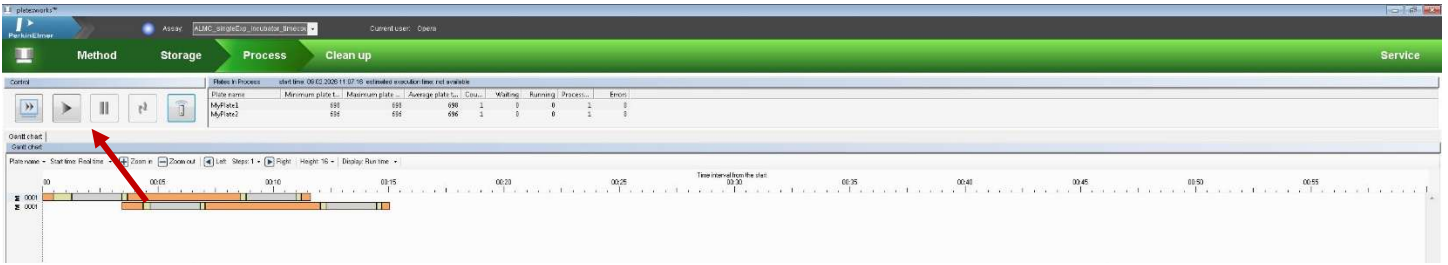
27. Click the *Process* chevron at the top of the screen.
 - a. Click the *Simulate assay* button (computer screen with double arrows).
 - b. In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows, changing the *PlateCount* variables to match the number of plates that you want to run for each experiment, the *Estimated_Read_Time* variables to match the recorded Experiment estimated read times from Harmony, the *Number_of_Timepoints* to the number of timepoints that you want, and the *Interval* to be how often you want each plate imaged in minutes, and then click *Start*. You should not have to change anything else in these windows for now, but it will show you what windows will pop up before your run.
 - c. If there are any errors, they will appear now and, sometimes, the software will ask you what to do.
 - d. If there are no errors, this generates a *Run report...* pop-up window that tells you how long the automation process will take.
 - e. The simulated time log in the software will show you what will happen during the automation. Ensure that it includes as many plates as you want it to with the proper experiments / imaging settings and that the timecourse looks correct. Clicking on the sections in the simulated time log will give you more details.



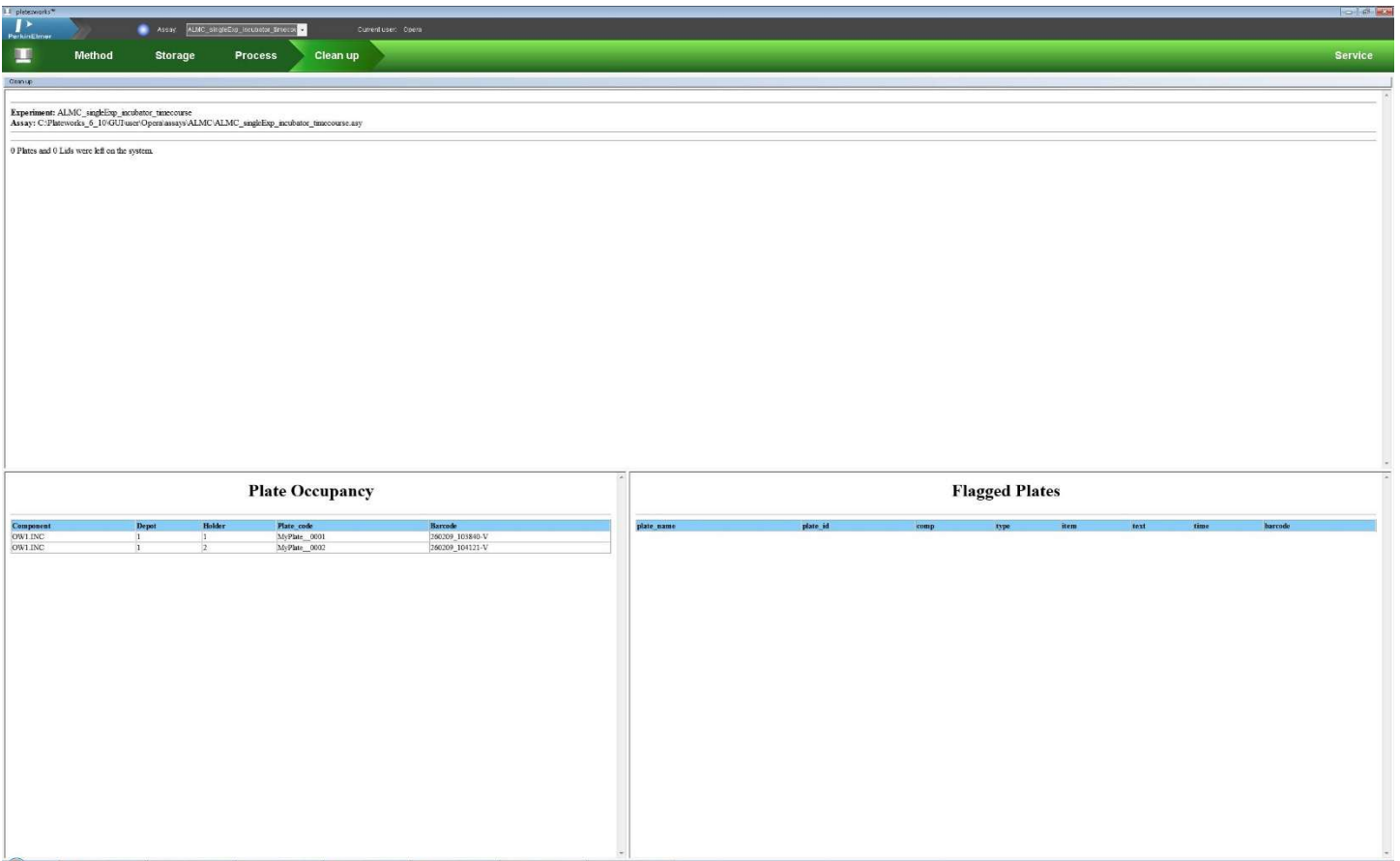
28. Fix any problems that arise and repeat the *Simulate assay* step. Save your automation by clicking the Phenix logo on the top-left of the screen and clicking *Save As...* and labeling it as *[Lab]_[initials]_description*. **Please, do not save over the original ALMC automation!**



