ClonePix™2

Mammalian Colony Picker

Software Version 1.6

Quick Start Guide

FORTÉBIO
Biologics by Molecular Devices

07MAN1181.F
May 2019

Product manufactured by Molecular Devices, LLC.
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Chapter 1: Introduction

ClonePix™ 2 Software controls the process of selective mammalian cell picking by the ClonePix 2 system. The software is designed to simplify the day-to-day interaction with the instrument. It permits multiple users to select and collect colonies of suspension cells from semi-solid medium or adherent colonies from liquid medium. The colony detection feature contains powerful algorithms for selecting cell colonies based on physical characteristics and proprietary multi-channel fluorescence technology for detecting protein secretion or production.

Your ClonePix 2 Mammalian Colony Picker is installed by a Molecular Devices Field Service Engineer. Use the following procedures to get started using your system. Additional information can be found in the *ClonePix 2 Mammalian Colony Picker User Guide*.

- Before You Start on page 7
- Powering On the System on page 9
- Loading Plates on page 11
- Preparing for a Pick Run on page 13
- Using Pick Run on page 15
- Powering Off the System and the Software on page 31
- Obtaining Support on page 5

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website, www.moleculardevices.com/service-support, has a link to the Knowledge Base, which contains technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, you can submit a request to Molecular Devices Technical Support.

You can contact your local representative or Molecular Devices Technical Support at 800-635-5577 (North America only) or +1 408-747-1700. In Europe, call +44 (0) 118 944 8000.

Please have your instrument serial number or Work Order number, and your software version number available when you call.

WARNING! BIOHAZARD. It is your responsibility to decontaminate components of the instrument before you return parts to Molecular Devices for repair. Molecular Devices does not accept items that have not been decontaminated where it is applicable to do so. If parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

Chapter 2: Before You Start

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Before you power on the ClonePix2 instrument and start the software, do the following:

- Verify that the instrument and compressor are plugged in.
- Verify that the correct picking pins are installed in the picking head for the type of cells to be picked.

  Tip: The picking pin type is engraved at the base of each pin. You might need to remove the pin from the head to confirm the pin type.

  - **F1 Picking Pins** (400µm internal diameter; X4961) for suspension cell picking from semi-solid medium.
  - **F2 Picking Pins** (700µm internal diameter; X4962) for adherent cell picking from liquid medium.

    Before a Pick Run, picking pins should be cleaned by sonication in a 2% solution of QuClean (K2505) and autoclaved. It is advisable to autoclave the Picking Pin Removal Key (X4948) at the same time.

    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.
- Verify that instrument bed is clear of obstructions and loose items.
- Verify that all motor tracks are free of obstruction.
- Verify that there are no obstructions to movement of the picking head.
- Verify that the main instrument axes, XY, are roughly in the center of their respective travel positions. The axes need to be away from their respective end-stops on start up so that they can reference themselves correctly to their home positions.
- Wipe the instrument bed with 70% ethanol using a lint-free cloth.
- Verify that no plates or cassettes are in the stackers.
- Verify that the **Emergency Stop** button on the front panel of the instrument is pulled out.
- Fill the ethanol feed bottle with 70% ethanol and empty the ethanol waste bottle, as needed.
- Separately autoclave the picking feed bottle and cap assembly, then fill the Picking Feed bottle with sterile deionized water and cap the bottle in a sterile tissue culture hood.
- Ensure that your picking waste bottle is empty before continuing.
- Verify that the enclosure front door is closed.

- Wait for approximately 2 minutes after the instrument is powered on before opening the software to ensure that all drives 'home' correctly. See Powering On the System on page 9.

WARNING! For safety reasons the front door interlock should never be interfered with or overridden. If the door is opened at any stage, this triggers the actuator head or germicidal lamp to stop until the door is closed.

Chapter 3: Powering On the System

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Before continuing, do as instructed in Before You Start on page 7.

Every time the instrument is used, the three axes sequentially run through their “Initialize drives” routine. This enables the drives to find their respective home positions. The system must complete this routine without interference to ensure that there is no damage to the instrument or its auxiliary equipment.

To power on the system:

1. Power on the compressor and ensure compressed air gauge is set to 80 psi (5.5 bar).
2. On the front panel of the instrument, push the **Start** button.
   The **Power On** light illuminates.

