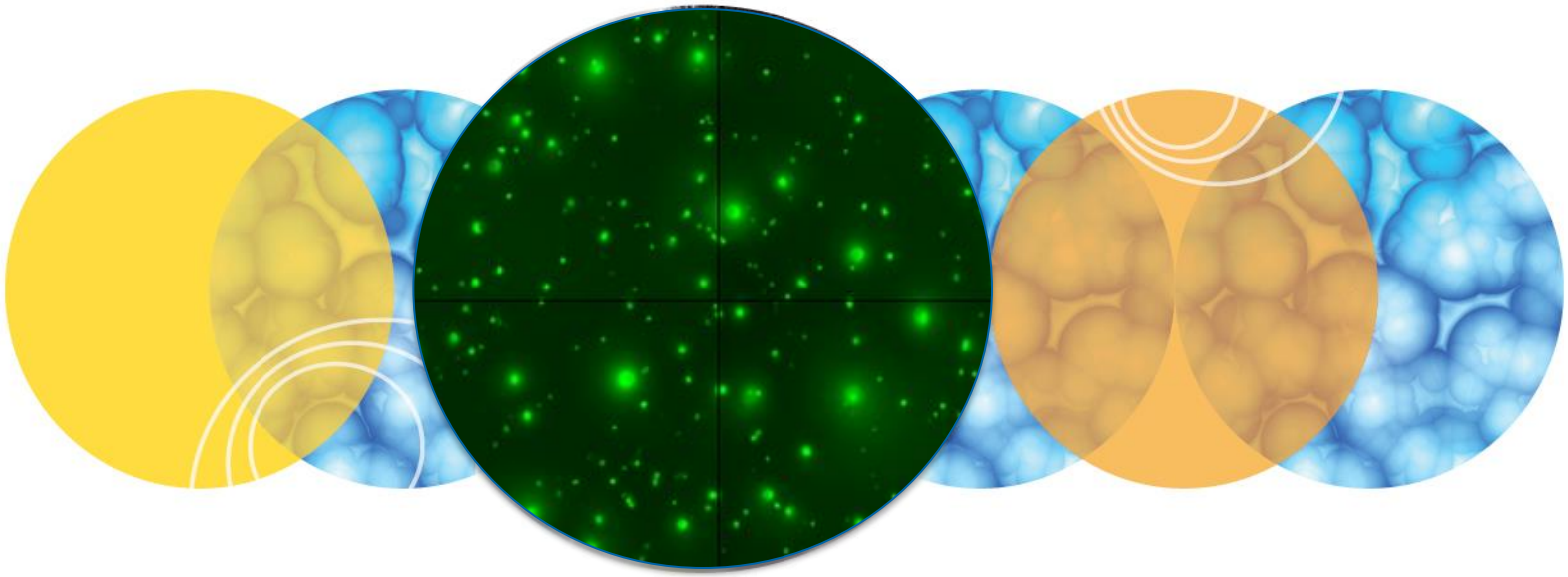

ClonePix 2 Training Guide

Preparing For & Setting Up A Pick Run

Date Revised 11/18/2016 Version C

MOLECULAR DEVICES
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Index

Chapter Purpose

The purpose of this chapter is to guide the user through the basics of setting up a pick run on the ClonePix2.

This guide does not include detailed descriptions around sample prep, statistics, etc. Please refer to corresponding chapters for details on these topics.

System Startup/Instrument Preparation

1. Ensure that the **robot** and **compressor** are plugged in.
2. Turn on the **compressor** and ensure compressed **air gauge** is set to **80 psi (5.5 bar)**.
3. Ensure **Emergency Stop** button on the front right of the instrument is **not** pressed in.
4. Switch **on** ClonePix 2. The **HEPA** filtration system works all the time that ClonePix 2 is on.
5. After approximately **2 minutes**, launch the **ClonePix 2 software** by double clicking on the icon.
6. Wipe out ClonePix 2 bed with **70% ethanol** or fresh **Sterilizing Agent** (SporKlenz https://us.vwr.com/store/catalog/product.jsp?product_id=4621746) using a lint-free cloth.
7. Fill the **ethanol feed bottle** with **70% ethanol** and empty the **ethanol waste bottle.**
8. Make sure that the correct **Picking Pins** are installed for the type of cells to be picked.
   - **F1 Picking Pins** (400 um internal diameter; X4961) for **suspension cell picking from semi-solid medium**.
   - **F2 Picking Pins** (700 um internal diameter; X4962) for **adherent cell picking from liquid medium**.

Picking Pin Preparation

- **Picking Pins** should be cleaned by **sonication in aQuClean** (K2505) and **autoclaved** prior to use.
  - It is advisable to autoclave the **Picking Pin Removal Key** (X4948) at the same time.
  - See training module on **Picking Head Maintenance OR General Maintenance** section of the **Robot Manual** for detailed instructions.

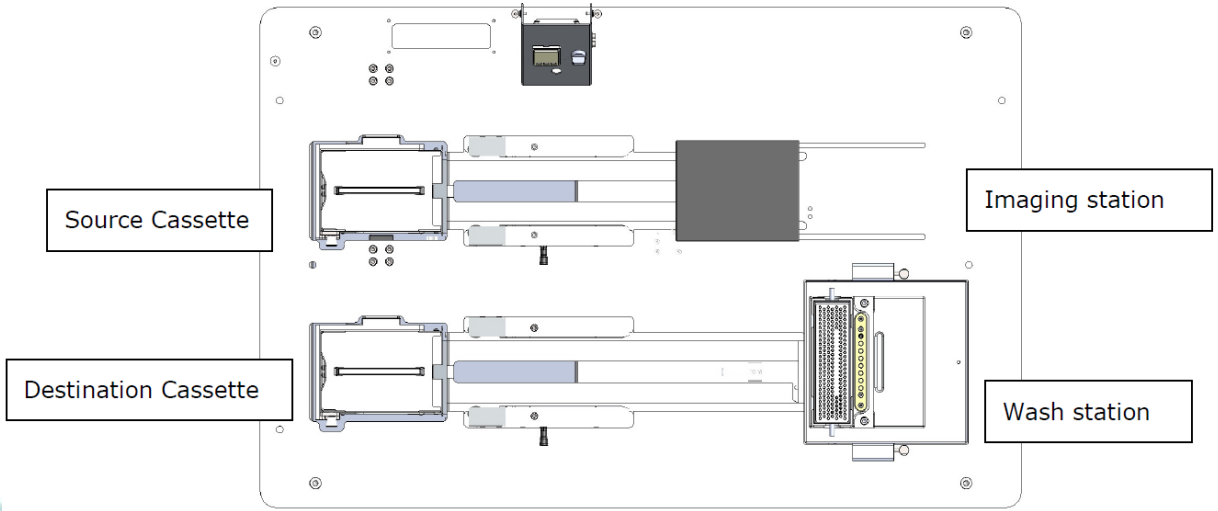
- If the **Picking Pins** need to be changed remove the **Picking Head** and swap the pins.
  - To do this, click on the **Picking Head Management** icon, then the **Replace Head** icon and follow the on-screen instructions.
  - Refer to the training module on **Picking Head Maintenance OR General Maintenance section of the Robot Manual** for guidance on how to remove and replace the head and the pins.

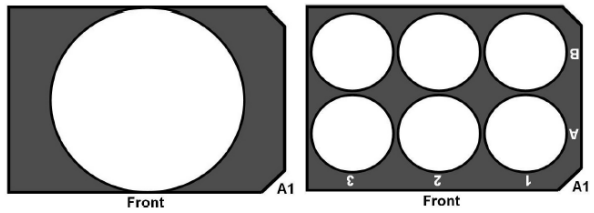
Loading Plates into Stackers

1. Load plates into the appropriate plate holders. Generally, **source plates** are loaded into **GREEN** holders, **destination plates** into **BLUE** holders.

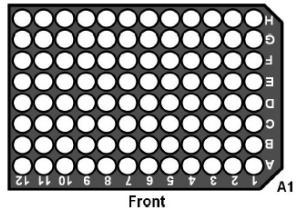
2. Raise the stacker cassettes either manually or via clicking on the Raise Source or Raise Destination buttons in the software.
   - ***Note: When the cassettes are placed in the source and destination stackers, they must be held firmly in place by the locking bolt on the front left hand side of the stacker systems. Failure to lock a cassette may cause a malfunction of the collection and/or return of the microplates.***

3. Place **plates + holders** into the appropriate **stacker cassettes**, with **lids on** and **well A1 in the front right-hand corner** (see below).
   - Each stacker holds a maximum of **10 plates**.
   - Plate holders must be placed **level** into the cassette and **all the way to the back of the cassette** to ensure correct positioning.





Orientation of source plates in ClonePix 2



Orientation of destination plates in ClonePix 2

Prepare for Pick Run

The **Prepare for Pick Run** process is designed to validate that:

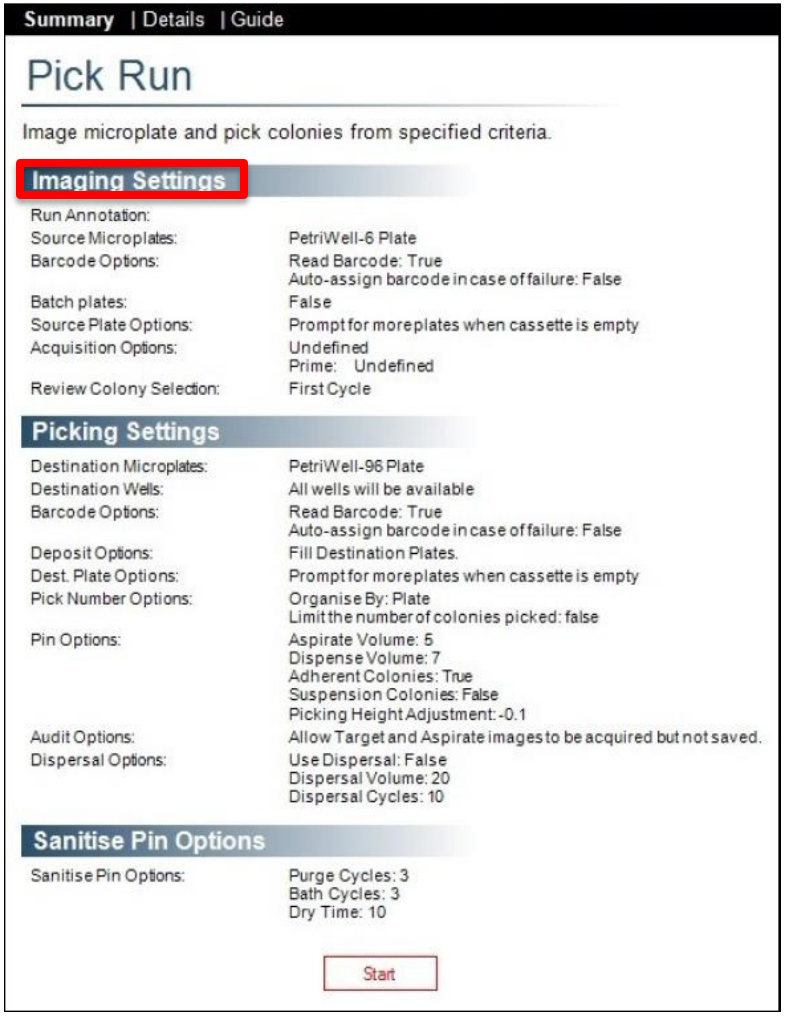
1) The **picking pins** are firing correctly,
2) the **camera, pins and microplates** are aligned, and
3) the **fluid system** is sterile and ready for use.

1) From the **Main Navigation Screen**, click on the **Prepare for Pick Run icon** and follow the on-screen instructions
.
2) When prompted to load a **source plate**, it is recommended that **a blank source plate of the type that will be used for the pick run** be used.

3) Follow the prompts to carry out the pin firing, alignment, and fluid handling tests. When the tests are all complete, click **Close Process** to return to the **Main Navigation Screen**.

Carrying Out A Pick Run: Imaging Settings

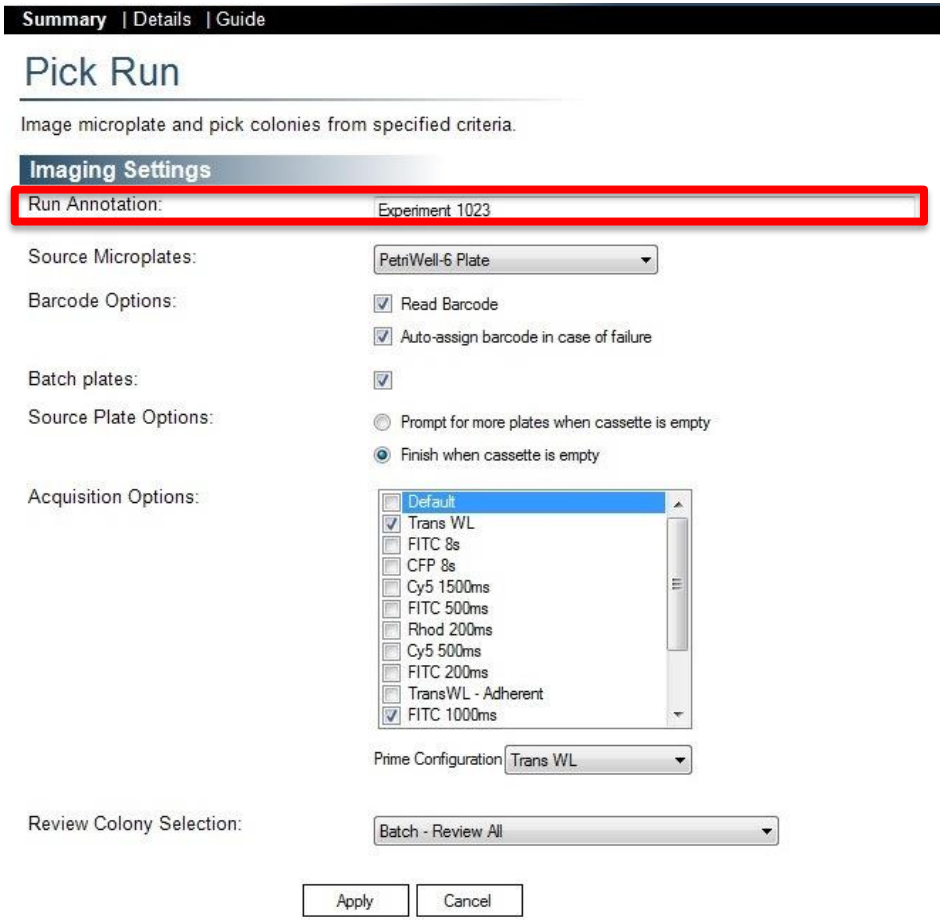
1) From the **Main Navigation Screen**, click on the **Pick Run icon. You will see the following screen appear.** Click on the **Imaging Settings** heading to edit the parameters within:



Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

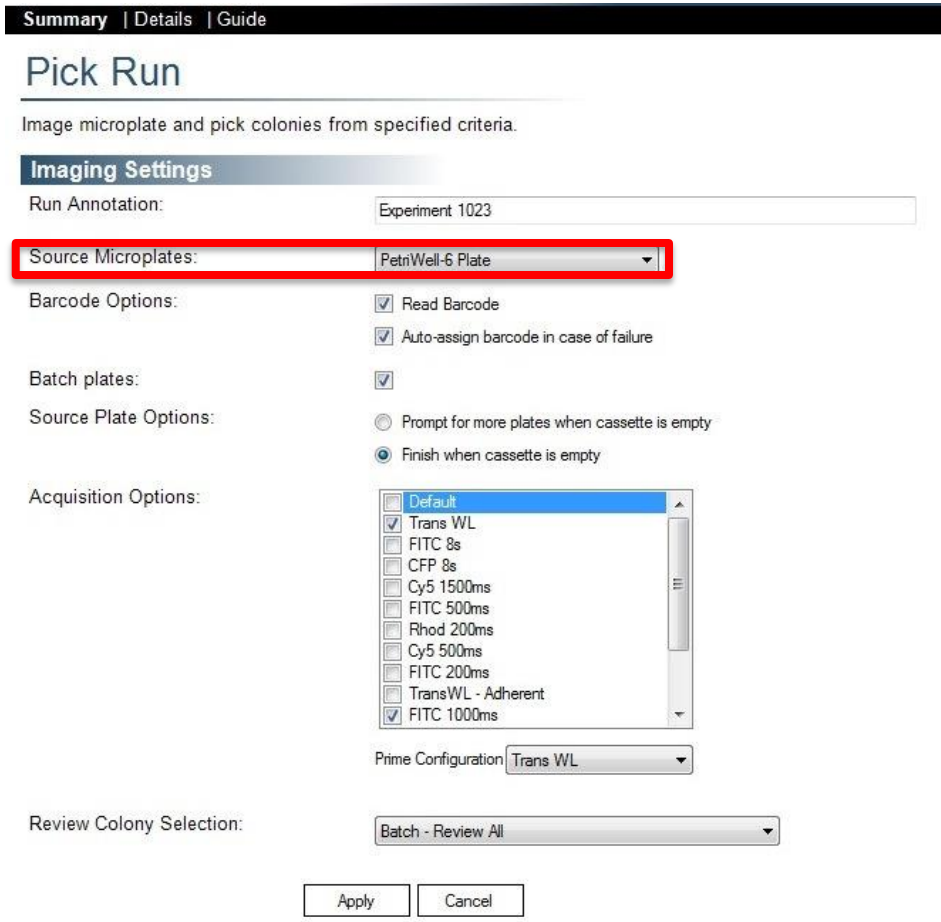
a) Run Annotation - Enter a name to identify this run.



Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

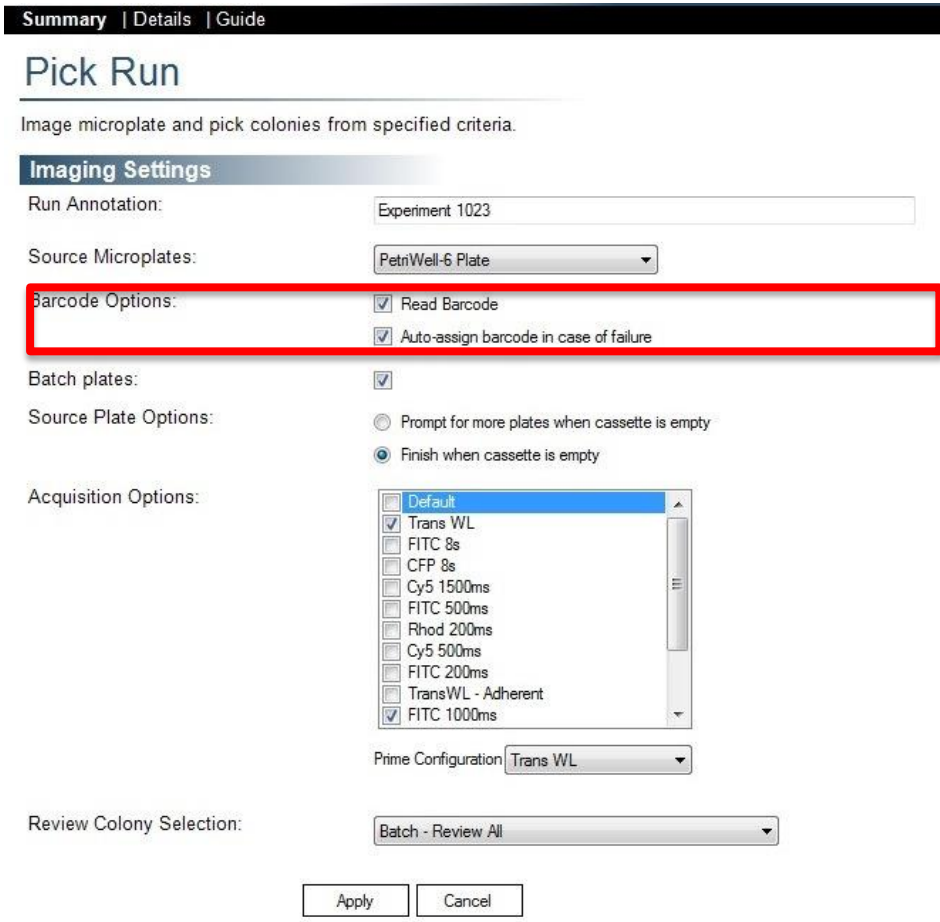
a) **Run Annotation** - Enter a name to identify this run.
b) **Source Microplates** – Select your source plate type from the dropdown menu.



Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

a) **Run Annotation** - Enter a name to identify this run.
b) **Source Microplates** – Select your source plate type from the dropdown menu.
c) **Barcode Options**
   * If your source plates are barcoded, select **Read Barcode**.
   * We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.



Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

a) **Run Annotation** - Enter a name to identify this run.
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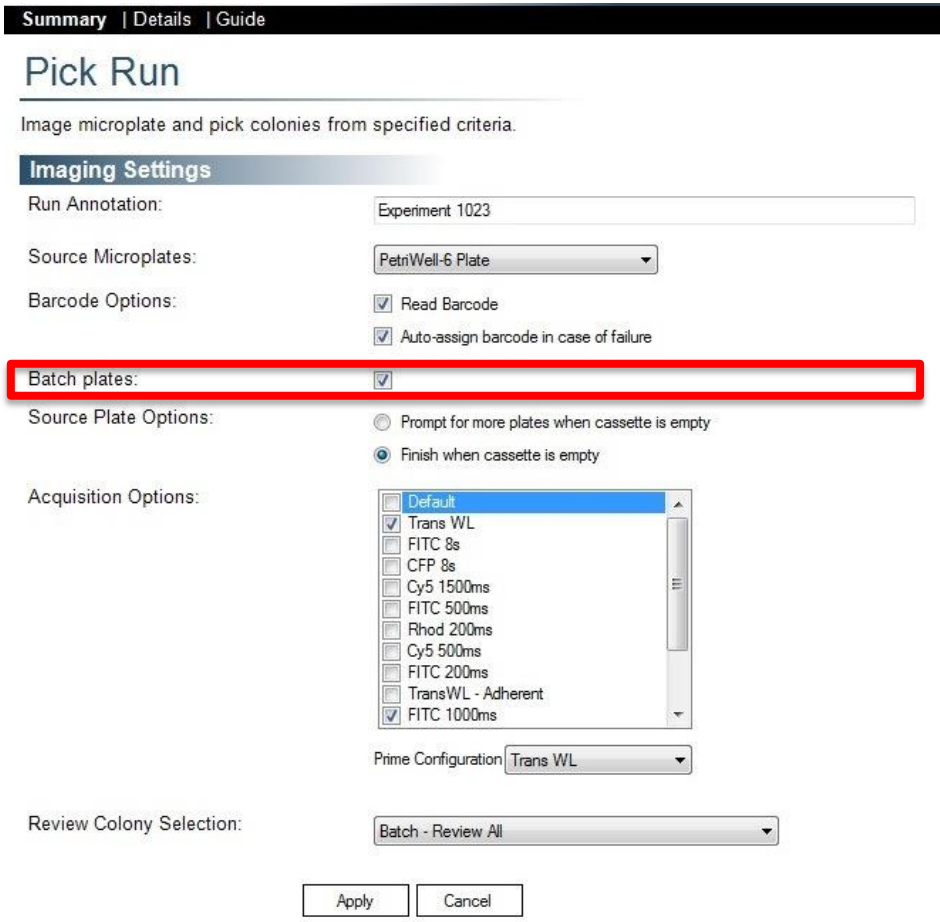
   * If your source plates are barcoded, select **Read Barcode**.
   * We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
d) **Batch plates**: Enable this option. This assumes that multiple plates containing the same sample are being loaded and they will be processed as a single experiment.



Carrying Out A Pick Run: Imaging Settings

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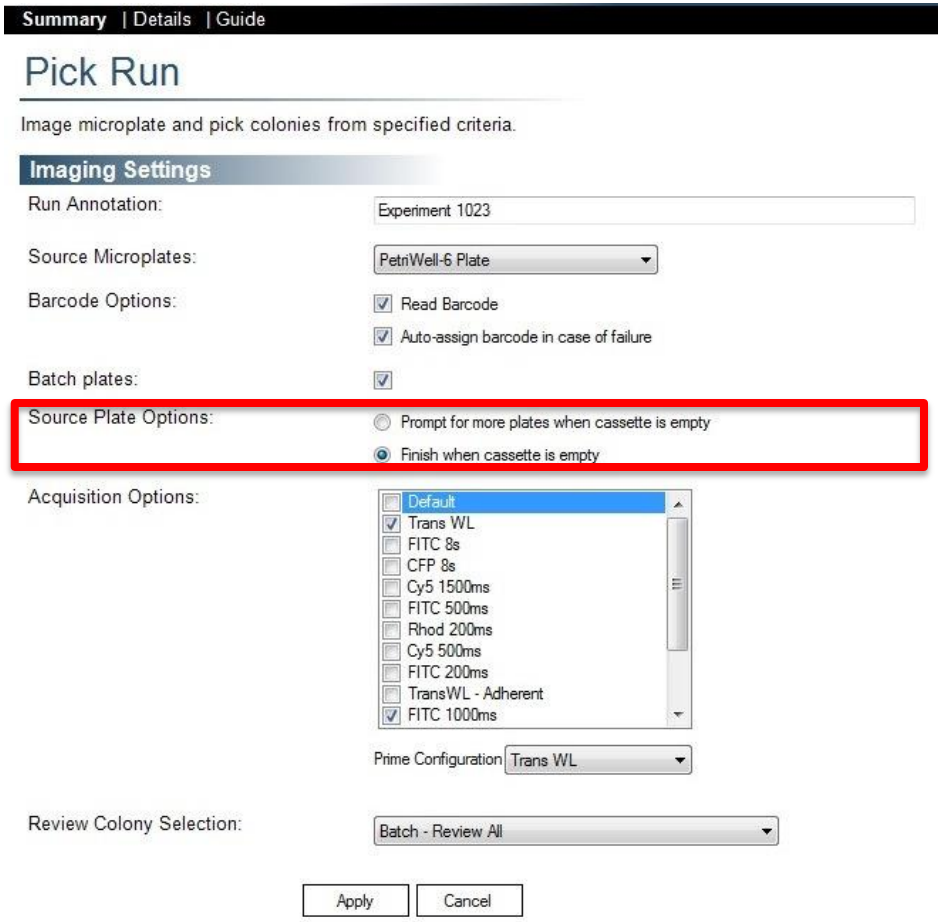
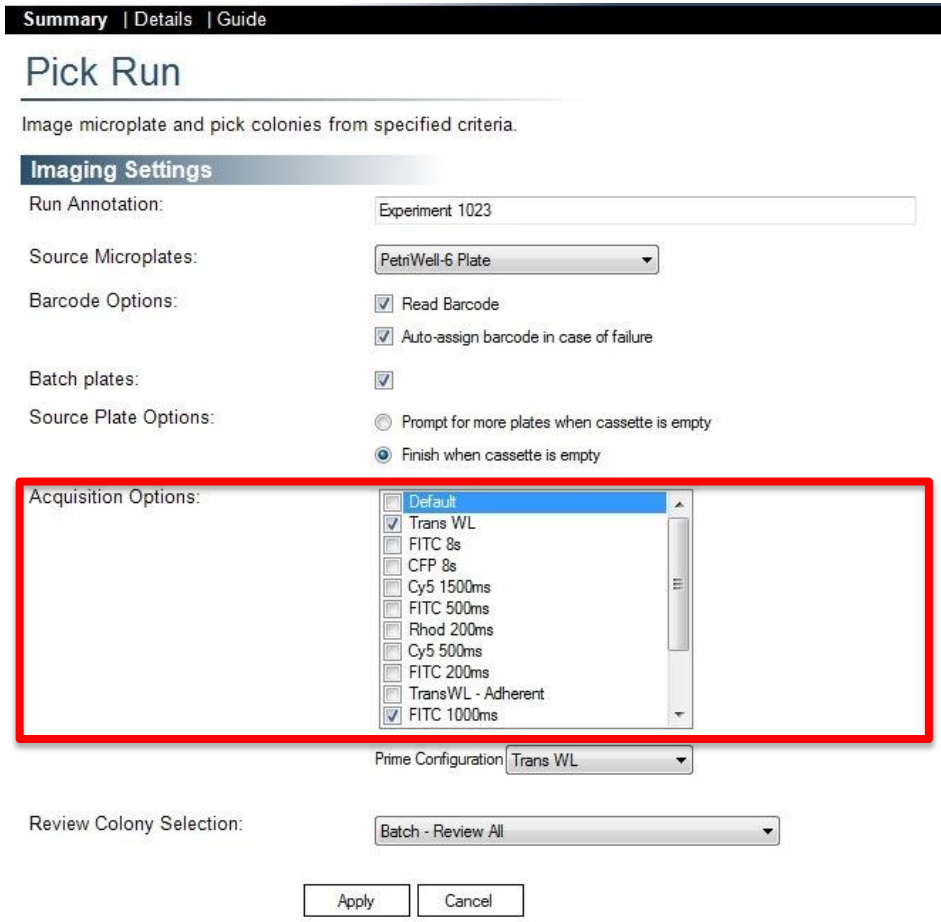
d) **Batch plates**: Enable this option. This assumes that multiple plates containing the same sample are being loaded and they will be processed as a single experiment.
e) **Source Plate Options**: Select **Finish when cassette is empty.**



Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

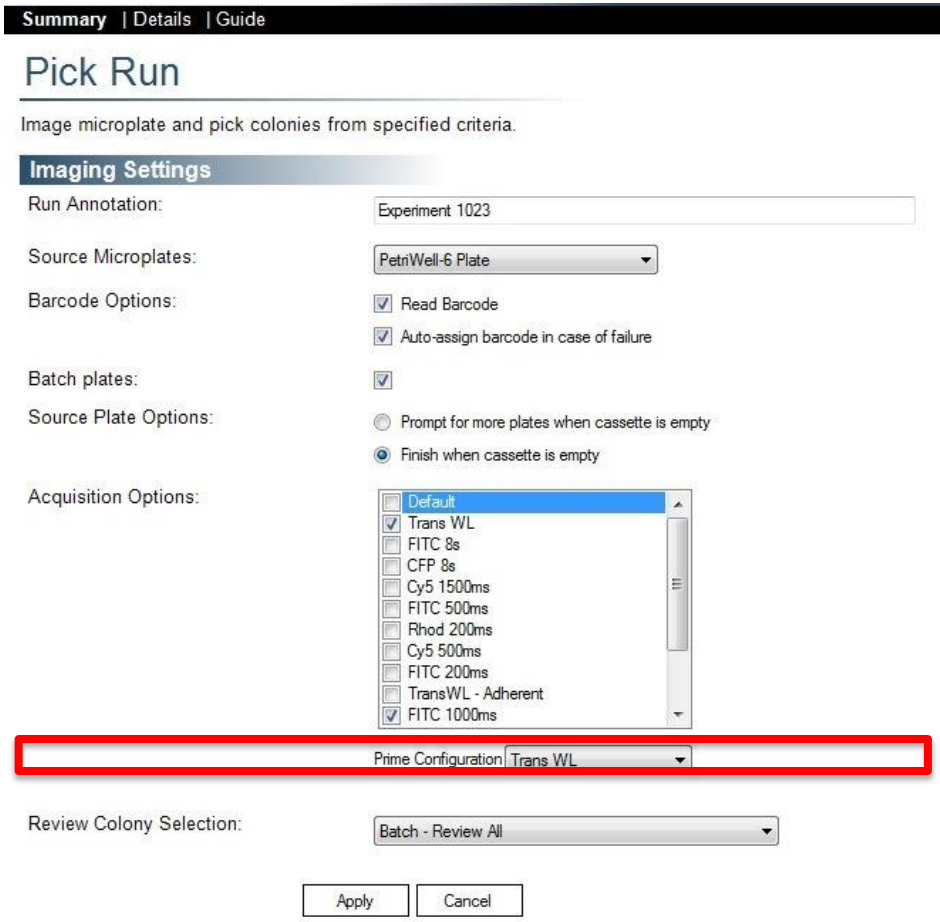
a) **Run Annotation** - Enter a name to identify this run.
b) **Source Microplates** – Select your source plate type from the dropdown menu.
c) **Barcode Options**
   * If your source plates are barcoded, select **Read Barcode**.
   * We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.
d) **Batch plates**: Enable this option. This assumes that multiple plates containing the same sample are being loaded and they will be processed as a single experiment.
e) **Source Plate Options**: Select **Finish when cassette is empty.**
f) **Acquisition Options:** This option provides a choice of which images to capture. If there are appropriate Image Acquisition options select them here. ***For instance, Trans WL is generally always selected, plus any fluorescence options.*** Note that specific acquisition options (such as exposure times) can also be created later.



Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

g) **Prime Configuration:** This is the acquisition option to be used for colony detection. Select the **Trans WL** option from the dropdown.

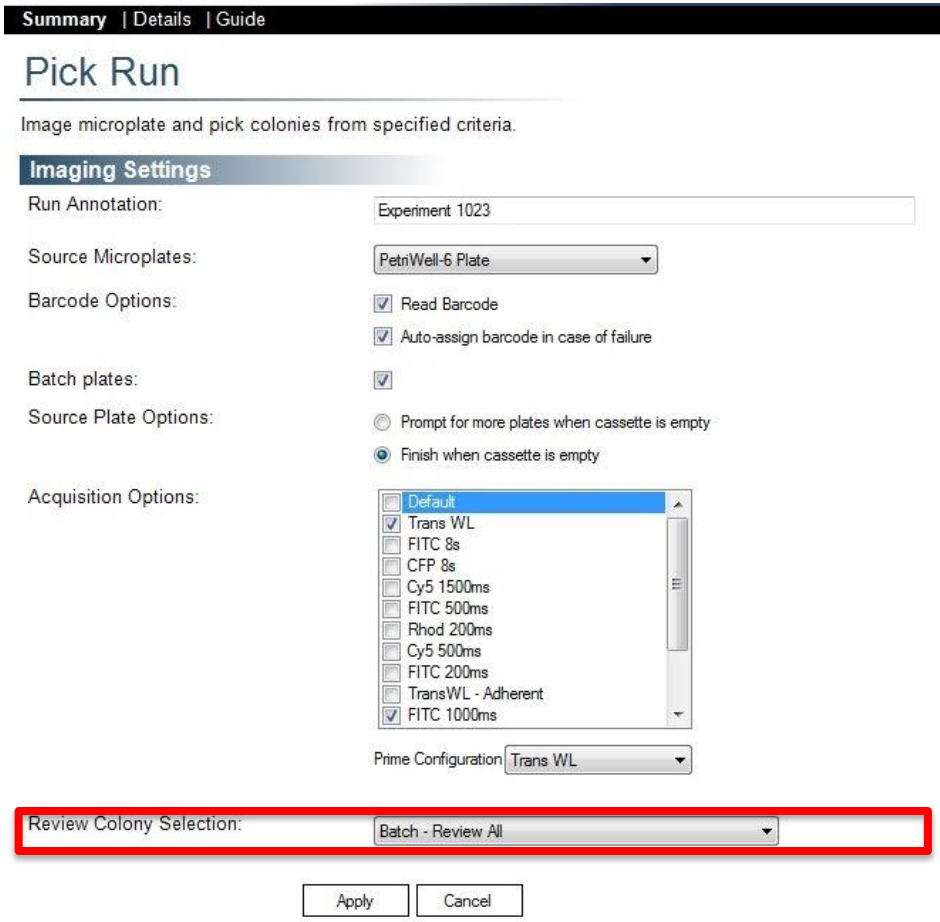


Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

g) **Prime Configuration:** This is the acquisition option to be used for colony detection. Select the **Trans WL** option from the dropdown.

h) **Review Colony Selection:** Select **Batch – Review All** from the dropdown.



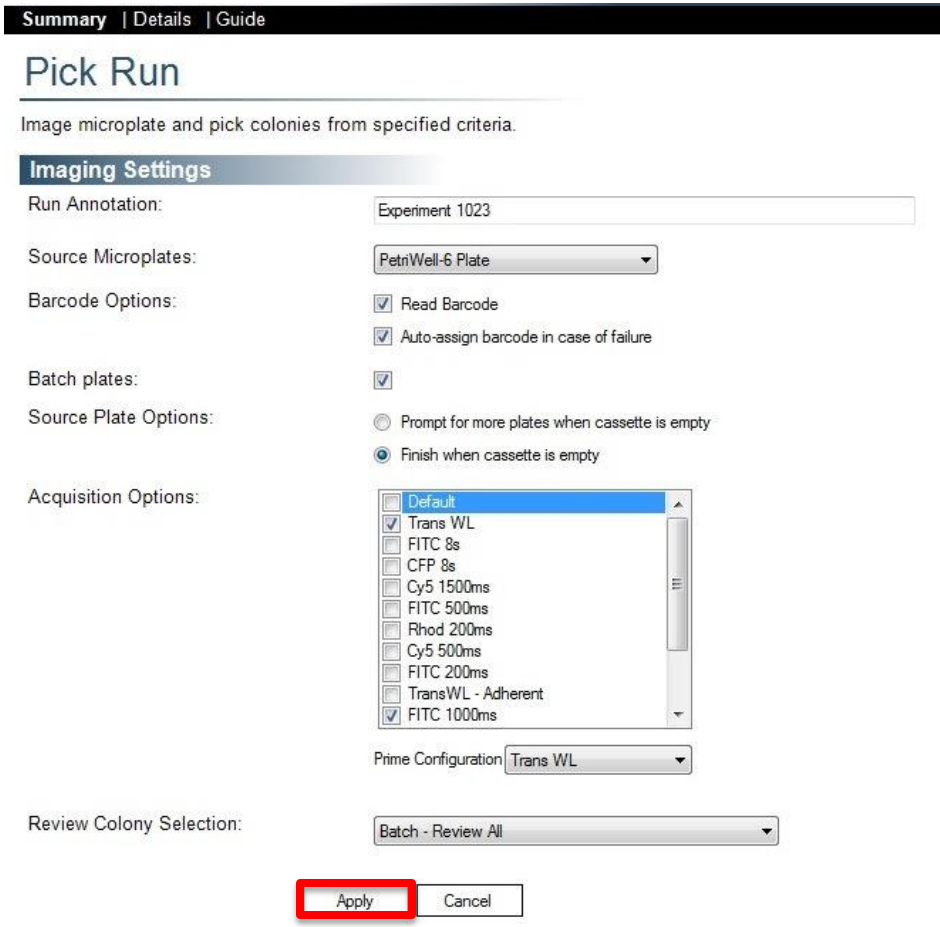
Carrying Out A Pick Run: Imaging Settings

2) Define your **imaging settings** within this dialog, then click **Apply.**

g) **Prime Configuration:** This is the acquisition option to be used for colony detection. Select the **Trans WL** option from the dropdown.

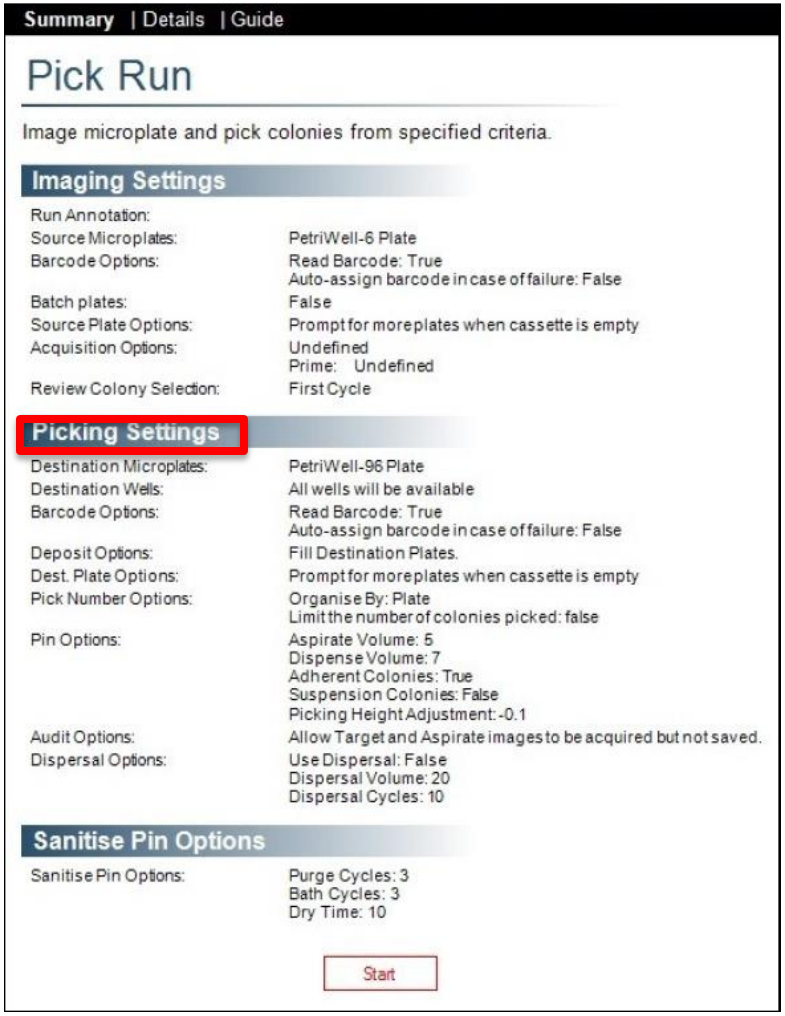
h) **Review Colony Selection:** Select **Batch – Review All** from the dropdown.

i) Click **Apply** to return to the main **Pick Run menu**.



