SINGER INSTRUMENTS
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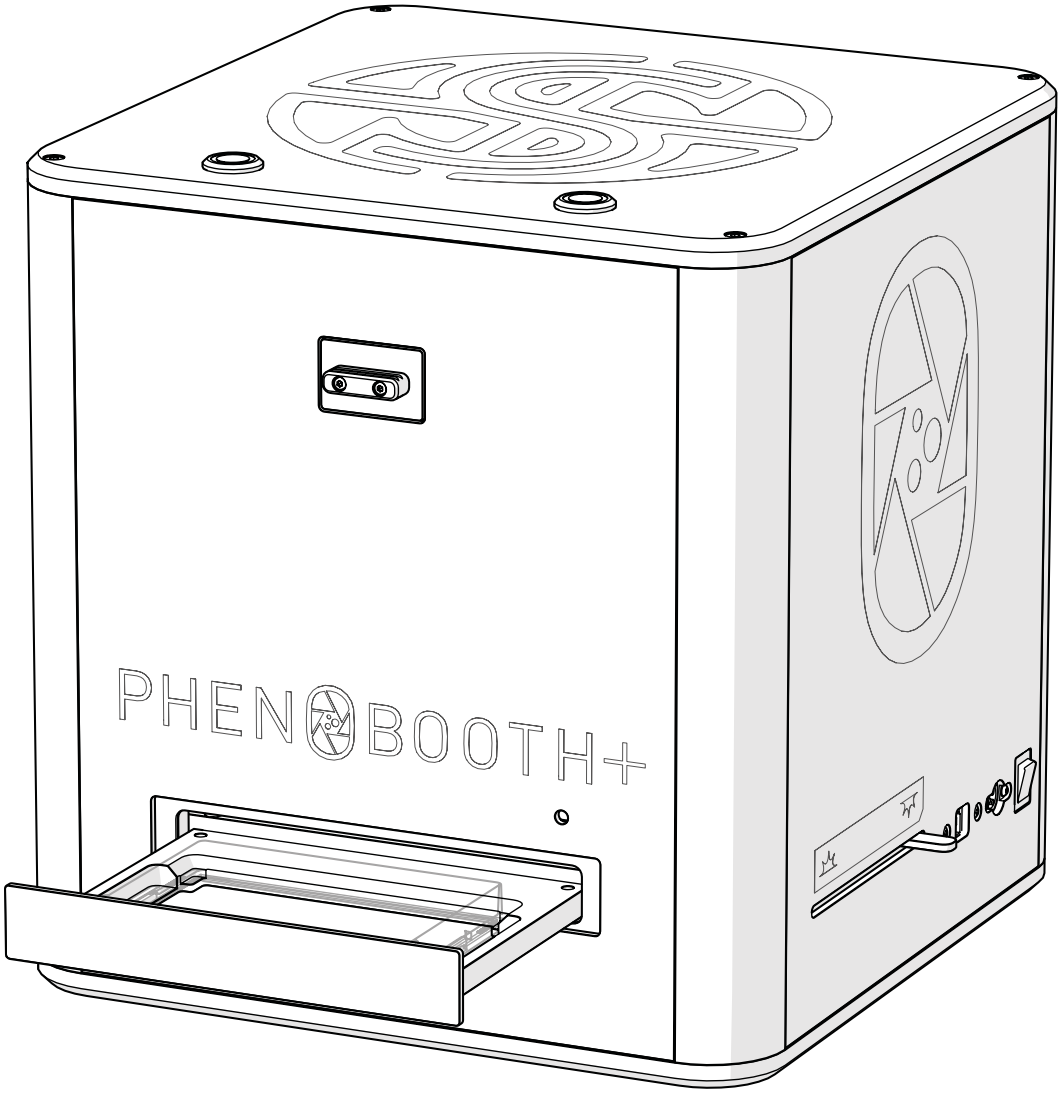
PHENOBOOTH+™

USER GUIDE v1.4



READ BEFORE YOU START!

- Remove plate lids before imaging. If lids are in place when the input tray is closed, the machine will crash and may be damaged.

- Keep your hands clear while the input tray is moving. Catching your hands in the input tray may cause injury.

- Ensure power supply is the correct way round. Plugging the power supply into the PhenoBooth+ upsidedown will cause damage to the machine. See p.10 for details.

- Avoid plugging the USB 3.0 cable in slowly. A common error with all USB 3.0 devices is that they are recognised as USB 2.0. This happens when the USB plug is inserted too slowly.

- Remove plate and petri adaptor before moving PhenoBooth+. If you need to move the PhenoBooth+ for any reason, make sure any plates are removed from the machine as well as the petri dish adaptor. These items can fall from the input tray and become lodged inside the PhenoBooth+.

- The PhenoBooth+ is not a microscope! It is a macroscopic CCD imaging workstation. As such it will not detect sub-cellular or low levels of expression. The in-house strains used to successfully test PhenoBooth's fluorescence detection have a fluorophore molarity of 1.84E-30 nM per cell. As such we cannot guarantee fluorescence detection below this value.

- Set agar height for different plate types. The PhenoBooth+ is capable of using a variety of different plates which can have different base thicknesses. To account for this, we ask users to input the distance from the top of the agar (trying to ignore the meniscus) to the bottom of the plate. See p.53 for details.

2. Before you start!

3. Introduction

5. OUT OF THE BOX
6. Anatomy & features
9. Unboxing & installation

11. LET'S START!
12. PhenoBooth+ hardware operation
PhenoBooth+ software set-up

14. COLONY COUNTING PROJECT
15. New project
16. Image acquisition
17. Pre-processing
19. Detection
20. Filtering
21. Export

22. COLONY SCREENING PROJECT
23. New project
24. Image acquisition
26. Pre-processing
27. Detection
28. Filtering
29. Analysis
30. Visualisation
31. Export

32. RCP PROJECT
33. New project
34. Image acquisition
36. Pre-processing & detection
37. Filtering
38. Stinger Export
39. ROTOR HDA / The Stinger

43. BEST PRACTICE
44. Software icons
46. Feedback light
47. Batch processing
48. History plug-in
49. Add new plate run
50. Import/export Project
52. Using filters
53. Setting Agar Height

54. Notes

PHENOBOOTH+™

INTRODUCTION

The PhenoBooth+ is a fast, semi-automated plate reader for imaging and analysis of cell colonies. The PhenoBooth+ can image standard SBS plates, Petri dishes and Singer PlusPlates.

USER GUIDE

Follow these instructions to get the most out of the PhenoBooth+. This guide outlines getting started and general operation, as well as software tutorials for each package.
The information in this guide relates to software version 2.22.0607.1

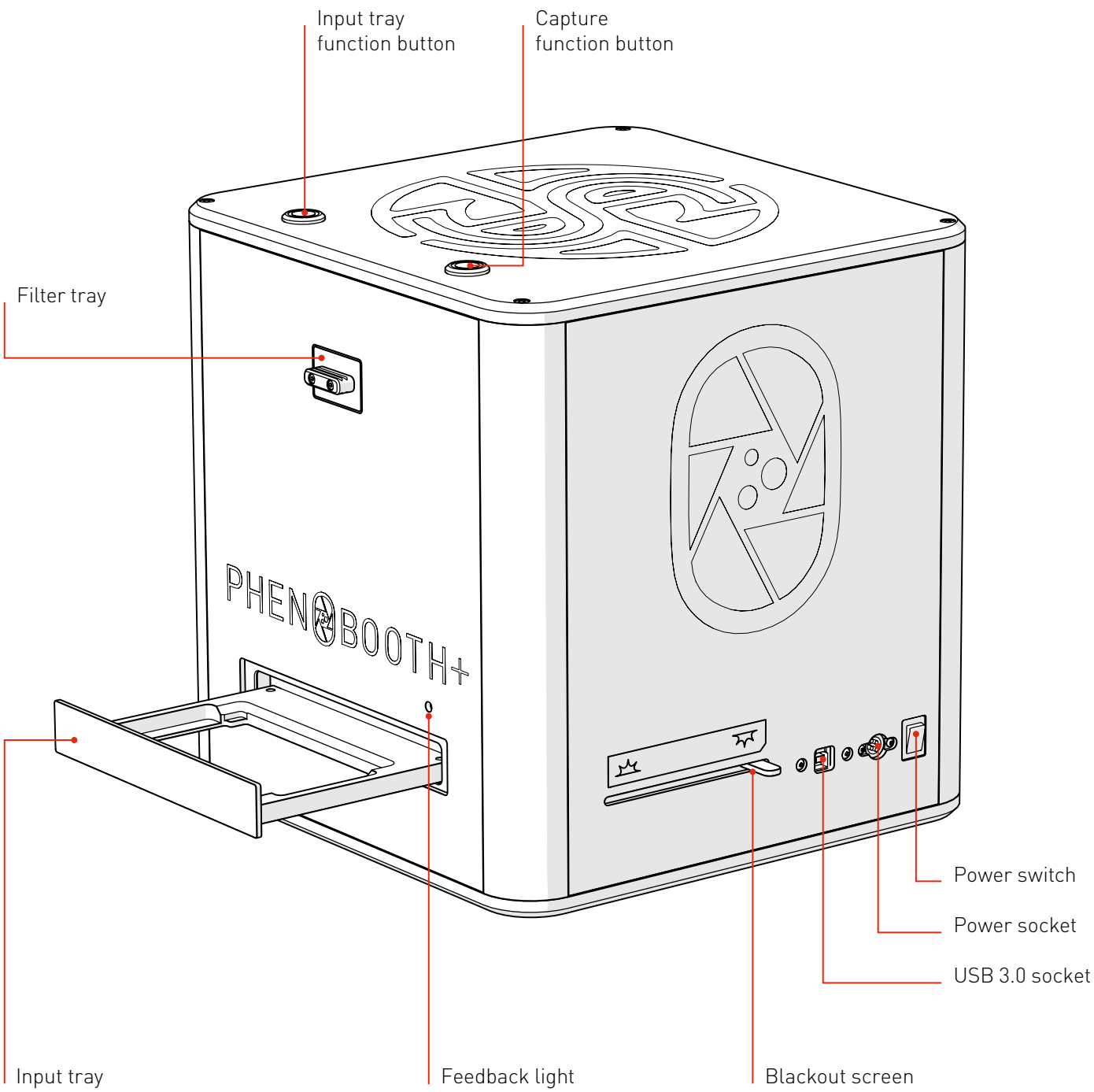
DISCLAIMER

At Singer Instruments, we are constantly seeking to improve our products and adapt them to the requirements of modern research techniques and testing methods. This involves modification to the mechanical structure and optical design of our instruments. Therefore, all descriptions and illustrations in this user guide, including all specifications are subject to change without notice.
To download the latest version of this user guide please visit:
singerinstruments.zendesk.com

OUT OF THE BOX

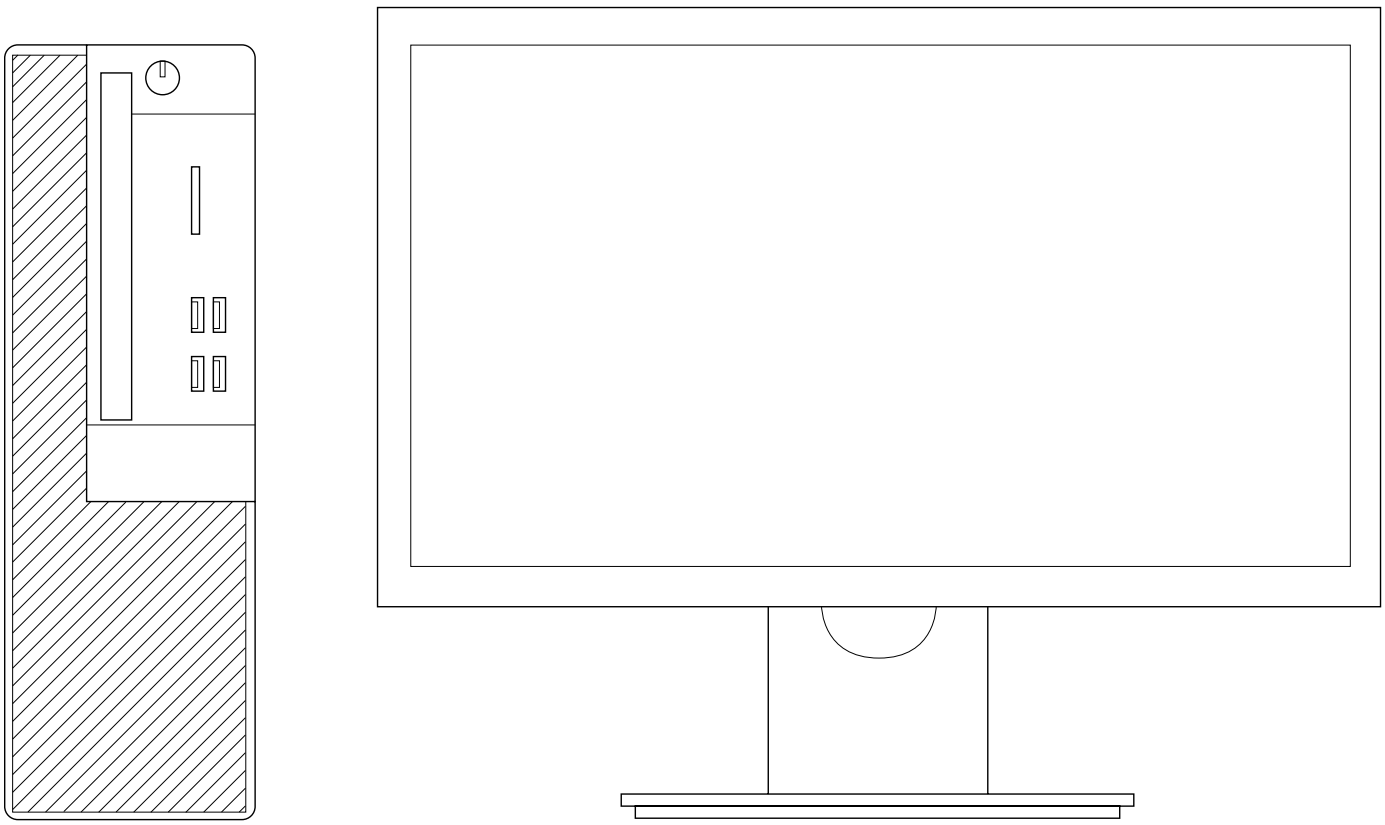
Find out what comes with the PhenoBooth+. We'll take you through the steps involved in unboxing and assembling ready for the exciting world of colony imaging!

PHENOBOOTH+

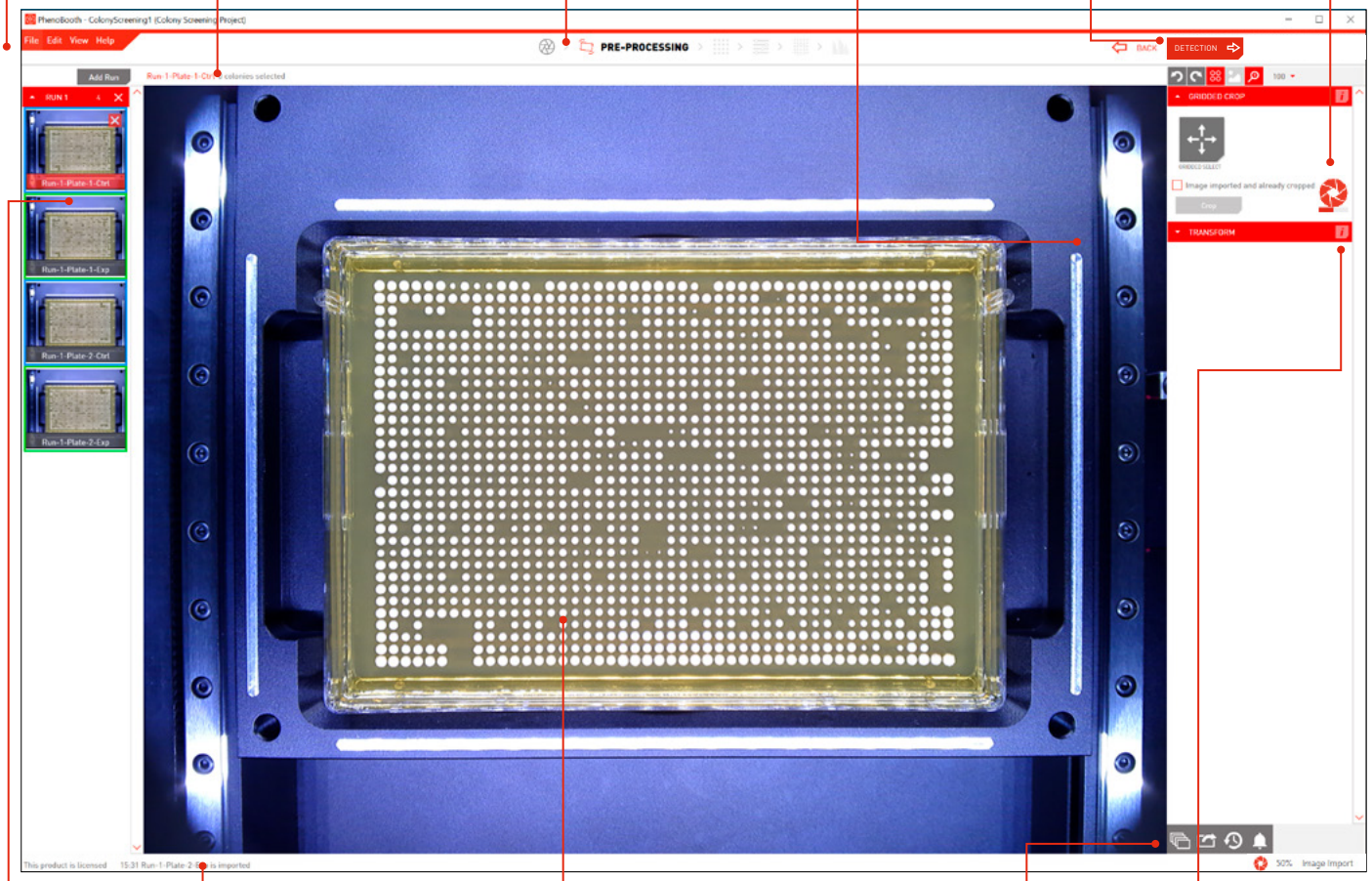

Input tray function button
Capture function button
Filter tray
PHENOBOOTH+
Power switch
Power socket
USB 3.0 socket
Input tray
Feedback light
Blackout screen

COMPUTER AND MONITOR

See Dell instructions in product boxes.



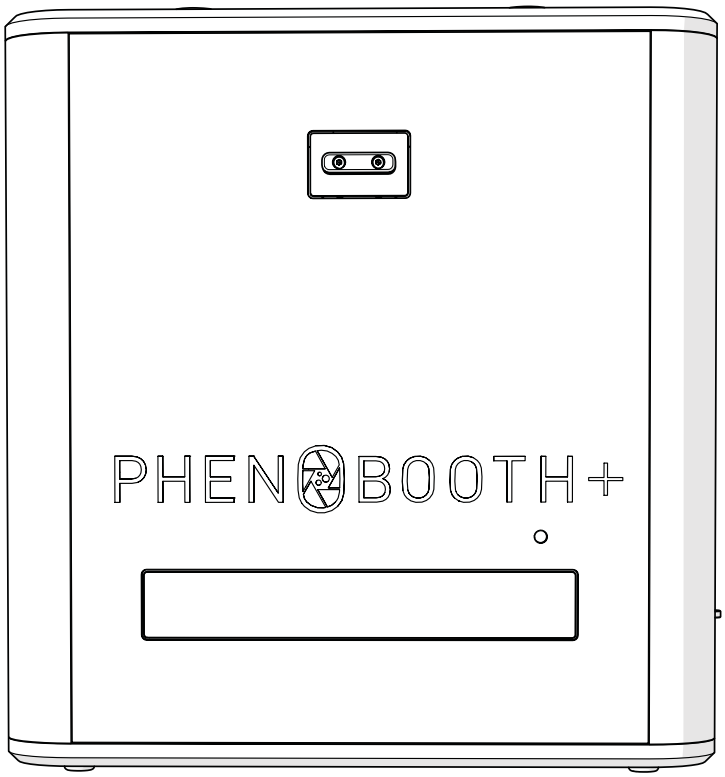
SOFTWARE


Menu bar
Plate information
Stage selection
Stage specific plug-ins
Progress bar
Next stage button
PRE-PROCESSING
BACK
DETECTION
GRIDDED CROP
TRANSFORM
Plate previews
Last task completed
Current plate
General Plugins
Plugin information

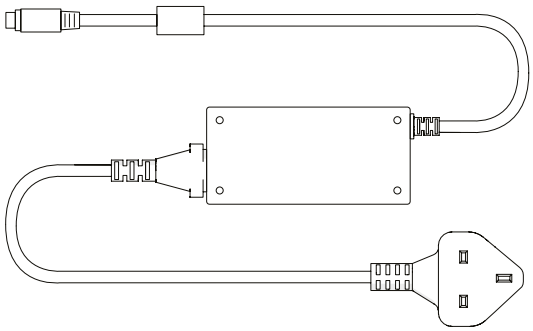
PHENOBOOTH+ BOX

1. PhenoBooth+
2. Power supply
3. 90mm petri adapter (optional extra)

1.



2.