   Tip: If the power to the system does not turn on, make sure that the door is closed and the **Emergency Stop** button is pulled out.

   The instrument cycles through various start-up processes indicated on the front indicator panel.
3. Power on the computer and wait for it to finish initializing.
4. From the computer desktop, double-click on the ClonePix 2 icon.

Chapter 4: Loading Plates

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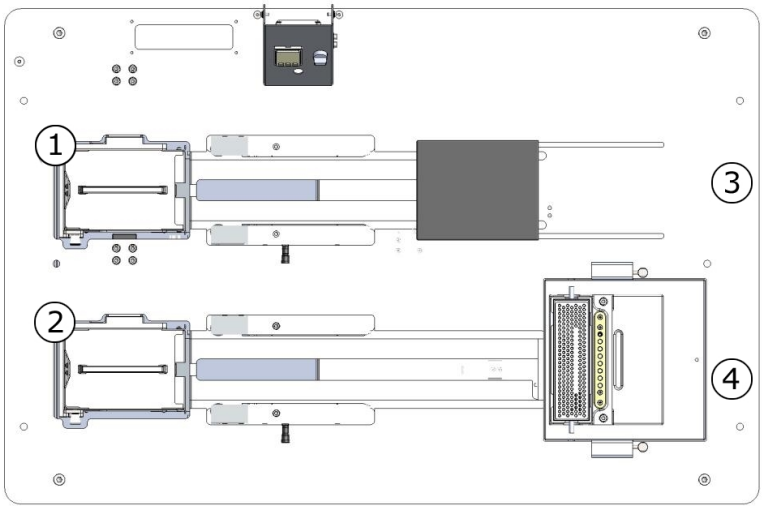
It is important to load plates correctly.

The Source plate stacker system is located in the rear of the instrument deck, and the Destination plate stacker system is in the front of the deck. Both the Source stacker and Destination stacker contain one removable cassette on the left side.



Number	Description
1	Source Stacker
2	Destination Stacker
3	Imaging Station
4	Wash Station

Each stacker holds a maximum of 10 microplates. Plates feed individually from the stacker using the plate holder onto the bed for automatic lid removal as the plate passes the plate lid lifter. At the end of the colony picking for a plate, the de-lidded plate returns to the stacker where the lid is replaced on the way back to the stacker cassette.

When loading plates do the following:

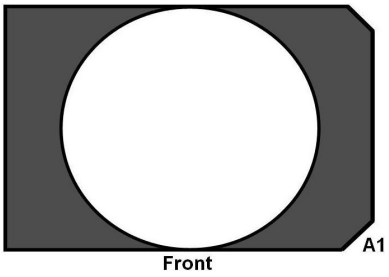
- Install each stacker cassette in the instrument and verify that the locking bolt holds the mechanism firmly.

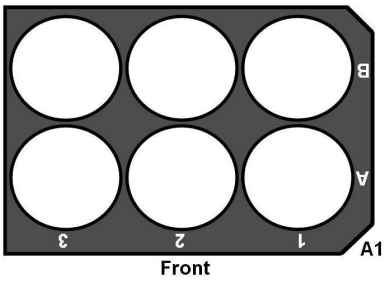
CAUTION! Failure to lock a stacker mechanism can cause a malfunction during the microplate transfer processes.

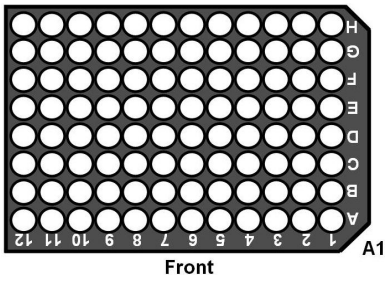
- Only load plates that match the plate type selected in the software.

CAUTION! You can damage the instrument loading a plate different than the plate type selected in the software.

- For proper plate orientation, load each plate, with a lid on and the A1 well in the front right corner, onto the appropriate coded plate holders. For guidelines, see Appendix F of the *ClonePix 2 Clone Picking System Software User Guide*.







- For proper positioning in the stacker, load plate holders with plates into a stacker levelly and flush with the back of the stacker mechanism.