Carrying Out A Pick Run: Picking Settings

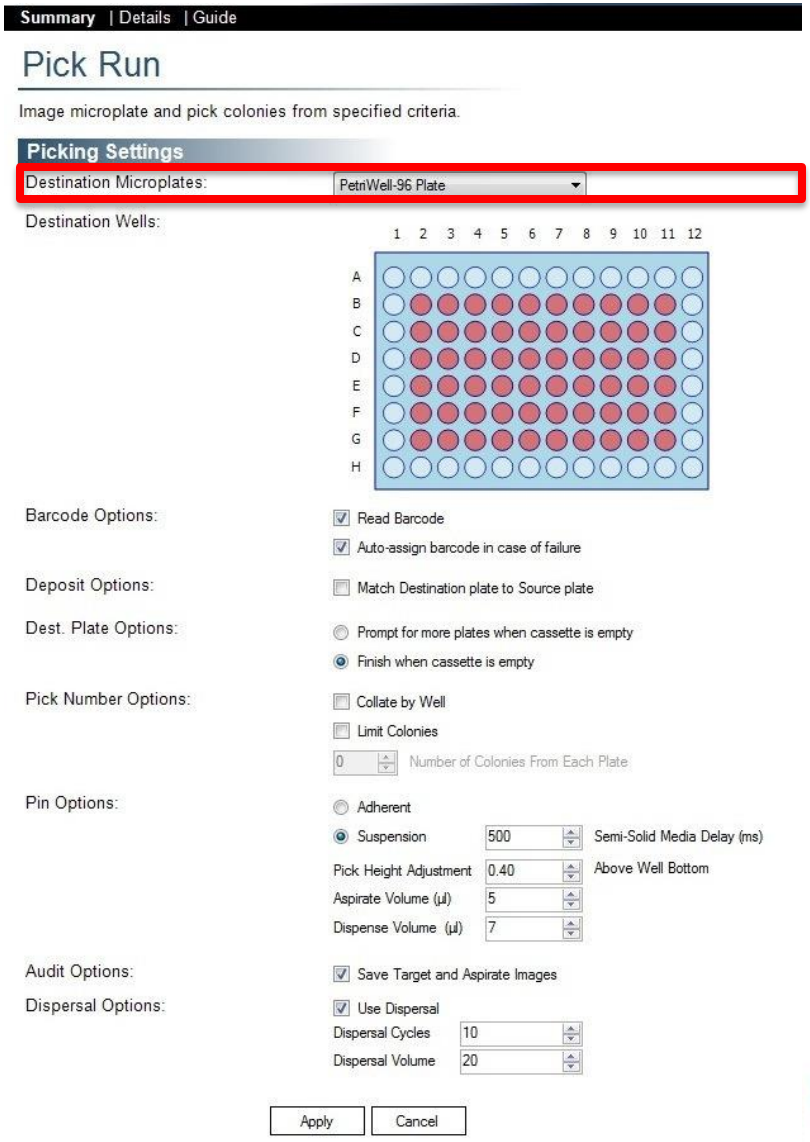
1) From the **Pick Run** menu, click on the **Picking Settings** heading to edit the parameters within:



Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply.**

a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.

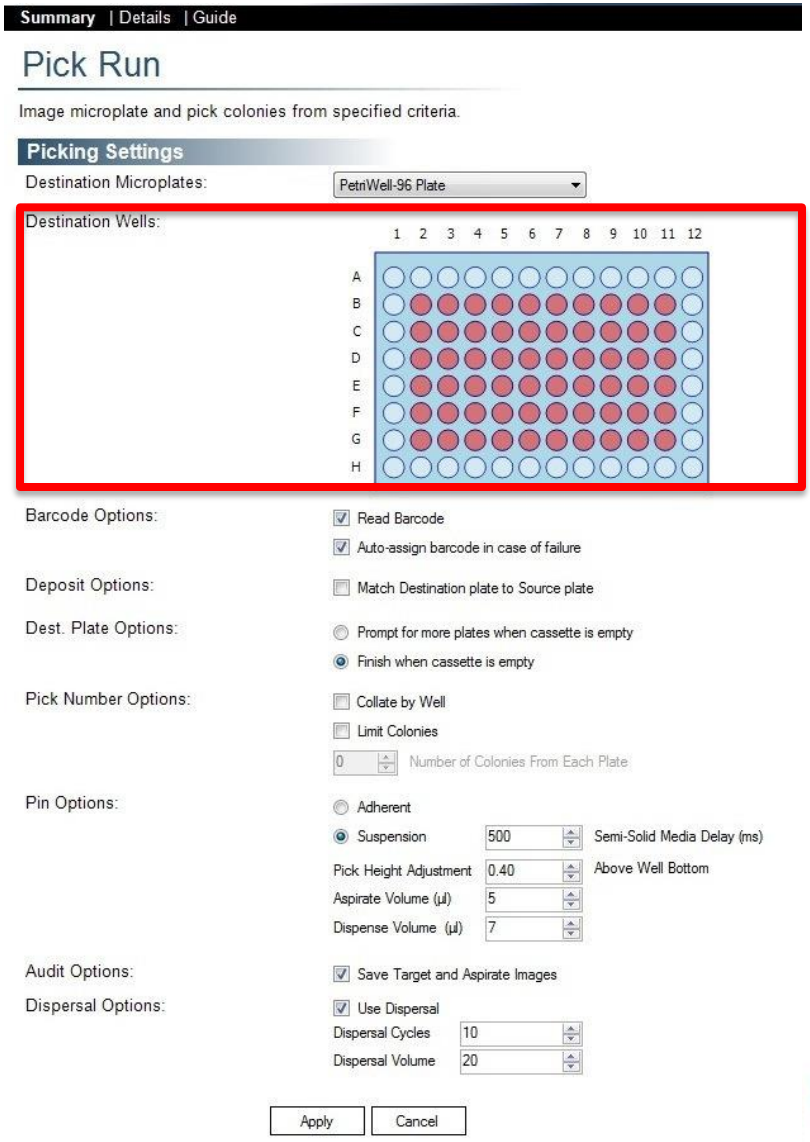


Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply.**

a) **Destination Microplates** – Select your 96-well destination plate type from the dropdown.

b) **Destination Wells** – Specify the wells into which colonies are going to be deposited. Either **left or right click** on the mouse to select or de-select wells. To deselect entire rows/columns, hold down the right mouse button and drag. All destination plates will be filled using this template.



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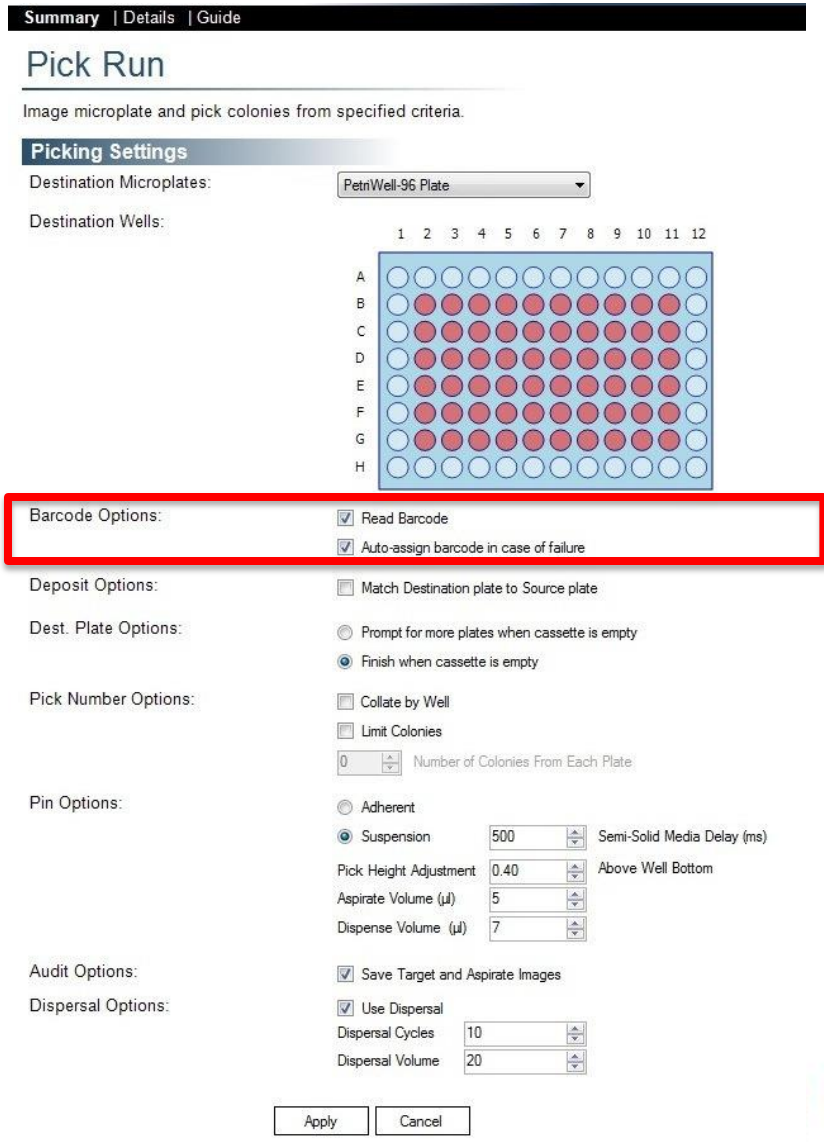
c) **Barcode Options**

* If your destination plates are barcoded, select **Read Barcode**.
* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.



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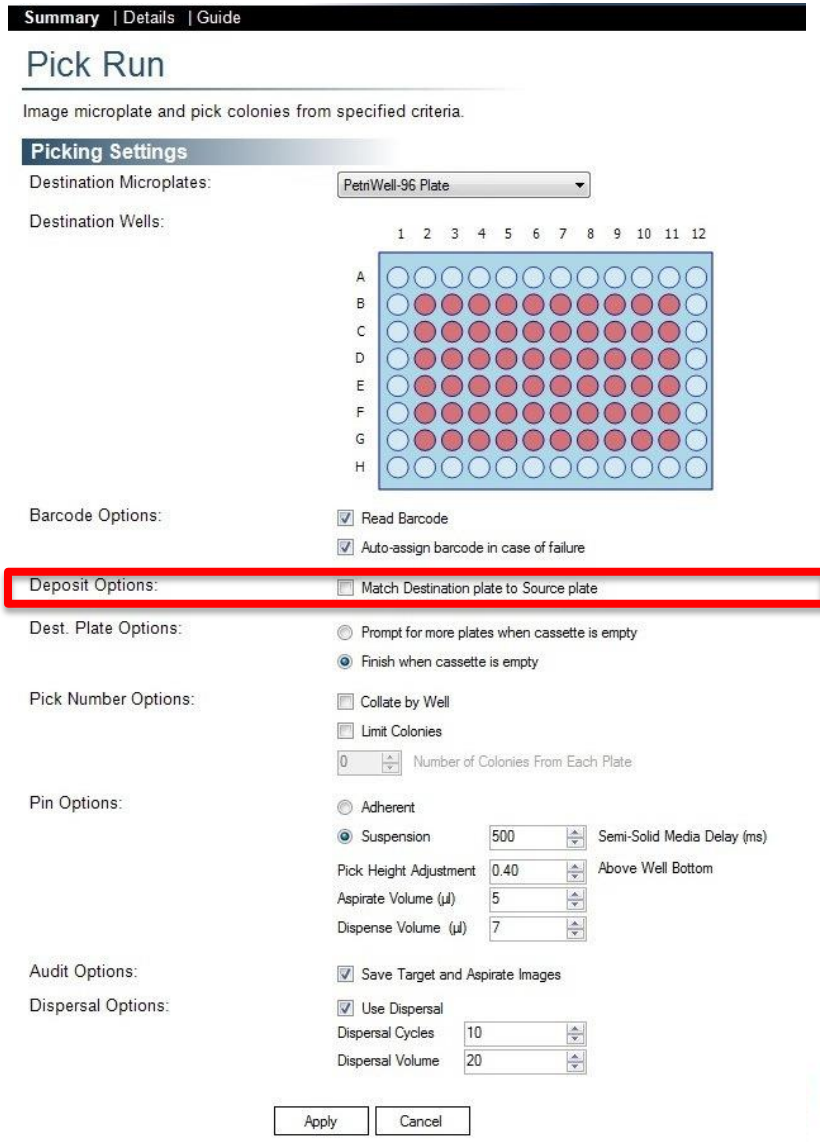
d) **Deposit Options**: Leave unselected.



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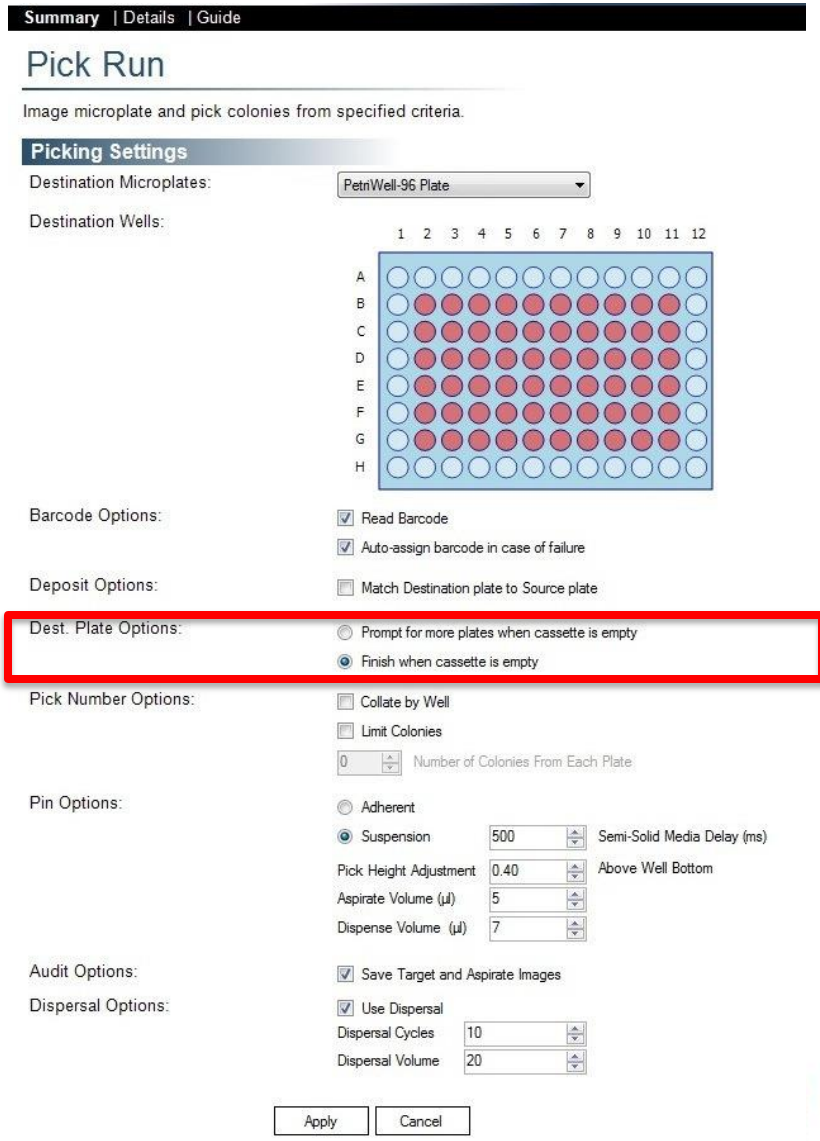
d) **Deposit Options**: Leave unselected.

e) **Destination Plate Options**: Select **Finish when cassette is empty.**



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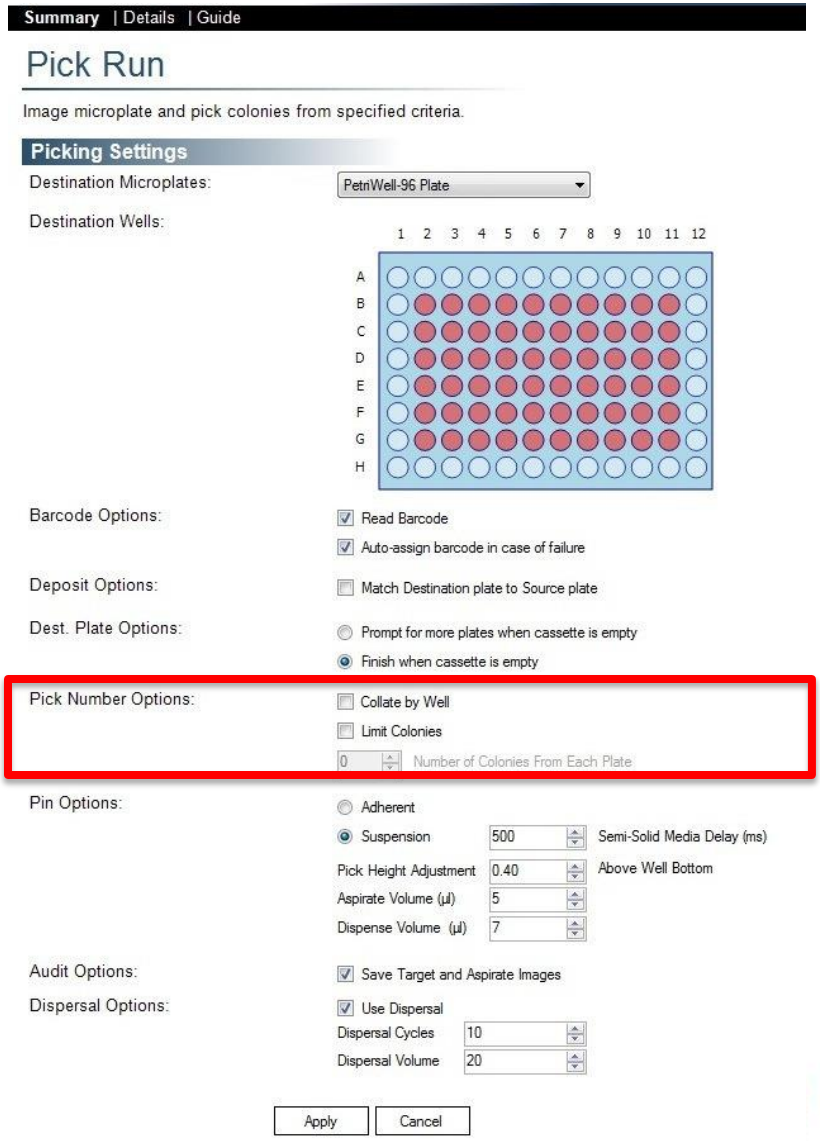
d) **Deposit Options**: Leave unselected.

e) **Destination Plate Options**: Select **Finish when cassette is empty.**

f) **Pick Number Options: Do not select any options here.** Leave as default (Collate by Plate).



Carrying Out A Pick Run: Picking Settings

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c) **Barcode Options**

* If your destination plates are barcoded, select **Read Barcode**.
* We also recommend you also select **Auto-assign barcode in case of failure** - if there are no barcodes on the plates, the ClonePix 2 will automatically assign a code.

