Quick Start Guide

BAB200

(ICTEM room 304B)

Imperial College London

Facility for Imaging by Light Microscopy

Observing Life As It Happens

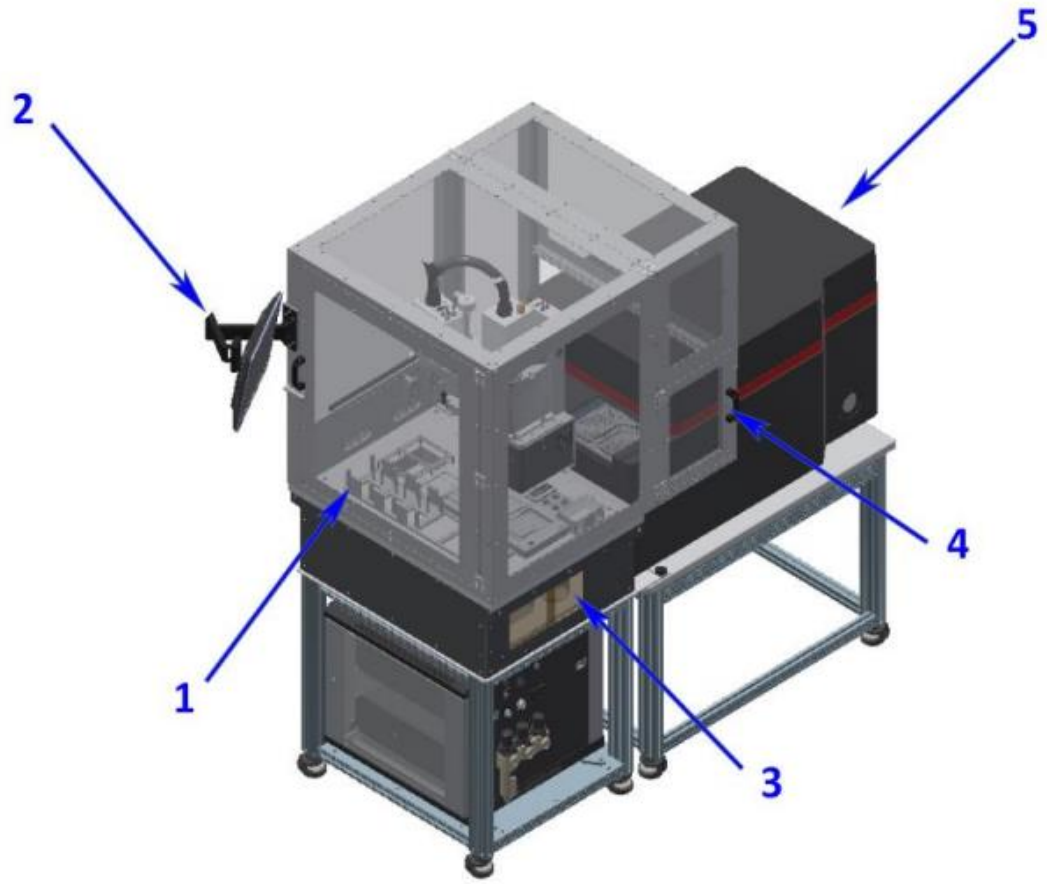
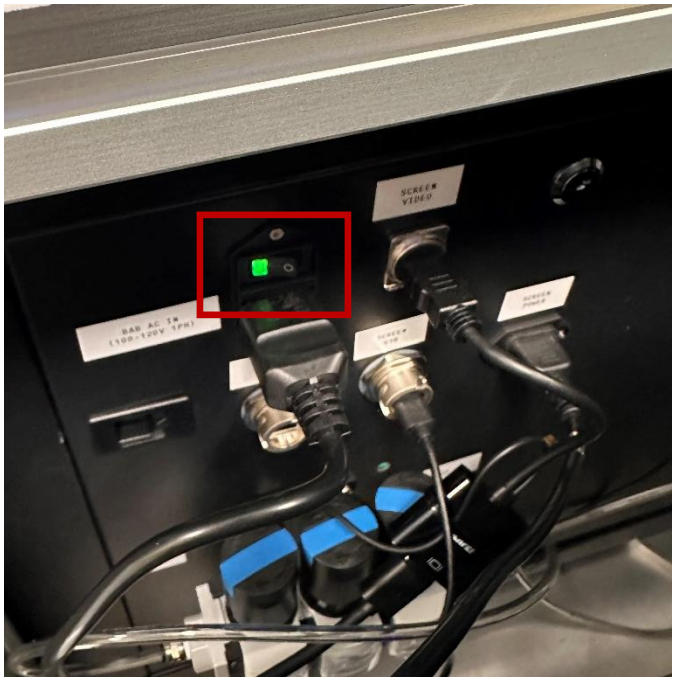


Figure 3: BioAssemblyBot® and Cell DIVE™ Hardware

1. Front Door
2. HMI (Human Machine Interface)
3. Liquid and tip waste
4. Side door
5. Cell DIVE™

Power ON

1. Switch on the BAB200 by pressing the power switch on the electrical enclosure. A green light will show up. Give the machine at least 8 minutes to completely start up. Once the machine status is green, you will be able to start operations.