Tip: When positioned properly within the stacker cassette, you will hear the magnet on the plate holder engage.

Chapter 5: Preparing for a Pick Run

Before starting a **Pick Run** process, make sure that your ClonePix 2 system is set up correctly.

The **Prepare for Pick Run** process must be done every time that your ClonePix 2 system is first powered on. For detailed procedures, see the *ClonePix 2 Mammalian Colony Picker Software User Guide*.

The **Prepare for Pick Run** process helps you to validate the following:

- The pins are firing correctly.
- The camera, pins and microplates are aligned.
- The fluid system is sterile and ready for use.

To do the **Prepare for Pick Run**:

1. With the software running, on the Main Navigation screen, click **Prepare for Pick Run**, and then follow the on-screen instructions.
2. When prompted to load a source plate, use an empty source plate type.

   Tip: After alignment using any configured plate type, your system is ready for use with any configured plate type.

3. When finished, click **Close Process** to return to the **Main Navigation** screen.

Chapter 6: Using Pick Run

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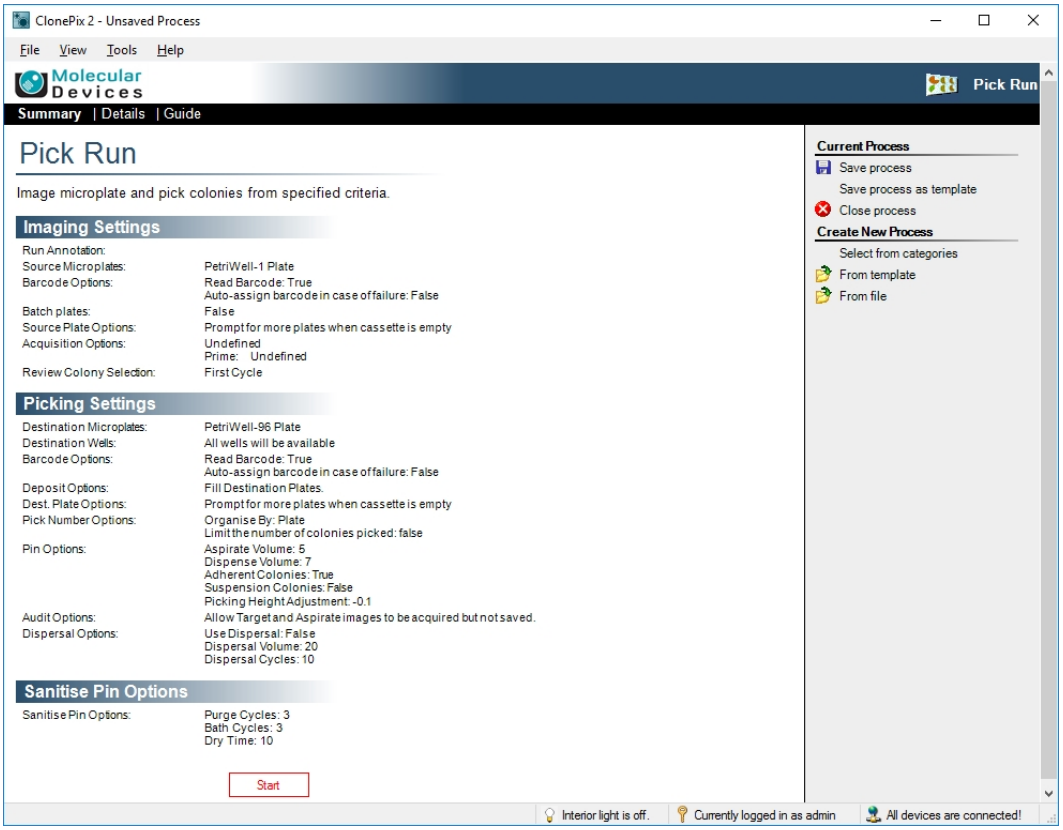
The **Pick Run** process guides you through your picking run.

The following procedure assumes that you have plates of cell colonies with fluorescent halos of secreted protein.