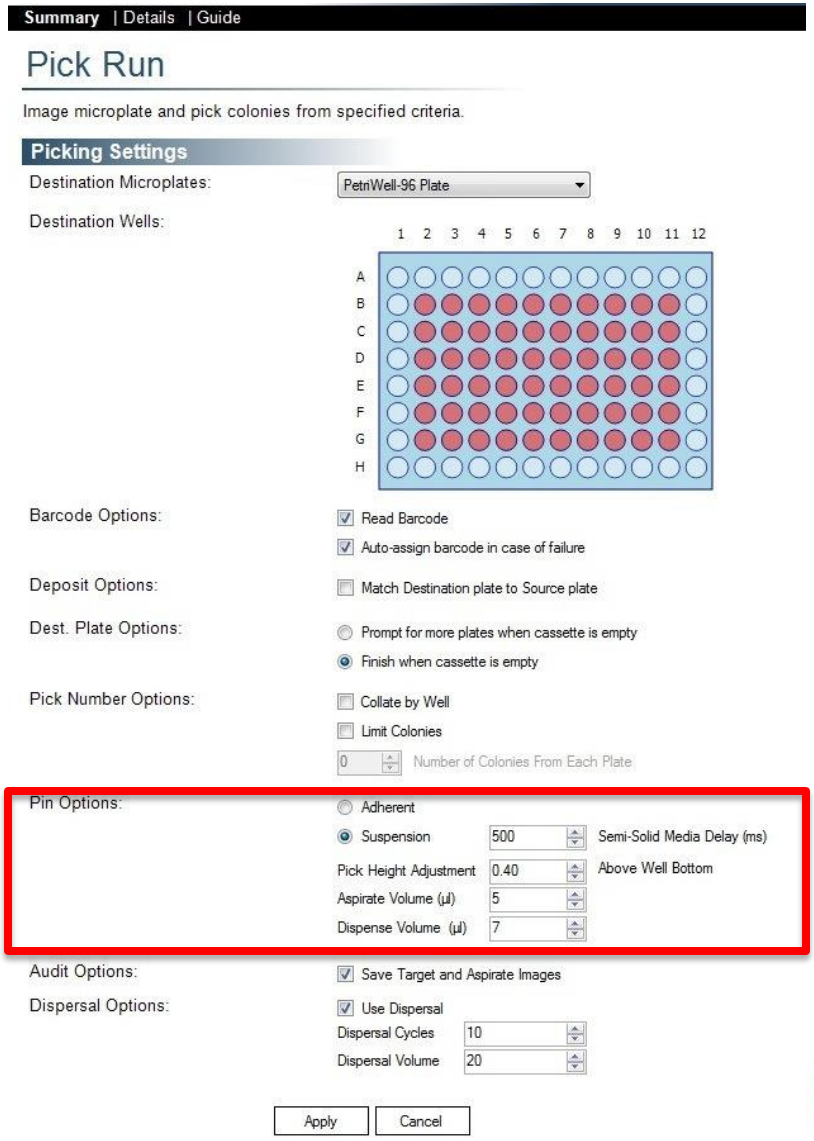
d) **Deposit Options**: Leave unselected.

e) **Destination Plate Options**: Select **Finish when cassette is empty.**

f) **Pick Number Options: Do not select any options here.** Leave as default (Collate by Plate).

g) **Pin Options**:

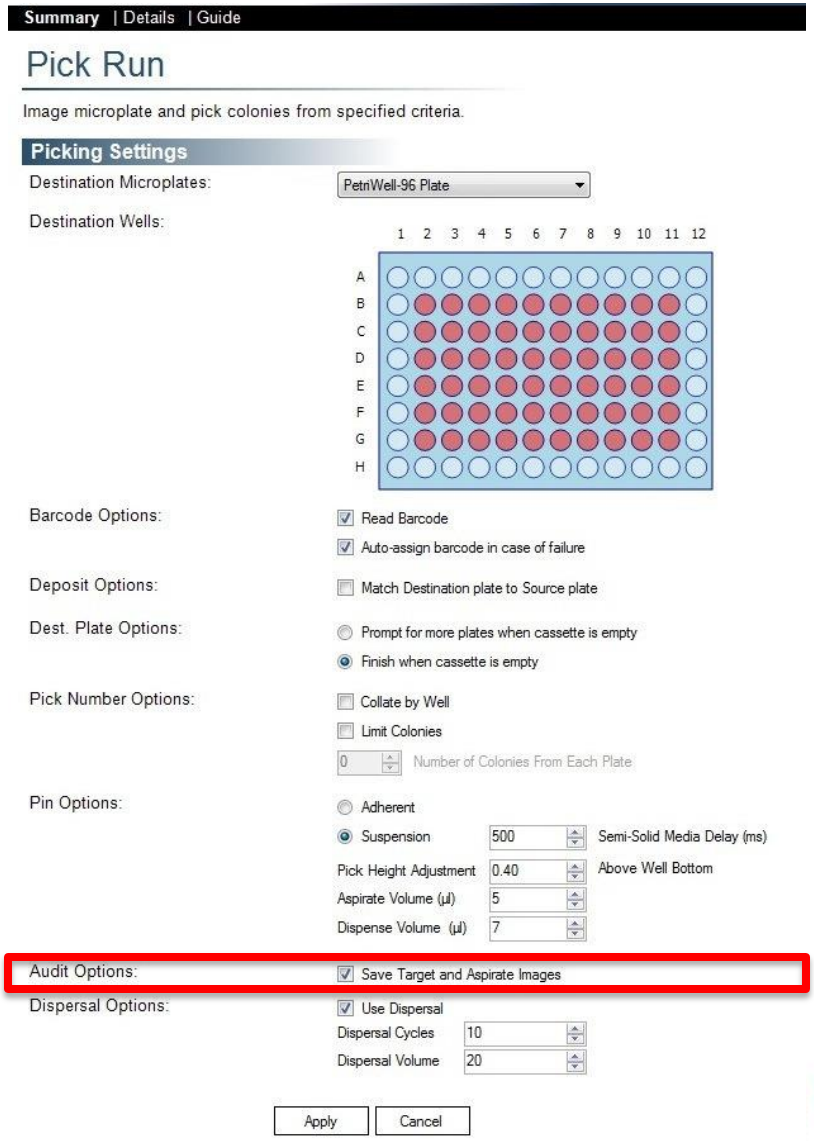
- Select the **type of colonies** that are going to be picked: **Suspension** or **Adherent**.
- **Pick Height Adjustment:** Leave as default.
- **Aspirate Volume:** Leave as default = **5 uL**
- **Dispense Volume**: Leave as default = **7 uL**



Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply.**

g) **Audit Options:** Tick this box to save the Target and Aspirate Images. **Note: This will slow down picking.**

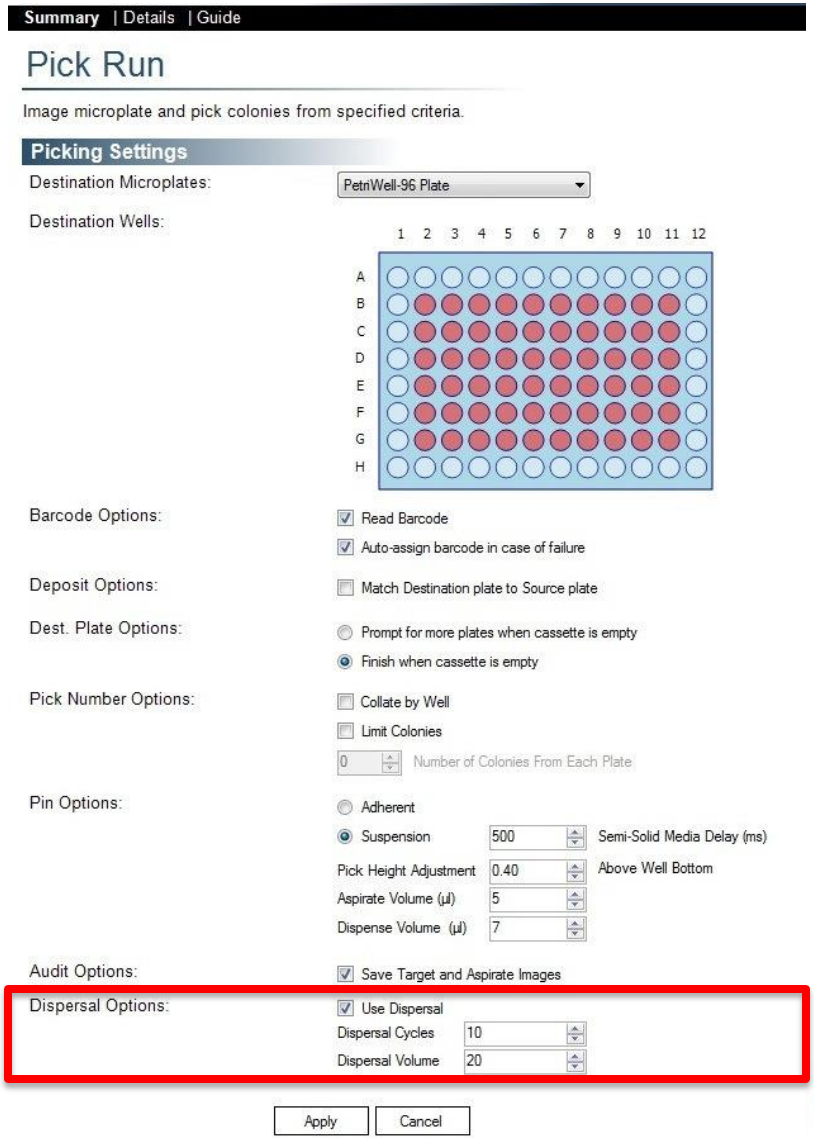


Carrying Out A Pick Run: Picking Settings

2) Define your **picking settings** within this dialog, then click **Apply.**

g) **Audit Options:** Tick this box to save the Target and Aspirate Images. **Note: This will slow down picking.**

h) **Dispersal Options:**
- **Use Dispersal**: Select to spread out colony cells after picking. *Do not select if intact*
- *colonies are required.*
- **Dispersal cycles:** Use **3-6** for **CHO** cells and **6-10** for **hybridomas**.
- **Dispersal volume**: Leave as default = **20 uL**.



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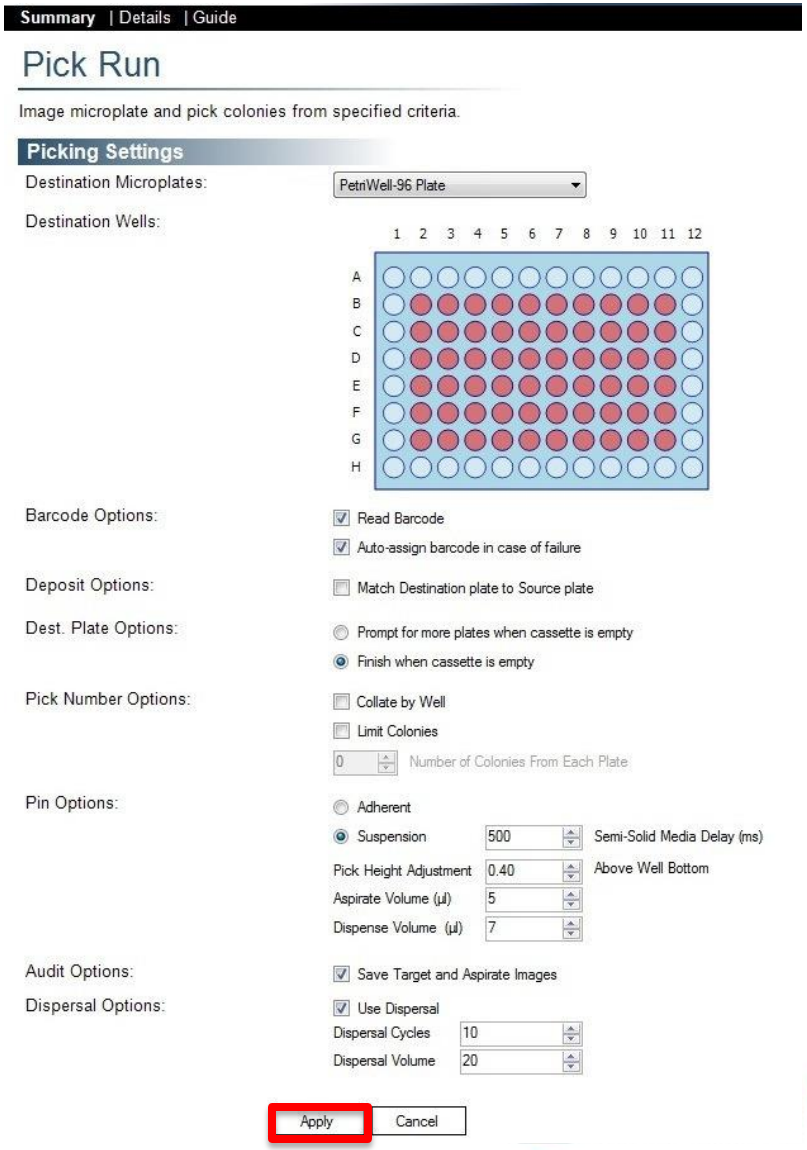
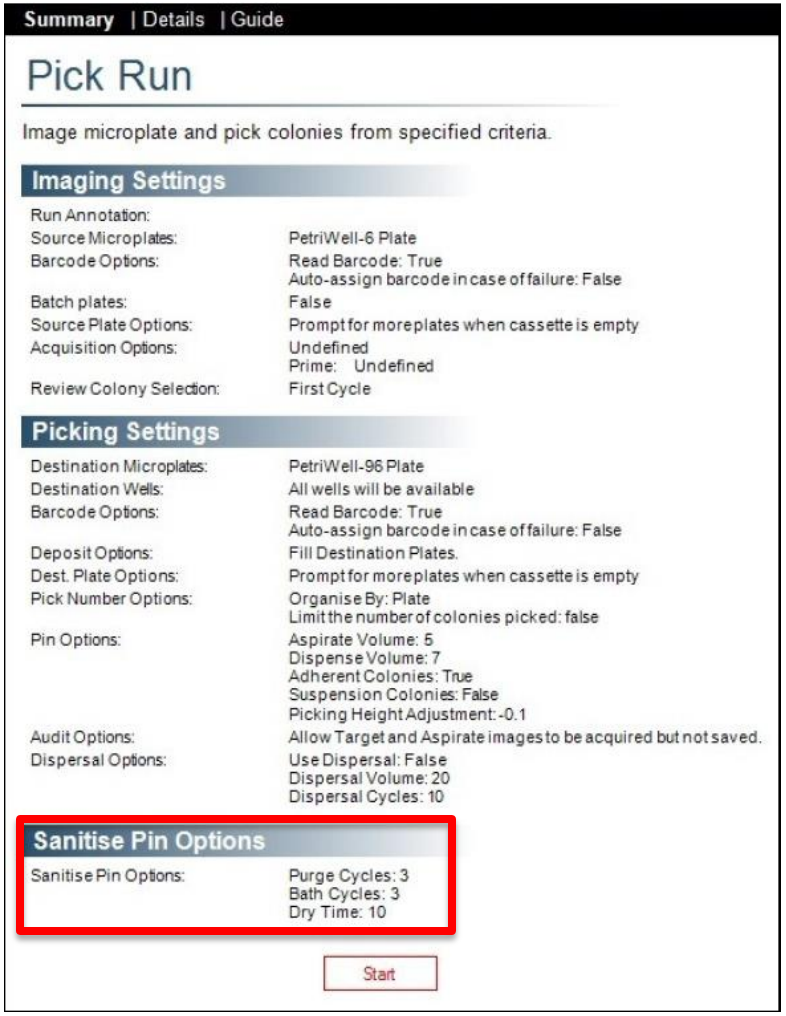
- **Dispersal cycles:** Use **3-6** for **CHO** cells and **6-10** for **hybridomas**.
- **Dispersal volume**: Leave as default = **20 uL**.

i) Click **Apply** to return to the main **Pick Run menu.**



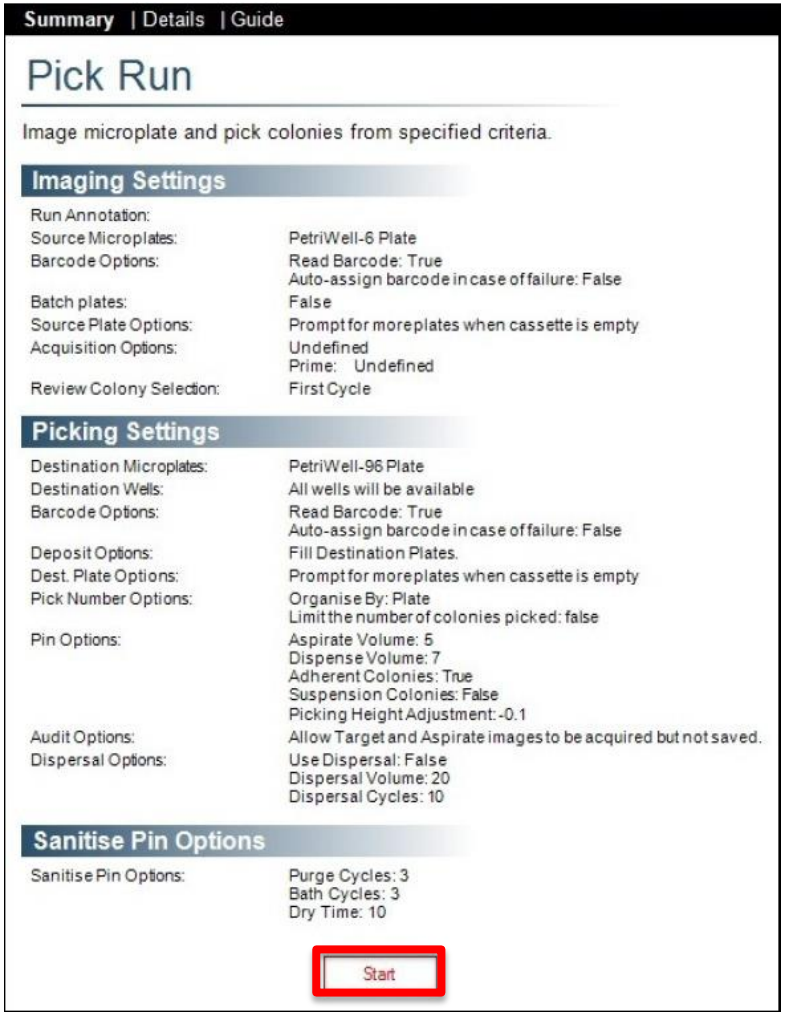
Carrying Out A Pick Run: Sanitise Pin Options

1) You will see **Sanitise Pin Options** at the bottom of the **Pick Run** main menu. **Leave these at the default settings**.



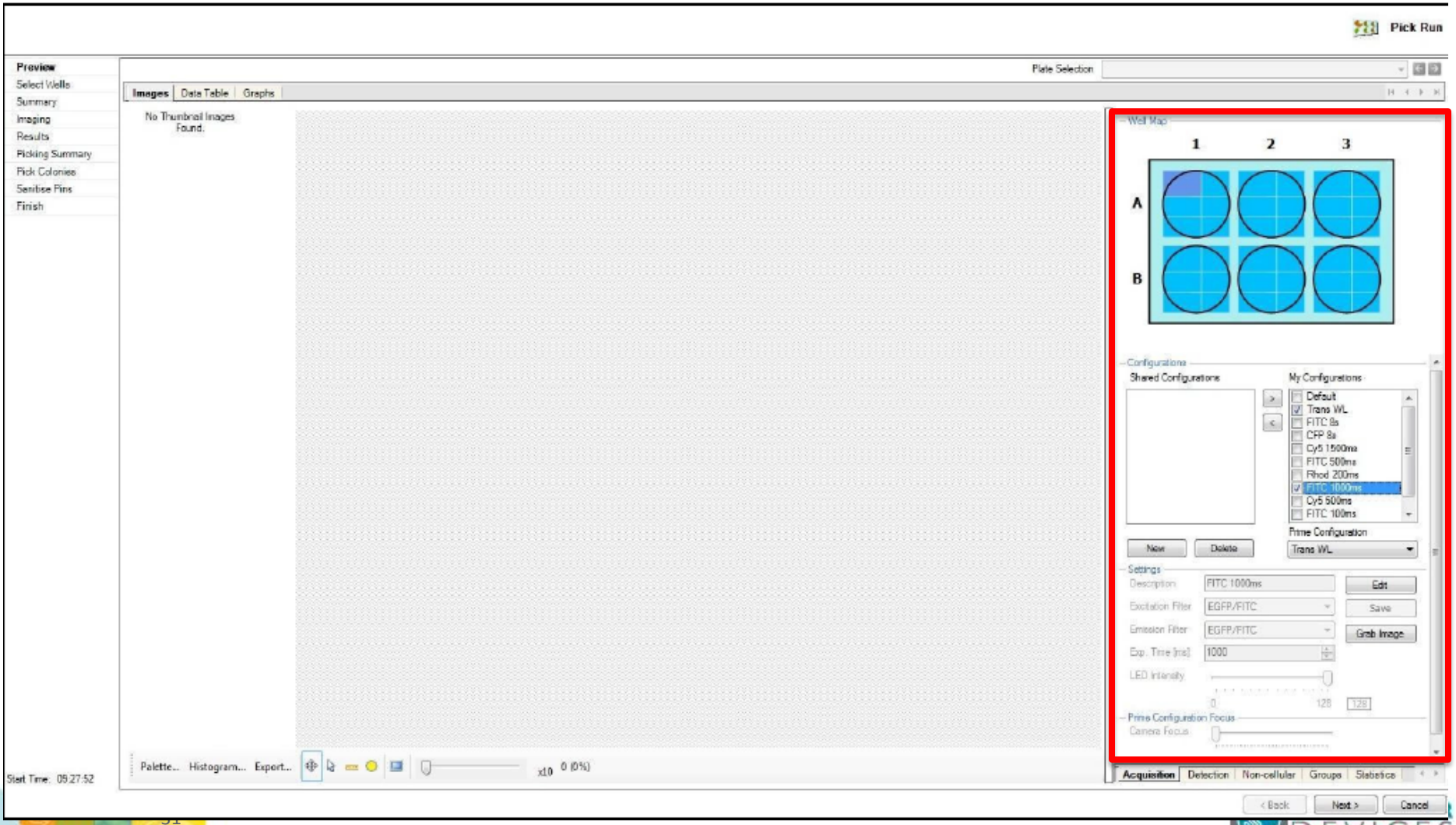
Carrying Out A Pick Run: Starting A Pick Run

1) Click the **Start** button at the bottom of the **Pick Run** main menu to begin your pick run.
2) When prompted, load the **cell colony plates** into the **source stacker cassette**.