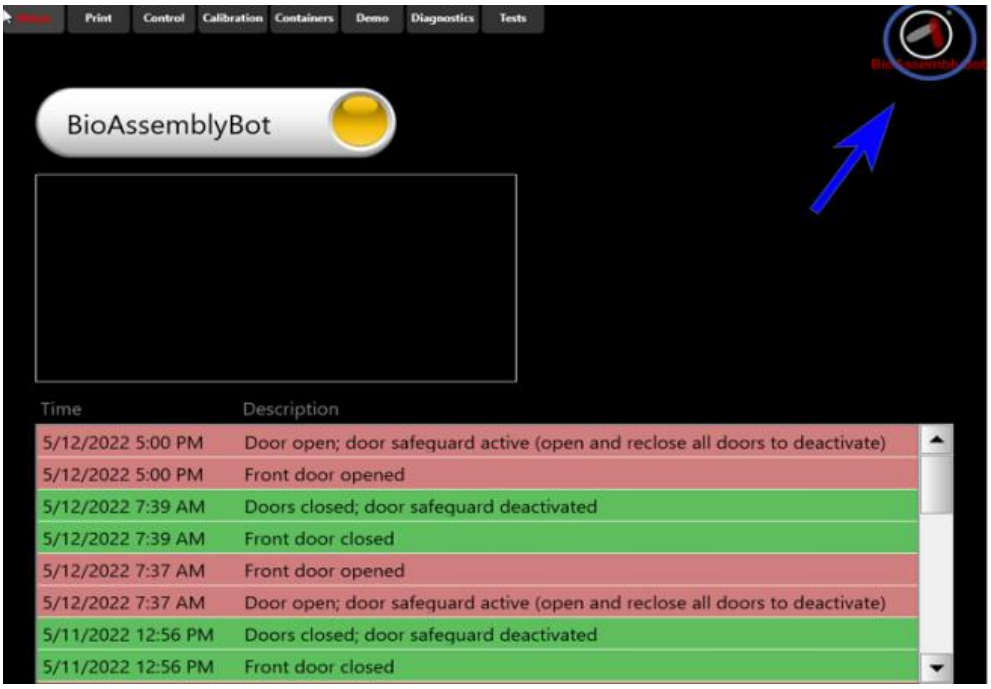


Emergency features

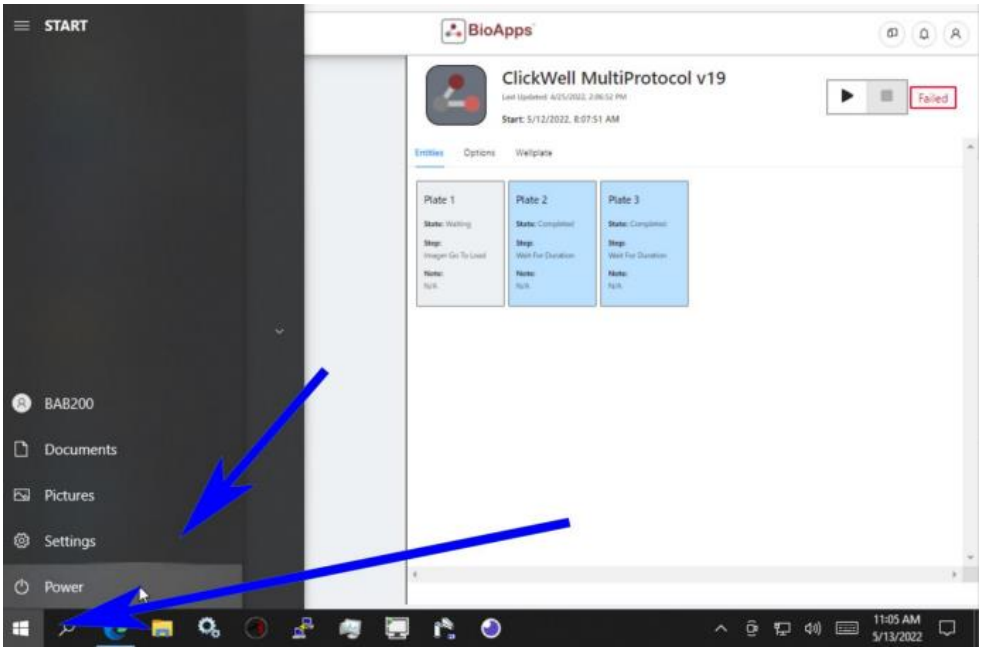
1. The BAB200 is equipped with an emergency stop push button, and safety door switch.
    a. Safety door: opening the front door latch (door) will cause the BAB 200 safety to be in an emergency stop status. Closing the latch, (door) will reset the safety status to a ready status.
    b. Emergency stop: pushing in (engaging) the emergency stop button, locks the push button in an emergency stop status. To disengage the emergency stop button twist it clockwise. The E-stop must be disengaged, and the door latch must be closed to move from emergency stop status to ready status.

Power OFF

1. Close the software on the touchscreen by pressing the BAB200 logo on the upper right-hand side of the screen.



2. Press the windows logo at the bottom left of the screen and select power, then shutdown. After 30 seconds, you can safely power off the machine. Switch off the BAB200 by pressing the power switch on the electrical enclosure.