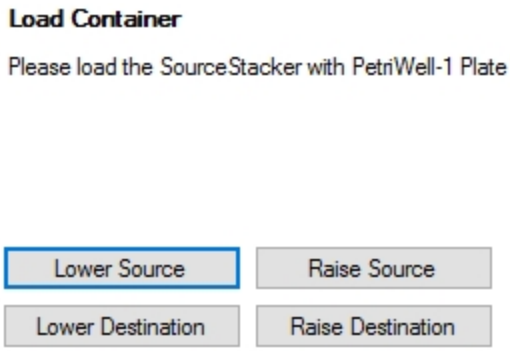
To begin a Pick Run

1. Before you continue, if needed, make setting changes. On the **Pick Run > Start** screen, you can change the following settings:
   - Imaging Settings on page 16
   - Picking Settings on page 18
   - Sanitize Pin Options on page 20



2. To start the run process, click **Start**.

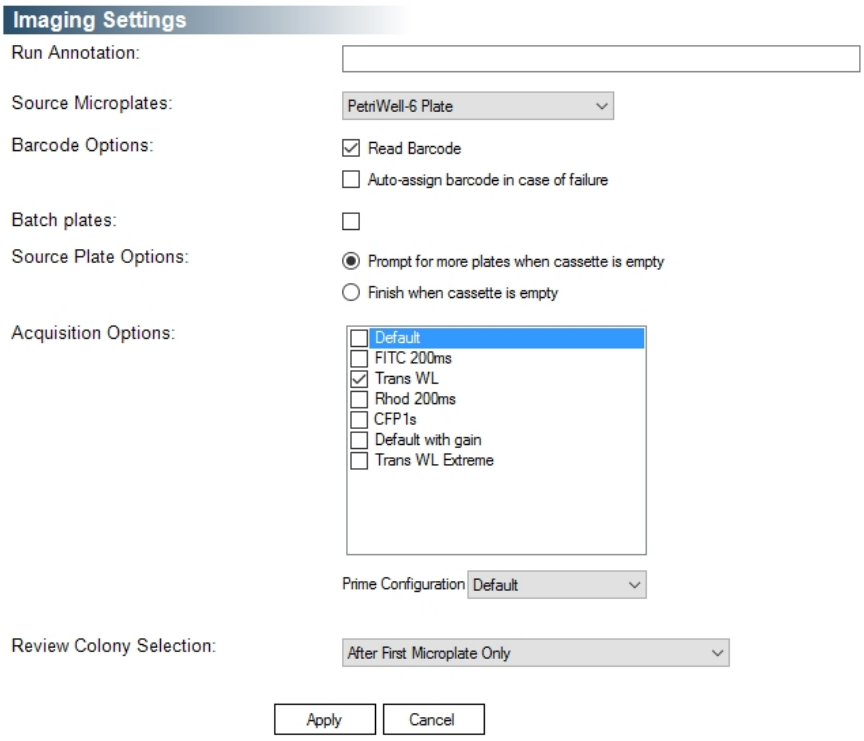
3. When **Load Container** appears, load the source plates into the source stacker.



4. If needed, to raise or lower a stacker, click the appropriate software button.
5. Click **Next**.

Imaging Settings

To edit **Imaging Settings**, click the **Imaging Settings** title, or alternatively, click the **Details** tab or click the **Guide** tab.



You can change the following:

- **Run Annotation**—Type a name to identify the run.
- **Source Microplates**—Select the source well plate type.

- **Barcode Options**—Select one of the following options:
  - **Read Barcode**—Activates the source plate barcode reader.

    Note: Must be activated for a **Batch Pick Run**.

    Tip: Using barcodes is recommended. Barcodes must be placed on the front short side of the microplate (A1 end).

  - **Auto assign barcode in case of failure**—Automatically generates a unique plate identifier if the barcode fails to read.
    If this check box is not selected, the system requests an identifier to be typed manually.

    Note: Compatible barcodes for the system are 39, 93, and 128.