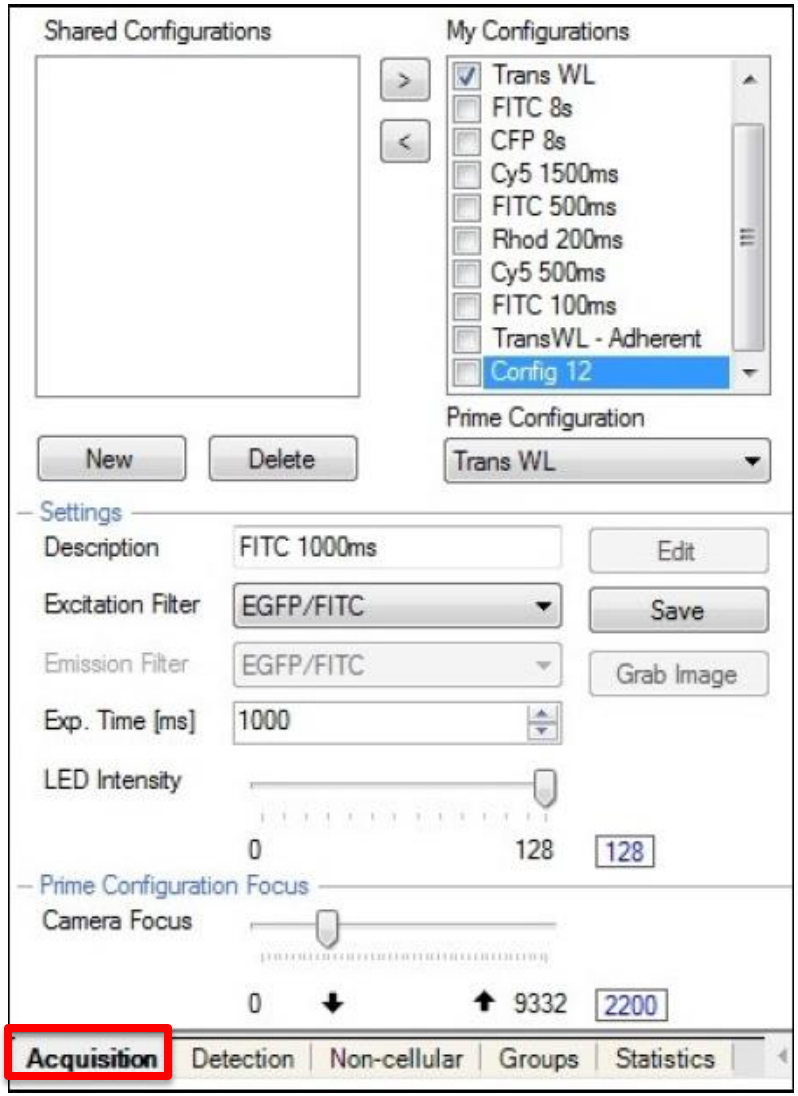
Carrying Out A Pick Run: Define Image Acquisition Settings

3) The **Preview** screen appears. Here you will first define your **image acquisition, colony detection, and groups to pick settings**.



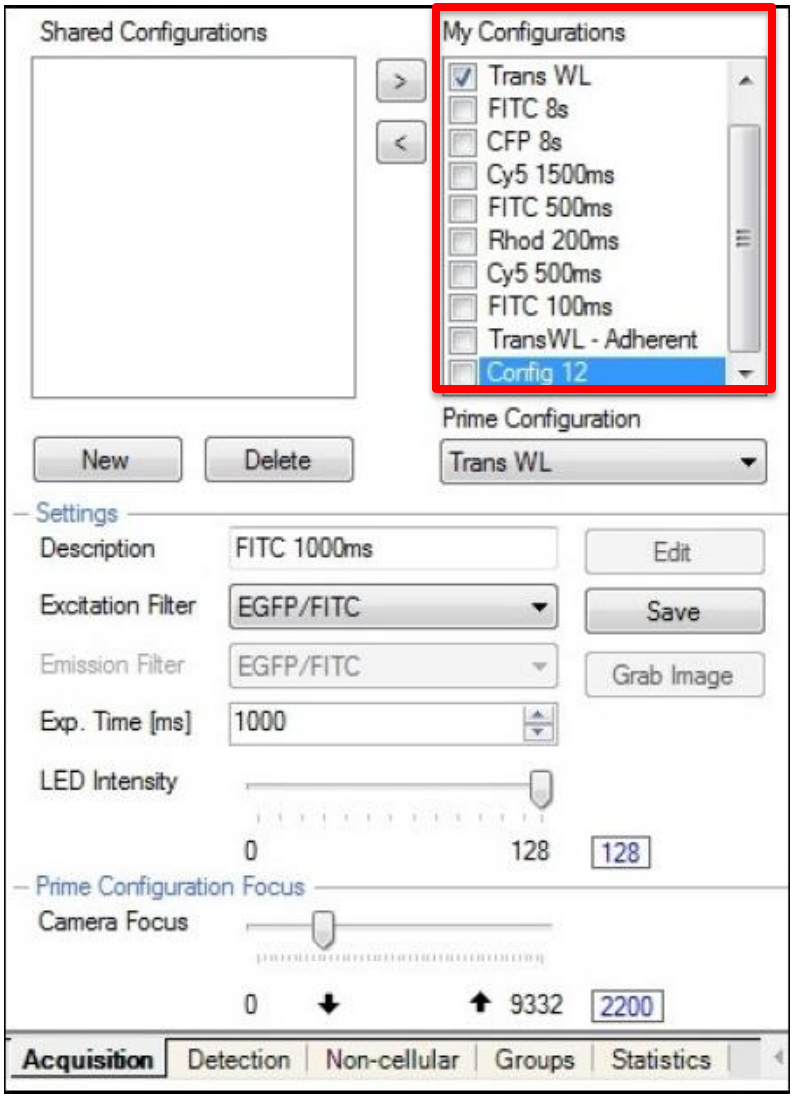
Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   a) Click on the **Acquisition** tab.



Carrying Out A Pick Run: Define Image Acquisition Settings

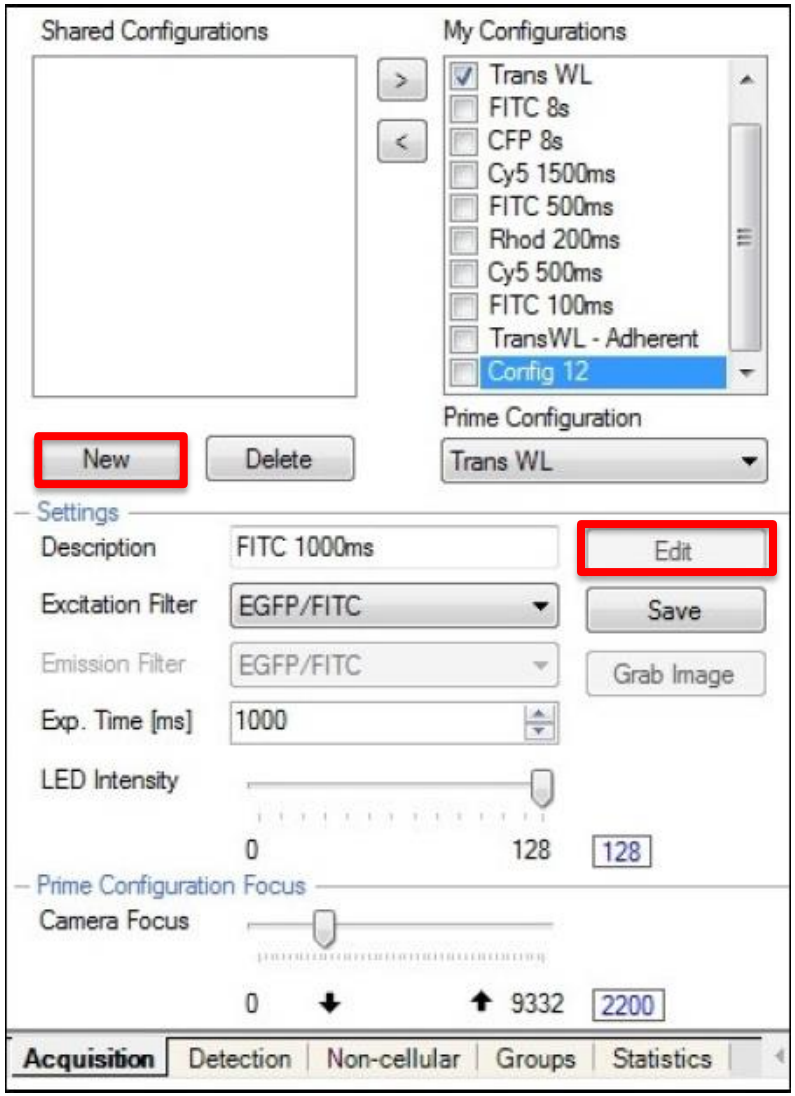
3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   a) Click on the **Acquisition** tab.
   b) If the desired acquisition option is not available in the **My Configurations** box, new Acquisitions options will need to be set up (see example screenshot on right).



Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   a) Click on the **Acquisition** tab.
   b) If the desired acquisition option is not available in the **My Configurations** box, new Acquisitions options will need to be set up (see example screenshot on right).
   c) Click **New** and then click **Edit**. The following settings should work for most scenarios:

Description	White Light	FITC 1s
Excitation Filter	WHITELIGHT (TRANS)	EGFP/FITC
Exp. Time (ms)	200	1000
LED Intensity	3	128
Camera Focus	2200	2200

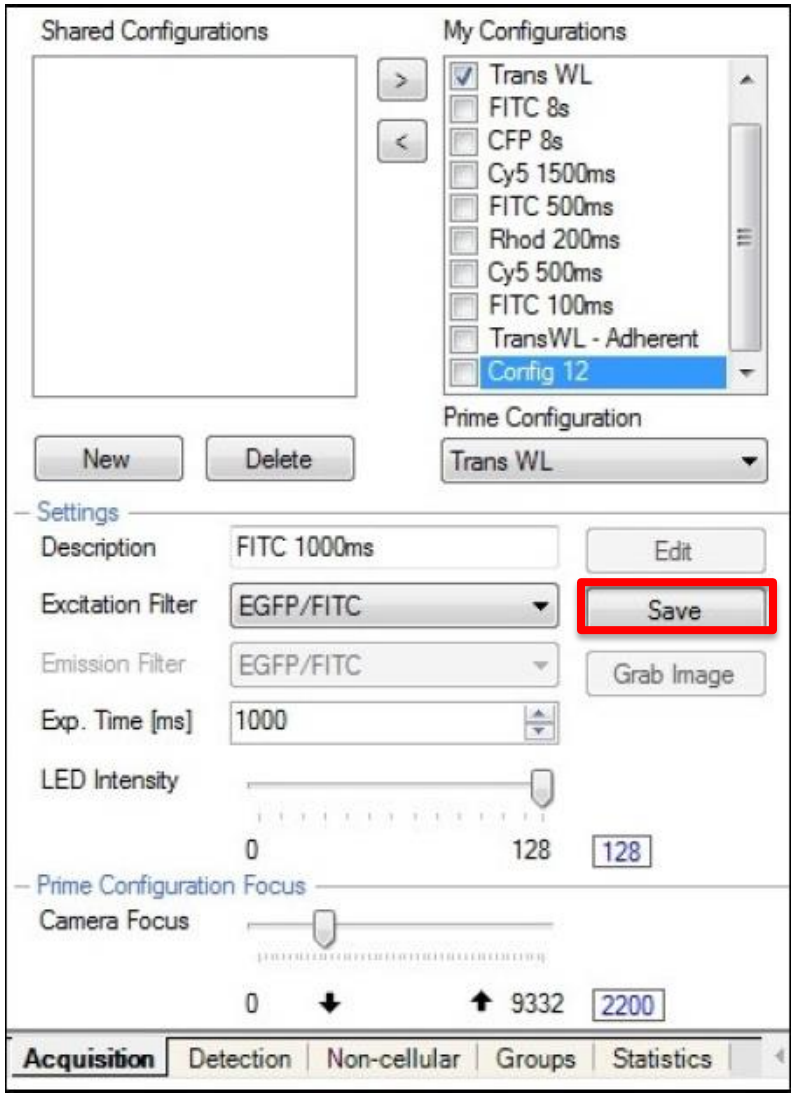


Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   a) Click on the **Acquisition** tab.
   b) If the desired acquisition option is not available in the **My Configurations** box, new Acquisitions options will need to be set up (see example screenshot on right).
   c) Click **New** and then click **Edit**. The following settings should work for most scenarios:

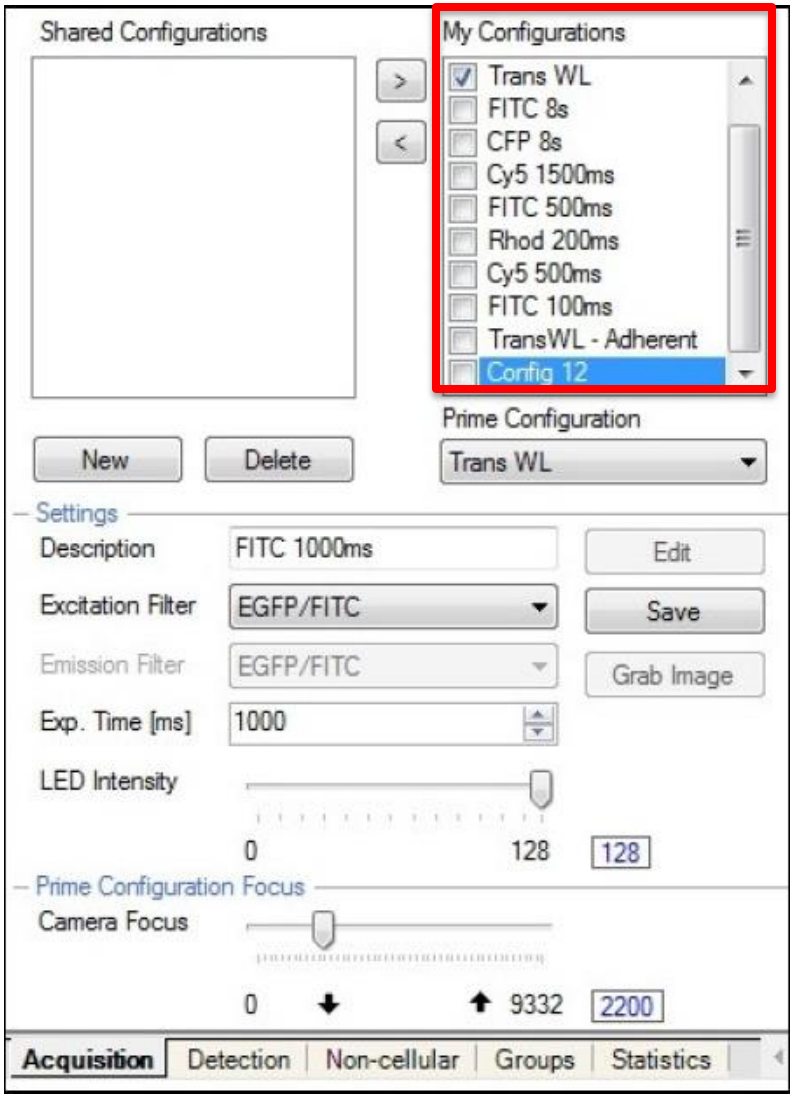
Description	White Light	FITC 1s
Excitation Filter	WHITELIGHT (TRANS)	EGFP/FITC
Exp. Time (ms)	200	1000
LED Intensity	3	128
Camera Focus	2200	2200

   d) Click **Save** to store each option.



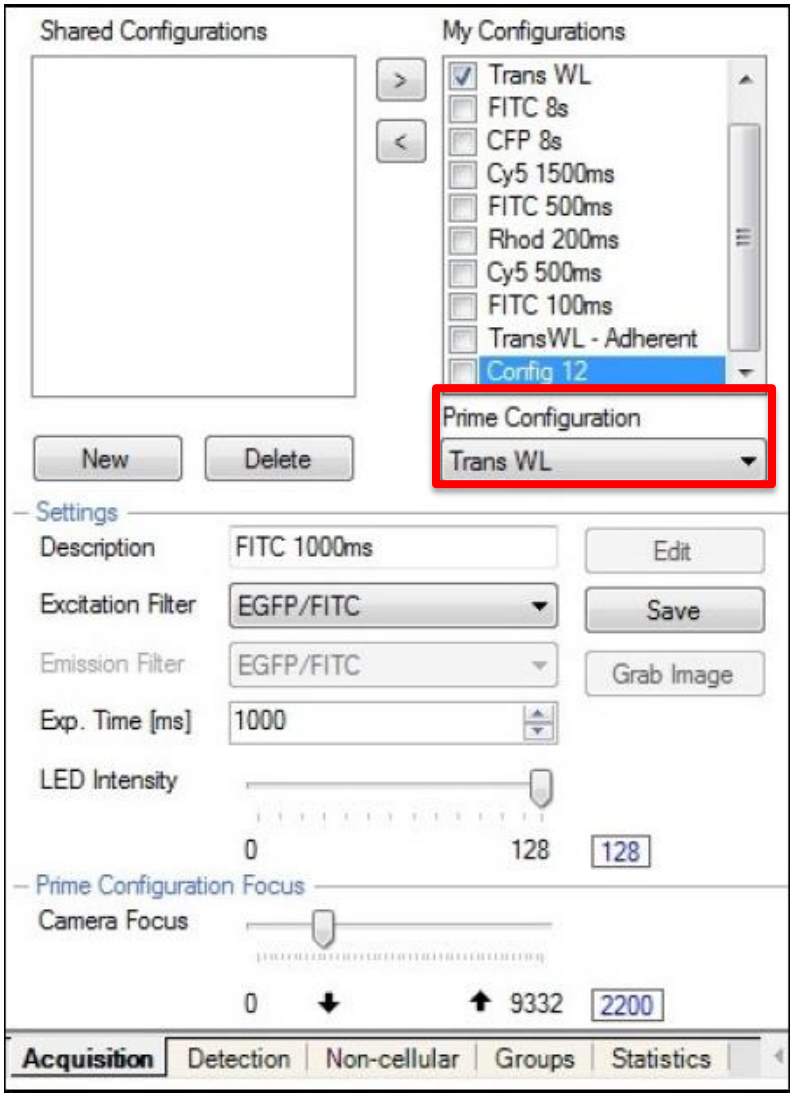
Carrying Out A Pick Run: Define Image Acquisition Settings

3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   e) The new acquisition options will now be displayed under **My Configurations**. Ensure that **TransWL** and **FITC 1000ms** options are selected.
   Note: Do not delete the Default option – it is required for other ClonePix 2 functions.