BAB200 tools

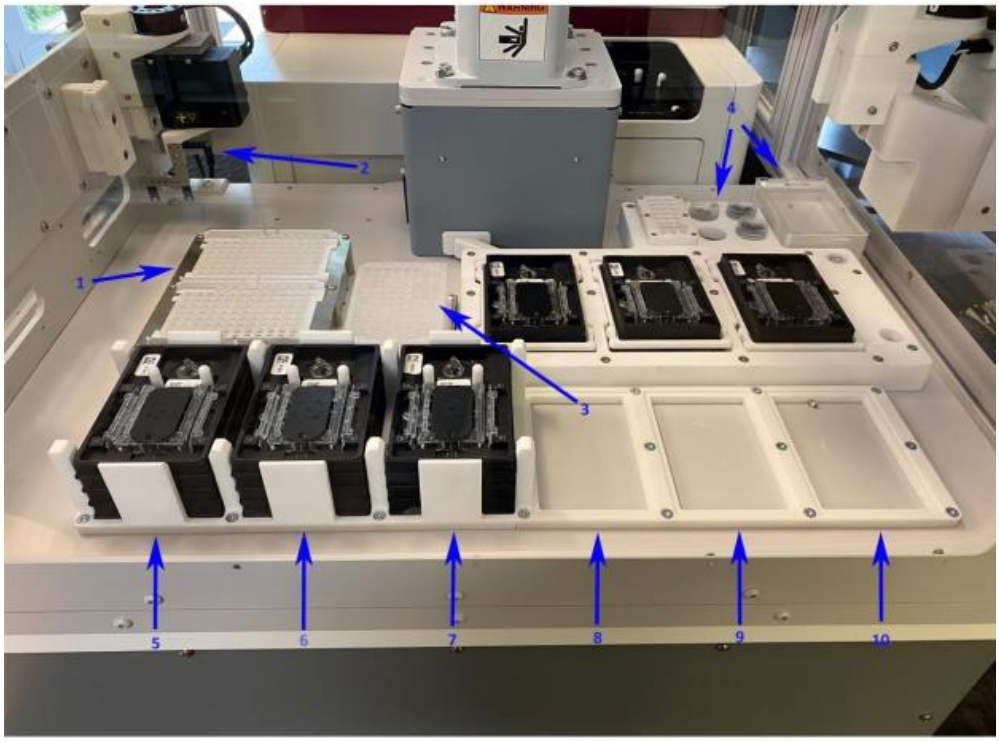
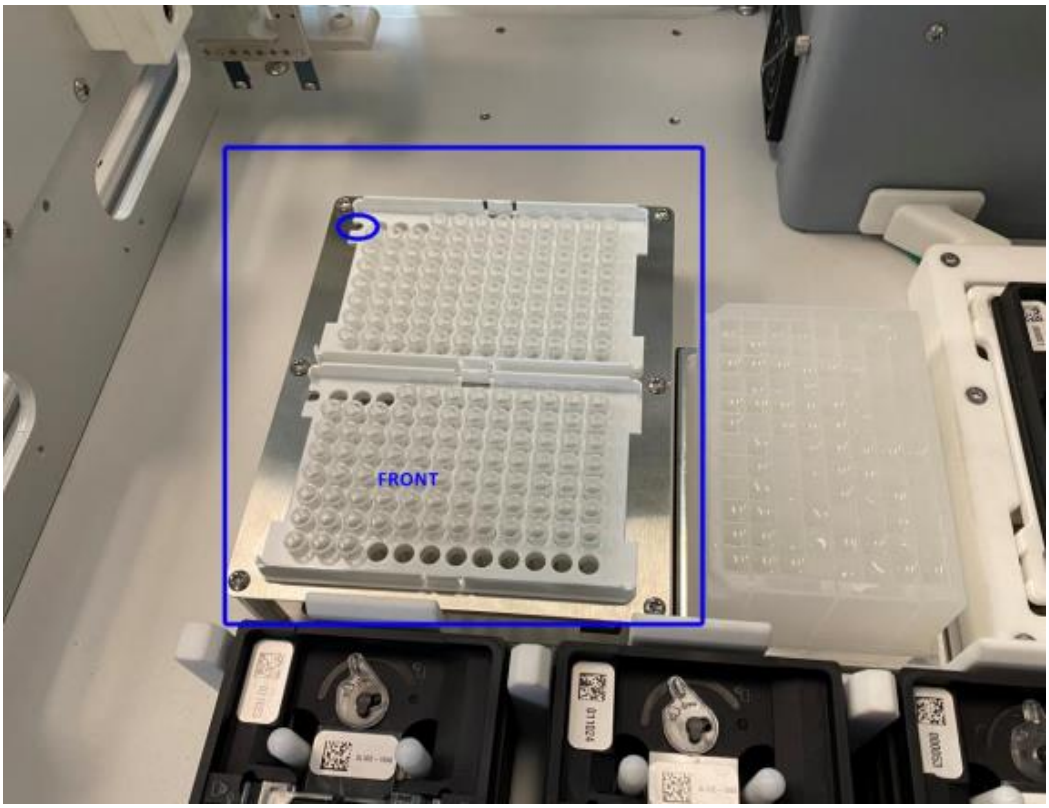


Figure 4: Inside the front door

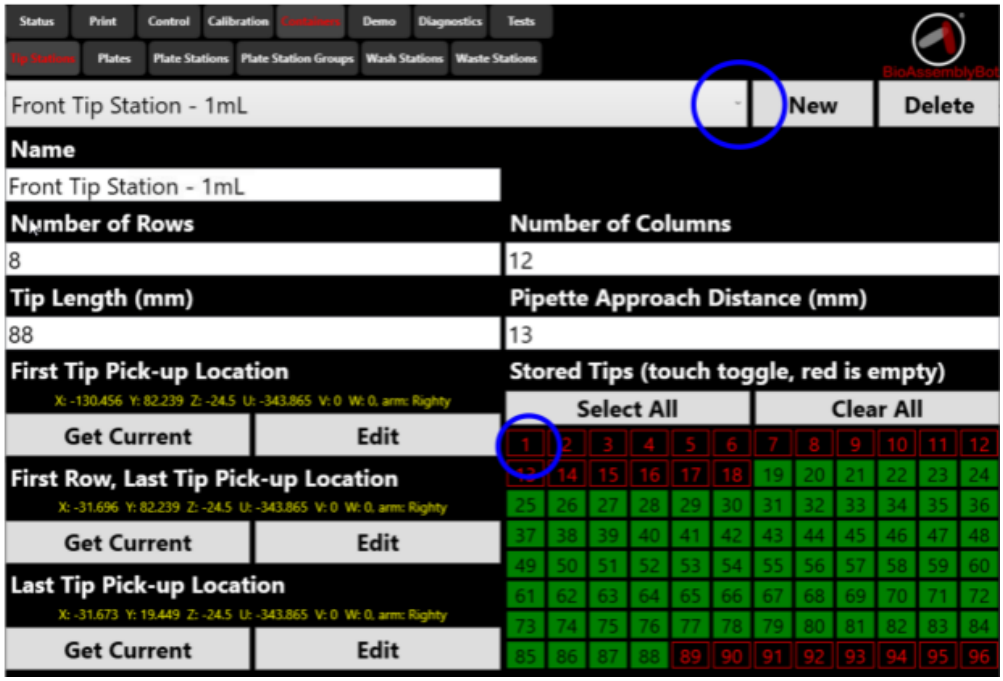
1. Pipette tip storage rack.
2. Tool bays (Front: Pipette, Rear: Pick and Place).
3. Chilled deep well plate location.
4. Liquid storage locations.
5. Source location 1 (up to 5 plates).
6. Source location 2 (up to 5 plates).
7. Source location 3 (up to 5 plates).
8. Destination location 1.
9. Destination location 2.
10. Destination location 3.