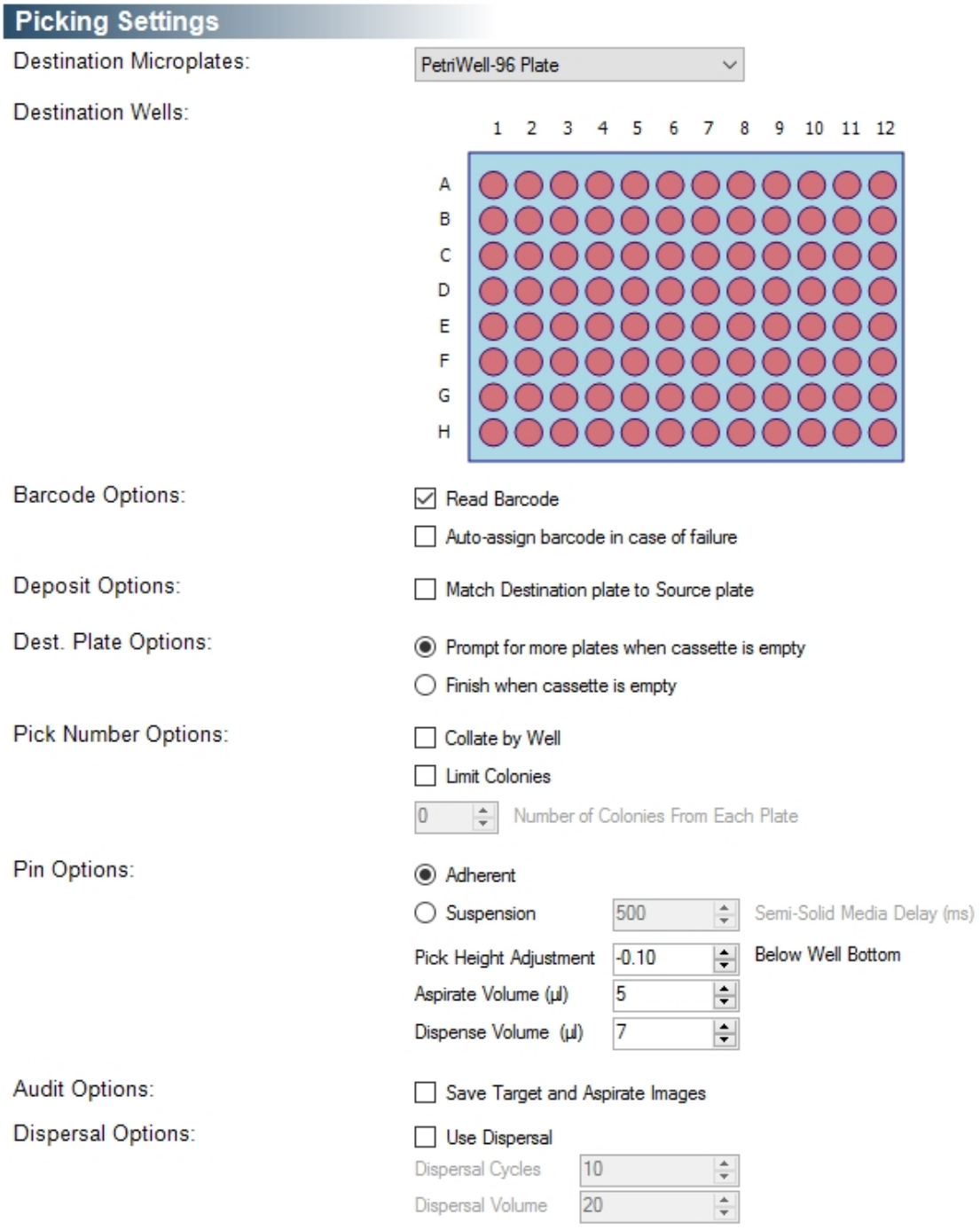
- **Batch Plates**—Selects the **Batch Pick Run** option where all plates in the cassette are imaged and statistics are generated as an aggregate for all plates. The colony selection for the whole series of plates can be reviewed and adjusted before picking.
- **Source Plate Options**—Select one of the following options:
  - **Prompt for more plates when cassette is empty**—Prompts you for more plates to be added to the cassette after all the available plates are imaged in the cassette.
  - **Finish when cassette is empty**—Ends the imaging process after all the available plates are imaged in the cassette.
- **Acquisition Options**—Select from the list of acquisition options that have already been created. Your selection can be further modified in **Preview**.
- **Prime Configuration**—Use for colony detection. Typically, this is the white light acquisition option.
- **Display Results**—Select to display the results at the end of the run.