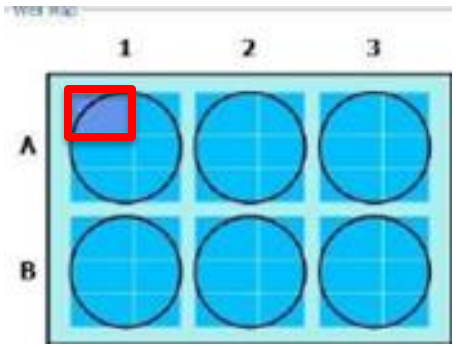
Carrying Out A Pick Run: Define Image Acquisition Settings

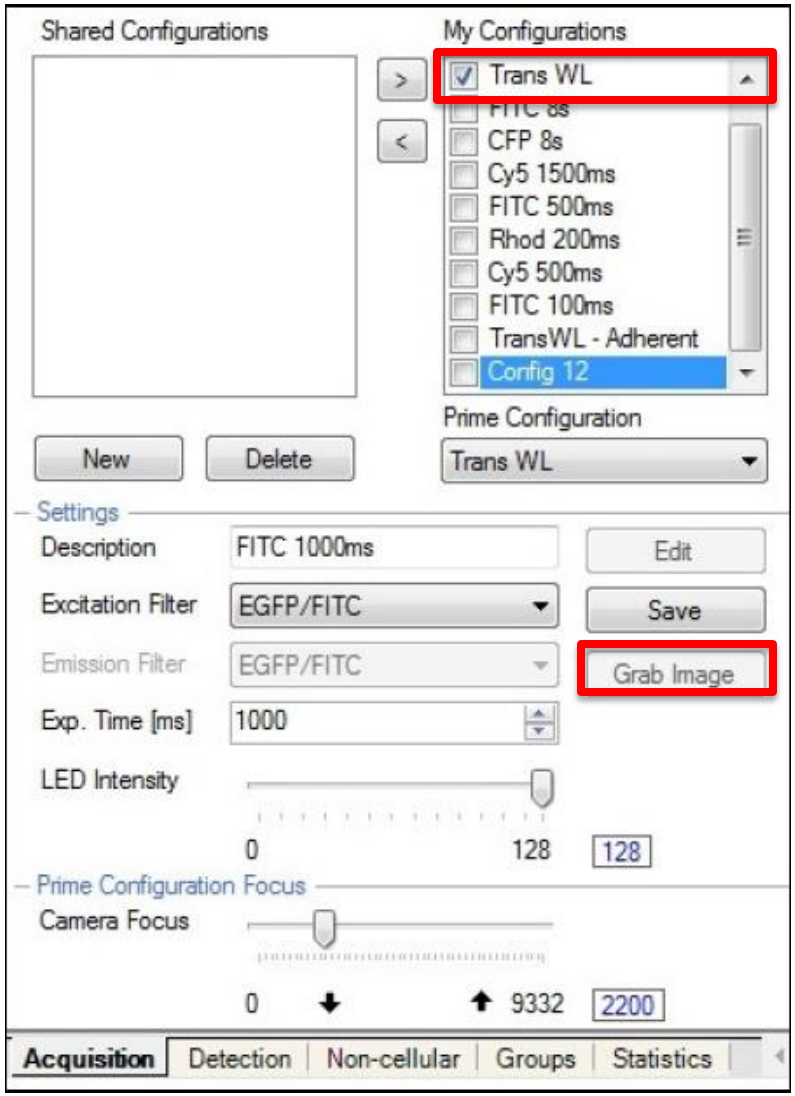
3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   e) The new acquisition options will now be displayed under **My Configurations**. Ensure that TransWL and FITC 1000ms options are selected. *Note: Do not delete the Default option – it is required for other ClonePix 2 functions*.
   f) Ensure that the **Prime Configuration** is set to **TransWL**. This is critical for correct colony detection.



Carrying Out A Pick Run: Define Image Acquisition Settings

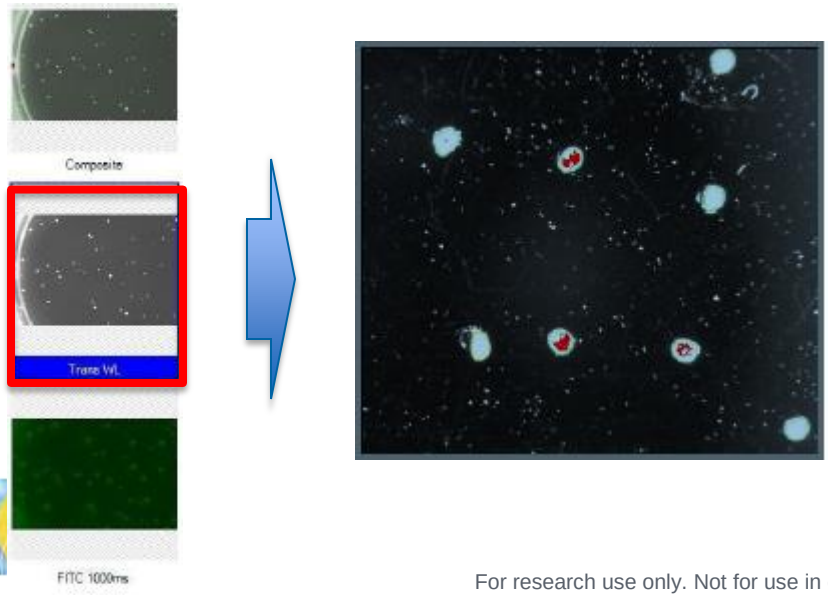
3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   e) The new acquisition options will now be displayed under **My Configurations**. Ensure that **TransWL** and **FITC 1000ms** options are selected. *Note: Do not delete the Default option – it is required for other ClonePix 2 functions.*
   f) Ensure that the **Prime Configuration** is set to **TransWL**. This is critical for correct colony detection.
   g) To test each setting, select it in the **My Configurations** list and click **Grab Image** which will capture images for the area currently highlighted in the **Well Map**, or click on another area on the **Well Map** to display a new image.

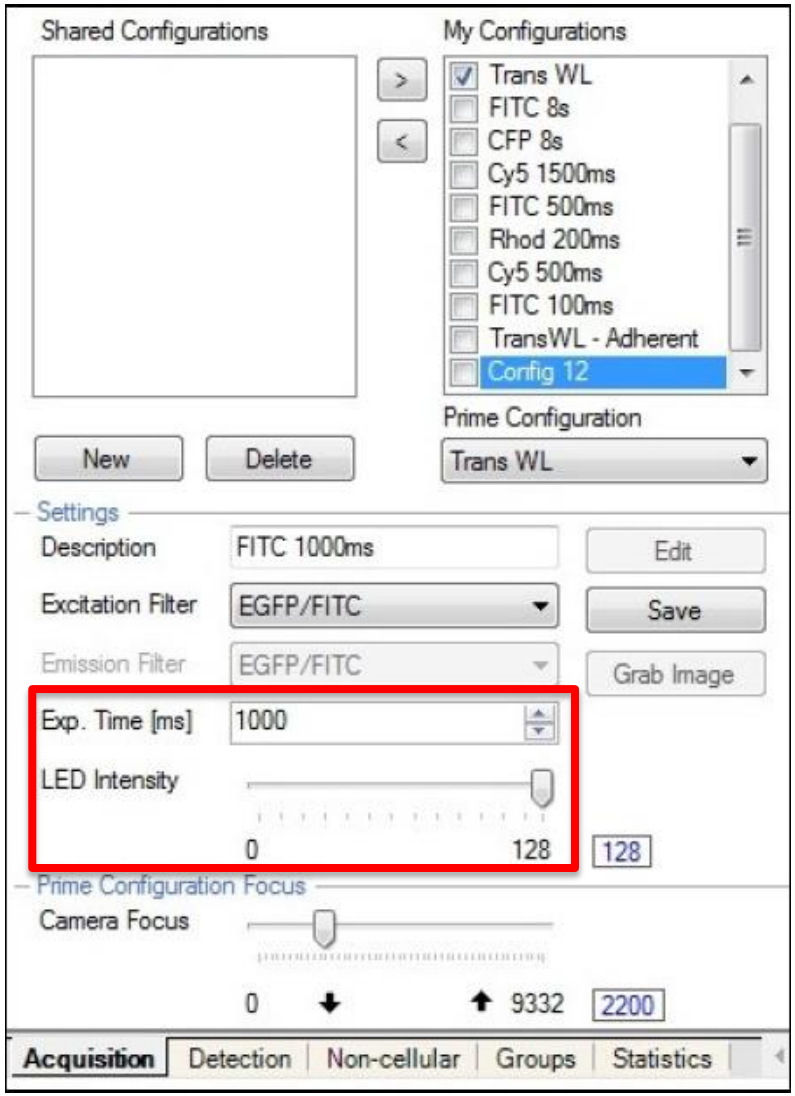




Carrying Out A Pick Run: Define Image Acquisition Settings

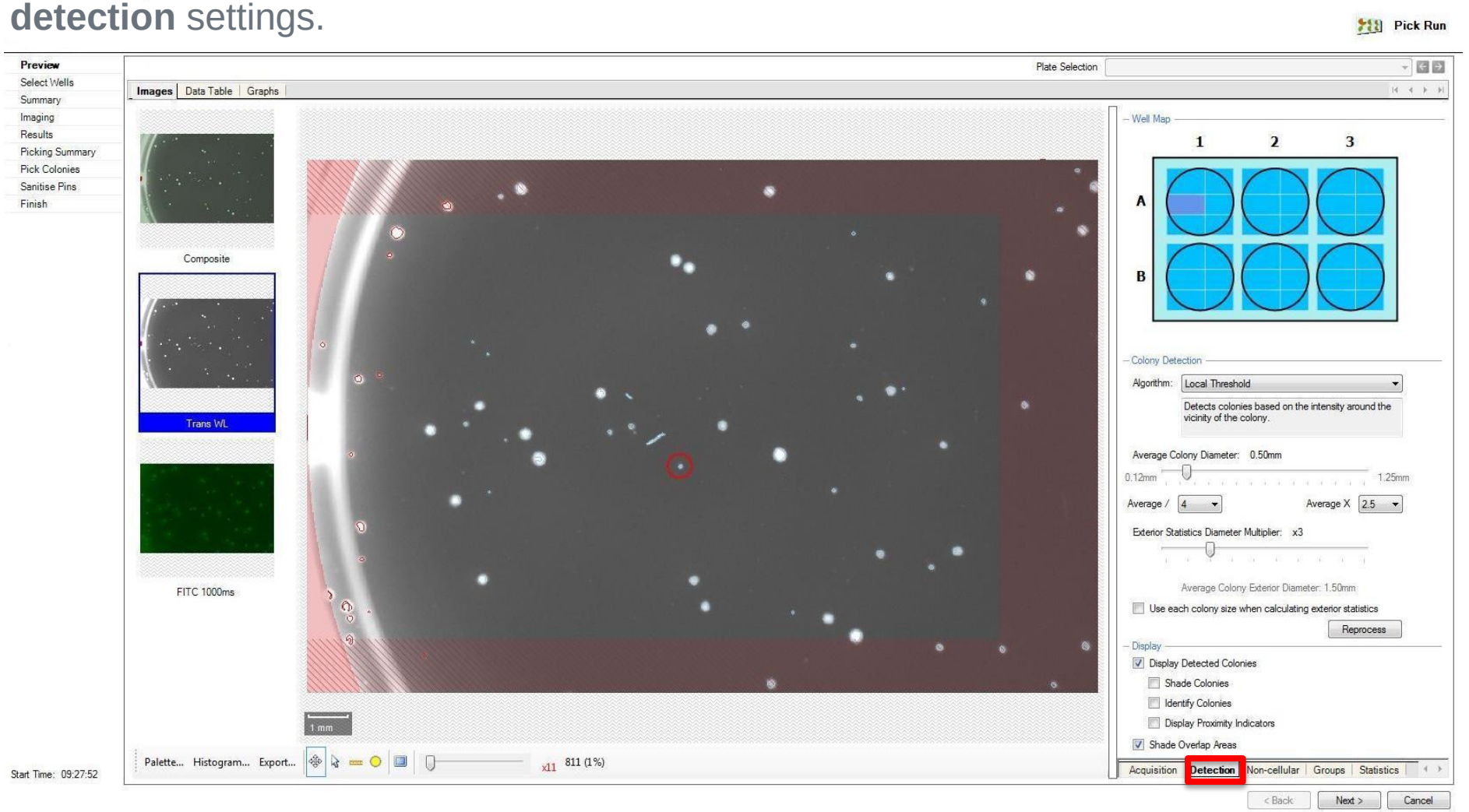
3. The **Preview** screen appears. Here you will first define your **image acquisition settings**.
   h) There will now be a main image with 3 thumbnails to the left named **Composite**, **TransWL** and **FITC 1000ms**.
   - Toggle between the thumbnails to inspect the images.
   - **Red pixels on the image indicate that it is overexposed**. In this case, lower either the **exposure time** or **LED Intensity** and **Grab Image** again. Adjust until there are no red pixels.





Carrying Out A Pick Run: Define Colony Detection Settings

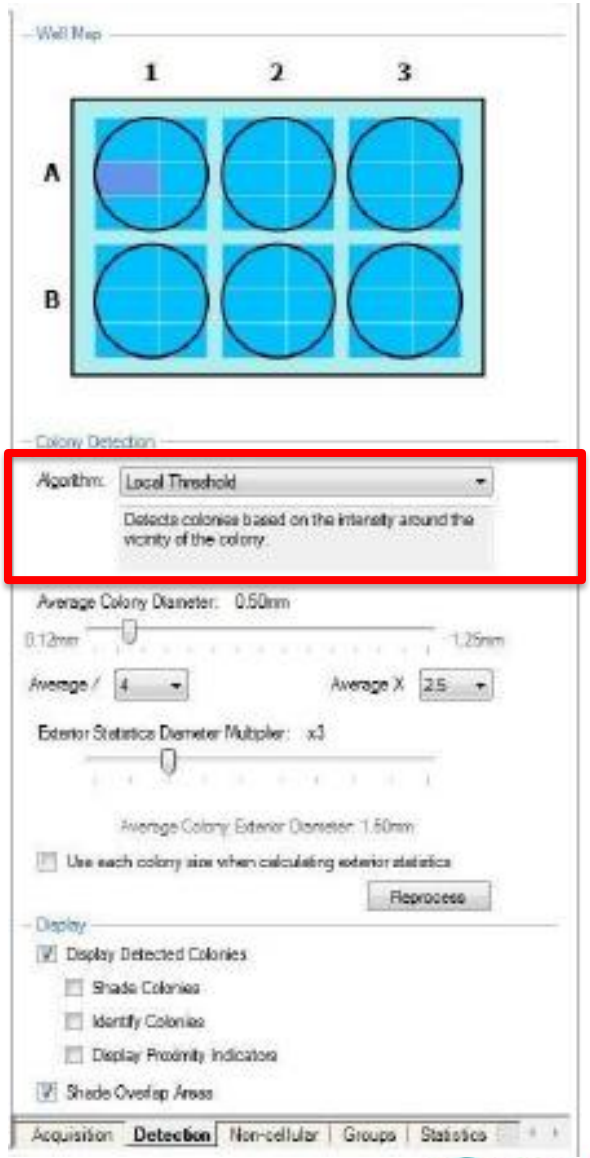
4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection** settings.



Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

a) Set **Algorithm** to **Local Threshold** from the drop-down menu.

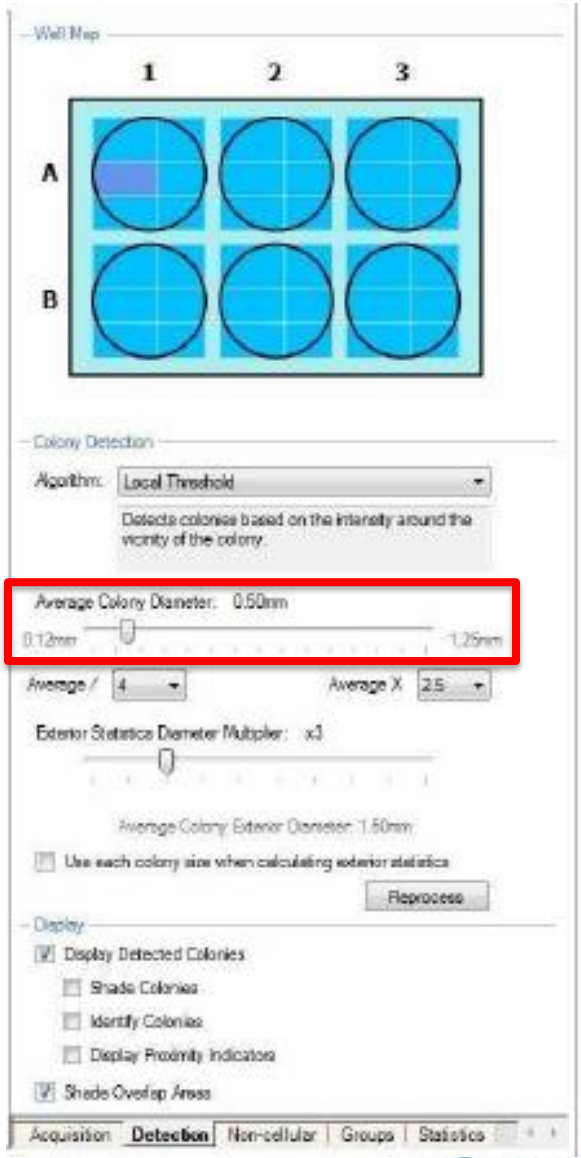


Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

a) Set **Algorithm** to **Local Threshold** from the drop-down menu.

b) Using the **White Light** image, set **the Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25 and 0.70mm** depending on the size of the



Carrying Out A Pick Run: Define Colony Detection Settings

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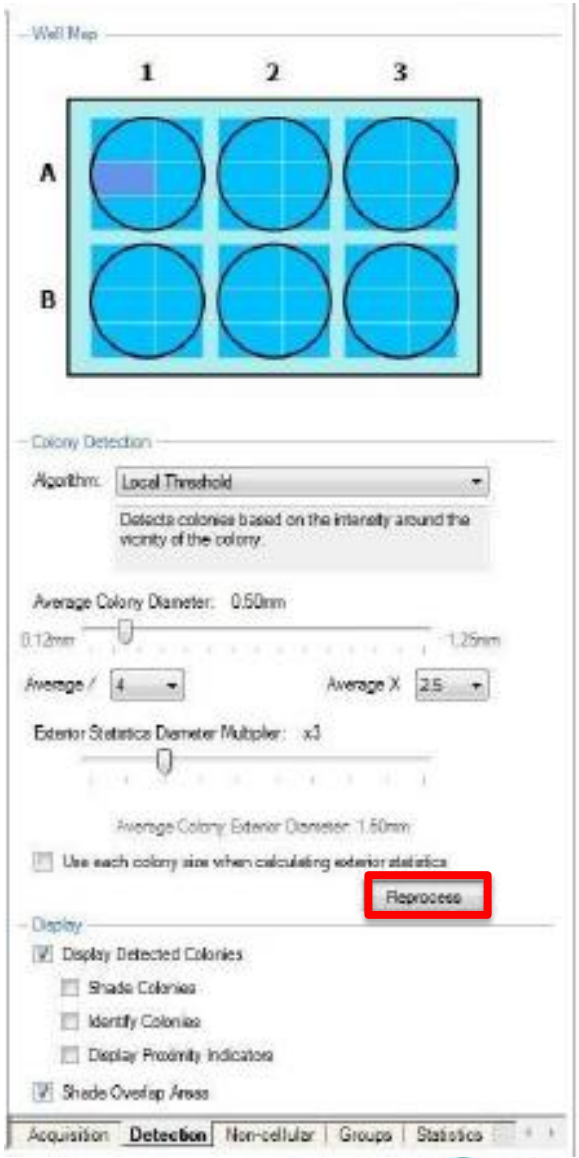
b) Using the **White Light** image, set **the Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25 and 0.70mm** depending on the size of the colonies.

c) Click **Reprocess** after moving the **slide bar** to apply the changes.