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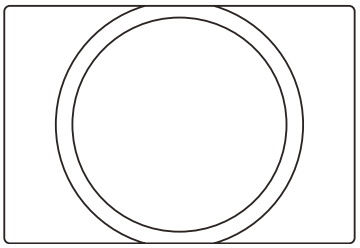
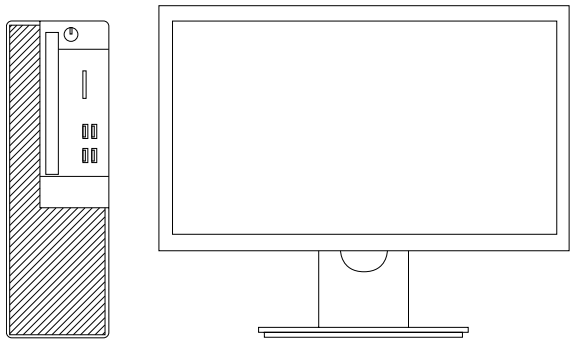


ADDITIONAL ITEMS

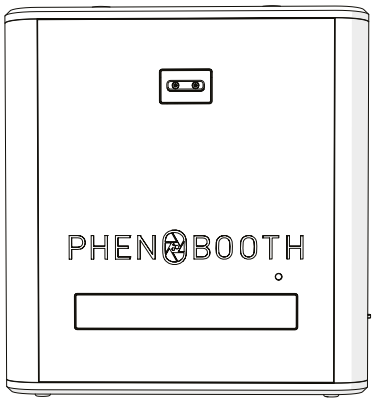
4. Monitor box (includes Dell monitor, stand and connections)
5. Computer box (include Dell computer, power cable, mouse and keyboard

1



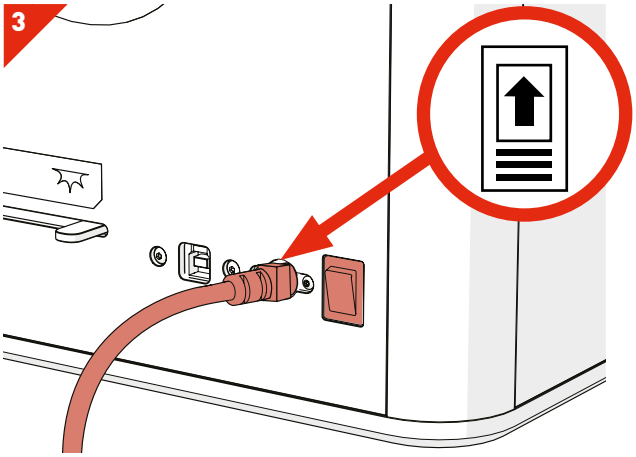
· Set up the computer and monitor as details in their instructions.

2



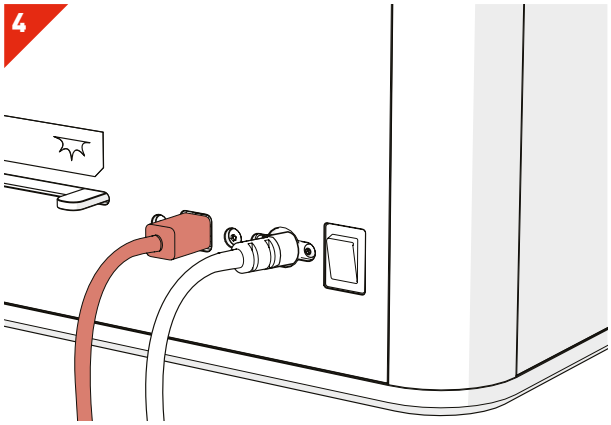
· Carefully remove the PhenoBooth+ from the box and stand on your workspace.
· HEAVY: May require 2 people.

3



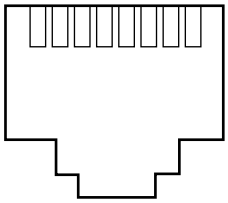
· Insert PhenoBooth+ power supply and turn on the power switch.
· WARNING: Make sure the arrow diagram is on top of the cable. Inserting the power supply upsidedown will cause damage to the PhenoBooth+.

4



· Insert the USB cable into the PhenoBooth+ USB 3.0 socket and to the MCI USB 3.0 socket.

5



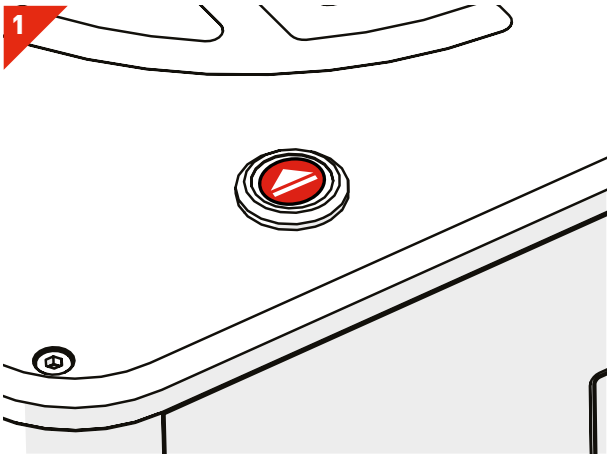
· For future software updates you will need to connect the computer to the internet via the LAN socket. See computer instructions.

LET'S START!

I'm ready to start!

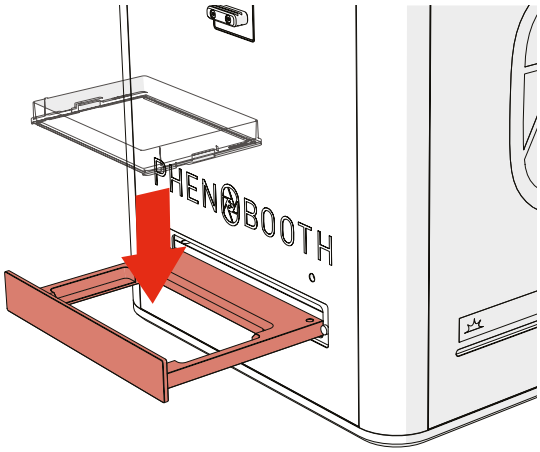
Here is a quick run-through of how to use the PhenoBooth+ and get started with the software — it's all very simple!

1



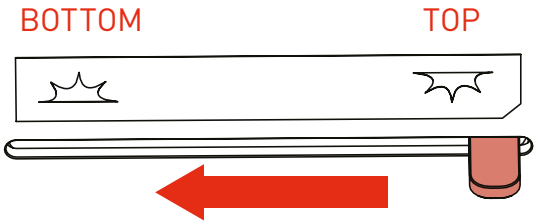
- Press the input tray function button to open the input tray.

2



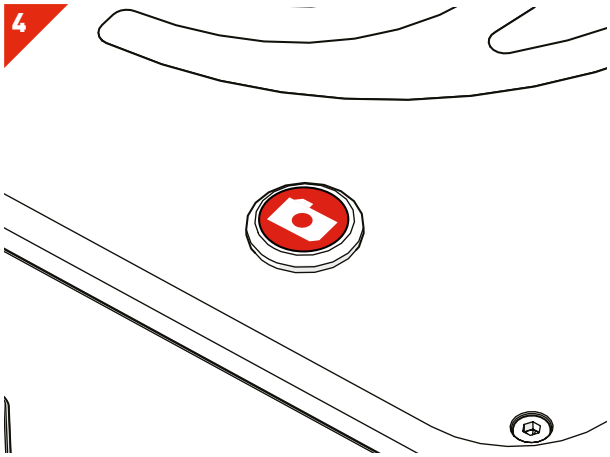
- Remove the lid from your plate and place the plate into the input tray.
- WARNING: Ensure plate is nestled correctly and lid is removed to avoid a hardware crash.

3



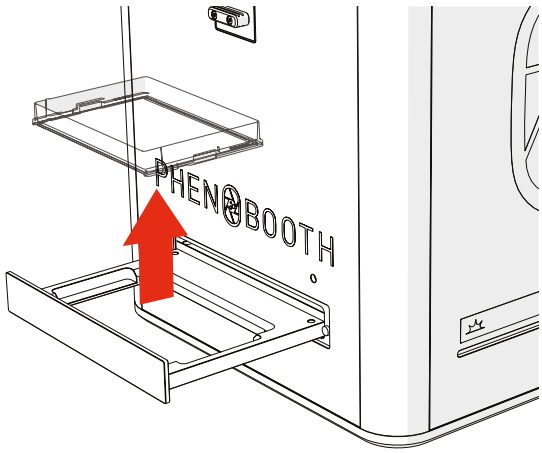
- Choose between top or bottom lighting by sliding the blackout screen (left for bottom and right for top).

4



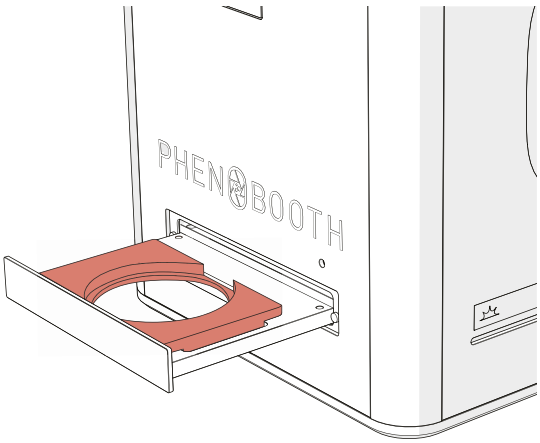
- Press the capture function button to image the plate.
- Follow the software instructions.

5



- Press the input tray function button to open the input tray, remove your plate and replace the lid.
- Repeat these steps for the rest of your plates.

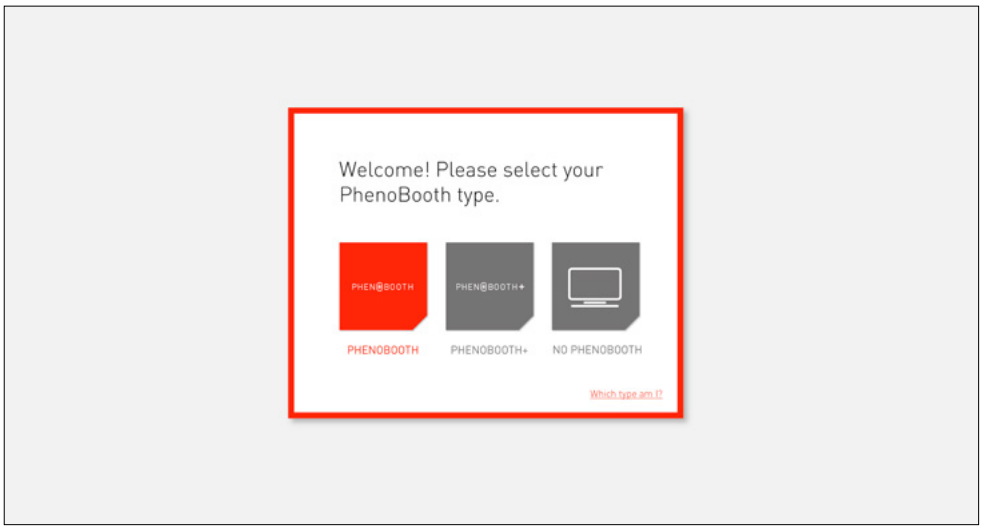
6



- If using petri dishes, you will need to use a petri dish adaptor. Place the adaptor into the input tray.
- Remove the petri dish lid and insert into the adaptor.

PHENOBOOTH+ SOFTWARE SET-UP

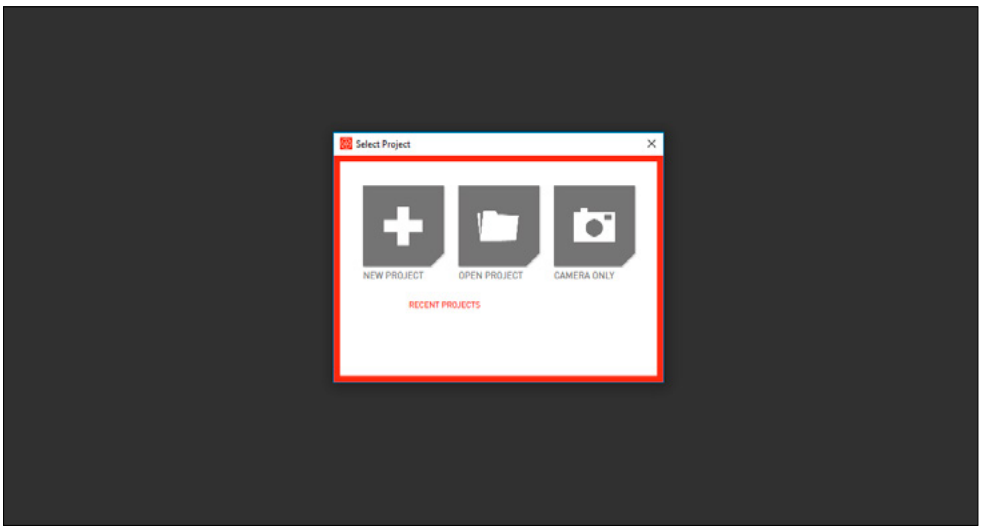
1



- PhenoBooth+ replaced the original PhenoBooth in October 2019. In order for your software to work properly, you'll need to select your PhenoBooth type:

- PhenoBooth: For machines delivered after October 2019
- PhenoBooth+: For machines delivered after October 2019. If you're unsure, the label on the front of the machine will tell you which type you have.
- No PhenoBooth: For software installed on a stand alone computer.

2

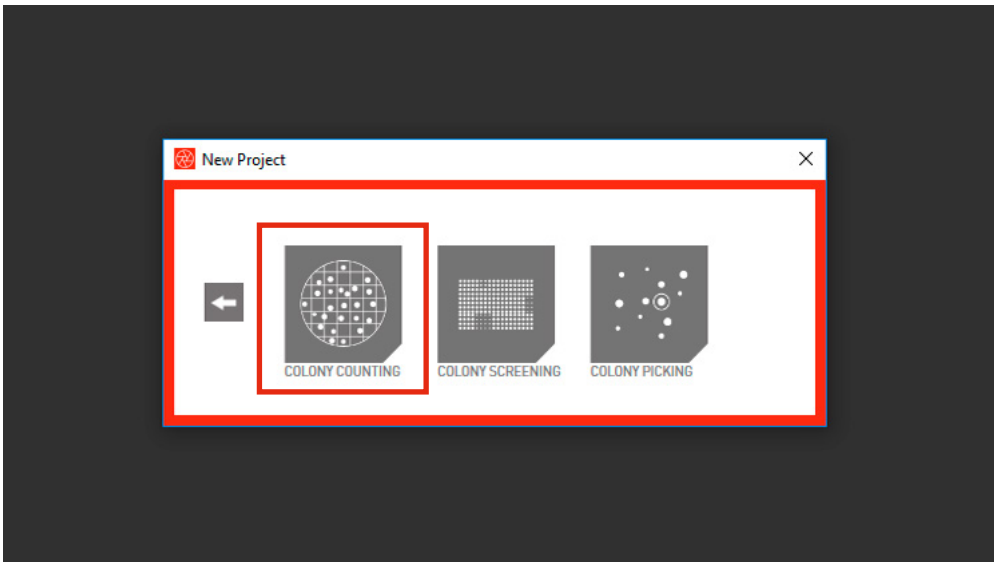


- Now you're all ready to start. Simply click to select a new project or open an existing one.
- If you just want to take a photo of a plate, with no analysis, select Camera Only.

COLONY COUNTING PROJECT

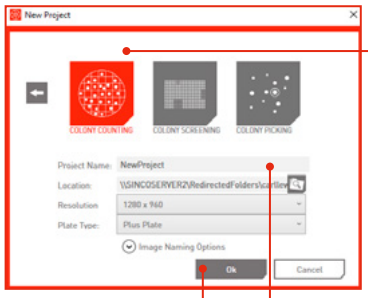
Colony Counting is the basic package included with your PhenoBooth+. There are 5 stages in the Colony Counting package: Image Acquisition, Pre-processing, Detection, Filtering and Summary.

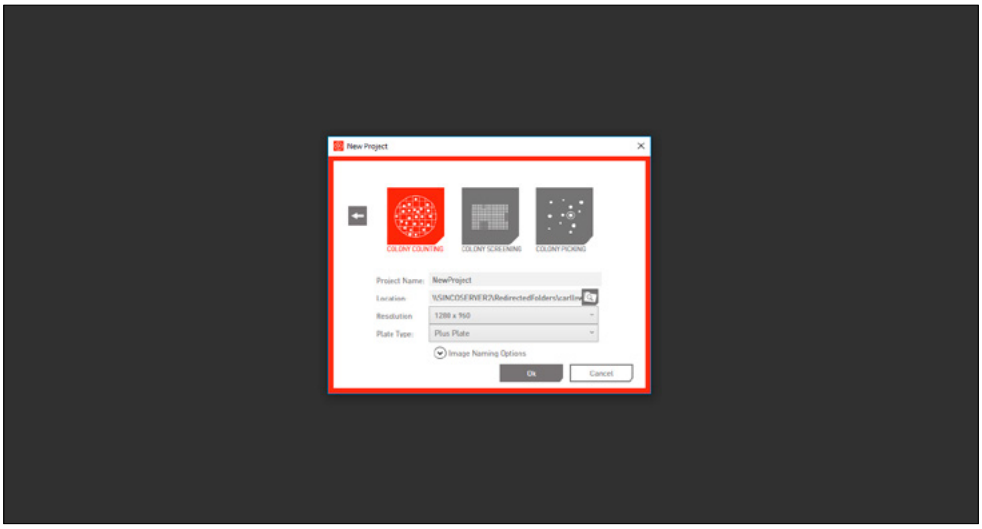
Start a new project then select Colony Counting.



COLONY COUNTING - NEW PROJECT

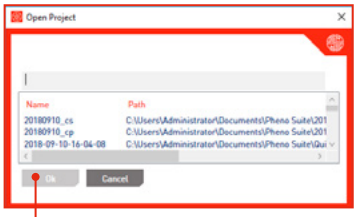
1

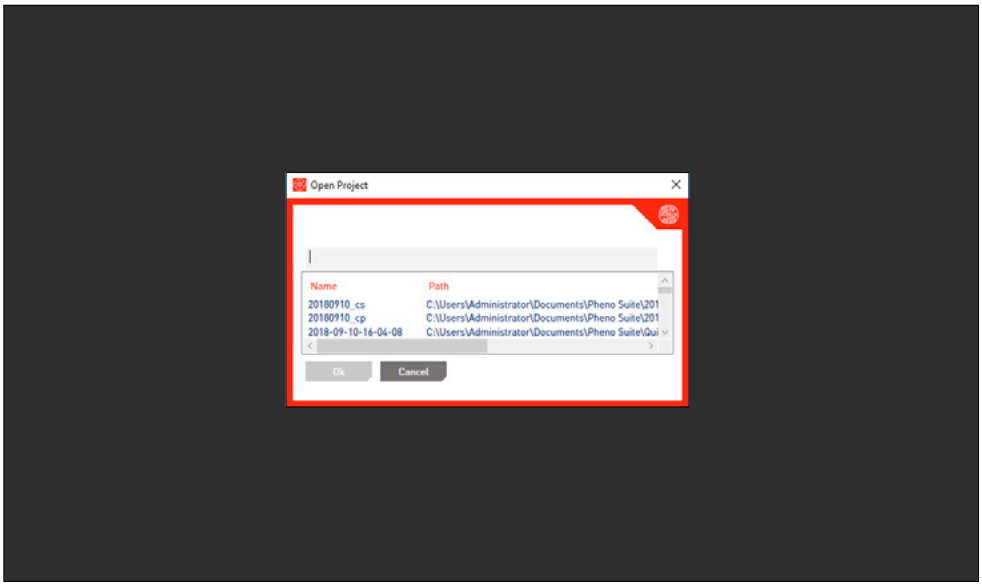




- Open the PhenoBooth+ software and select New Project.
- Select the Colony Counting Icon. Icons will vary depending on which software packages you have purchased. Your chosen project type is highlighted in red.
- Name your project, select a save location, choose an image resolution and select your plate type.
- There are also a few optional naming settings you can use to customise your plate images.
- Click OK to launch project.

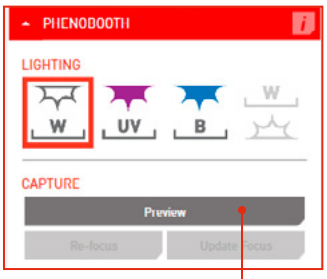
2

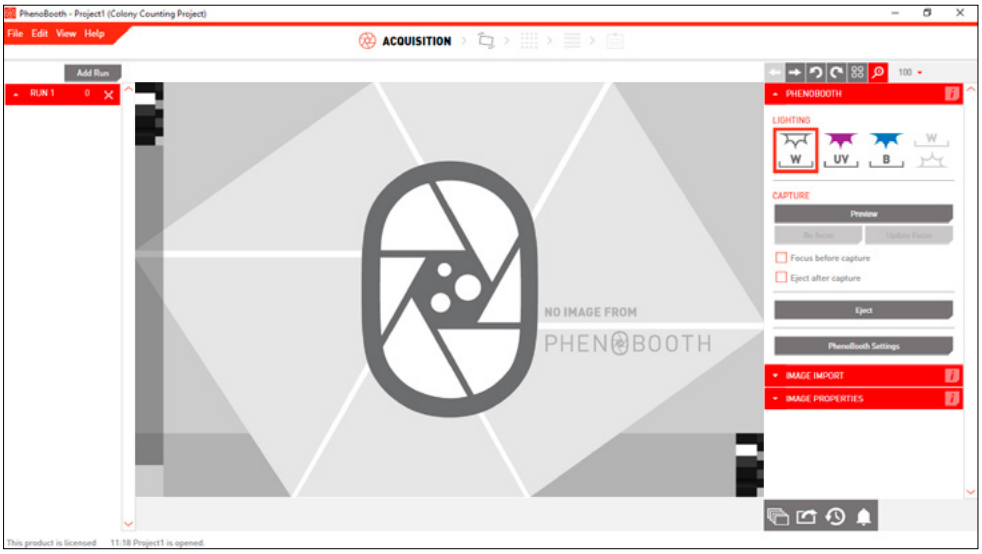




- If you want to open a previous project select Open Project and pick from the list of existing projects.
- Click OK to launch project.