29. Click the *Run assay* button (single large play button) to start the automation.
- In the *Assay Wizard* (green pop-up window), click *Next* to move through the windows.
 - Change the value for the *PlateCountExp1* variable = [number of plates in Experiment 1].
 - Change the value for the *PlateCountExp2* variable = [number of plates in Experiment 2].
 - Change the value for the *Estimated_Read_Time_Exp1* variable = [estimated read time for Experiment 1 from Harmony].
 - Change the value for the *Estimated_Read_Time_Exp2* variable = [estimated read time for Experiment 2 from Harmony].
 - Change the value for *Number_of_Timepoints* variable = [number of timepoints in timecourse].
 - Change the value for *Interval* = [how often you want each plate imaged in minutes].
 - Change the *Experiment-ID* and the *Description* to fit your experiment.
 - Click *Start*.
 - There will be some beeps from the incubator and some machine noises before anything happens. This is normal and not a problem. 😊
 - After the process is complete, the system will generate a *Run report...* pop-up window that tells you how long the automation process took and where the logfiles are.

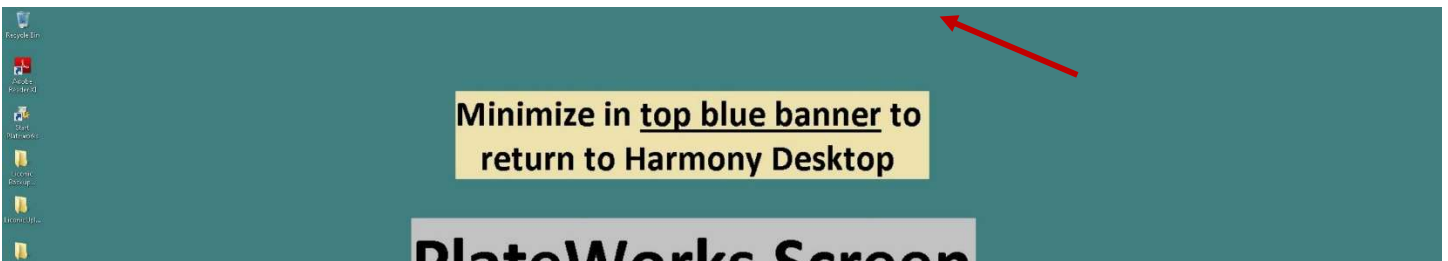


30. In the *Clean up* chevron, make sure there are no plates in the Phenix and remove your plates from the incubator.



Look at and save your images

31. In the blue bar at the top of the screen, exit the remote session by clicking the 'X' symbol.



32. On the Harmony computer, re-open the *Harmony* application. **In the *Run Experiment* tab, change the *Control to Local*.** The images should be displayed in the *Run Experiment* tab.