1. **Loading tips**



   a. **Replacement:** There are two tip stations within the workstation (front and rear). To replace the tip tray, lift the old tray and place the new tray in. Insert the tip tray in the correct orientation, with the tab on the side of the tray oriented towards the upper left.
   b. To change tip status in the software, select **Containers** and **Tip Stations**. There are 2 tip stations (front and rear). Select the front tip station by using the drop-down arrow. 96 tip locations are represented in the bottom right of the screen. Tip locations with a tip are colored green. Either click the "**Select All**" button or individually press each tip location to select, and location will change from red to green once selected, indicating a tip is in that position on the workstation. Once tip selection is complete, repeat for the Rear Tip

Station.



2. Loading ClickWells

a. Insert up to 5 ClickWells in each of the 3 source locations.

b. Change ClickWell status in the BAB200 software: select **Containers** and **Plate Stations**. There are 3 plate stations (Source 1 - 3). Enter how many plates are in each. Select the first source plate station with the drop-down arrow. Highlight the number of plates in the Source 1 Plate Station. Repeat

for the other two Source positions. The Completed Plate Stations (located to the right of the Source Plate Stations) should be set to 0 to reflect the absence of plates in that location.

3. Loading reagents

a. Reagent locations:

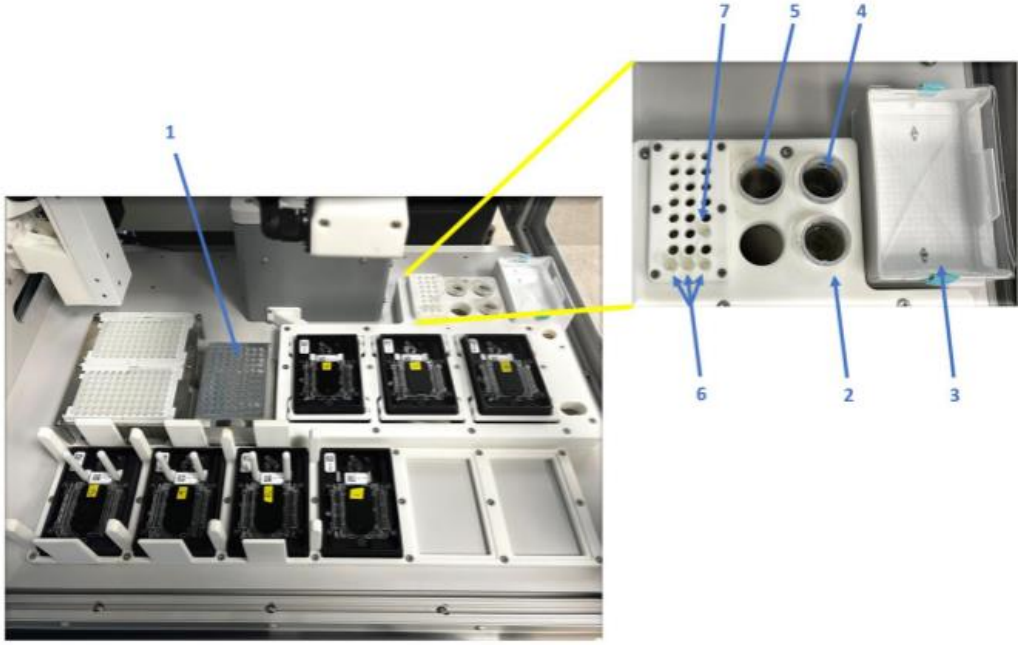


Figure 11: Deck Reagent Layout

Location 1: Deep well plate (See loading instructions below).

Location 2: Mounting Media (Standard ClickWell; 50% Glycerol, 4% Propyl Gallate)

Location 3: Tween Wash Buffer

Location 4: (10 mL 0.5M $NaHCO_3$ + 35 mL ddH_2O)

Location 5: (optional for second round of dye inactivation)- Dye Inactivation Solution: Diluted Sodium Bicarbonate (10 mL 0.5M NaHCO3 + 35 mL ddH2O)

Location 6: Reusable tips for Wash/Waste/DAPI

Location 7: Foil punch reusable tip

b. Reagents required:

Reagents	Concentration	Storage
Hydrogen Peroxide	H2O2 (30%)	Fridge in room 312
Water and Sodium bicarbonate	H2O and NaHCO3 (0.1 M NaHCO3)	Shelf
PBS wash buffer	0.01% Tween 20 in 1X PBS	Shelf
Antibody diluent	3% BSA in 1X PBS	Shelf
Direct conjugates	3% BSA in 1X PBS	Provided by user
Primary antibody diluent	3% BSA in 1X PBS	Shelf
Secondary antibody diluent	3% BSA in 1X PBS	Shelf
Primary antibodies	3% BSA in 1X PBS	Provided by user
Secondary antibodies	3% BSA in 1X PBS	Provided by user
Mounting media	50% Glycerol in 1X PBS	Shelf
DAPI Staining solution	100 ug/mL DAPI in 1X PBS	Shelf

c. Consumables:

Consumables	Storage
Square 2 mL Deep Well Plate	Shelf
Pierceable Membrane	Shelf
1 mL Tips for BAB200	Shelf
Reagent WASH Reservoir	Shelf

4. Reagent plate layout for Direct Conjugate AB Staining Workflow:

This workflow is used for the full round automation of a Cell DIVE workflow. Each antibody is stored separately diluted in 50 ul of Antibody Diluent Buffer (Columns 2-5). Column 1 contains 50 µl of Antibody Diluent Buffer. Column 1 and the four antibody dilutions (Columns 2-5) are combined prior to addition to the ClickWell for a final staining volume of 250 ul. Columns 11 and 12 are filled with 1.5 ml of DAPI (see the Cell DIVE User Manual for details).