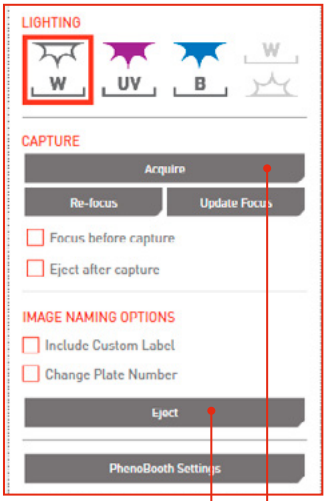
1





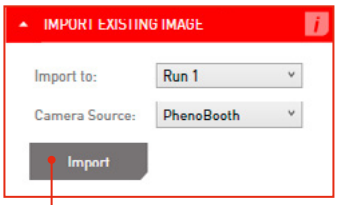
- There are two options for acquiring an image: PhenoBooth+ or import an existing image.
- Select the desired method from the plug-in panel on the right of the screen.
- If using the PhenoBooth+ camera, you can preview the image by pressing Preview.

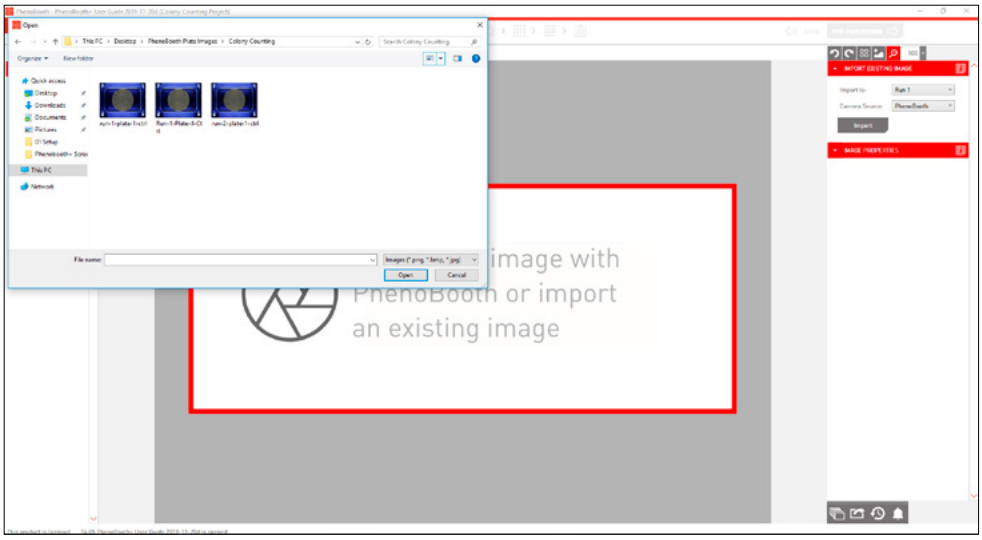
2



- If using the PhenoBooth+, click on that tab and select your desired light settings. Plate names are automatically generated.
- Insert a plate into PhenoBooth+ and press Acquire.
- Eject will allow you to remove the plate.
- Repeated until all of your plates have been captured.
- ➡ moves onto the pre-processing stage.

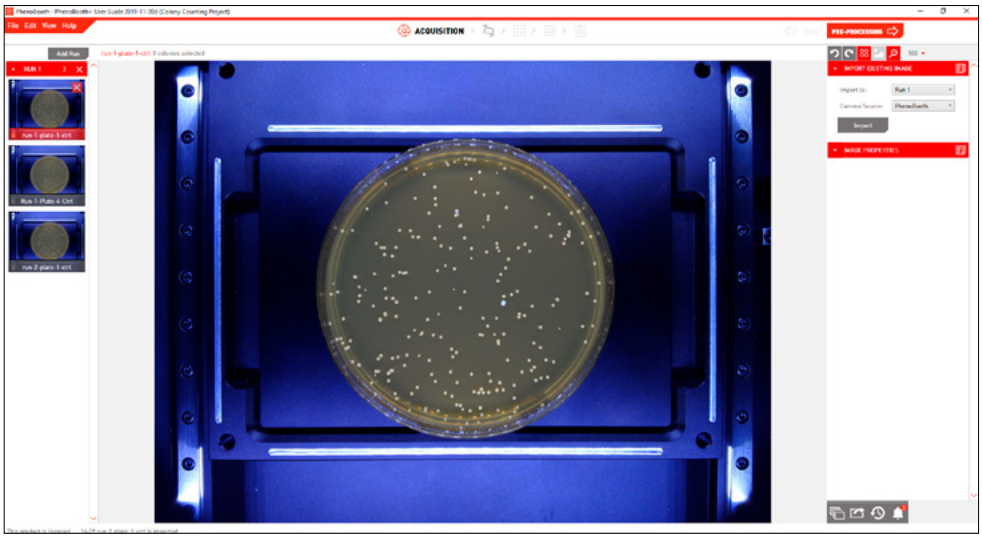
3





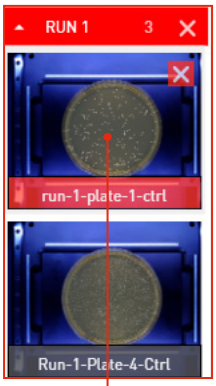
- If using existing images, click the Image Import tab. Here you can select which run the plate images will be associated. If this is your first run then Run 1 will be the only option. See p.49 to add new runs.
- Click Import to select images from your computer.

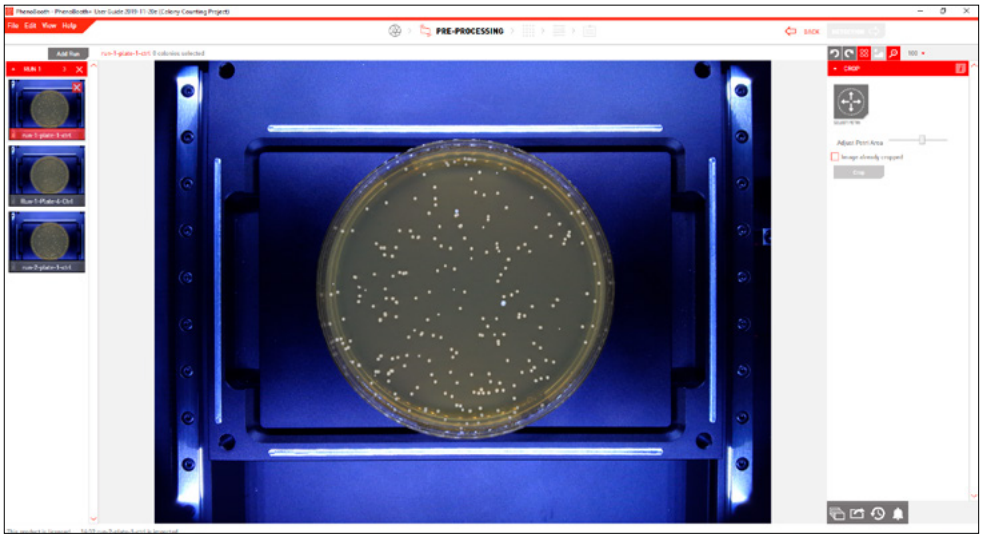
4



- Your captured or imported images now appear on the left of the screen. The currently selected plate is highlighted with red.
- Once you've finished capturing or importing images, use the next stage button to move onto the pre-processing stage.

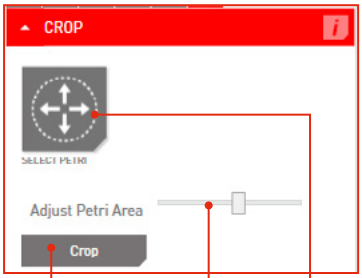
5

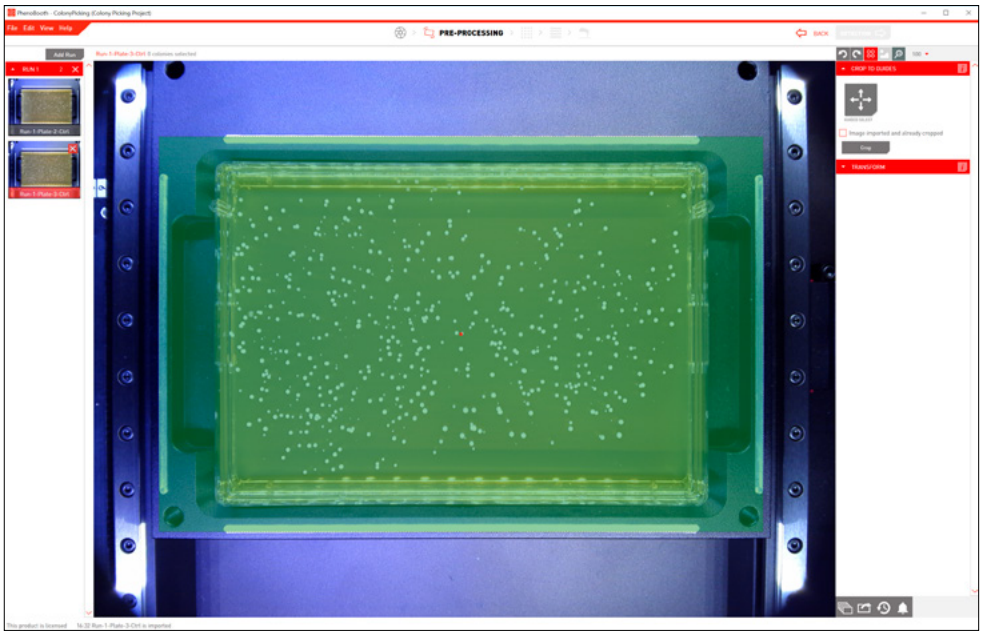




- Select which image to process first. If using the batch process option, this plate's settings will be applied to the rest of the plates in the run. Learn more about batch processing on p.47.

6

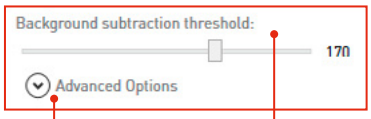


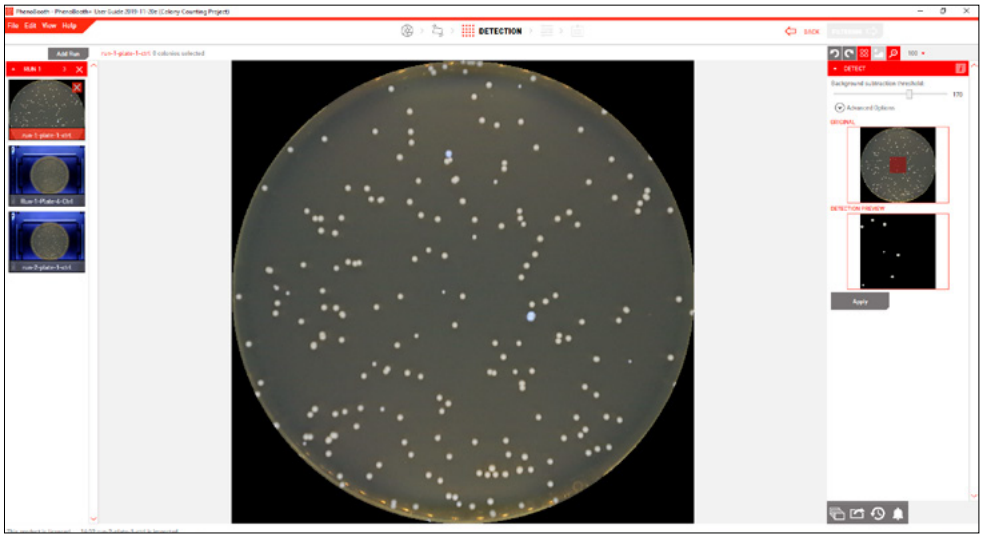


- Click on Select Petri to automatically detect the plate area. You can adjust the plate area using the Petri Slider.
- If using a PlusPlate, press the Select button and drag over the colony area of the plate.
- Click Crop to remove the edge of the plate and beyond.
- You will automatically move onto the filtering stage.

COLONY COUNTING - DETECTION

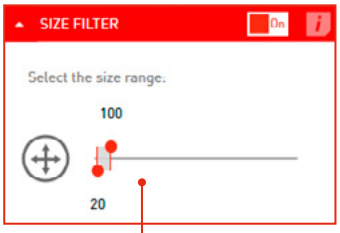
7

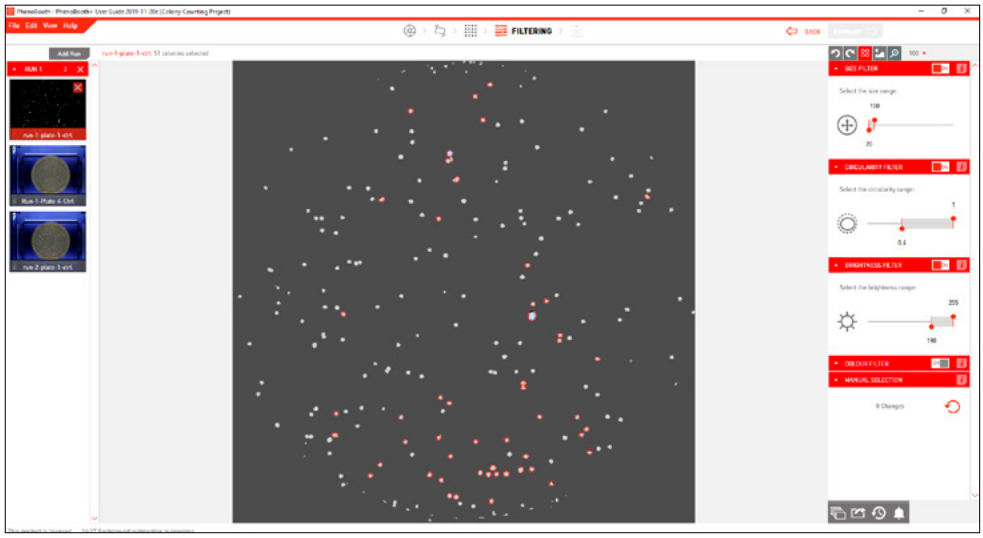




- In order to generate colony information, the background needs to be removed.
- Use the slider to adjust the filter intensity if necessary.
- If you have colonies that touch you can use the colony separation tool found in the advanced options.
- Check that all the smaller colonies are visible in the preview window then click Apply.
- Once applied, you will automatically move onto the filtering stage.

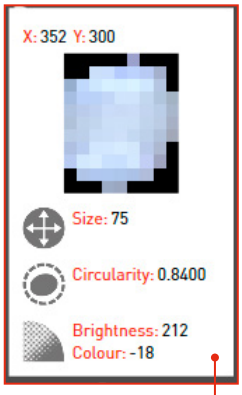
8

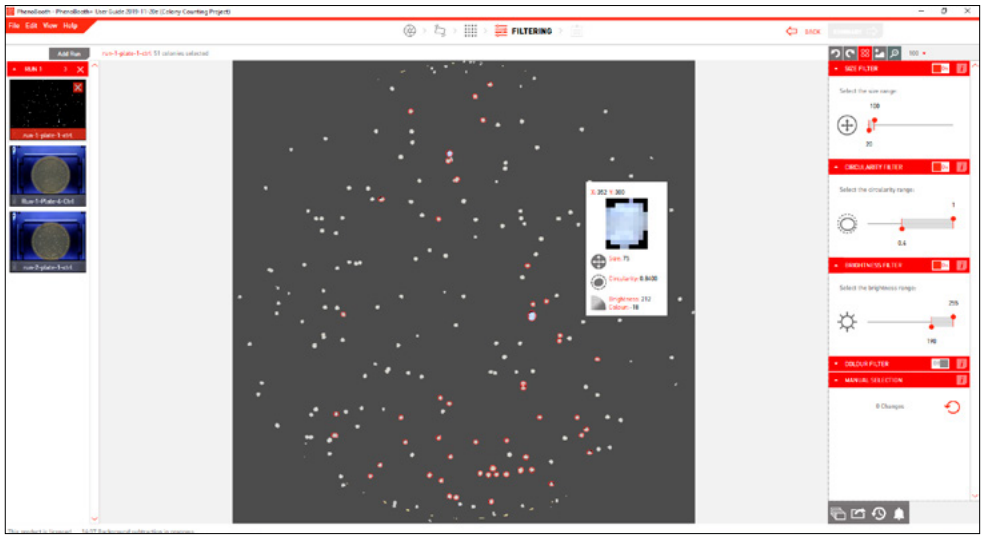




- Detected colonies are highlighted with a red circle. turns highlights on or off.
- Use the filter options to set minimum and maximum values for size, circularity, brightness and colour for colony detection. You can use the size filter to discard items that are too large or small to be colonies.
- Colonies can also be manually selected by simply clicking on a colony to select or deselect it.

9

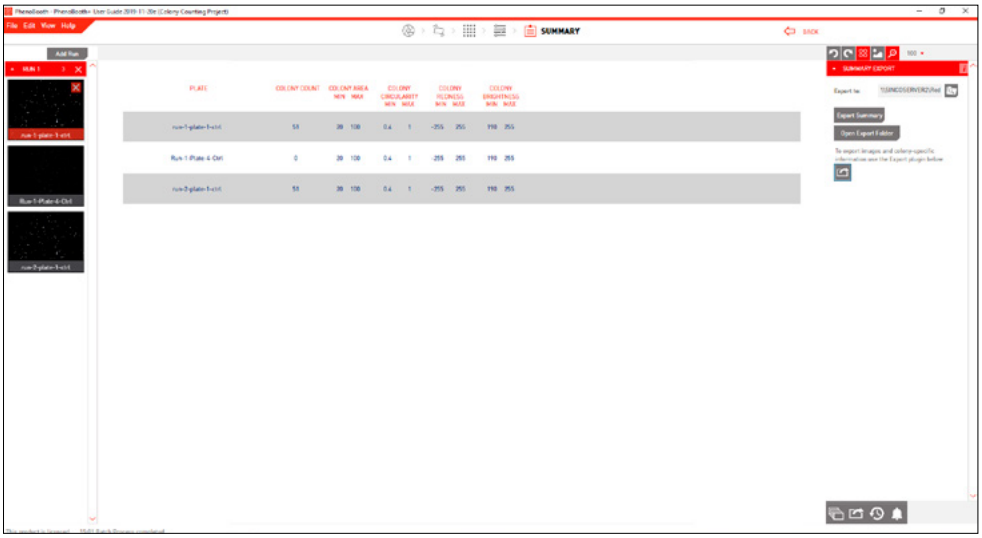


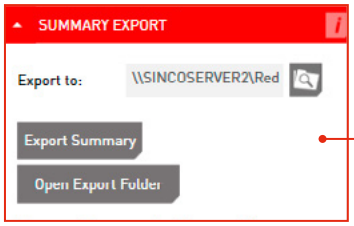


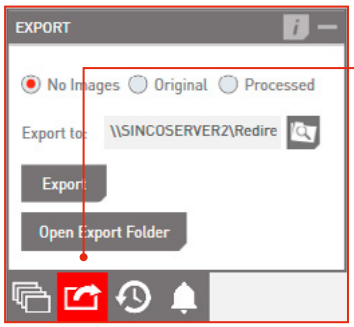
- Hover over a colony to see it's size, circularity, brightness and colour properties in the Colony Info card.
- You can batch process the rest of your plates with the same options using the instructions on p.47.
- After processing all of your plates you can use the next stage button to move onto the summary stage.
- NOTE: All plates must be processed before you can advance to the summary.

COLONY COUNTING - SUMMARY

10







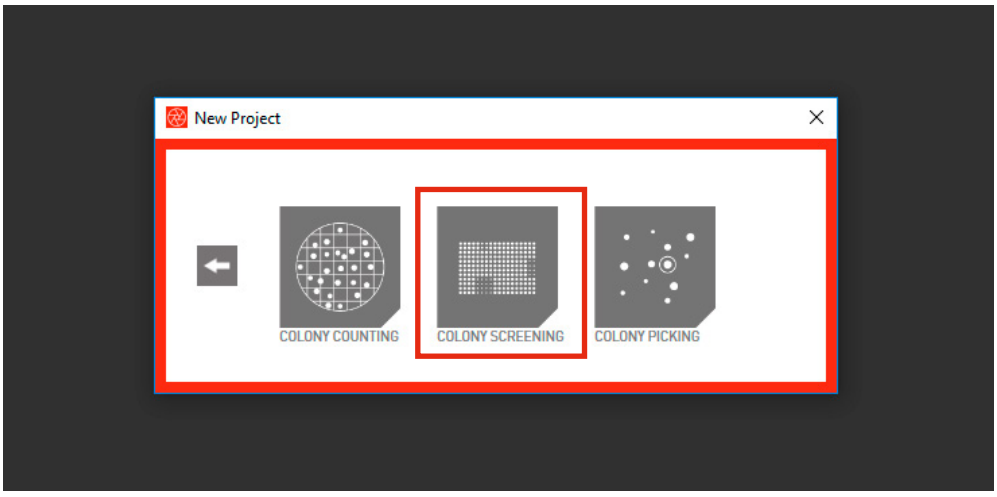
- The Summary Export plugin will export the data on the summary screen.
- For a full export of your workflow, click the Export icon on the bottom right. You can choose to export no images, processed images or the original images.
- The export includes: plate image (if selected), summary file and colony data table.
- The Summary File contains colony counts for all plates as well as colony imaging and filtering options information.
- The Colony Data Table contains position and properties of all detected colonies on all plates.