Carrying Out A Pick Run: Define Colony Detection Settings

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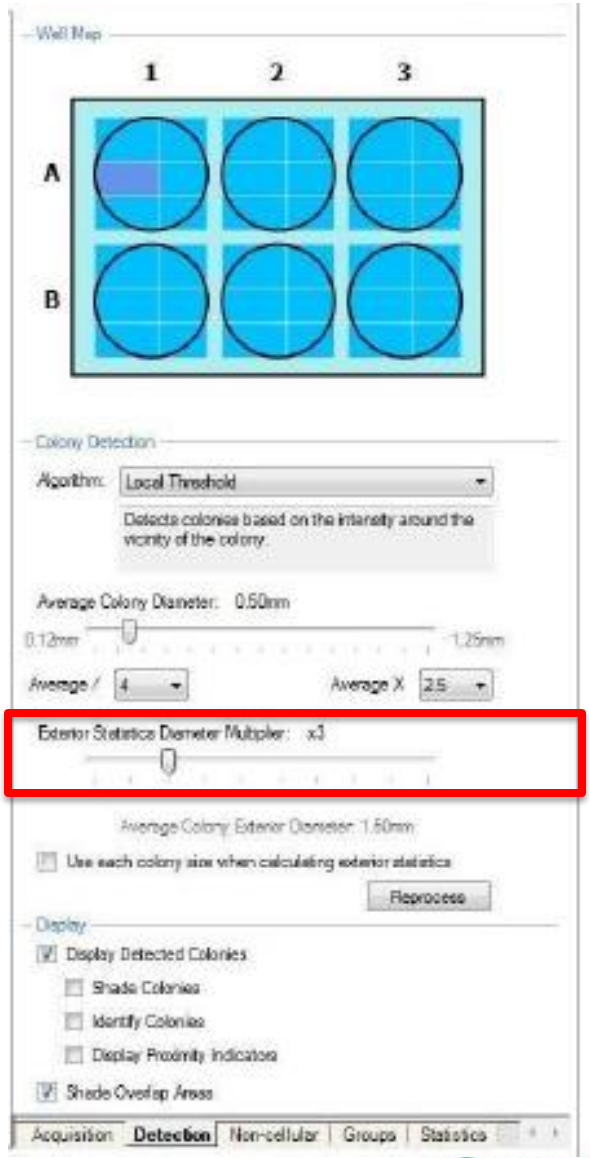
c) Click **Reprocess** after moving the **slide bar** to apply the changes.

d) Leave **Exterior Statistics Diameter Multiplier** at the default setting (**x3**).



Carrying Out A Pick Run: Define Colony Detection Settings

4) From the **Preview** screen, click on the **Detection** tab to define your **colony detection settings**.

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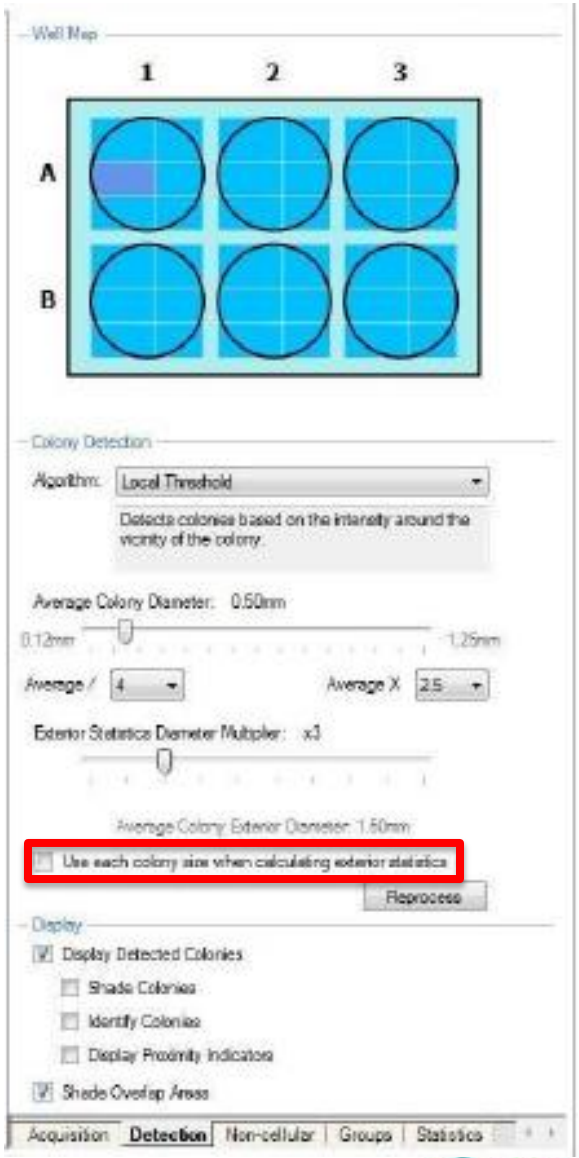
b) Using the **White Light** image, set **the Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25 and 0.70mm** depending on the size of the colonies.

c) Click **Reprocess** after moving the **slide bar** to apply the changes.

d) Leave **Exterior Statistics Diameter Multiplier** at the default setting (**x3**).

e) Select **Use each colony size when calculating exterior statistics**.



Carrying Out A Pick Run: Define Colony Detection Settings

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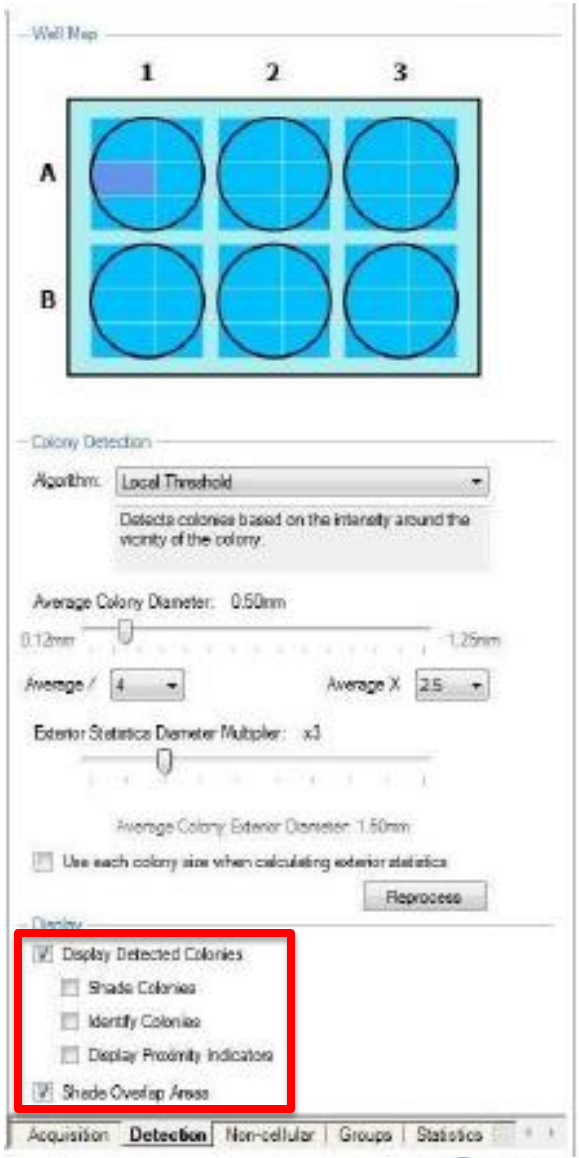
b) Using the **White Light** image, set **the Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25 and 0.70mm** depending on the size of the colonies.

c) Click **Reprocess** after moving the **slide bar** to apply the changes.

d) Leave **Exterior Statistics Diameter Multiplier** at the default setting (**x3**).

e) Select **Use each colony size when calculating exterior statistics**.

f) Leave **Display settings** as **default** (only **Display Detected Colonies** and **Shade Overlap Areas** selected).



Carrying Out A Pick Run: Define Colony Detection Settings

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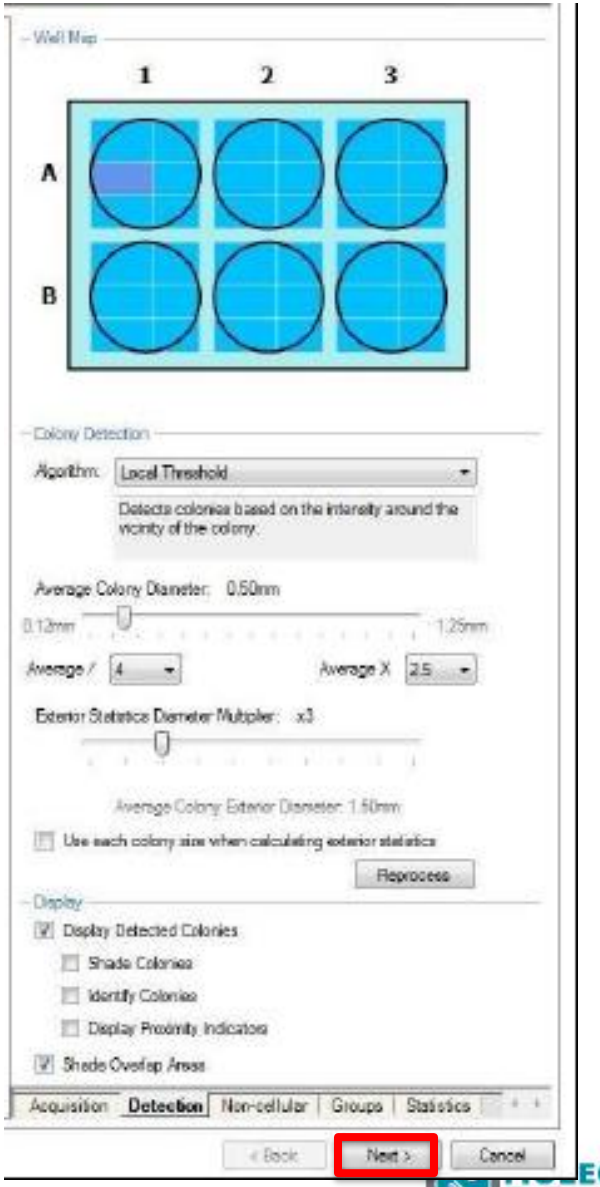
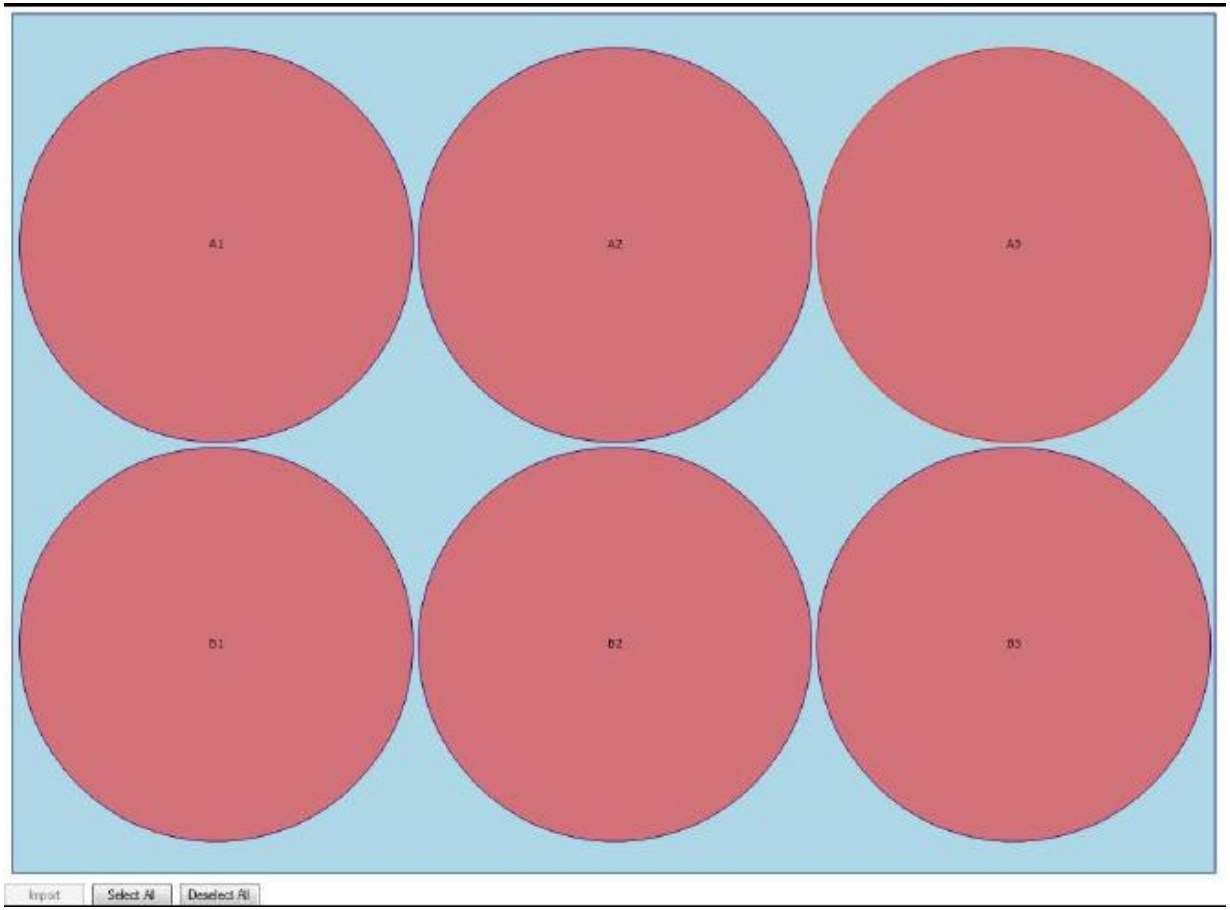
a) Set **Algorithm** to **Local Threshold** from the drop-down menu.
b) Using the **White Light** image, set **the Average Colony Diameter** to a size that best detects the colonies. This will probably be between **0.25 and 0.70mm** depending on the size of the colonies.
c) Click **Reprocess** after moving the **slide bar** to apply the changes.
d) Leave **Exterior Statistics Diameter Multiplier** at the default setting (**x3**).
e) Select **Use each colony size when calculating exterior statistics**.
f) Leave **Display settings** as **default** (only Display Detected Colonies and Shade Overlap Areas selected).
g) Click **Next** to proceed.



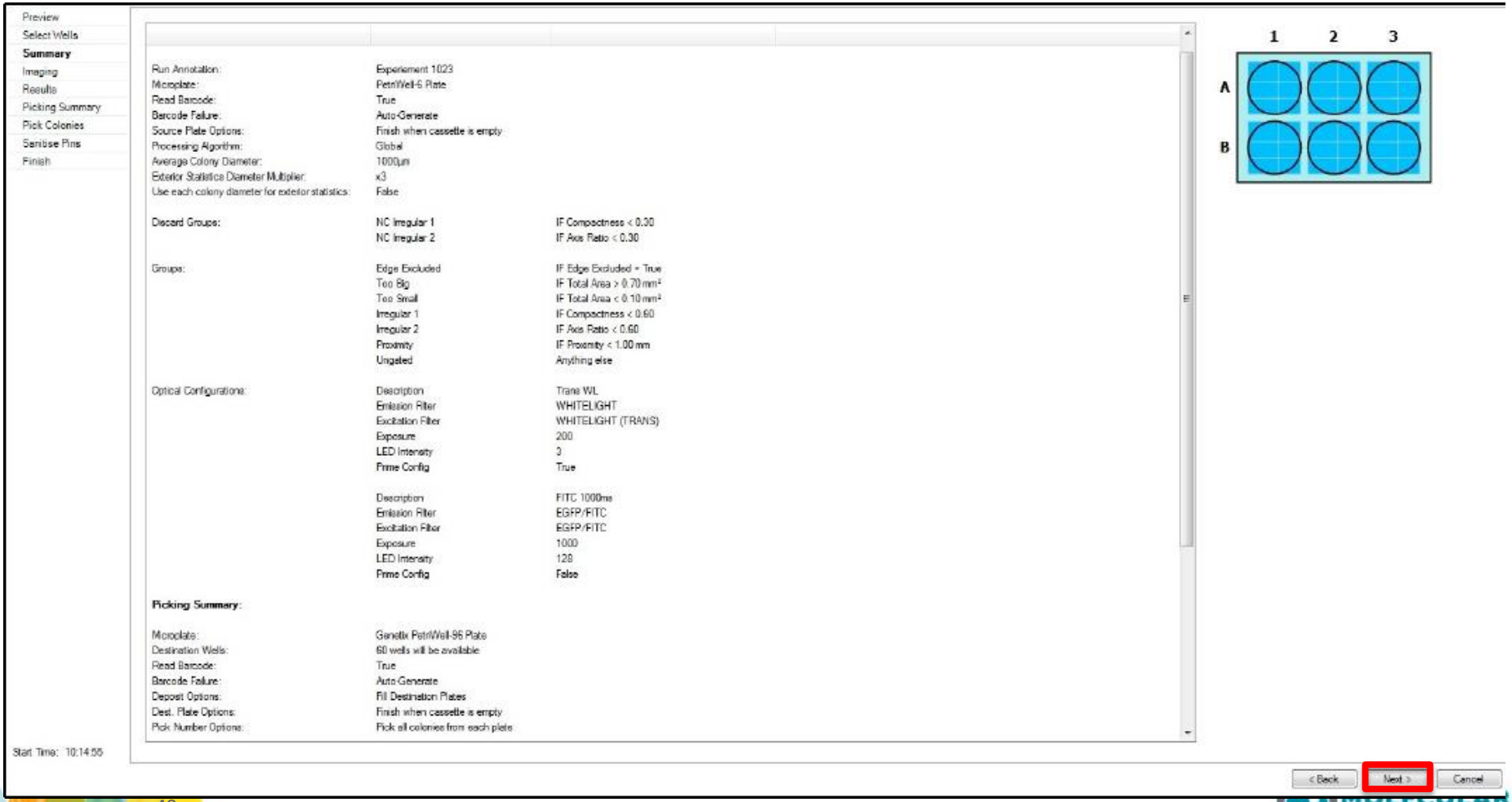
Carrying Out A Pick Run: Select Wells

5) The **Select Wells** window appears. Click on your source plate wells to select which wells to pick from – selected wells will be highlighted in **pink** as shown below. The **Select All** or **Deselect All** buttons can also be used to add or removed wells to be imaged & picked. Click **Next** to proceed.



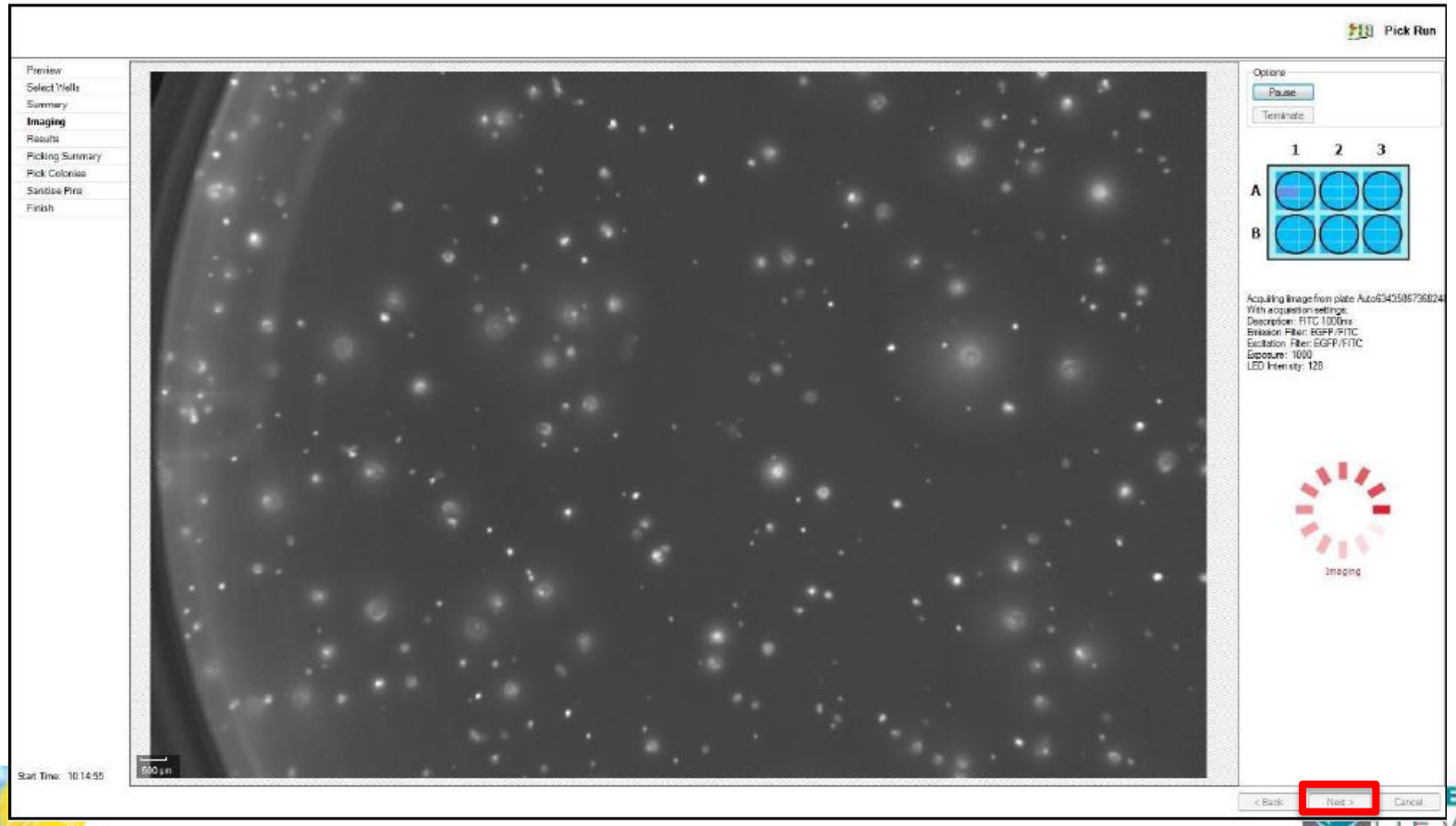
Carrying Out A Pick Run: Summary

6) The **Summary** window appears. This window provides a summary of the imaging and picking parameters that will be used for the run. This is a good point to check all settings are correct before proceeding to imaging. Click **Next** to proceed.