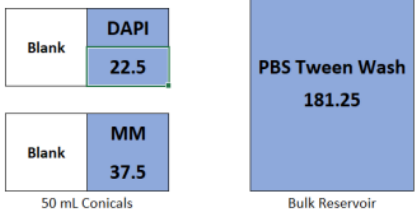
Required reagents and volumes (assume 15 slides and no dead volume or waste)

- PBS tween wash –182 mL, room temp
- Mounting Media –38 mL, room temp
- Ab diluent and antibodies - (in a deep well multi-well plate), 4°C, (Table 2)
- DAPI Staining Solution - (in a deep well multi-well plate), 4°C, (Table 2)

Pipette Tip Usage Guidance (1 mL tips)

- To process 3 active ClickWells through this workflow requires 16 pipette tips
- To process 15 ClickWells through this workflow requires 48 tips

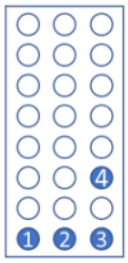
Bulk reagent layout (volumes in mL, dead volume included)



Deep-Well plate Total Volume*

Ab Dil	800 uL
Abs	800 uL each

*Extra volume (enough for one extra slide) included to account for pipetting errors, etc.



Deep-well reagent plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL		
B	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL		
C	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL		
D	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL		
E	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL		
F	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL		
G	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL		
H	Ab Dil 50 uL	Ab1 50 uL	Ab2 50 uL	Ab3 50 uL	Ab4 50 uL							

Well allocation per slide

	Wells
	Ab Sol'n
Slide 1	A1-A5
Slide 2	B1-B5
Slide 3	C1-C5
Slide 4	D1-D5
Slide 5	E1-E5
Slide 6	F1-F5
Slide 7	G1-G5
Slide 8	H1-H5
Slide 9	A1-A5
Slide 10	B1-B5
Slide 11	C1-C5
Slide 12	D1-D5
Slide 13	E1-E5
Slide 14	F1-F5
Slide 15	G1-G5

5. Reagent Plate Layout for Dye Inactivation Workflow

This workflow is used for one or two steps of dye inactivation. Columns 2 and 4 contain 300 ul of 30% hydrogen peroxide for the first cycle. If 2 steps of dye inactivation are desired, columns 1 and 3 contains 300 µl of 30% hydrogen peroxide. Columns 11 and 12 are filled with 1.5 ml of DAPI (see the Cell DIVE User Manual for details). Cover reagent plate with a pierceable membrane.

Required reagents and volumes (assume 15 slides and no dead volume or waste)

• PBS wash –163 mL, room temp

• Mounting Media –38 mL, room temp•NaHCO3 & H2O solution –54 mL, room temp

• H2O2 –(300 µl per well in a deep well multi-well plate), 4°C, (Table 3)

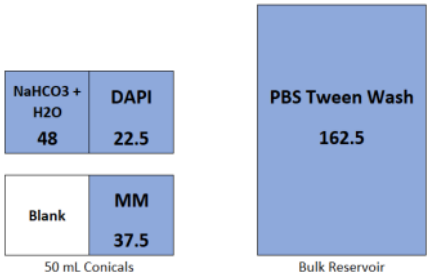
• DAPI Staining Solution 22.5ml

Pipette Tip Usage Guidance (1 mL tips)

• To process 3 ClickWell plates through this workflow requires 18 pipette tips

• To process 15 ClickWell plates through this workflow requires 63 tips

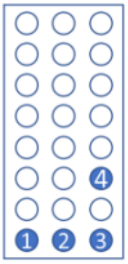
Bulk reagent layout (volumes in mL, dead volume included)



Deep-Well plate Total Volume**

H_2O_2	4.8 mL

**Extra volume (enough for one extra slide) included to account for pipetting errors, etc.



Deep-well reagent plate layout (Mix wells must be empty)

	1	2	3	4	5	6	7	8	9	10	11	12
A											H2O2 300 uL	H2O2 300 uL
B											H2O2 300 uL	H2O2 300 uL
C											H2O2 300 uL	H2O2 300 uL
D											H2O2 300 uL	H2O2 300 uL
E											H2O2 300 uL	H2O2 300 uL
F											H2O2 300 uL	H2O2 300 uL
G											H2O2 300 uL	H2O2 300 uL
H						MIX	MIX	MIX	MIX	MIX	H2O2 300 uL	MIX

Well allocation per slide

	Wells	
	H2O2	Mix
Slide 1	A11	H6
Slide 2	B11	H6
Slide 3	C11	H6
Slide 4	D11	H7
Slide 5	E11	H7
Slide 6	F11	H7
Slide 7	G11	H8
Slide 8	H11	H8
Slide 9	A12	H8
Slide 10	B12	H9
Slide 11	C12	H9
Slide 12	D12	H10
Slide 13	E12	H10
Slide 14	F12	H12
Slide 15	G12	H12

6. Reagent plate layout for Full Round Workflow:

This workflow is used for the full round automation of a Cell DIVE workflow. Each antibody is stored separately diluted in 50 ul of Antibody Diluent Buffer (Columns 2-5). Column 1 contains 50 µl of Antibody Diluent Buffer. Column 1 and the four antibody dilutions (Columns 2-5) are combined prior to addition to the ClickWell for a final staining volume of 250 ul. Columns 11 and 12 are filled with 1.5 ml of DAPI (see the Cell DIVE User Manual for details).

Required reagents and volumes (assume 15 slides and no dead volume or waste)

- PBS tween wash –294mL, room temp
- Mounting Media –76 mL, room temp
- Ab diluent and antibodies - (in a deep well multi-well plate), 4°C, (Table 2)
- DAPI Staining Solution – 38ML

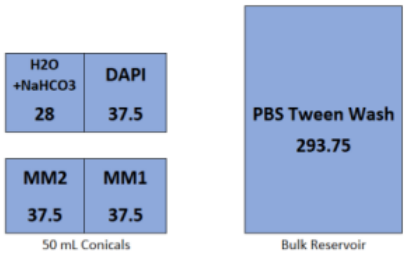
Pipette Tip Usage Guidance (1 mL tips)

- To process 15 ClickWells through this workflow requires:

o 60 tips in racks (12 tips for 3 CW, 60 tips for 15 CW)

o 4 reusable tips in tip storage

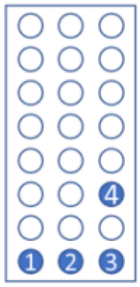
Bulk reagent layout (volumes in mL, dead volume included)



Deep-Well plate Total Volume*

Ab Dil	800 uL
Abs	800 uL each
H_2O_2	2.4 mL

*Extra volume (enough for one extra slide) included to account for pipetting errors, etc.