COLONY SCREENING PROJECT

The Colony Screening package includes all the plug-ins you need for colony screening, such as size and circularity filters, plate normalisation, gene mapping, control / experiment comparison and heat map visualisation.

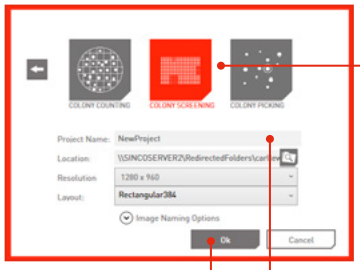
There are 6 stages in the Colony Screening package: Image Acquisition, Pre-processing, Detection, Filtering, Analysis and Visualisation.

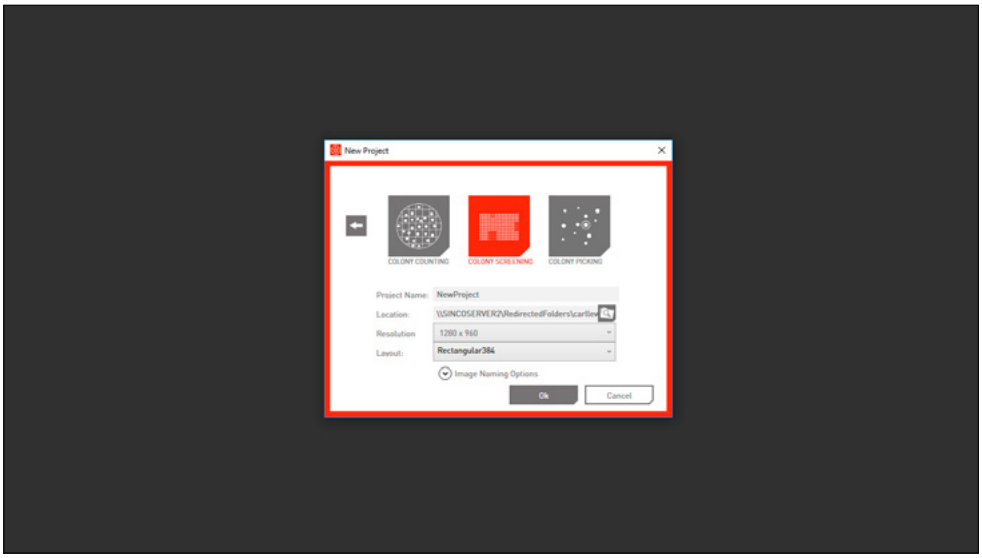
Start a new project then select Colony Screening.



COLONY COUNTING - NEW PROJECT

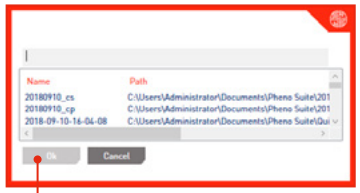
1

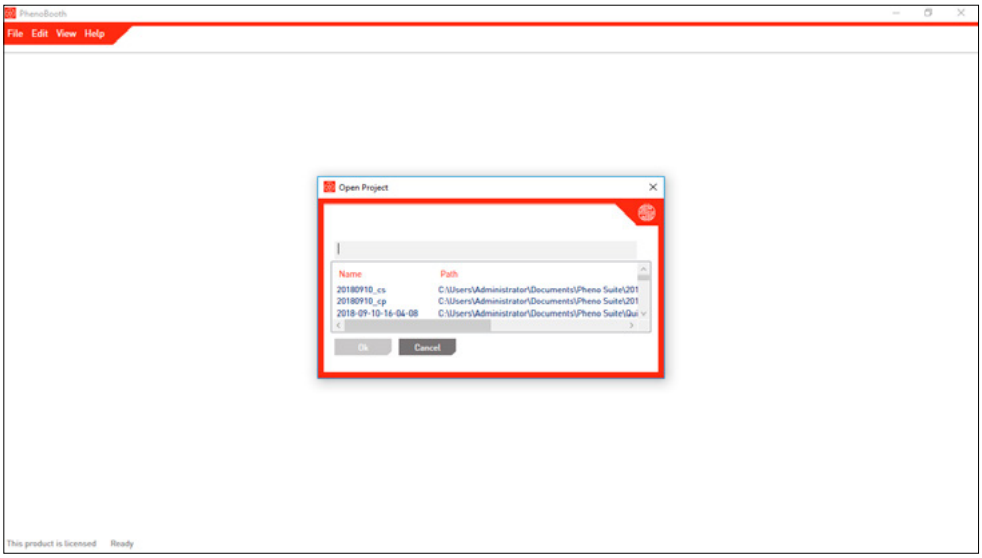




- Open the PhenoBooth+ software and select New Project.
- Select the Colony Screening Icon. Icons will vary depending on which software packages you have purchased. Your chosen project type is highlighted in red.
- Name your project, select a save location, choose an image resolution and your layout.
- There are also a few optional naming settings you can use to customise your plate images.
- Click OK to launch project.

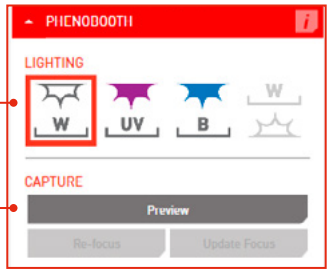
2

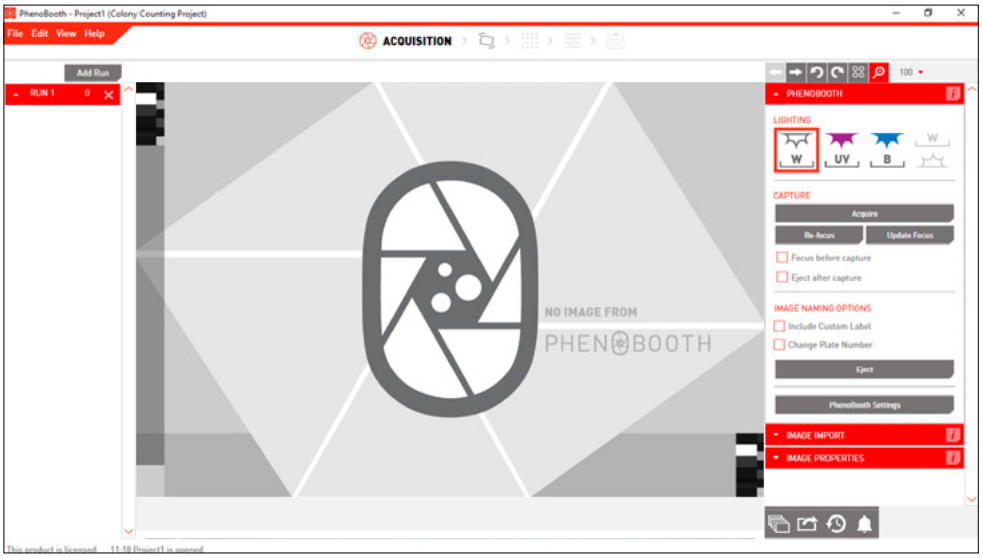




- If you want to open a previous project select Open Project and pick from the list of existing projects.
- Click OK to launch project.

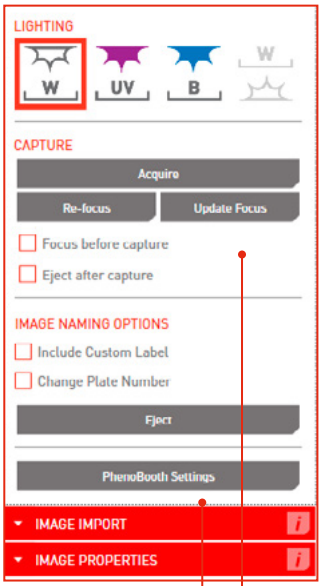
1

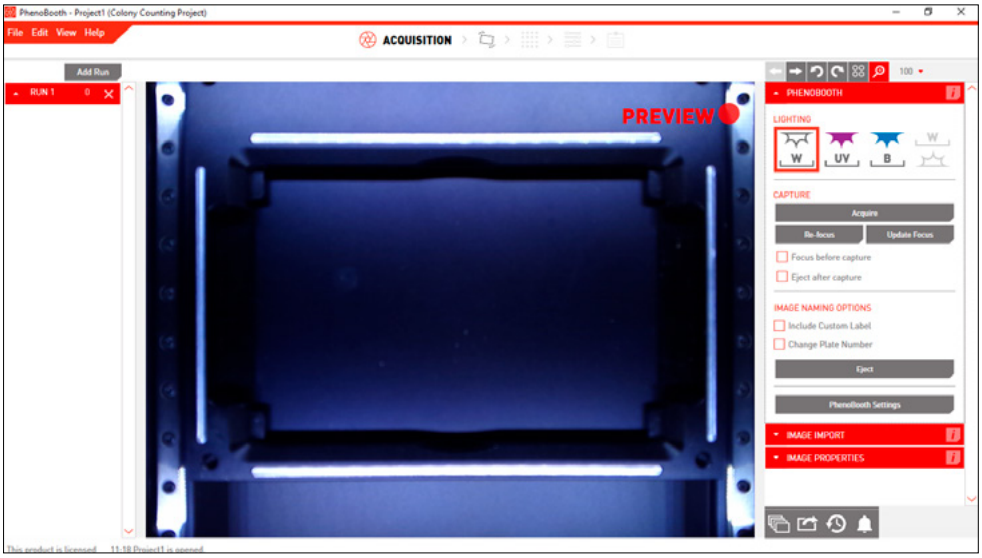




- There are two options for acquiring an image: PhenoBooth+ or import an existing image.
- Select the desired method from the plug-in panel on the right of the screen. Press Preview to preview the image.

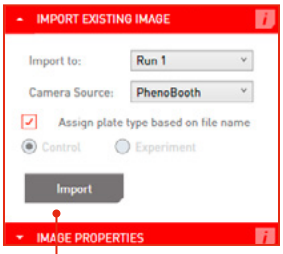
2

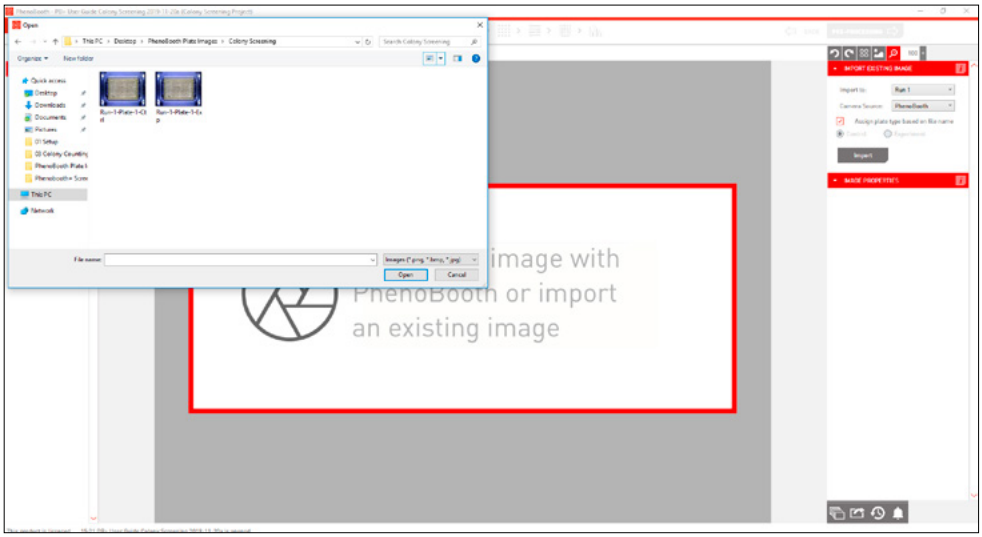




- If using the PhenoBooth+, click on that tab and select your desired light settings and select either Control or Experiment. Plate names are automatically generated.
- Insert a plate into PhenoBooth+ and press Acquire.
- Eject will allow you to remove the plate.
- Repeated until all of your plates have been captured.
- ➡ moves onto the pre-processing stage.

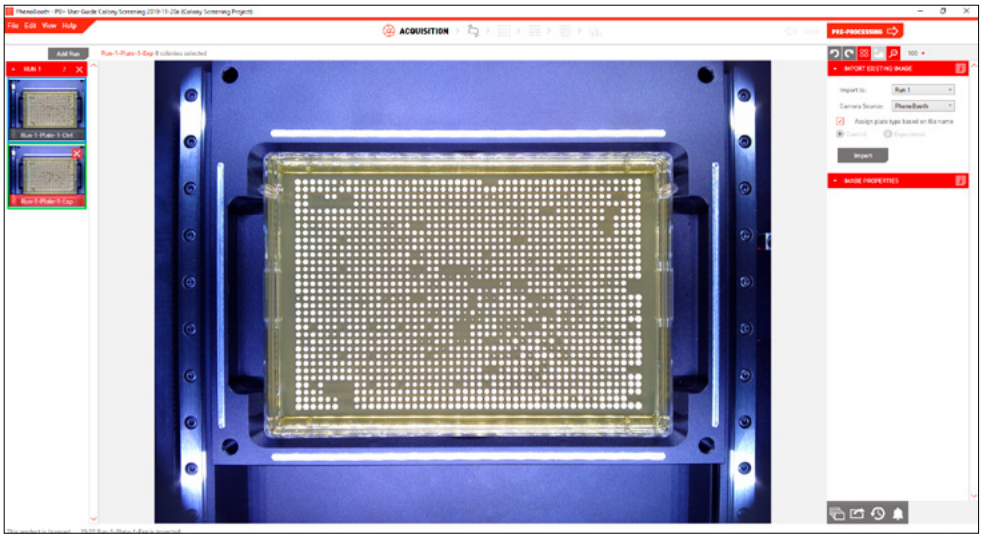
3





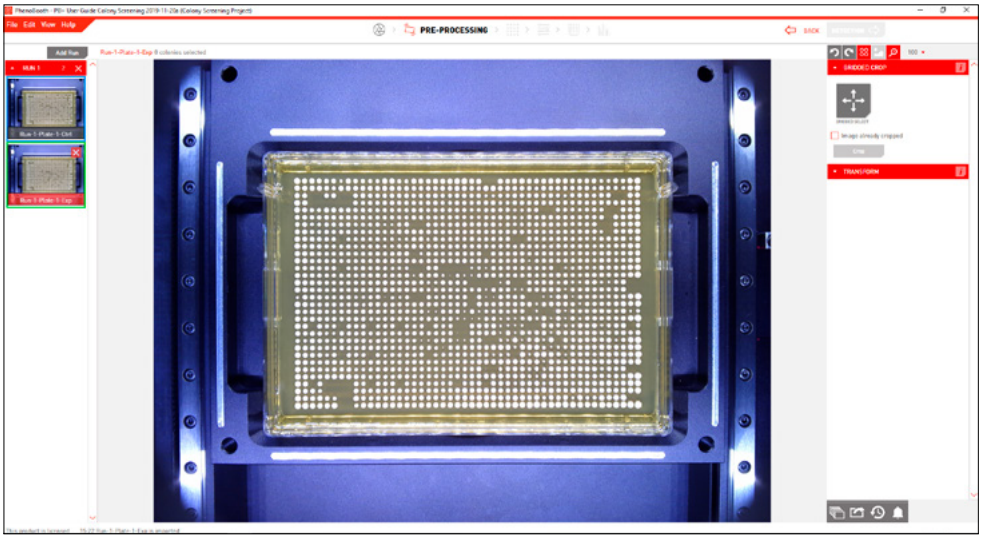
- If using existing images, click the Image Import tab. Here you can select which run the plate images will be associated. If this is your first run then Run 1 will be the only option. See p.49 to add new runs.
- Select whether the image is a control or experiment, or allow the PhenoBooth+ software to assign based on name. For example: *run-1-plate-1-ctrl* or *run-6-plate-9-exp*.
- Click Import to select images from your computer.

4



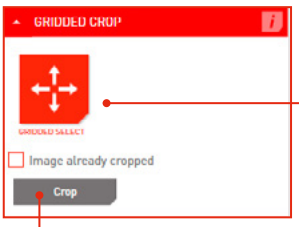
- Your captured or imported images now appear on the left of the screen. The currently selected plate is highlighted with red. Control plates have a blue border and experiment plates have a green border.
- Once all plates have been captured or imported, use the next stage button to move onto the pre-processing stage.

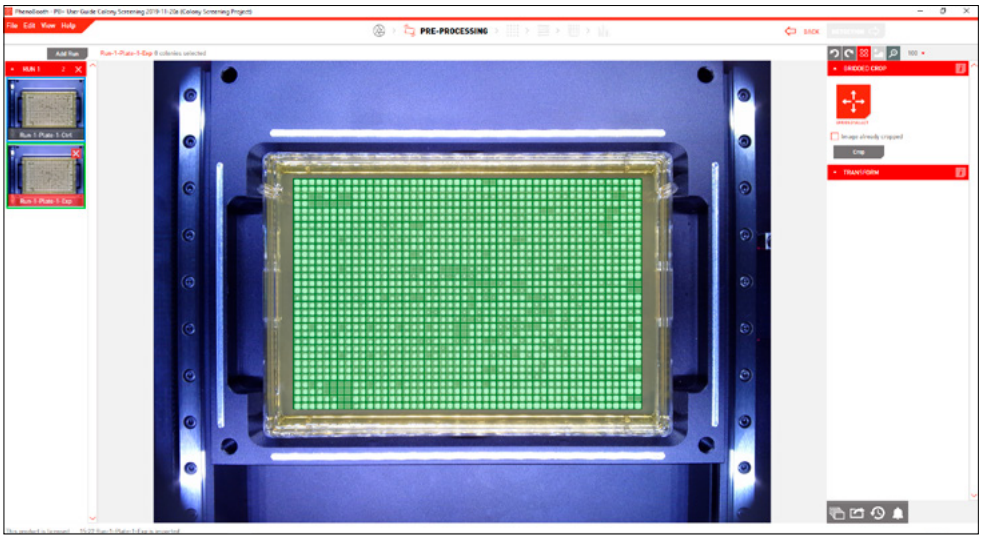
5



- Select which image to process first. If using the batch process option, this plate's settings will be applied to the rest of the plates in the run.

6

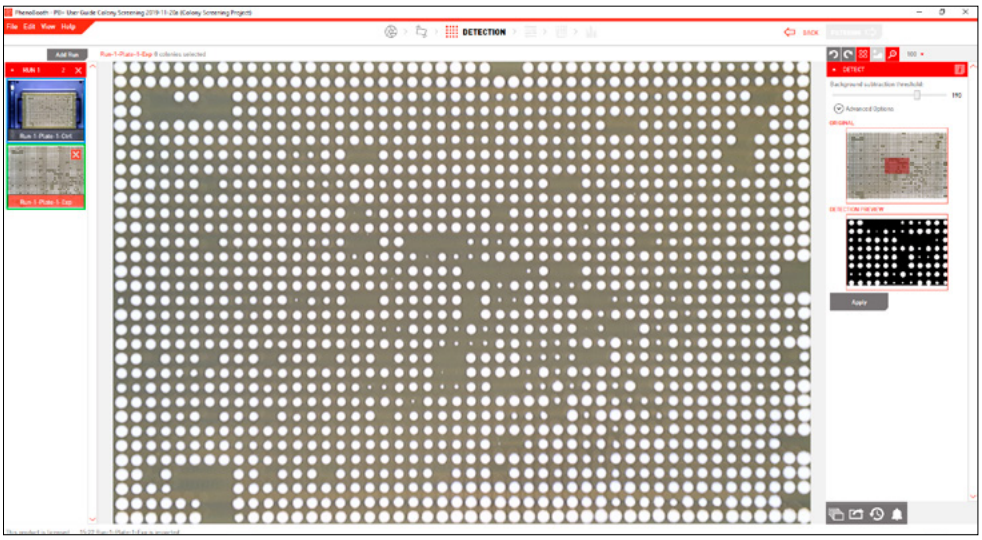


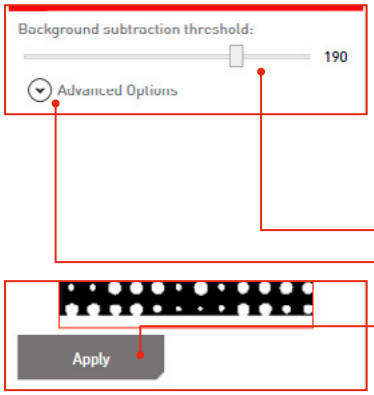


- In the Crop plug-in select Gridded Select.
- The grid will automatically overlay the plate image. You can also manually drag this grid over the plate with the mouse.
- Press Crop to remove the edge of the plate and beyond.
- Once cropped, you will automatically move to the detection stage.