33. Save your images and their metadata using *Write Archive...* as normal.

Troubleshooting

Problem: You get an error regarding *Set Init Storage* when moving from a *Method* chevron to the *Storage* chevron.

Possible solution:

1. In the *Storage* chevron, follow the steps to add your plate(s) and set the other positions to unused.
2. If you go back to the *Method* chevron, in the *Entry* tab, you should see the *Set_Init_Storage* function updated to match your current experimental settings.
3. As long as they are correct, continue on! If they are not, update them, double-check your settings in the *Storage* chevron, and continue on.

Problem: *Simulate assay* button is greyed out and cannot be clicked.

Possible solution:

1. Turn off the robot and the computer by pressing the red button underneath the robot cage (behind the cabinet door). It may take a few minutes for the robot and computer to fully shut down.
2. Restart the robot and the computer by pressing the same red button. It may take a few minutes for the robot and computer to turn on again. Let everything load before trying anything.
3. User: Automation Logged On
Password: Automation
4. Click the *PlateWorks* app on the Desktop and try the process again.

Problem: Simulation gives you an error regarding the timing.

Possible solution: Increase the Timeout error tolerance in the OPA (component) | Execute (function) step.

Problem: Your plate type does not exist in the drop-down list.

Possible solution:

1. On the Plateworks computer, find the *Plateworks Tools* app and open it.
2. Navigate to the Plate Types list by clicking *Lab Tools* → *LabTool*.
3. In this list, check the plate(s) that you need and save the file. Click *No* for the two following pop-up windows.

Problem: Your plate type does not exist in the *LabTool* plate list.

Possible solution:

1. On the Plateworks computer, find the *Plateworks Tools* app and open it.
2. Navigate to the Plate Types list by clicking *Lab Tools* → *LabTool*.
3. In this list, find a similar plate type and click the *Copy* button (two white pages overlapping) to copy this plate type.
4. Double click the copy to open it.
5. Change the values to match those on the plate specifications sheet from the plate manufacturer.
6. Adjust the gripper values by trial and error:
 - a. Gripper height = -1 (do not change this value)
 - b. Lift gripper height varies between plates, so test a few different values.
7. Click *Apply*, then *Apply & Quit*, and then *Save*. Click *No* for the following two pop-up windows.
8. In the *Plateworks* app, in the upper-right corner, click *Service*.
9. In the *Service* window, right-click *ROB* → *Direct Mode On*.
10. In the new window, click the *Service* tab. On the left, click *RACK* (even though your eventual automation will not be from the fixed plate rack). In the middle section, click the drop-down next to *Plate type* and select the new plate type that you just added.
11. Put your **empty** plate in the fixed plate rack in the bottom position.
12. In the window, click *Get Plate*. The robot should move to pick up the plate. Look at how far up the sidewall of the plate the robot grippers are. Then click *Put Plate*. The robot should move to put the plate back in the fixed plate rack. Make sure the robot arm does not drop the plate into the rack. If either seems not secure or not ideal, go back to Step 6 and change the *lift gripper height*.

13. Repeat until the new plate type seems securely held by the robot.

Problem: ROB cannot be initialized. Emergency stop button was pressed.

Possible solution: There is a handheld controller that is hanging on the side of the clear cage around the robot near the loading door to the Phenix. The large red button near the top of the handheld controller sometimes gets pressed on accident. Pull this large red button to un-press it and try re-initializing the robot.

Problem: The incubator is alarming.

Possible solution: Pull up the Liconic Incubator manual on the Plateworks Desktop and search for the error code or follow the instructions below.

7.9 Alarm System

The StoreX has a combined acoustic / visual alarm system. An alarm occurs when one or more abnormal statuses occur or when interaction is required. When the system is operating properly there is no alarm. The various alarms are distinguished by the alarm signal duration and the pause between the signals. The following alarms are defined:

Signal / Pause (time in seconds)	Alarm	Comment, Action
0.1 / 2.4	No Initialization	StoreX not initialized. Press knob for initialization.
0.1 / 0.3	Gate Timeout	Check Handling position. Press knob to close Gate.
0.2 / 0.4	Glass Door Timeout	Close Glass Door or press knob to cancel alarm.
0.3 / 30	Water Level Low	Refill water or press knob for at least 3 seconds to cancel alarm.
0.5 / 0.5	Access collision	Occurs when Glass Door is opened and shovel is extracted. This alarm indicates high risk of damage to the handler. Do not rotate carousel. Check shovel position before moving stackers. Use extreme caution as shovel may be trapped in stacker.
0.1 / 0.1	System Busy Access	Glass Door is opened during a remote access. Close Glass Door and wait for remote access to be finished.