Deep-well reagent plate layout

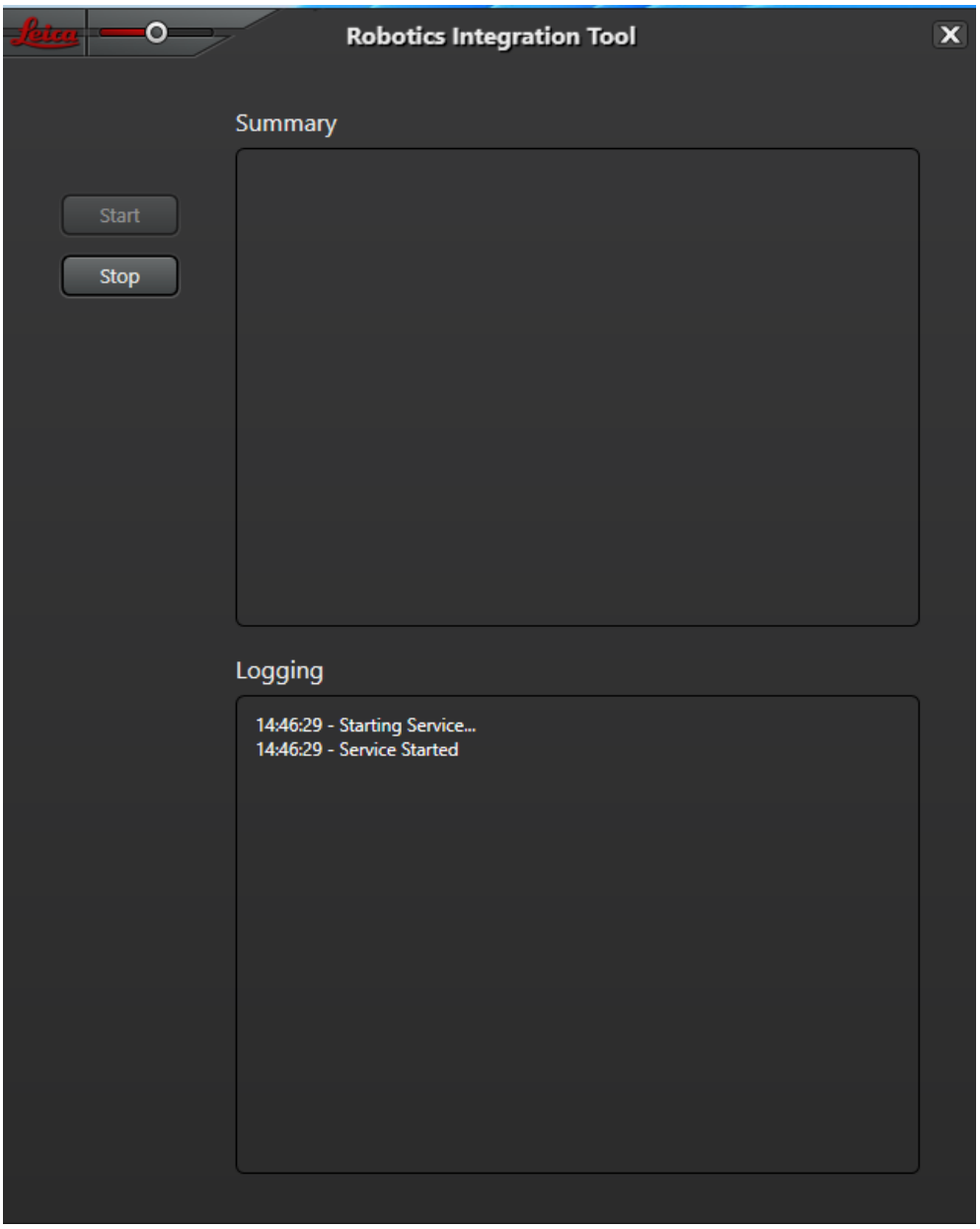
	1	2	3	4	5	6	7	8	9	10	11	12
A	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	H2O2 150 uL	H2O2 150 uL
B	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	H2O2 150 uL	H2O2 150 uL
C	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	H2O2 150 uL	H2O2 150 uL
D	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	H2O2 150 uL	H2O2 150 uL
E	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	H2O2 150 uL	H2O2 150 uL
F	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	H2O2 150 uL	H2O2 150 uL
G	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	H2O2 150 uL	H2O2 150 uL
H	Ab Dil 50 uL	Ab 1 50 uL	Ab 2 50 uL	Ab 3 50 uL	Ab 4 50 uL	MIX	MIX	MIX	MIX	MIX	H2O2 150 uL	MIX

Well allocation per slide

	Wells		
	Ab Sol'n	H2O2	Mix
Slide 1	A1-A5	A11	H6
Slide 2	B1-B5	B11	H6
Slide 3	C1-C5	C11	H6
Slide 4	D1-D5	D11	H7
Slide 5	E1-E5	E11	H7
Slide 6	F1-F5	F11	H7
Slide 7	G1-G5	G11	H8
Slide 8	H1-H5	H11	H8
Slide 9	A1-A5	A12	H8
Slide 10	B1-B5	B12	H9
Slide 11	C1-C5	C12	H9
Slide 12	D1-D5	D12	H10
Slide 13	E1-E5	E12	H10
Slide 14	F1-F5	F12	H12
Slide 15	G1-G5	G12	H12