COLONY SCREENING - DETECTION

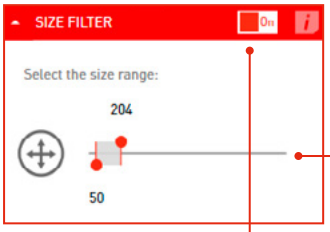
7

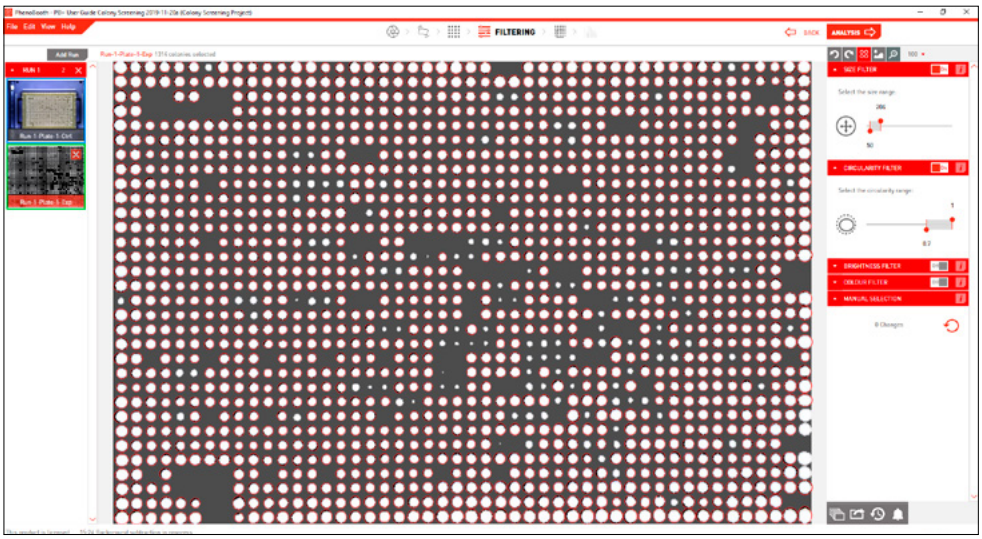




- In order to generate colony information, the background needs to be removed.
- Use the slider to adjust the filter intensity if necessary.
- If you have colonies that touch you can use the colony separation tool found in the advanced options.
- Check that all the smaller colonies are visible then click Apply.
- Once applied, you will automatically move to the filtering stage.

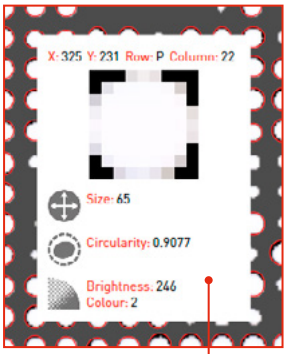
8





- Detected colonies are highlighted with a red circle. turns highlights on or off.
- Use the filter options to set minimum and maximum values for size, circularity, brightness and colour for colony detection. You can use the size filter to discard items that are too large or small to be colonies.
- Turn filters on and off using the toggle button.
- Colonies can also be manually selected by clicking simply clicking on a colony to select or deselect it.

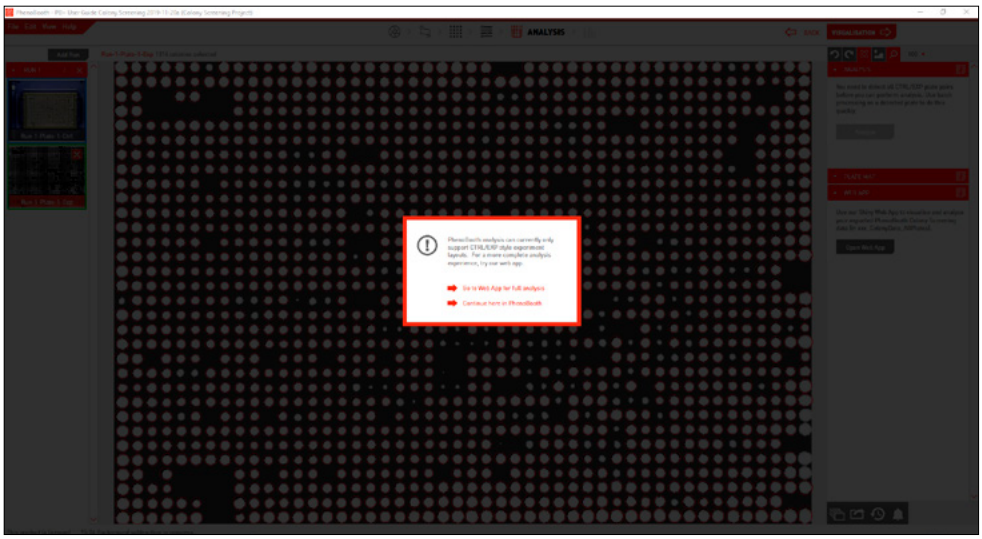
9

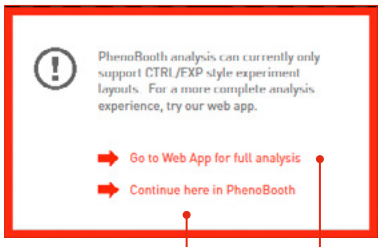


- Hover over a colony to see it's size, circularity and colour properties in the Colony Info plug-in tab.
- Now is the best time to batch process the rest of your plates with the same options using the instructions on p.47.
- Use the next stage button to move to the analysis stage.

COLONY SCREENING - ANALYSIS

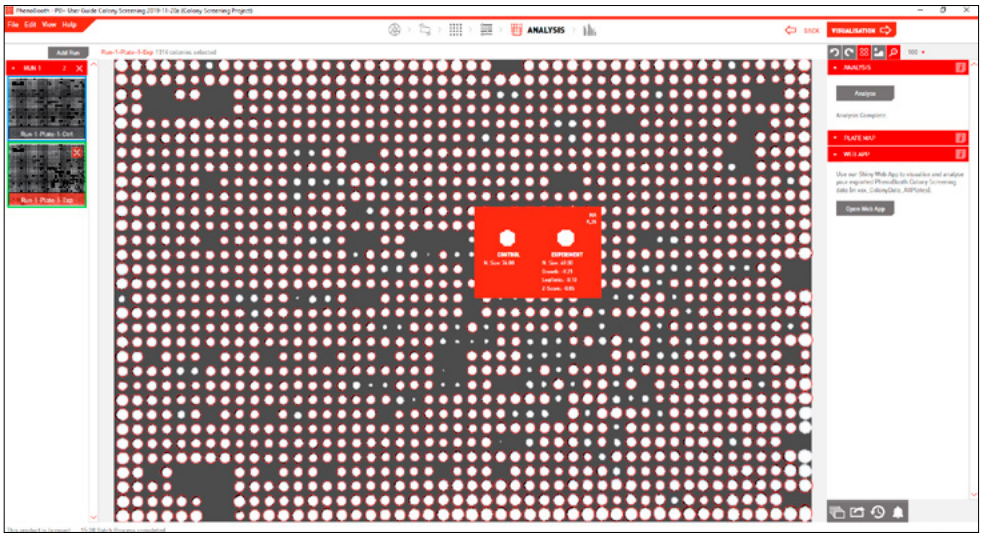
10

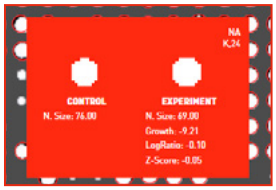




- The PhenoBooth+ software will handle simple control/experiment comparison. For more thorough analysis we have an online app.
- To move to the web app, select the Web App option from the pop-up window. You will be prompted to export your project at this stage.
- Click the Continue line to stay in PhenoBooth+.

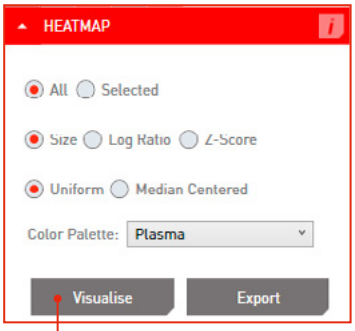
11

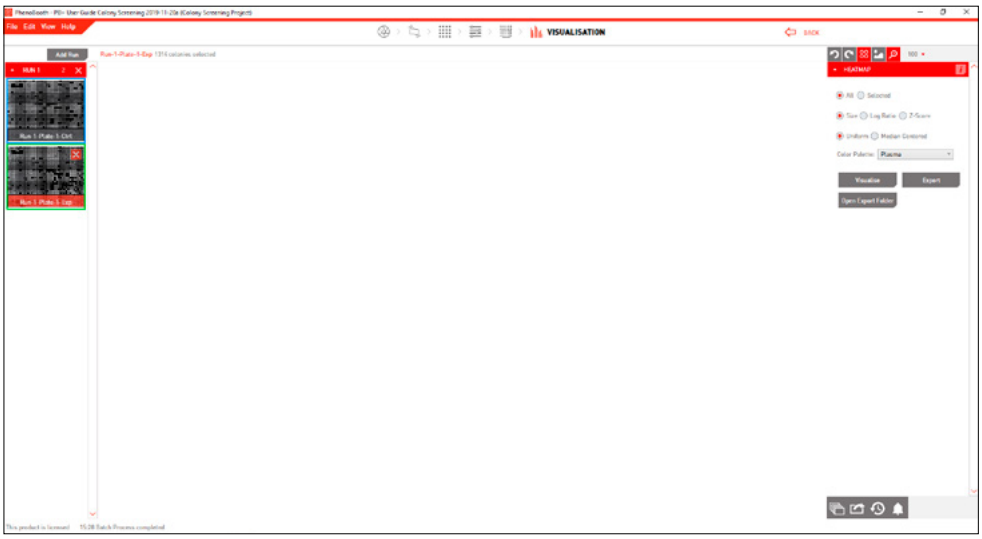




- To analyse colony growth difference between your current plate and it's control/ experiment plate, select the Analysis tab and click Analyse.
- NOTE: To analyse you must first process all plates. To do this use the batch process instructions on p.47.
- Place the cursor over a colony to view the growth information for the control and experiment. Colony co-ordinates and gene information (if available) are shown in the top right corner of the info box. You can upload or select a plate map using the Plate Map plugin.
- Use the next stage button to move to the visualisation stage.

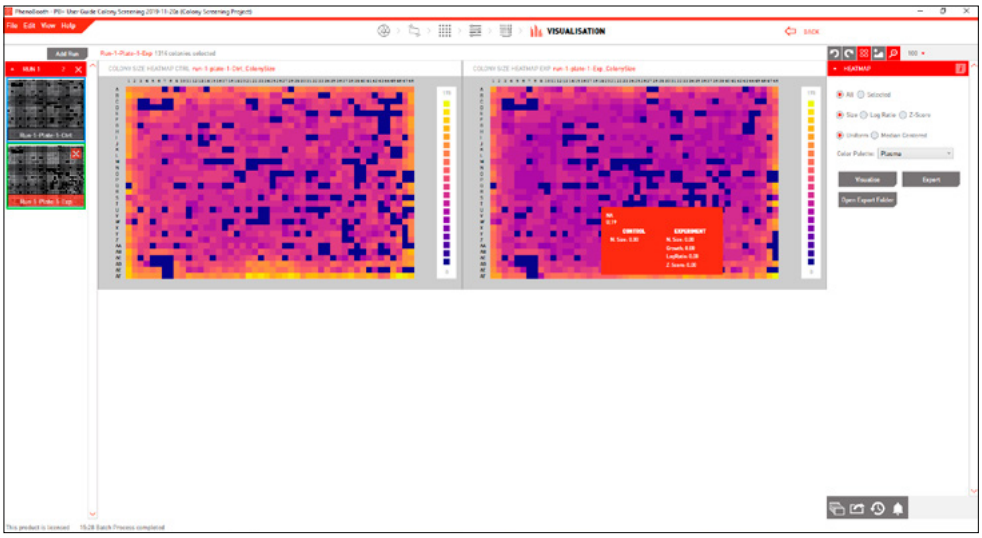
12





- To view heat maps of Size, Log Ratio or Z-score, choose your visualisation options from the Heat Map tab, then click Visualise.
- You can select to visualise all of your plates or just a selection.

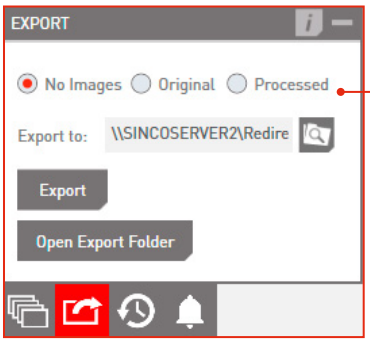
13

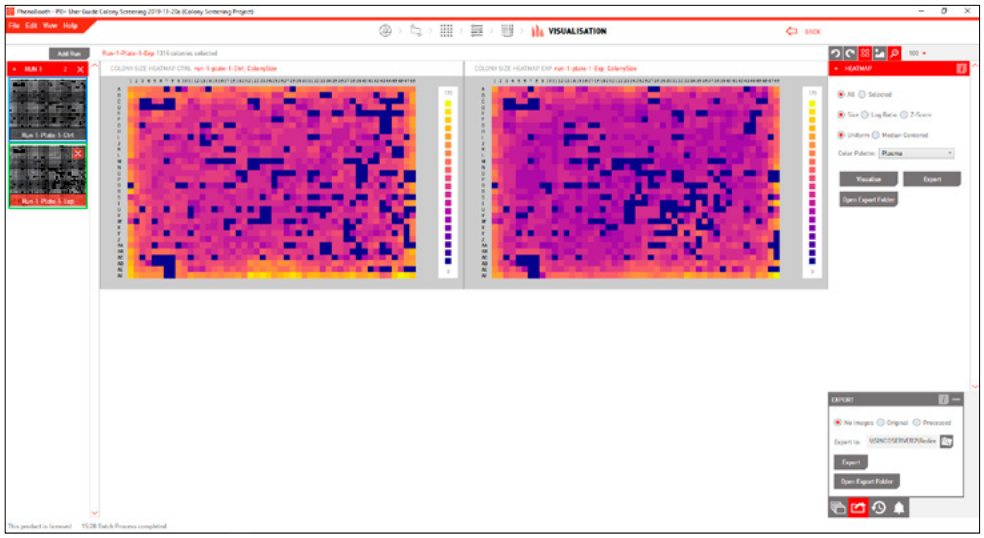


- Boom! You can now see heat maps for your selected plates. Hover over the map to see the colony comparison info.

COLONY SCREENING - EXPORT

14





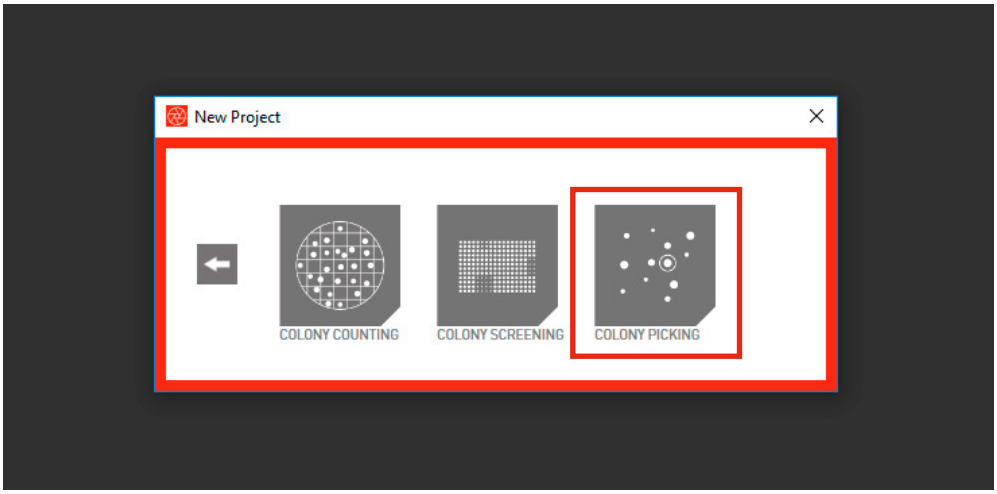
- The heatmap image can be exported in from the Heatmap plugin
- To export your entire project, use the Export tab. You can choose to export no images, processed images or the original images.
- Image formats available are: .bmp, .jpeg, .png or .tiff.
- The export includes: plate image (if selected), summary file, colony data table and colony data/analysis screen.
- The Summary File contains colony counts for all plates as well as colony imaging and filtering options information.
- The Colony Data Table contains position and properties of all detected colonies on all plates.

COLONY PICKING PROJECT

The Colony Picking package allows you to select individual colonies and export their coordinated to be picked using the Singer ROTOR. This section will guide you through the Colony Picking package for selection as well as the ROTOR software steps for picking.

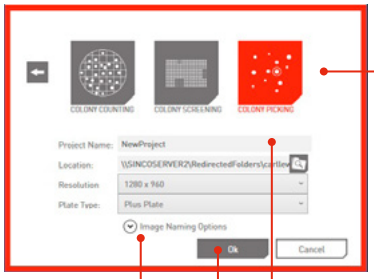
There are 5 stages in the Colony Picking package: Image Acquisition, Pre-processing, Detection, Filtering and Stinger Export.

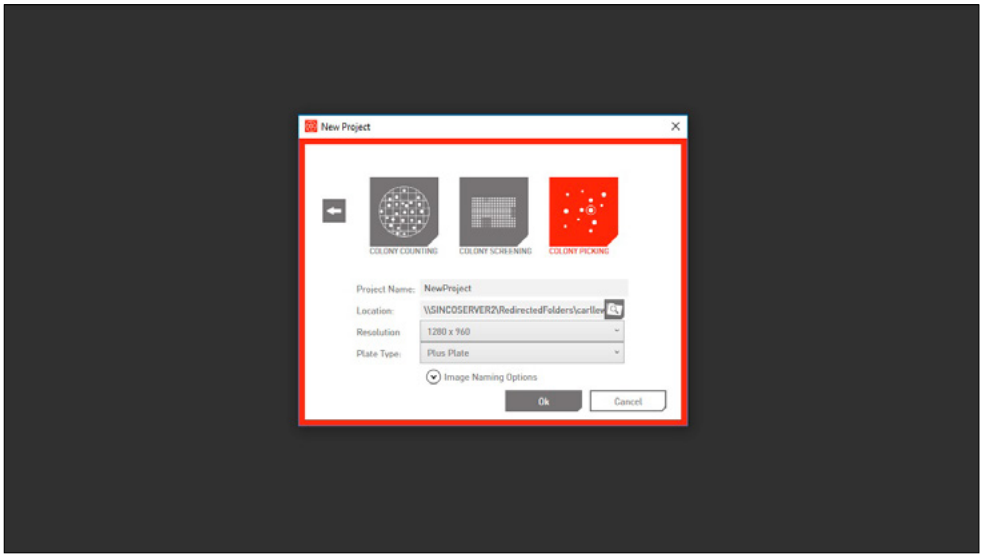
Start a new project then select Colony Picking.



COLONY COUNTING - NEW PROJECT

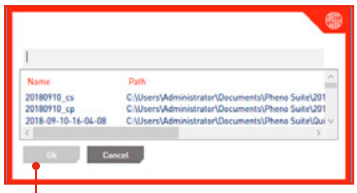
1

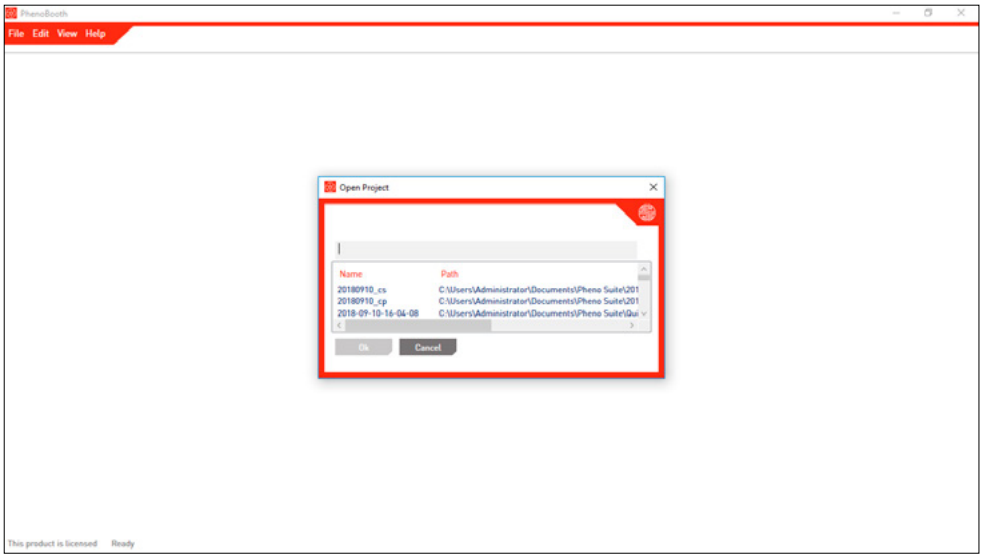




- Select the Colony Screening Icon. Icons will vary depending on which software packages you have purchased. Your chosen project type is highlighted in red.
- Name your project, select a save location and choose an image resolution and plate type.
- There are also a few optional naming settings you can use to customise your plate images.
- Click OK to launch project.