Carrying Out A Pick Run: Imaging

7) The **Imaging** window appears. This screen displays the images that are being captured as imaging is taking place. The selected wells will be imaged in sequence for each of the acquisition options selected. The images will then be processed together for colony detection according to the defined settings. Once imaging is complete, click **Next** to proceed.



Carrying Out A Pick Run: Results Review

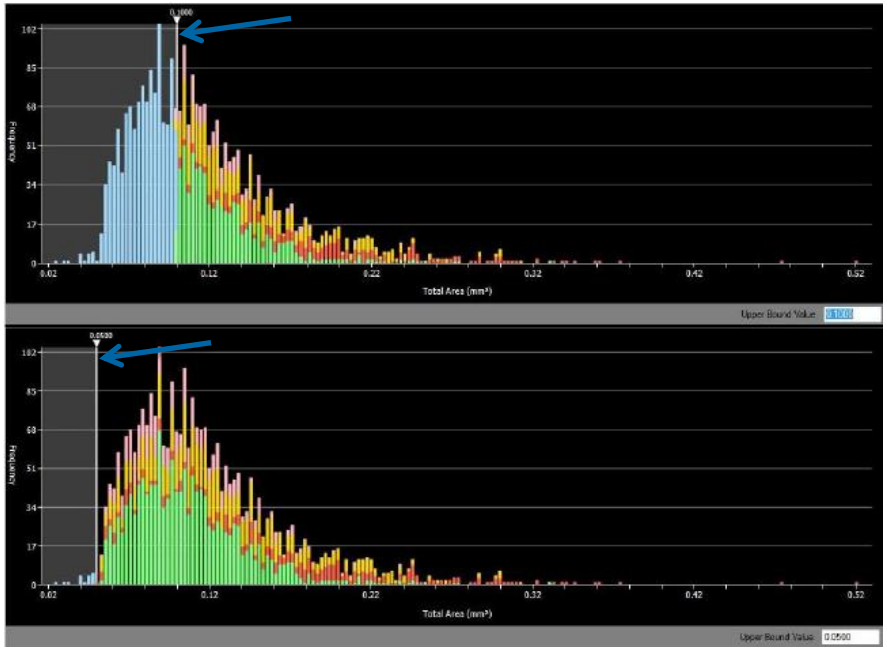
8) The **Results** window appears. This screen displays the results of the imaging run/colony analysis. Click on the **Graphs** tab (upper left), then the **Groups** tab (lower right) to proceed with defining colonies to pick.

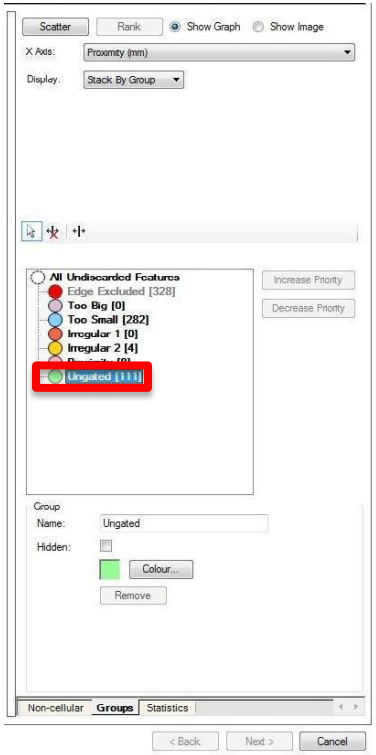
Carrying Out A Pick Run: Refining Default Groups of Colonies

9) Any default **group** can be altered but this may compromise clonality and viability. Where possible it may be best to leave the groups with the default values (with exception of the **Too Small** cutoff, see below).

a) Groups can be edited by **double clicking** on the desired **group** and **moving the default gate**.

b) For Example: the **Too Small** group cut-off point can be reduced by double clicking on the group (which will display the current cut-off value) and then **dragging the slide bar on the histogram** to 0.05 or the desired value.





Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

a) To further isolate only the colonies in the **Ungated** group that have highest associated FITC fluorescence, click **All Undiscarded Features** to display all the colonies on the histogram.

Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

a) To further isolate only the colonies in the **Ungated** group that have highest associated FITC fluorescence, click **All Undiscarded Features** to display all the colonies on the histogram.

b) Select **[FITC] Exterior Mean Intensity** from the **Histogram drop-down** menu.

Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

a) To further isolate only the colonies in the **Ungated** group that have highest associated FITC fluorescence, click **All Undiscarded Features** to display all the colonies on the histogram.

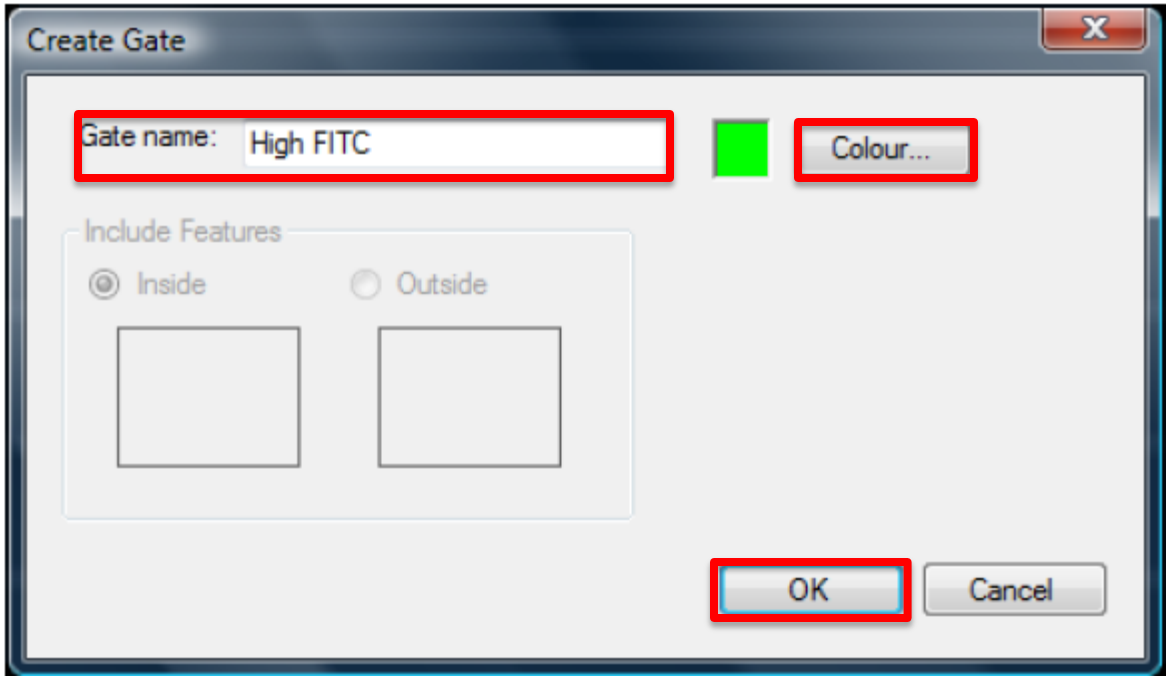
b) Select **[FITC] Exterior Mean Intensity** from the **Histogram drop-down** menu.

c) Using the **Add new line** gate tool, draw a gate on the histogram by **clicking on the histogram where the cut-off point will be and then drag the gate off the right end of the histogram – Click and Drag**.

Carrying Out A Pick Run: Defining Groups of Colonies to Pick

10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

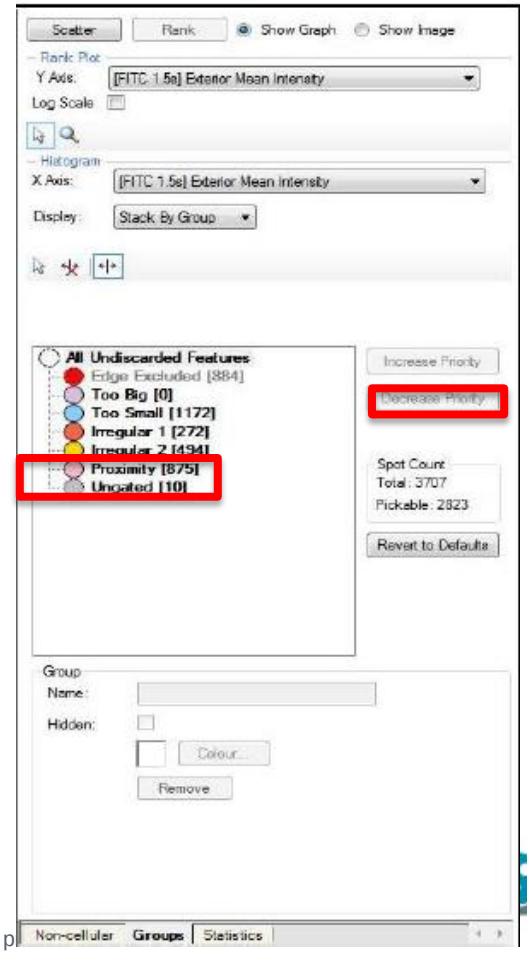
d) The **Create Gate** window will appear where it is possible to name the group by typing for example ‘High FITC’ in the **Gate name** field and assign a group color by clicking on the **Colour button**. Click **OK** to complete this process.



Carrying Out A Pick Run: Defining Groups of Colonies to Pick

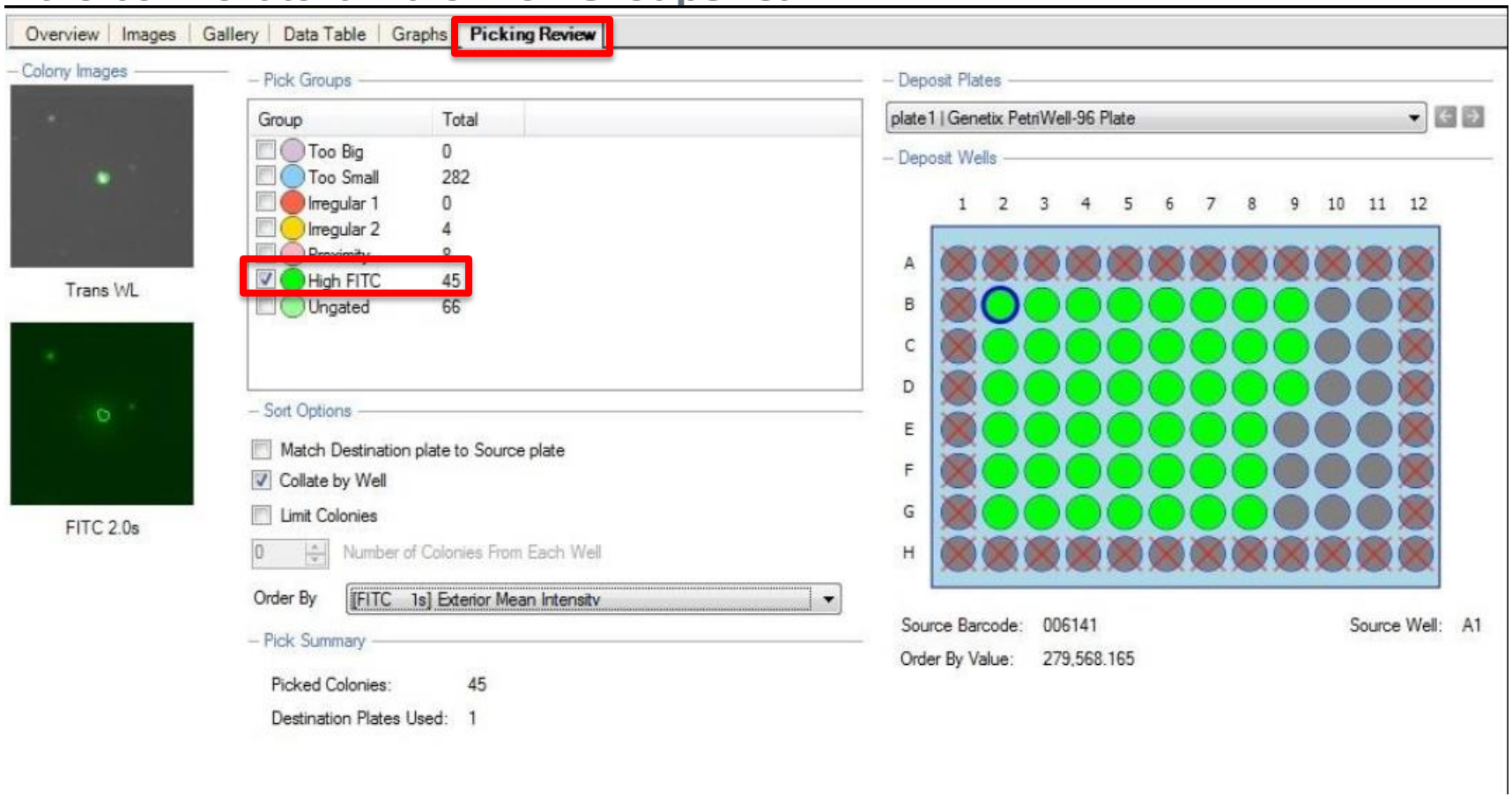
10) Colonies in the Ungated group are those that have good size, shape and distance from other colonies.

e) You will now be returned to the main graph window. Click on your **newly-created group in the All Undiscarded Features list** and then click on the **Decrease Priority** button until your new group sits just **above the Ungated group** in the list, then click **Next** to proceed.



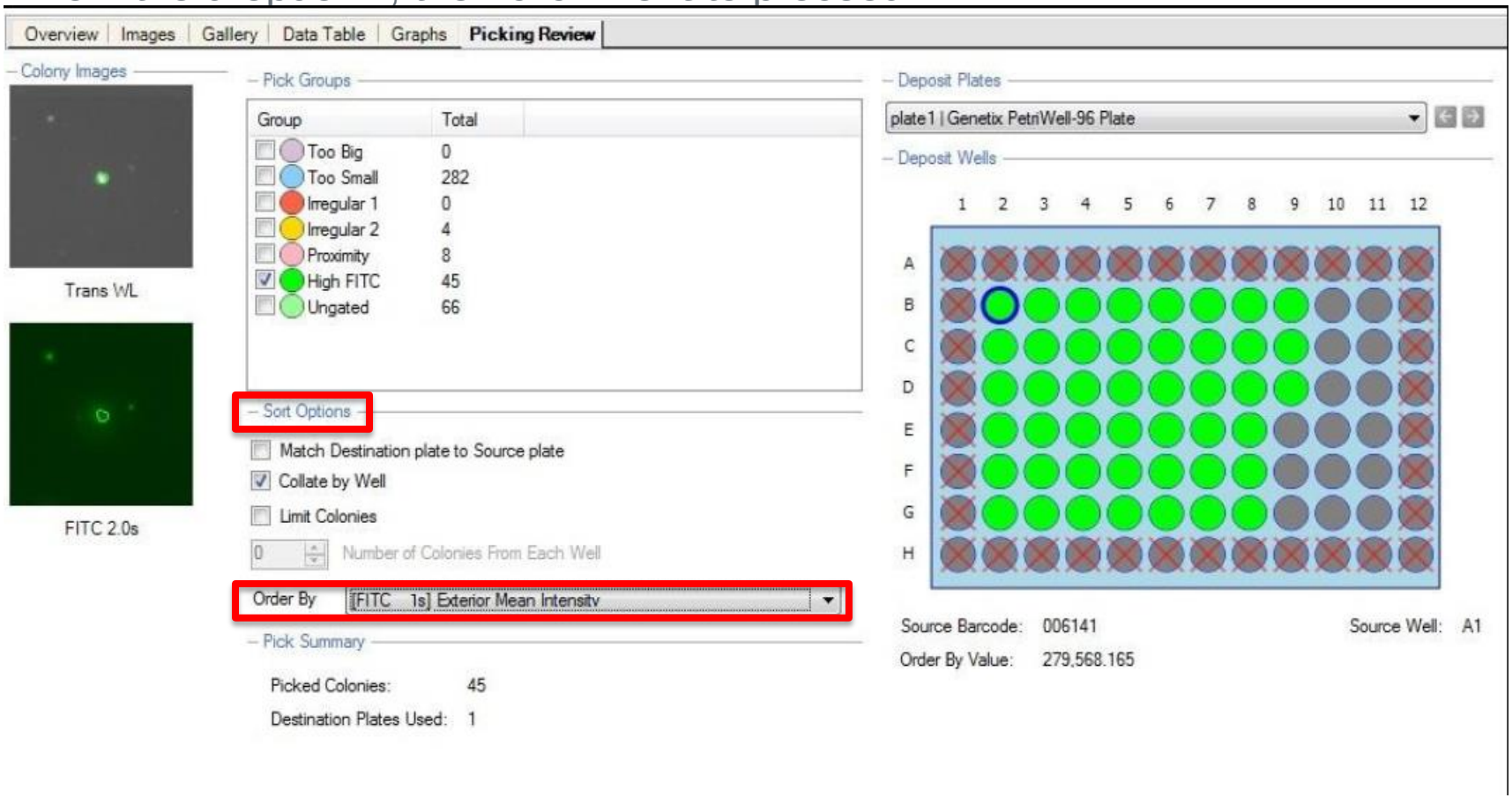
Carrying Out A Pick Run: Picking Review

11. The **Picking Review** window appears.
    a) In the **Picking Review tab**, select only the **High FITC group** by checking the box next to it in the **Pick Groups** list.



Carrying Out A Pick Run: Picking Review

11. The **Picking Review** window appears.
    b) Under **Sort Options**, select **Order By [FITC 1s] Exterior Mean Intensity** from the dropdown, then click **Next** to proceed.



Carrying Out A Pick Run: Proceed With Picking

12) The pick run will now be initiated.
    a) When prompted, load the **destination plate(s) into the destination stacker cassette** (the number of destination plates required will be displayed in the **Pick Summary** on the **Picking Review** tab).
    b) Click **Next** to proceed.
    c) The picking step will proceed automatically until all colonies in the selected group have been collected.

Finishing the Picking Process

12) Once the pick run has completed:
    a) Click **Finish** to return to the **Pick Run process top page**.
    b) Click **Close Process** to return to the **Main Navigation Screen**. If settings have not been saved previously a prompt will warn of this and allow settings to be saved.
    c) To view the results of the picking run, click on the **Review Results** icon.

ClonePix 2 Instrument Shut Down Procedure

13) Please follow these steps to shut down the ClonePix 2 instrument after a pick run:
    a) Exit from the ClonePix 2 application by selecting **Exit** from the **File** menu on the main setup screen.
    b) Close down Windows – Click the **Start** menu at the bottom of the screen then click **Shut Down**.
    c) Wait for computer to switch off completely.
    d) Turn the instrument off by pressing the **Stop** button on the front of the system.
    e) Turn the power off at the mains.

Support Resources

- Go to the HELP menu within ClonePix 2 Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- Request Support: http://mdc.custhelp.com/app/ask or via email support@moldev.com
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Biotherapeutics Products → ClonePix Instruments

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