  Tip: If this option is not selected, the run ends, and results can be viewed in **Review Results**. See Review Results Process on page 28.

To save your settings, click **Apply**.

Picking Settings

To edit Picking Settings, click the **Picking Settings** title, or alternatively, click the **Details** tab or click the **Guide** tab.



You can change the following:

- **Destination Microplates**—Select the type of destination well plates.
- **Destination Wells**—Specify the deposit wells to be used. All destination plates are filled using this template.

  Tip: Left-click and drag to select a group of wells. Right-click and drag to deselect a group of wells.

- **Barcode Options**—Select one of the following options:
  - **Read Barcode**—Activates the source plate barcode reader.

    Note: Must be activated for a **Batch Pick Run**.

    Tip: Using barcoded source and destination plates is recommended. Barcodes must be placed on the front short side of the microplate (A1 end).

  - **Auto assign barcode in case of failure**—Automatically generates a unique plate identifier if the barcode fails to read.
    If this check box is not selected, the system requests an identifier to be typed manually.

    Note: Compatible barcodes for the system are 39, 93, and 128.

- **Deposit Options**—When not selected (default), all destination plate wells are sequentially filled. When selected, the destination plate is matched to the source plate such that the completion of picking from each source plate prompts the return of the current destination plate.
- **Destination Plate Options**—Select one of the following options:
  - **Prompt for more plates when cassette is empty**—Prompts you for more plates to be added to the cassette after all of the available plates are imaged in the cassette.
  - **Finish when cassette is empty**—Ends the imaging process after all of the available plates are imaged in the cassette.
- **Pick Number Options**—Colonies can be picked by source plate or by source well. By default, picking continues as if all wells of the current plate contain the same sample and are organized by source plate. Select one of the following options:
  - **Collate by Well**—When selected, the system picks all specified colonies from well A1 of the source plate first and then proceeds to the next well. Select this option when wells of a multi-well source plate contain different samples.
  - **Limit Colonies**—Limits the number of colonies picked from each plate or well.

- **Pin Options**—Select one of the following options:
  - **Adherent or Suspension**—Select the **Adherent** option for collection of colony monolayers grown in liquid culture. Select the **Suspension** option for collection of colonies from semi-solid medium. By default, there is a 500ms delay for Suspension picking to permit the colonies to be fully collected from the semi-solid medium.
  - **Pick Height Adjustment**—The pick height is automatically adjusted for optimal Adherent or Suspension picking (by default these are 0.1 mm below and 0.4 mm above well bottom, respectively).
  - **Aspirate Volume**—Recommended volume is 5µL.
  - **Dispense Volume**—Recommended volume is 7µL.
- **Audit Options**—Saves the target and aspirate images before and after picking a colony. If this option is selected, all images are saved. If this option is not selected, you can toggle the images on and off while picking but the images are not saved.

  Note: This option slows picking dramatically.
- **Dispersal Options**—Selecting dispersal separates the cells of a picked colony by aspirating and dispensing within the destination plate. If dispersal is required, the following parameters can be set:
  - **Dispersal Cycles**—3 to 6 for CHO cells and 6 to 10 for hybridomas. Small colonies in chemically defined media can be left undispersed.
  - **Dispersal Volume**—Maximum volume that can be dispersed is 20µL.

To save your settings, click **Apply**.

Sanitize Pin Options

Leave the default settings.