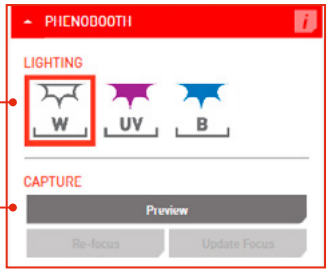
2

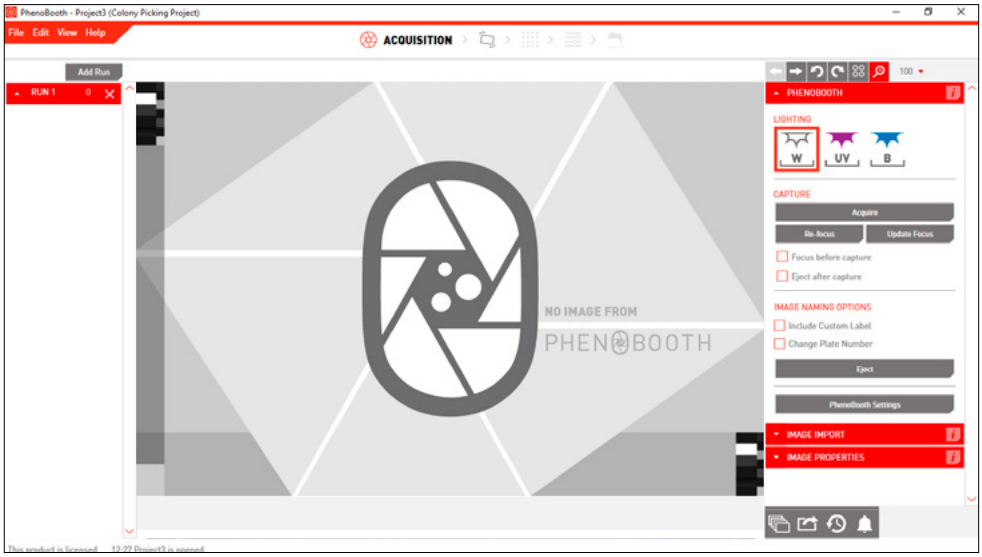




- If you want to open a previous project select Open Project and pick from the list of existing projects.
- Click OK to launch project.

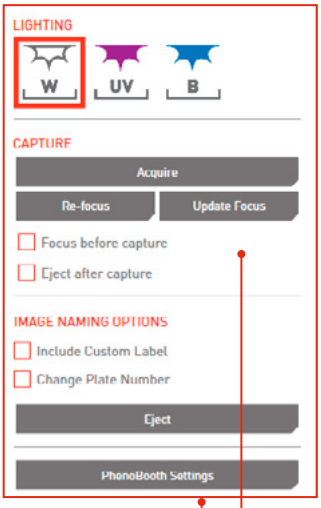
1

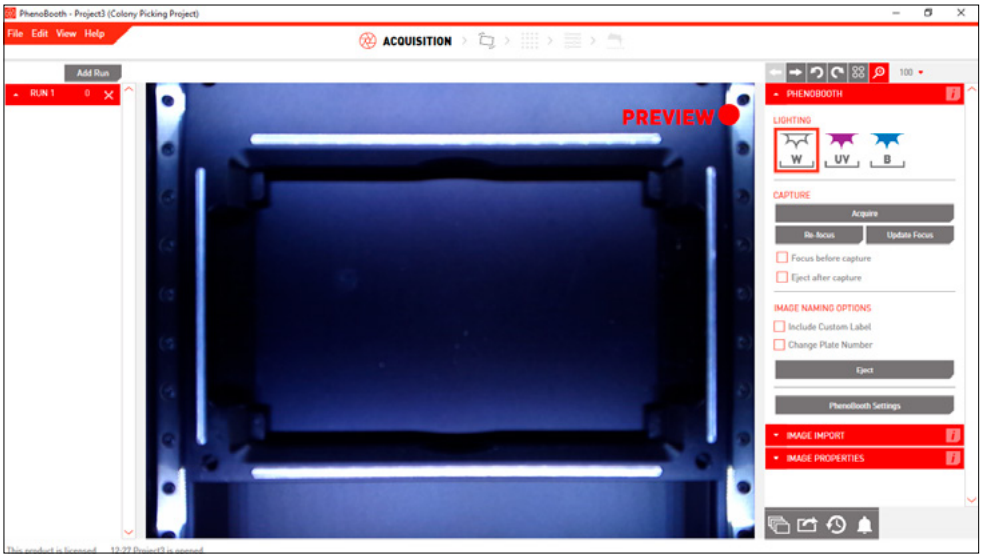




- There are two options for acquiring an image: PhenoBooth+ or import an existing image. However, using imported images for colony picking is not recommended because the plate may have changed since the photo was taken, compromising accuracy.
- Select your lighting settings and press Preview to preview a live image from PhenoBooth+.

2

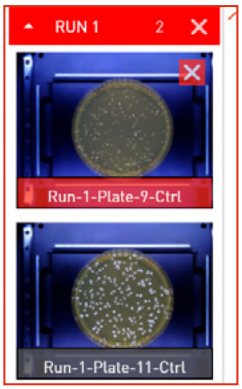


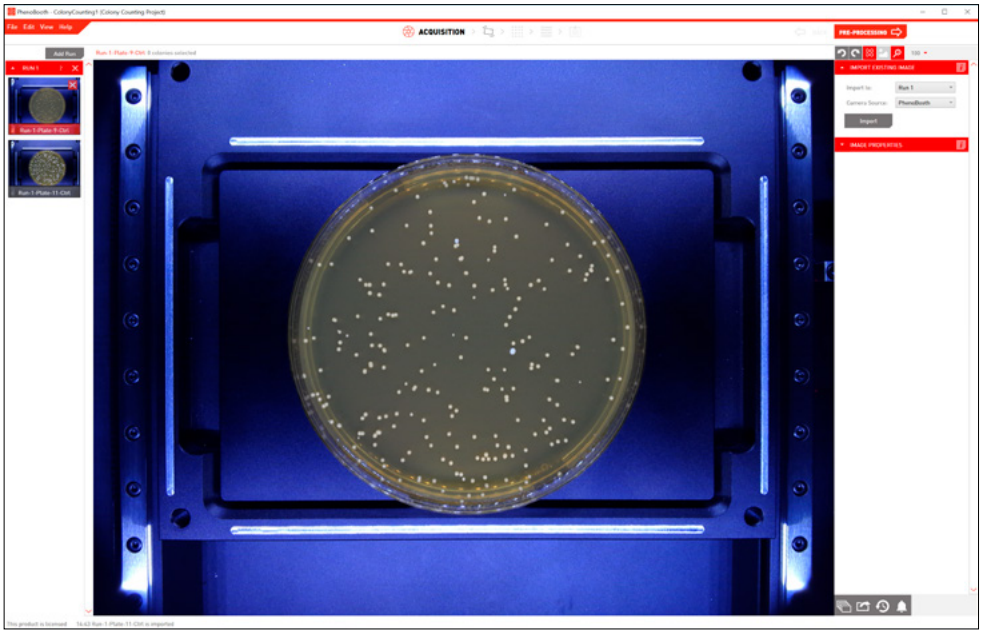


- If using the PhenoBooth+, click on that tab and select your desired light settings. Plate names are automatically generated.
- Insert a plate into PhenoBooth+ and press Acquire.
- Eject will allow you to remove the plate.
- Repeated until all of your plates have been captured.
- ➡ moves onto the pre-processing stage.

COLONY PICKING - IMAGE ACQUISITION

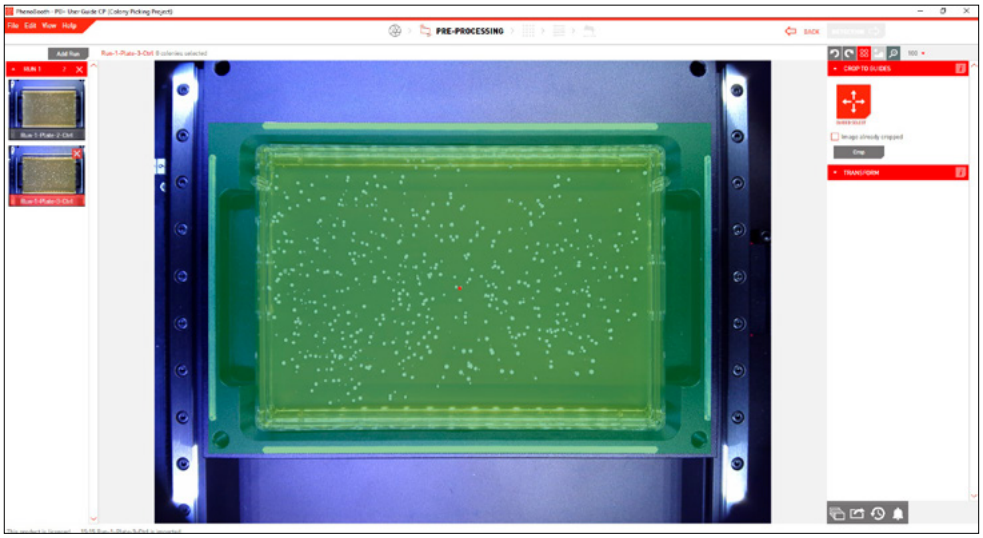
3

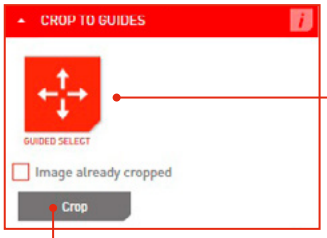




- Your captured images now appear on the left of the screen. The currently selected plate is highlighted with red.
- Use the next stage button to move to the pre-processing stage.

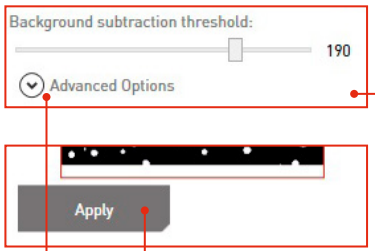
5





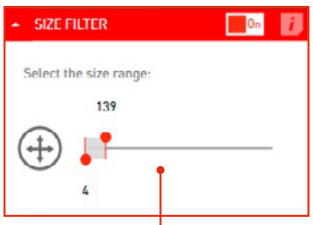
- In the Crop plug-in select Guided Select.
- The grid will automatically add a crop preview to the plate guides.
- Press Crop to remove unnecessary background.
- You will automatically move to the Detection stage.

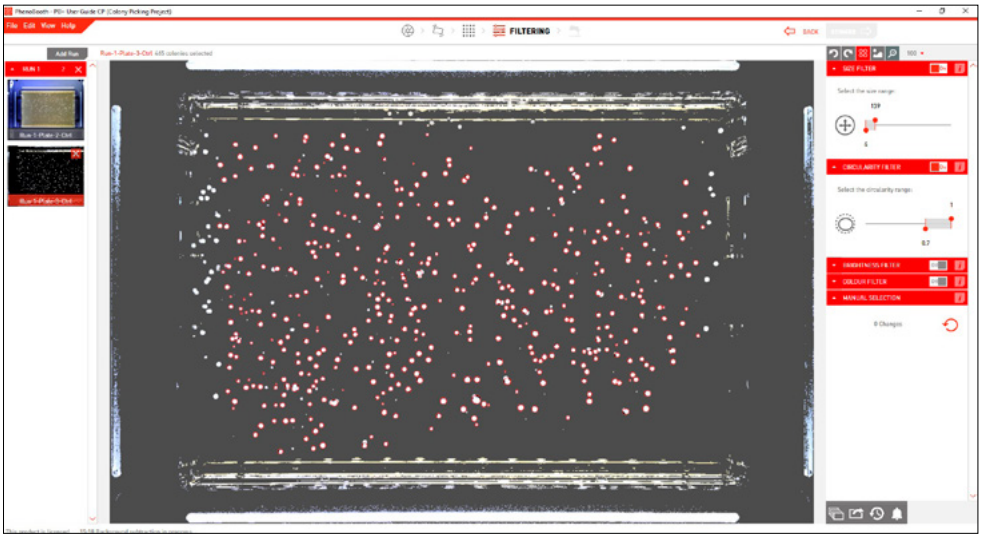
6



- The Background Subtraction plug-in is used to remove the plate background.
- Use the preview window to view colonies. Move the threshold bar if necessary to adjust the filter intensity.
- If you have colonies that touch you can use the colony separation tool found in the advanced options.
- When you're happy with the preview image, press Apply to remove the background.
- You will automatically move to the Analysis stage.

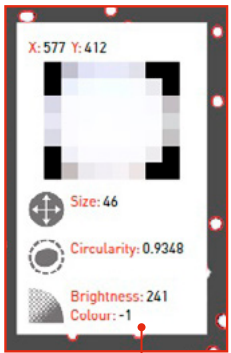
7





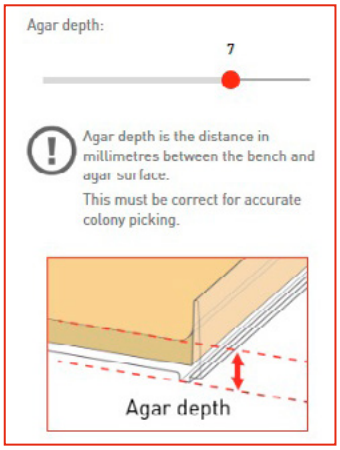
- Detected colonies are highlighted with a red circle. turns highlights on or off.
- Use the filter options to set minimum and maximum values for size, circularity, brightness and colour for colony detection. You can use the size filter to discard items that are too large or small to be colonies.
- Colonies can also be manually selected by clicking on a colony to select or deselect it. Changes are displayed in the colony detection plugin tab.
- You can batch process the rest of your plates with the same options using the instructions on p.47.

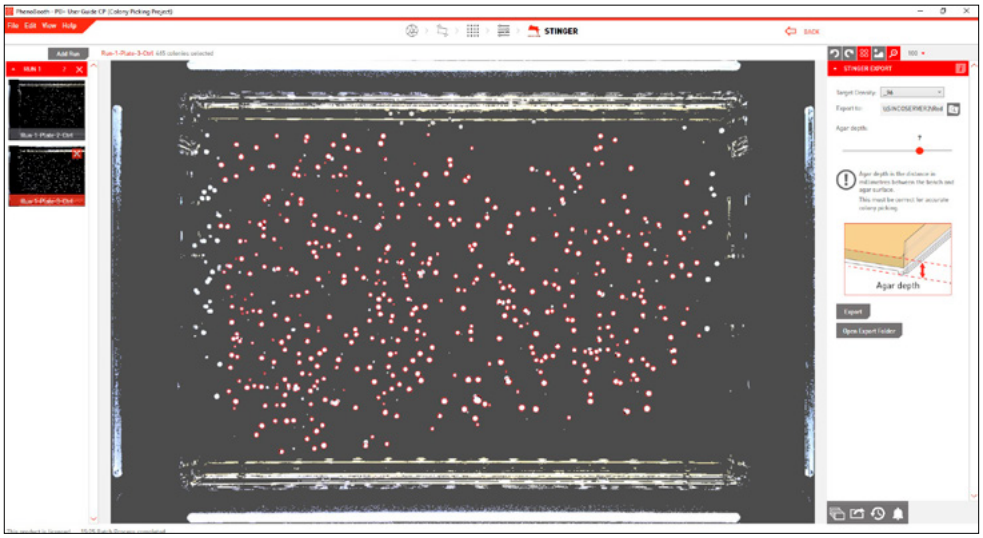
8



- Hover over a colony to see it's size, circularity and colour properties in the Colony Info plug-in window.
- Once all of your plates have been processed (see batch process instructions on p.47) use the next stage button to move to the Stinger stage.

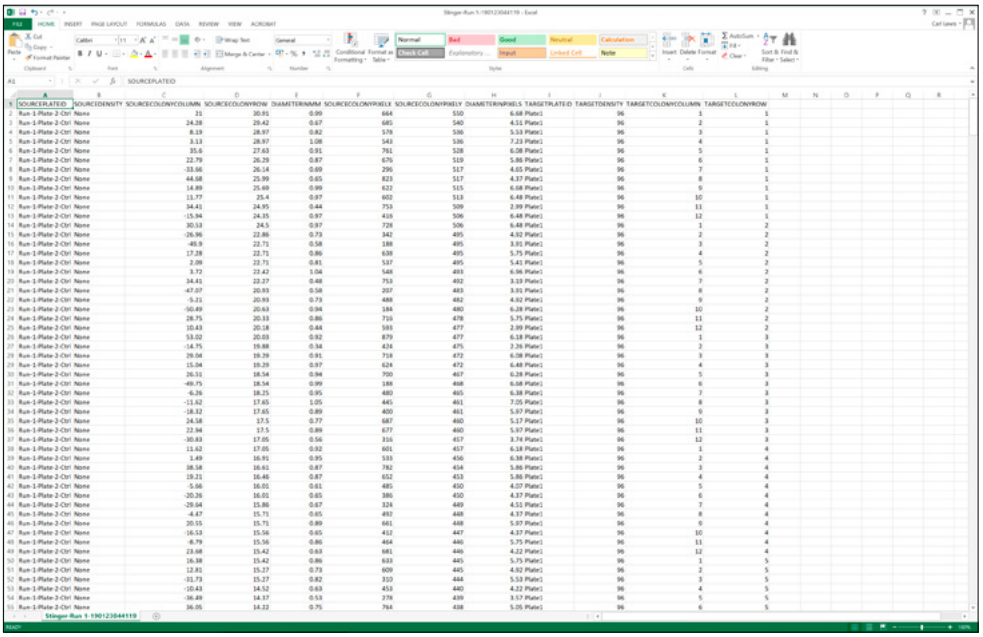
9



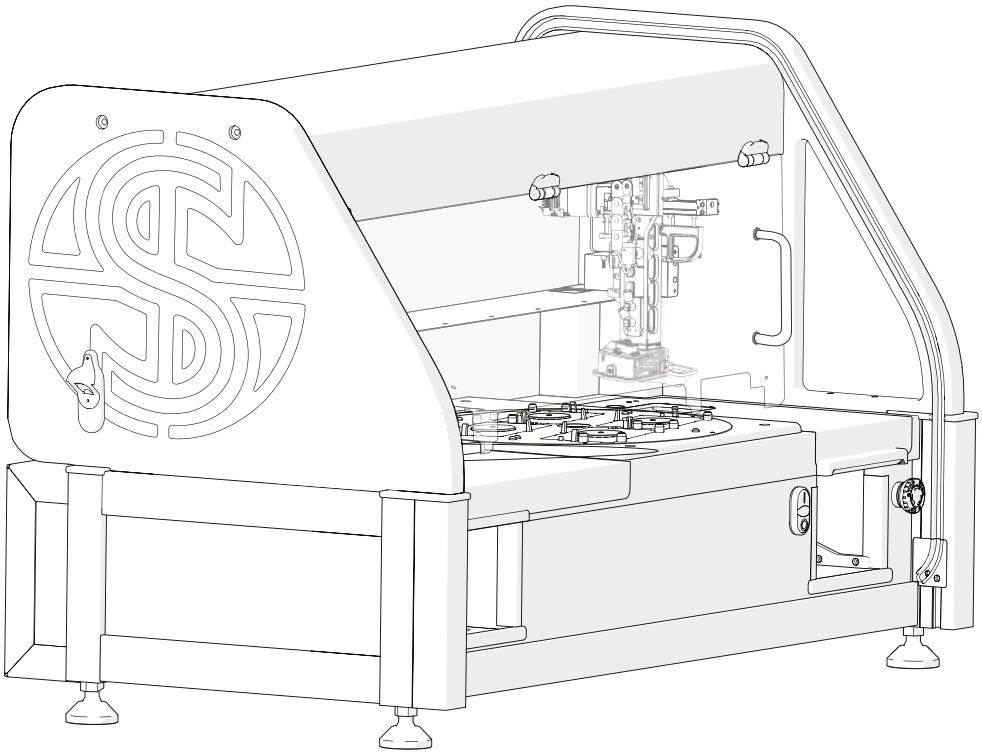


- Use the Stinger plugin to export colony locations for picking.
- Select the density you want the colonies arrayed in (96, 383, 1536 or 6144), select the file location.
- The depth of your agar will affect colony coordinates. Use the agar depth slider to set the correct depth.
- NOTE: Agar depth is the distance between the bench and the agar surface.
- The file will need to be saved to a USB stick so it can be transferred to the ROTOR HDA.

10



- The exported .CSV file contains all information on the selected colonies.
- It's now time to load this file into the ROTOR HDA.

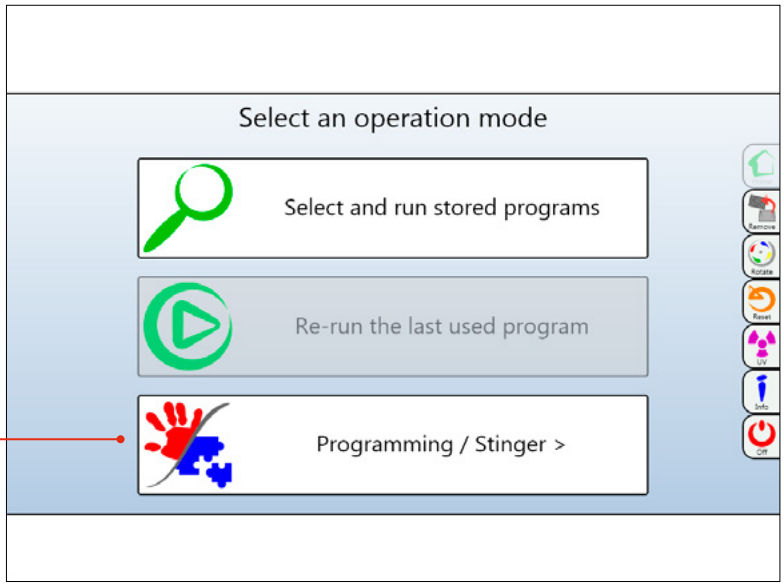


Now head over to the ROTOR HDA.