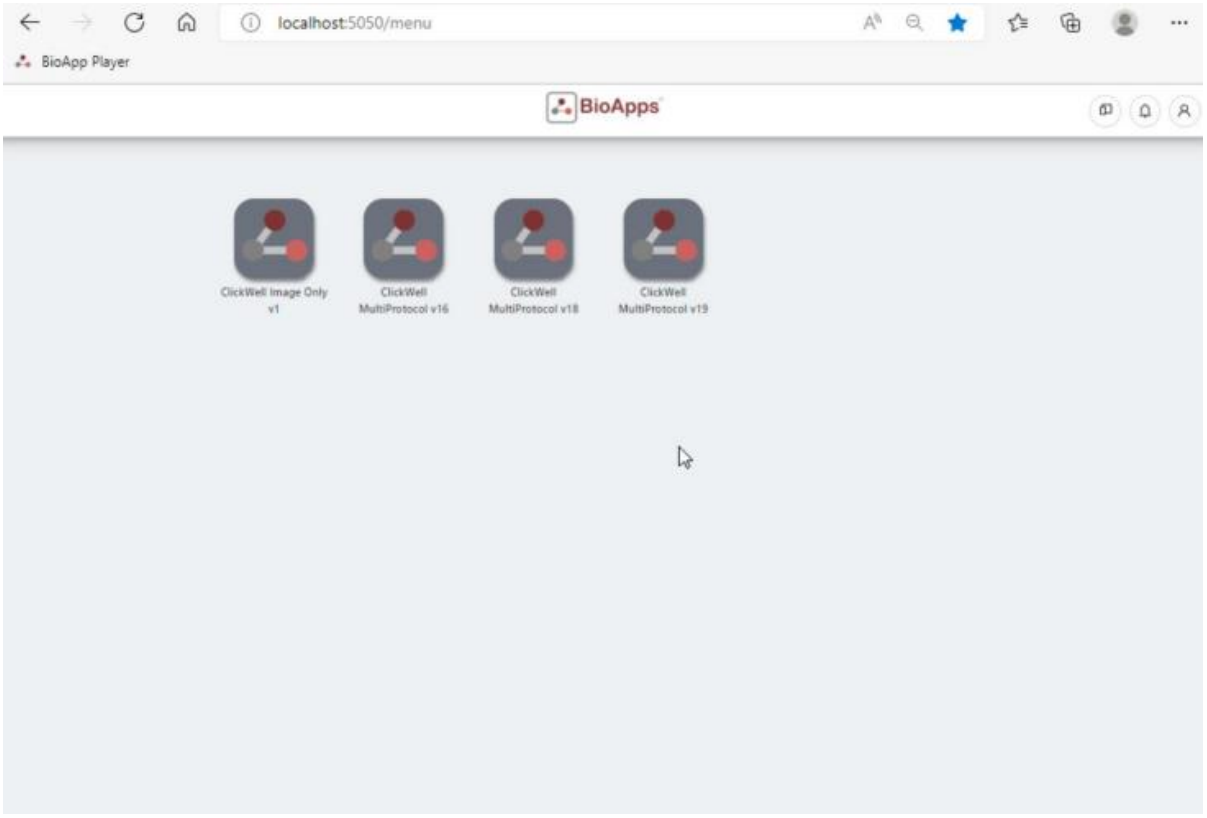
Enable Cell DIVE Imager:

1. Start the Cell DIVE™ Robotics Integration Tool by clicking the icon on the PC desktop. 



Configure the BioApp Player

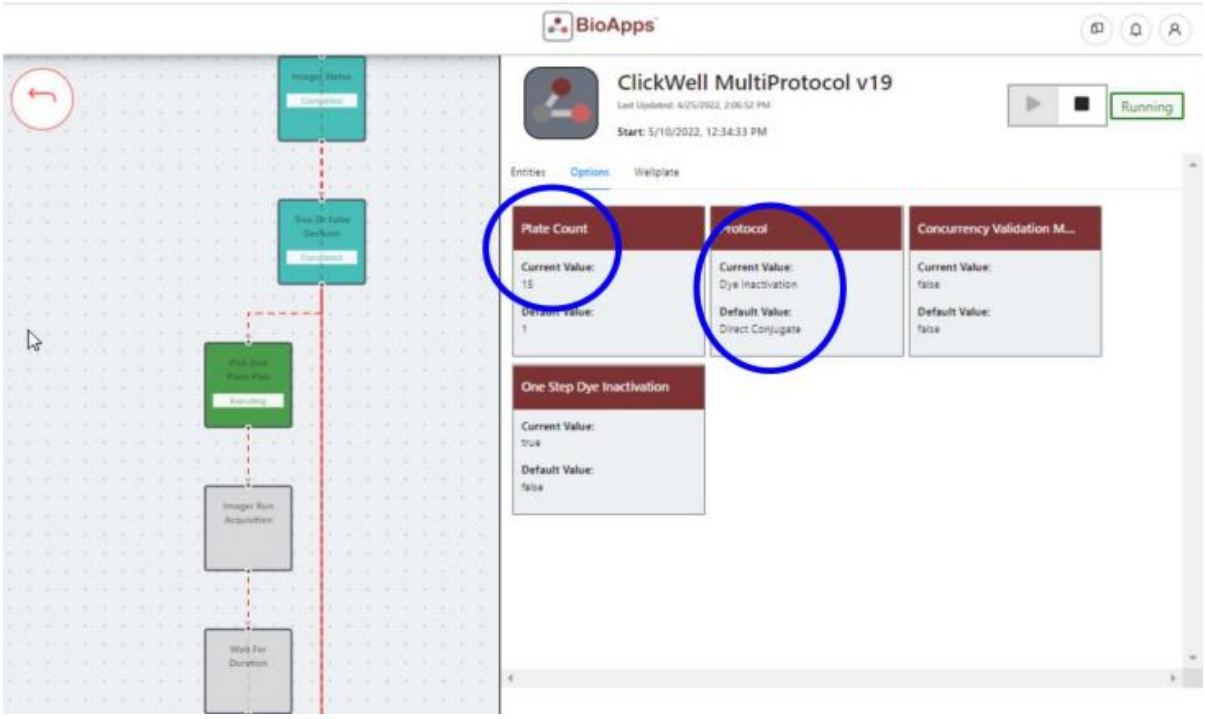
1. On the BAB200 touchscreen, swipe the screen to see the desktop. Open Microsoft Edge and click BioApp Player.