Please make sure The Stinger single colony picker is installed.

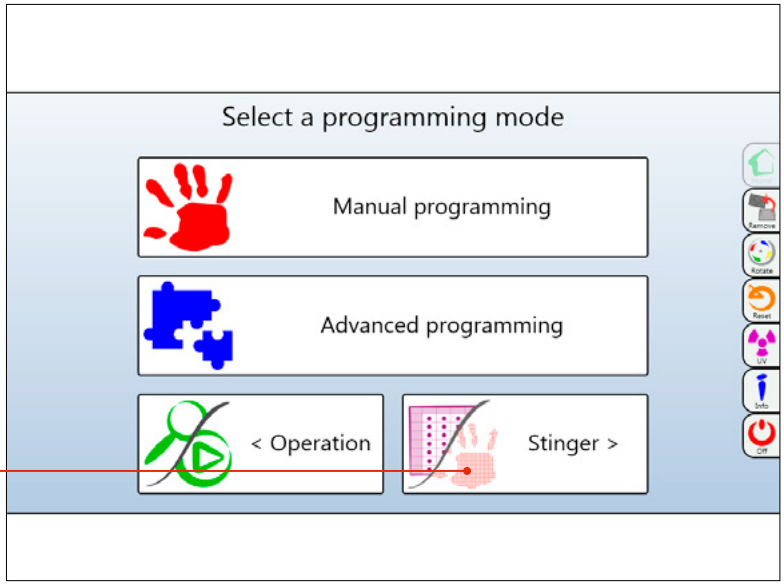
1

· Turn on the ROTOR and ensure that The Stinger is installed.
· On the ROTOR start-up screen you will be asked to select an operation mode. Select the Programming /Stinger option.



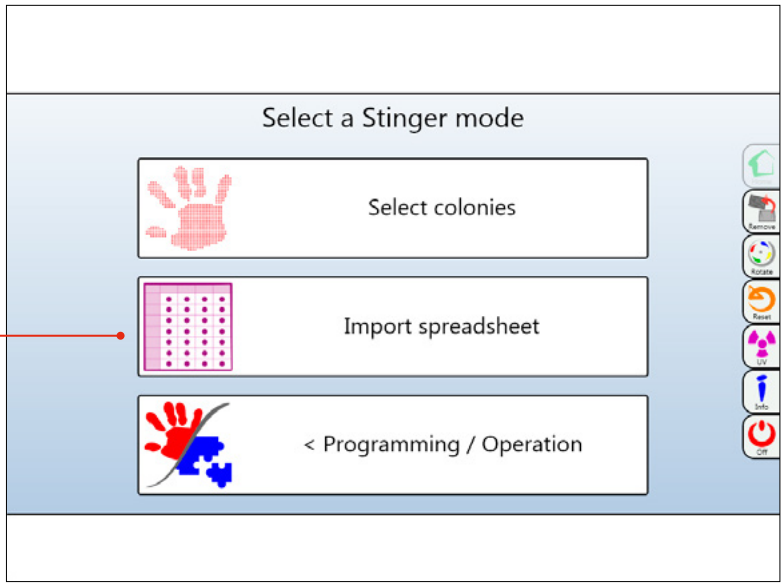
2

· Select the option labelled Stinger.



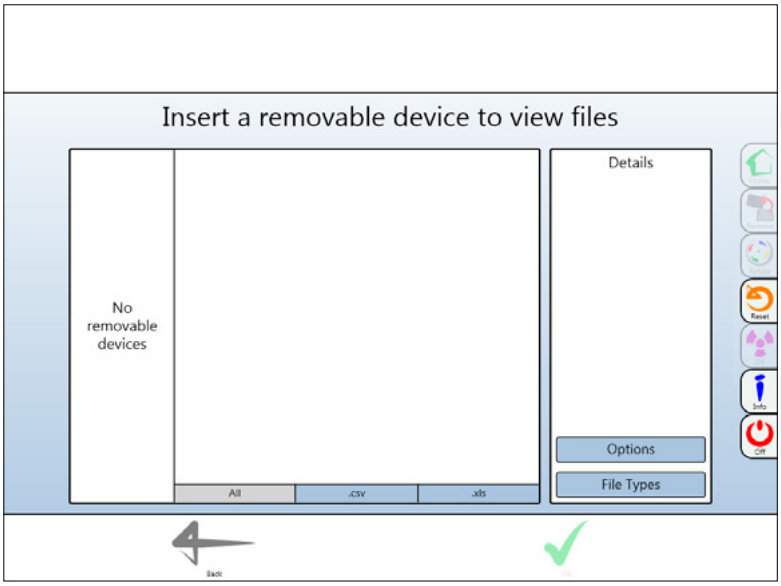
3

· On the Stinger Home Screen, select the Import Spreadsheet option.



4

· This screen will appear if no USB memory stick is inserted. Insert your USB memory stick now.

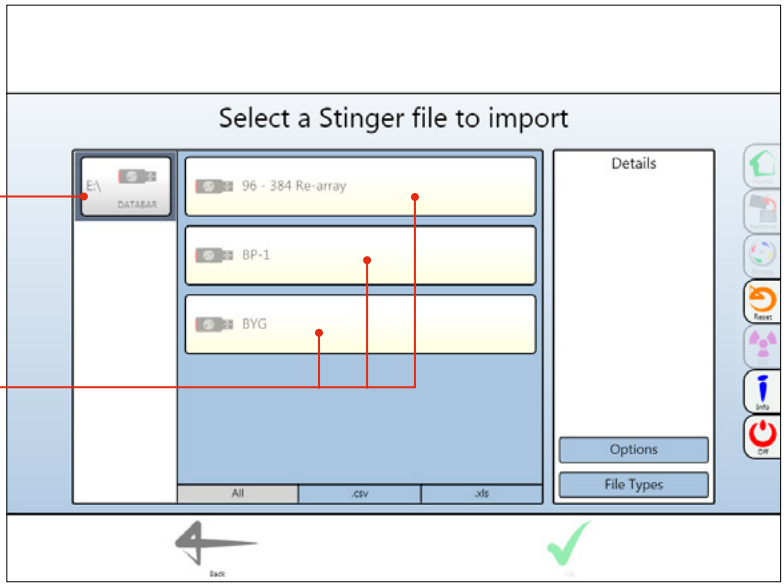


5

· Once your USB memory stick has been inserted, all .csv files in the root directory are displayed.

Memory stick

Files available

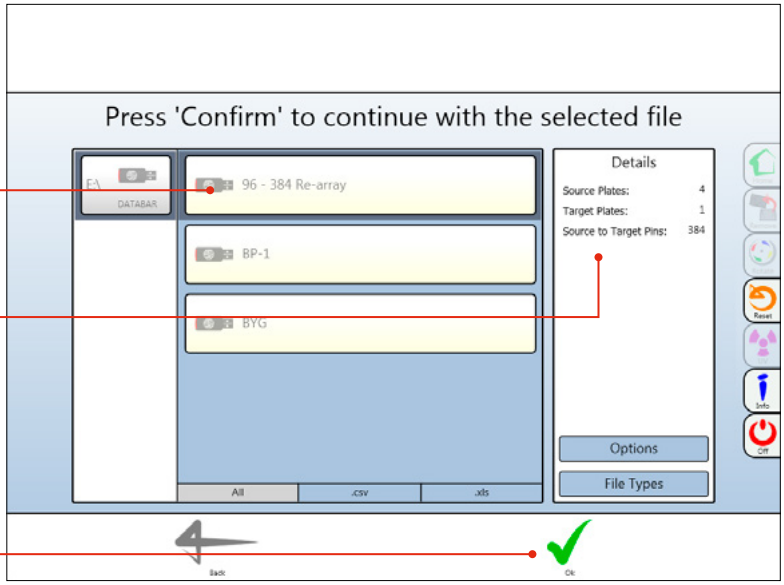


6

· Select the file you exported from PhenoSuite.

· Details of the file are displayed on the right.

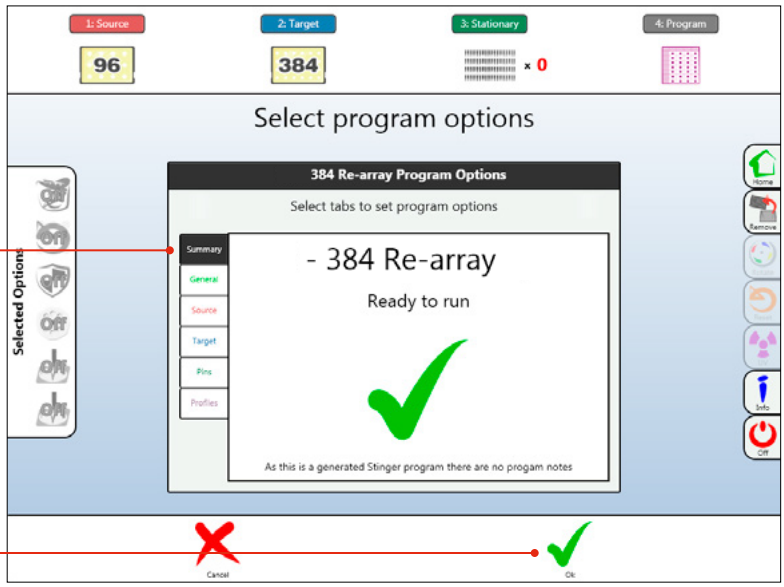
· Select OK to run chosen file.



7

· The final confirmation screen will appear. Click on the tabs to change advanced options if necessary.

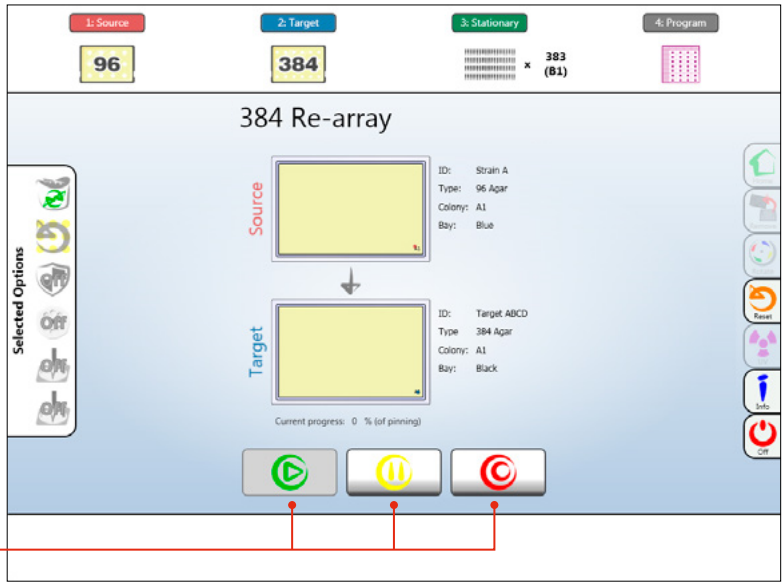
· Select OK to start program.



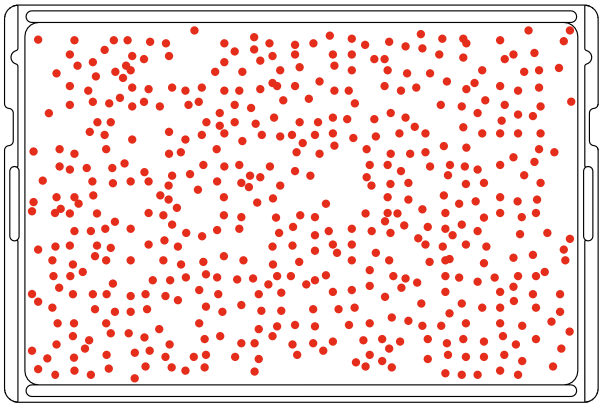
8

· Live program information will be updated throughout the operation.

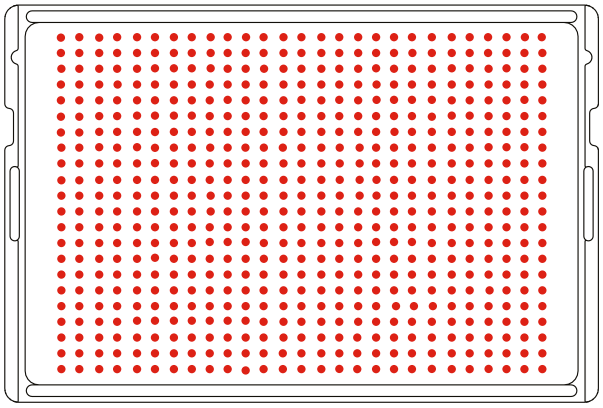
· Program: resume, pause, stop / abort.



SOURCE PLATE



TARGET PLATE



BEST PRACTICE

We want you to have a trouble-free time with your PhenoBooth+ so we've assembled some handy hints, tips and troubleshooting answers to keep you on the straight and narrow.

SOFTWARE ICONS

GENERAL TOOLS

UNDO. Undo the last process completed.

REDO. If you just used the undo tool, you can redo that process again with this button.

HIGHLIGHT COLONIES. Turn on or off detected colony highlights.

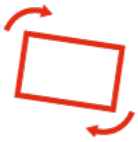
ZOOM. Use to zoom in on colonies of interest.

ZOOM PERCENTAGE. Selects zoom percentage of current plate.

STAGE NAVIGATION

IMAGE ACQUISITION.

ANALYSIS. Colony Screening package only.



PRE-PROCESSING.

VISUALISATION. Colony Screening package only.

DETECTION.

STINGER. Colony Picking package only.

FILTERING.

SOFTWARE ICONS

NOTIFICATIONS

CONNECT PHENOBOOTH+. PhenoBooth+ needs to be connected.

LIGHTING TIMED OUT. If left for too long the lighting goes on standby.

CAPTURING. PhenoBooth+ is taking an image.

CONNECTION PROBLEM. There is a problem with the PhenoBooth+ connection. Try reconnecting the USB 3.0 cable less slowly, see p.2.

TRAY CRASH. The input tray has crashed. Ensure plate is loaded correctly and the lid has been removed.

TRAY ACTIVE. Input tray is moving.

LIGHTING OPTIONS

WHITE TOP LIGHT. White, flat-field incident lighting.

UV TOP LIGHT. Ultra-violet, flat-field incident lighting.

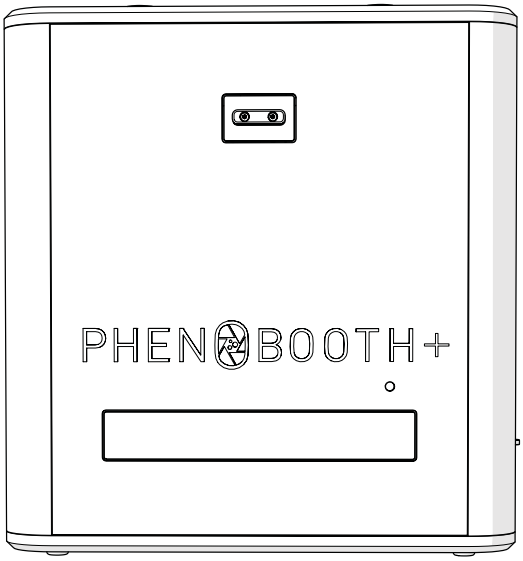
BLUE TOP LIGHT. Blue, flat-field incident lighting.

WHITE BOTTOM LIGHT. White, flat-field, transmitted light.

FEEDBACK LIGHT

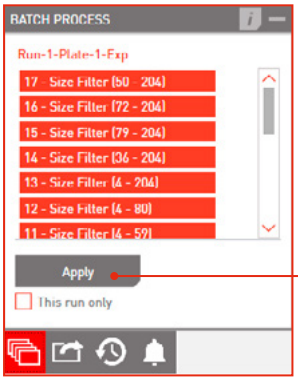
GREEN. All okay

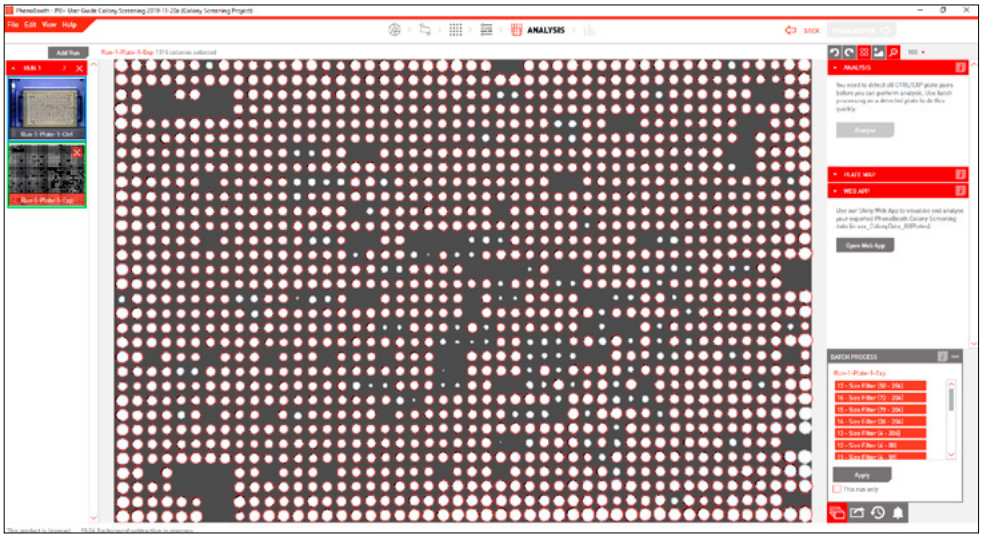
FLASHING RED FAST. Tray crash, Please wait while the tray automatically re-homes.



BATCH PROCESSING

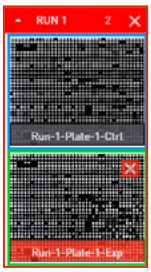
1

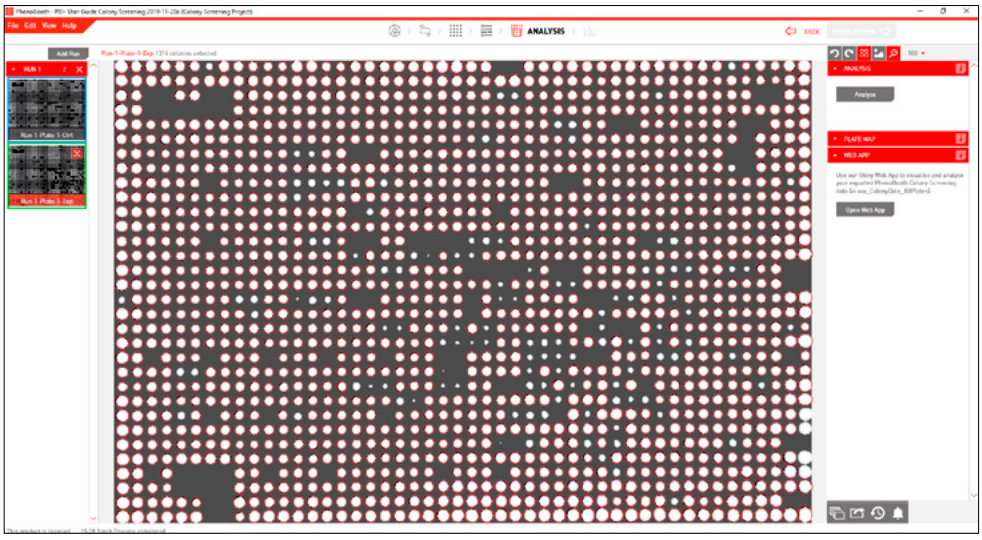




- Using the same settings for all plate images in a run will not only increase the speed of analysis but also ensure consistent and reliable information. A good time to batch process all of your plates is after all filtering has taken place.
- Click the Batch Process tab all processes are selected by default. Deselect any process you want to exclude.
- Click Apply and batch processing will begin. This may take a while for larger numbers of images.

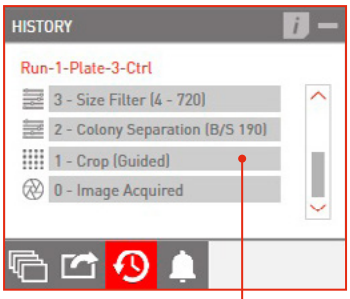
2

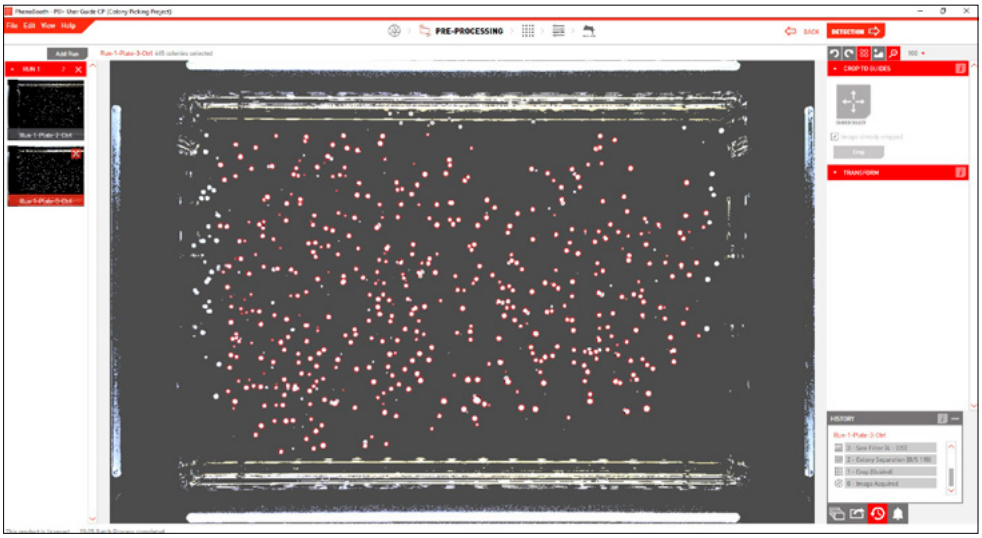




- All plate images in your current run have now been processed with the same options as your original image.
- You can see previews of the rest of your plates on the left of the screen. Click a plate to see a full screen view.

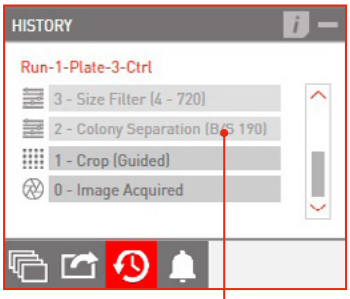
1





- If you want to undo the last change you made, simply click the undo button:
- If you want to go back further then use the History plug-in. Open the History plug-in tab and select the process you want to revert back to. The list is in chronological order with the most recent process at the top.
- In this example we're reverting back to when we cropped the image.

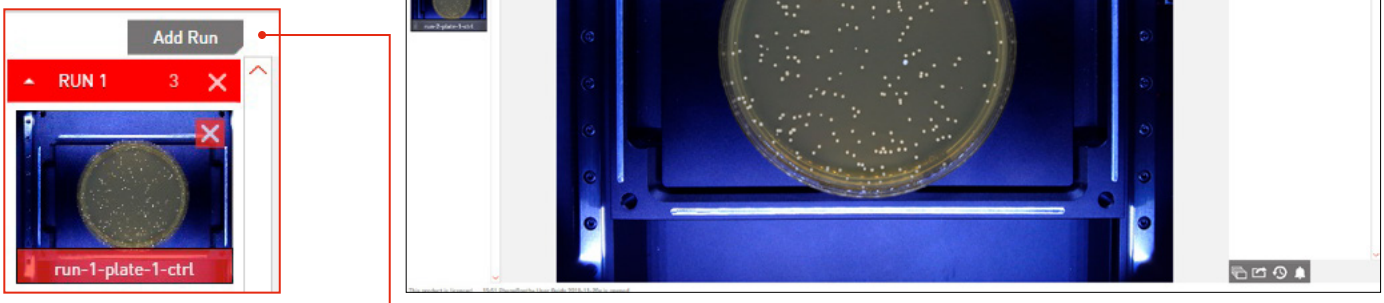
2



- Now, the last process applied to the image is crop. All processes after crop have been discarded.
- Discarded processes are marked as light grey in the History plug-in. You can revisit any of these processes by clicking on them, until you make a change to the plate image outside of the History plug-in.

ADD NEW PLATE RUN

1

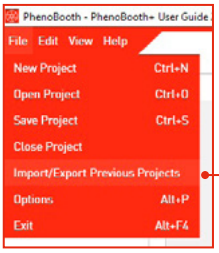


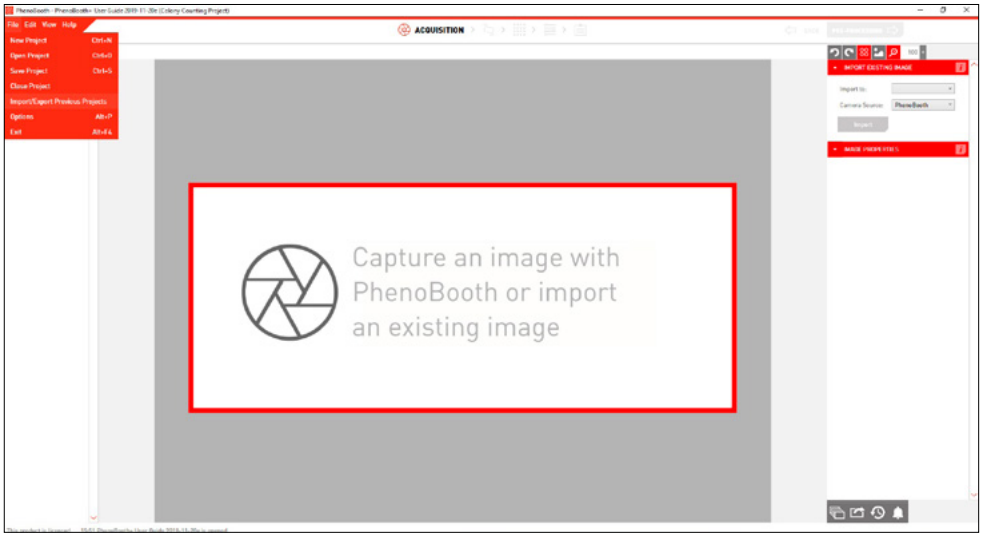
- Your plates can be organised into separate runs for different groups of experiments.
- To add a new run, simply click Add Run in the Plate Preview section on the left of the screen.

2

- When acquiring images, whether capturing using a PhenoBooth+ or importing existing images, you'll be asked to select which run you want your images to be filed under.
- Here you will see all the runs you have added so far.

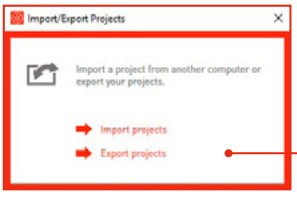
1

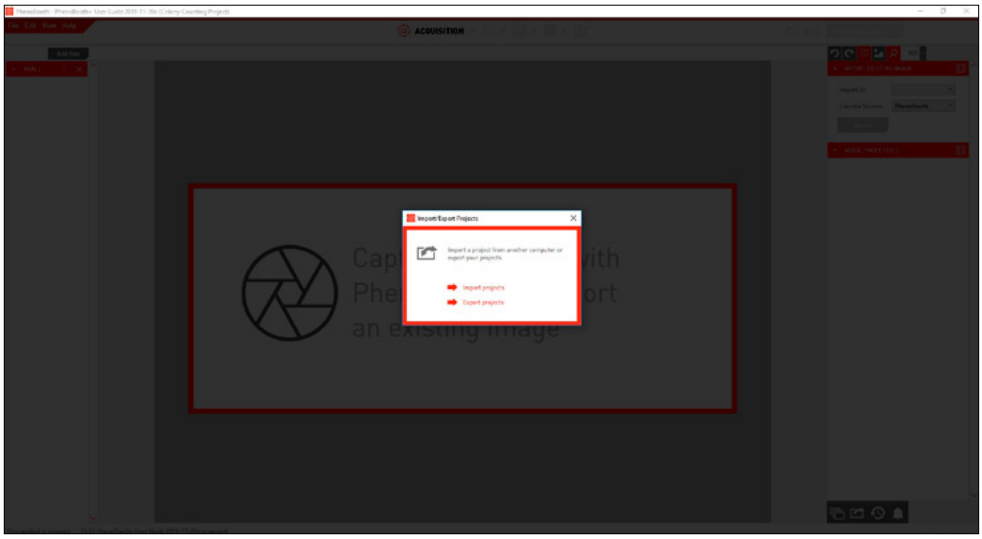




· PhenoBooth+ allows you to move projects from computer to computer. To do this, select the Import/Export Projects option in the File menu.

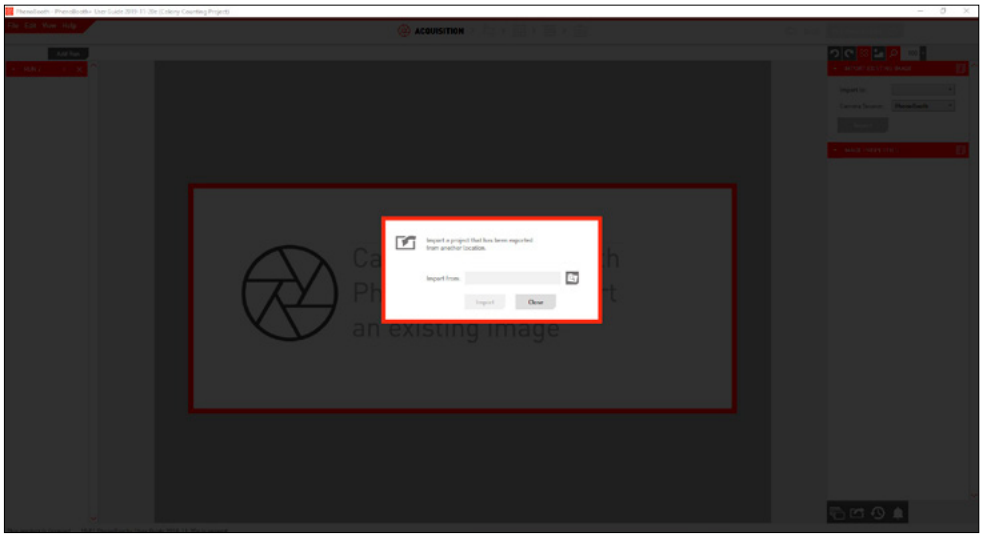
2





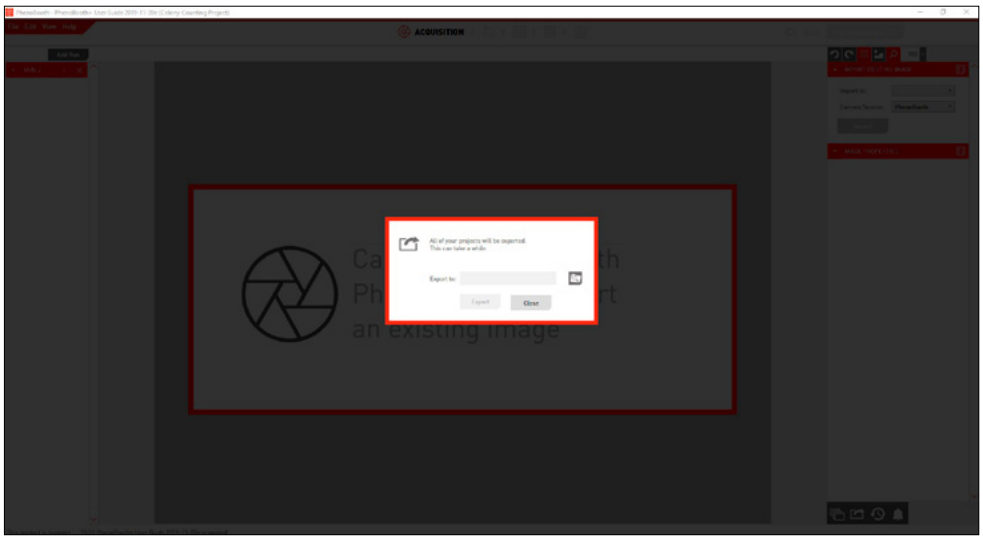
· In the pop-up window, select whether you want to import or export projects.

3



· For exporting, select the export location and then press Export.

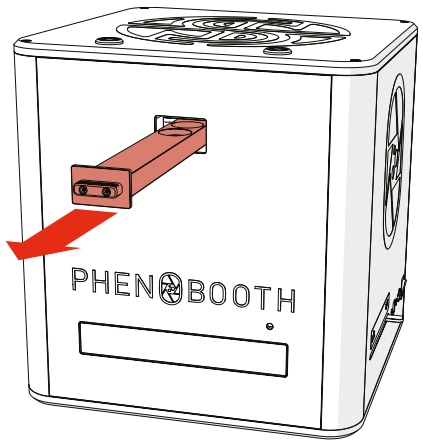
4



· For importing, select the import location and then press Import.

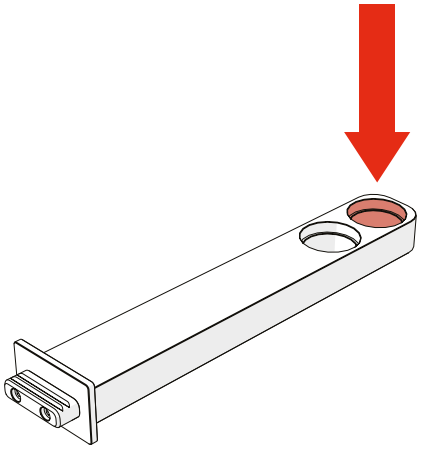
USING FILTERS

1



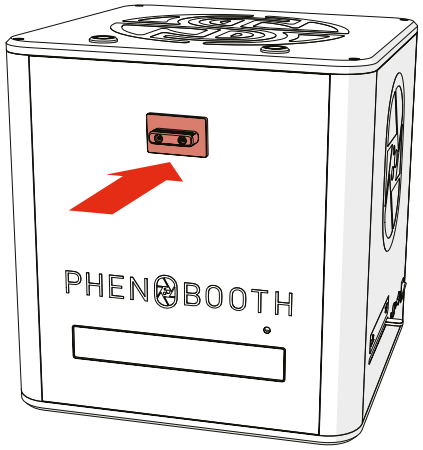
· Remove filter tray fully.

2



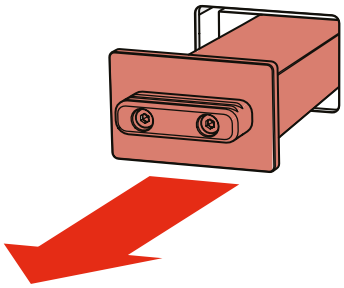
· Place the desired filter into the end filter slot.
· The PhenoBooth+ accepts standard 25mm circular filters with a maximum height of 5mm.

3



· Reinsert the filter tray fully until you feel the click. This will set the tray to the no filter position.

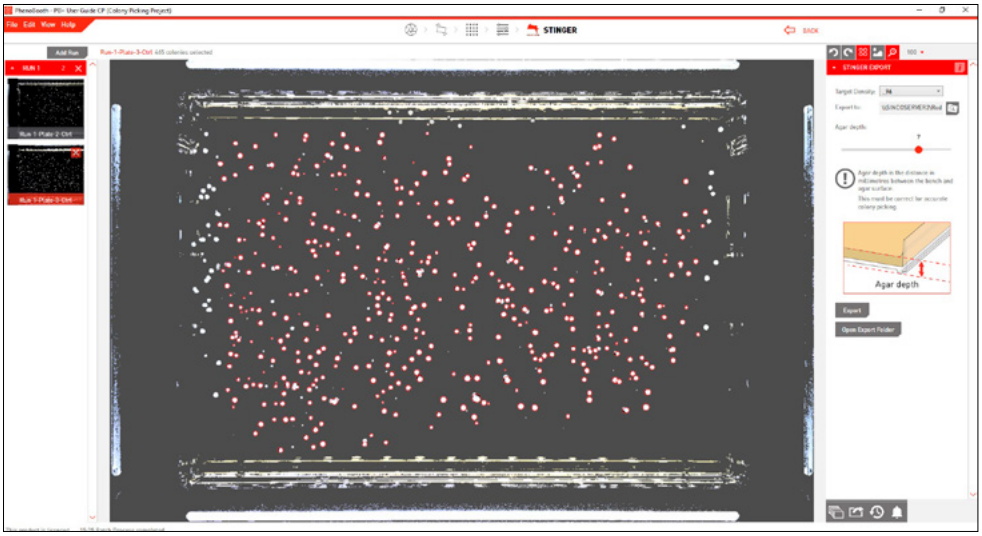
4



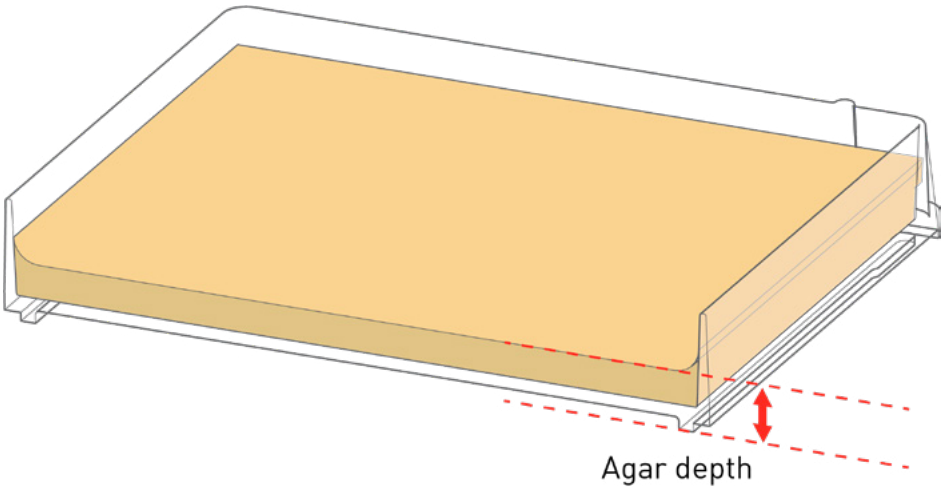
· To select the filter for use, pull the tray out slightly to the filter position — you will feel it click into place.

SETTING AGAR HEIGHT

1



- The PhenoBooth+ is capable of using a variety of different plates which can have different base thicknesses. To account for this, we ask users to set their agar height before exporting to The Stinger colony picker.
- Agar height is measured as the distance from the top of the agar (trying to ignore the meniscus) to the bottom of the plate. See the diagram below.



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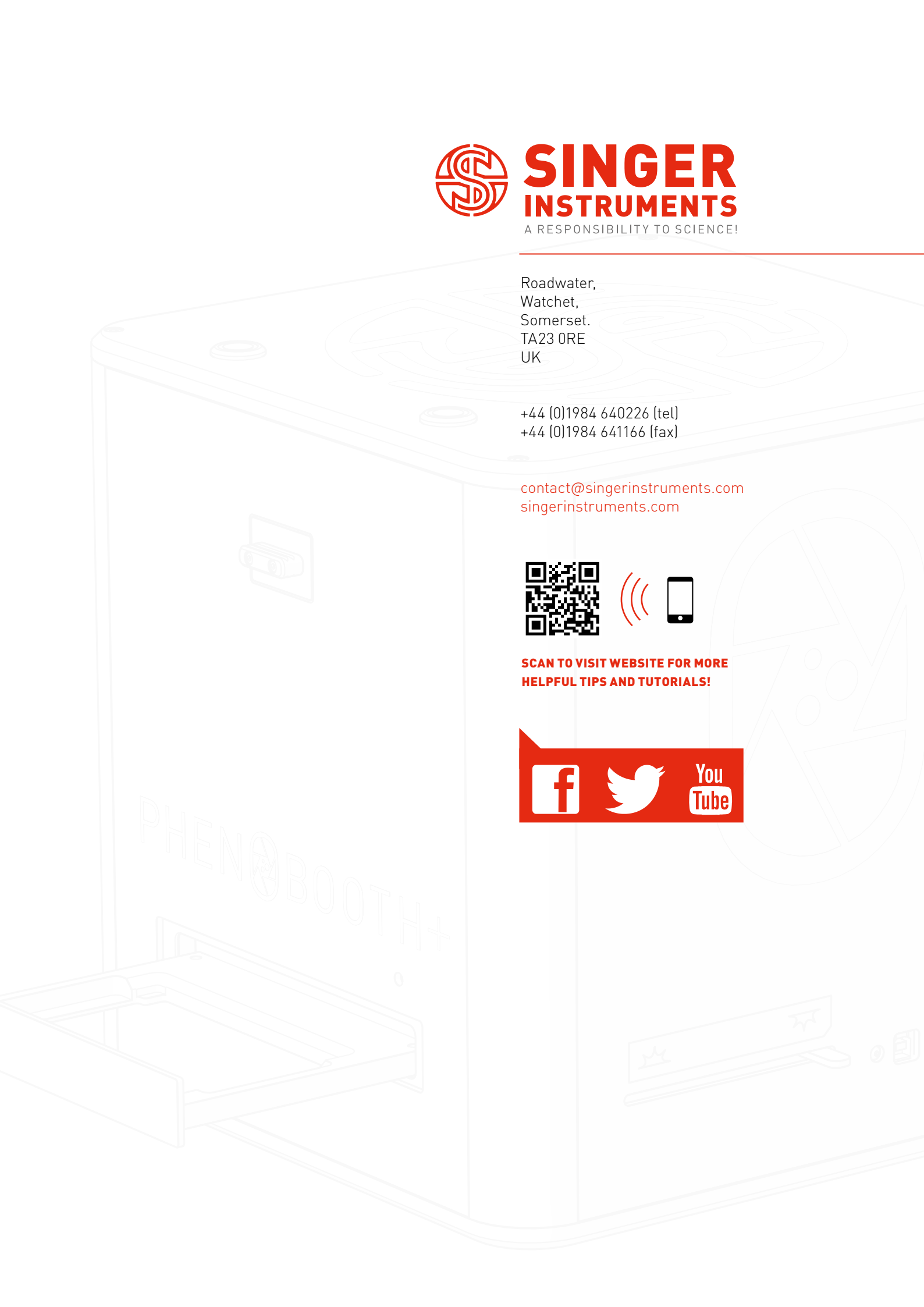
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