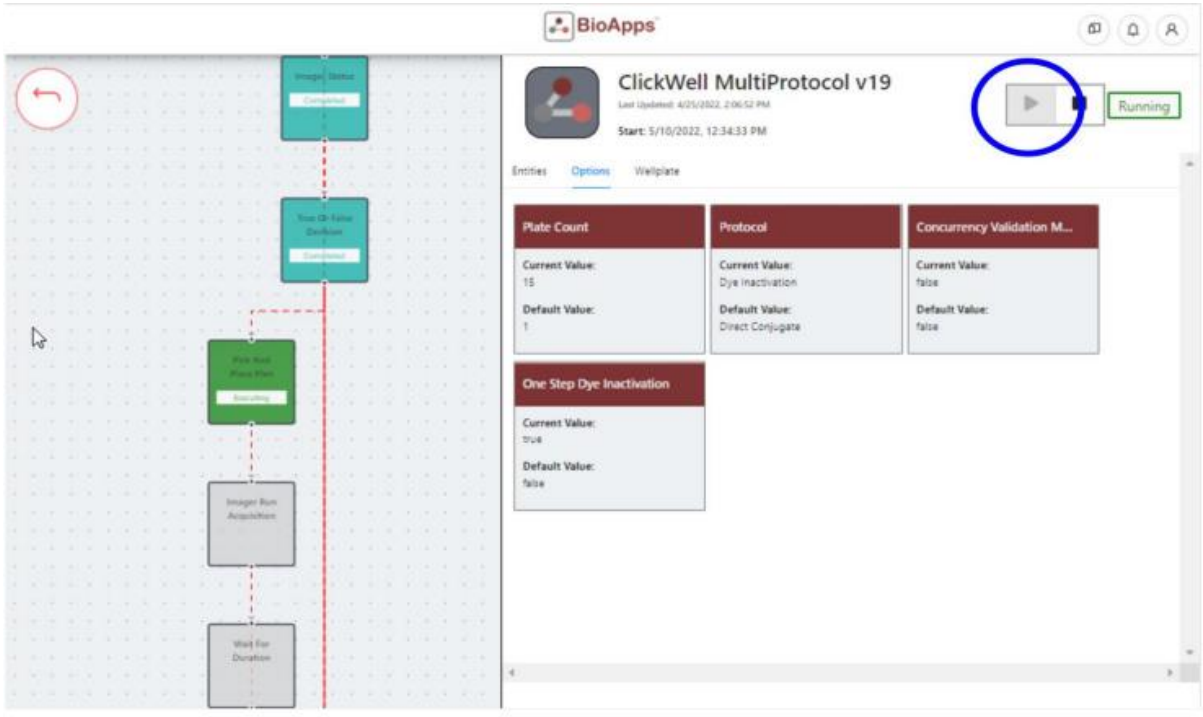
2. Select the BioApp to run. Image Only or ClickWell MultiProtocol.
   Image Only – Performs an imaging run with no staining operations.
   ClickWell MultiProtocol – Runs the full staining procedure.

3. Select the BioApp, then select the **Options tab**. Several boxes will appear on the screen. Select **Plate count** and enter the current plate count for the number of plates that are loaded into the Cell DIVE™ workstation. Next, select the **protocol**, either Direct Conjugation or Dye Inactivation. If Dye Inactivation is selected, choose whether inactivation will be done in one step or two steps (be sure that the dye

inactivation solution layout matches the entry in the BioApp).



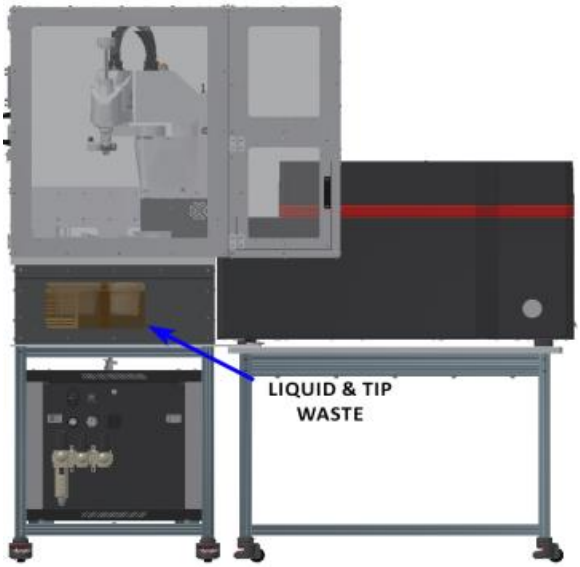
4. Start the BioApp. Once the **Protocol** is defined and **Plate Counts** are entered, press the **play button** in BioApp. **Ensure everything is loaded before starting, tips, plates, reagents, and the tip and reagent waste are not full.**



5. When using the Imager only protocol: selet **Plate Counts**, then **Save**, then **Play.** This will do one round of loading and unloading the imager with the plates from the source to the completed locations.
6. To stop the BioApp: press the stop button, or open a door, or press the e-stop button. Once a BioApp is stopped, you must reset the machine. Do the following steps below:
    a. If the pick and place tool is holding a plate – Use the control move interface or the gamepad to move the plate to a safe location (these interfaces are described more later). Once you are in a safe location, use the Control->Pick-and-Place screen Go Home button, to drop your plate. If you do not have a plate, you can simply move the tool back over the stage area. Once the tool is over the stage are, you can return it by going to the Control->Other screen and pressing return tool.
    b. If the Pipette tool is holding a Tip – Use the control move interface or the gamepad to move the tool to a safe location (these interfaces are described more later). Once you are in a safe location, open the door and remove the pipette tip. If you do not have a tip, you can simply move the tool back over the stage area. Once the tool is over the stage are, you can return it by going to the Control->Other screen and pressing return tool.
    c. Reset the machine – To reset for your next run, simply just follow the steps from earlier in this document, reload tips, plates, reagents, etc.+

Waste removal

The Liquid Waste and Tip Waste containers are Under the platform table behind the sliding doors.



To remove and empty waste from the Liquid Waste Container, slide the door open and turn the bottle counterclockwise to unscrew. Reverse the process to replace the bottle once emptied. Dispose of the liquid waste down the sink.

The Tip Waste container is to the left of the Liquid Waste container. To empty the Tip Waste Container, slide out the container and dispose of tips. Replace the emptied bin. Dispose of the used tips into the biohazard waste bin.