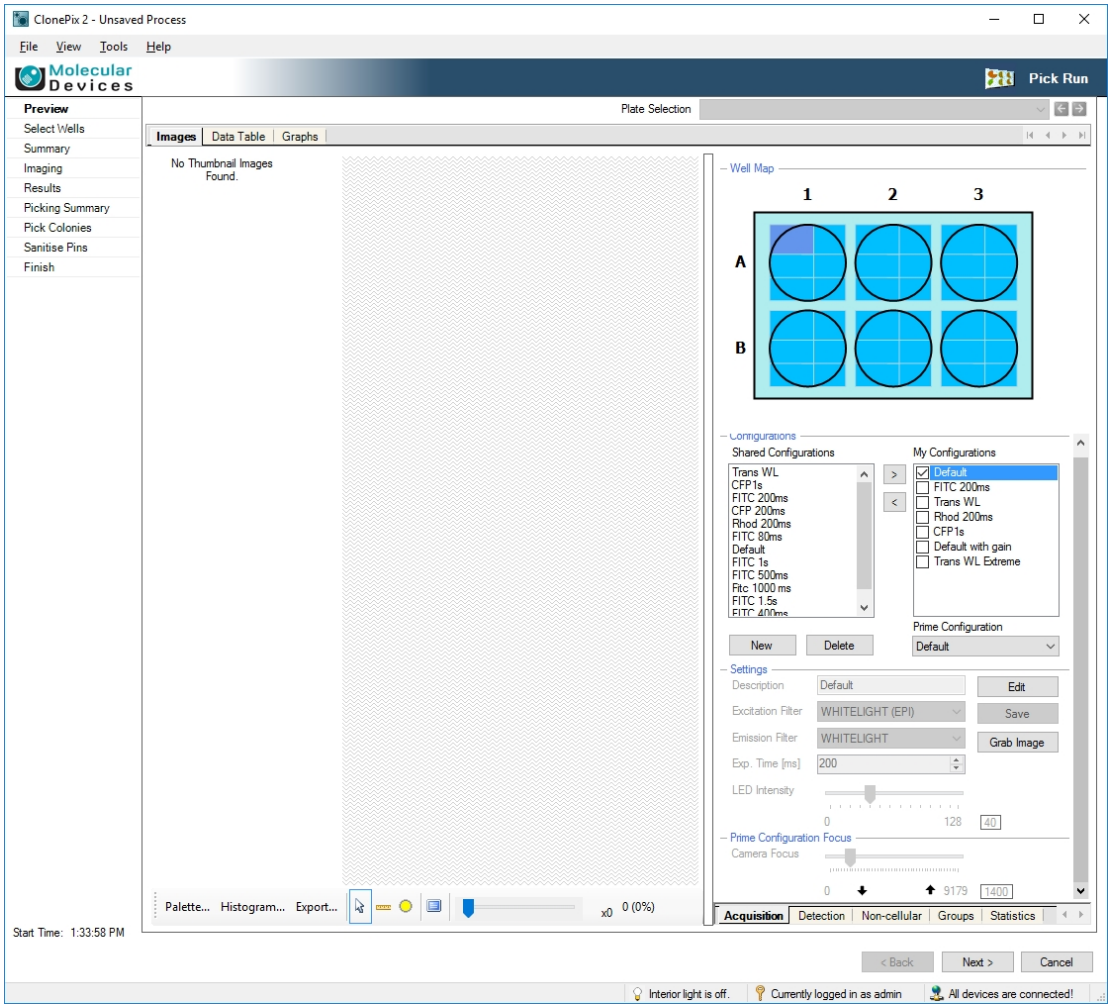
Start Pick Run

See To begin a Pick Run on page 15.

Preview

The **Preview** screen appears at the start of the image acquisition and colony detection set up.

The first screen that appears in Preview mode is the **Images** tab screen. This initially appears as a gray screen until an image is selected on the well map to acquire the images.



Set Up Acquisition Settings

1. Click the **Acquisition** tab.
2. In **My Configurations**, if you need to create a new acquisition option for selection, click **New**, and then click **Edit**. Use the following settings:

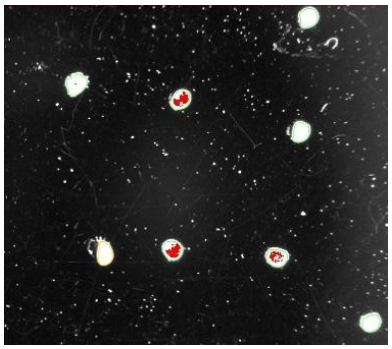
Description	White Light	FITC 1s
Excitation Filter	WHITELIGHT (TRANS)	EGFP/FITC
Exp. Time*	200 (SBIG) 75,000 (Basler)	1,000 (SBIG) 375,000 (Basler)
LED Intensity	3	128
Camera Focus	2200	2200
* The time value depends on which camera is installed in your system. The SBIG camera uses ms, and the Basler Pylon camera uses µs.		

3. To store the new option, click **Save**.

4. Select **TransWL** and **FITC 1000ms** options.

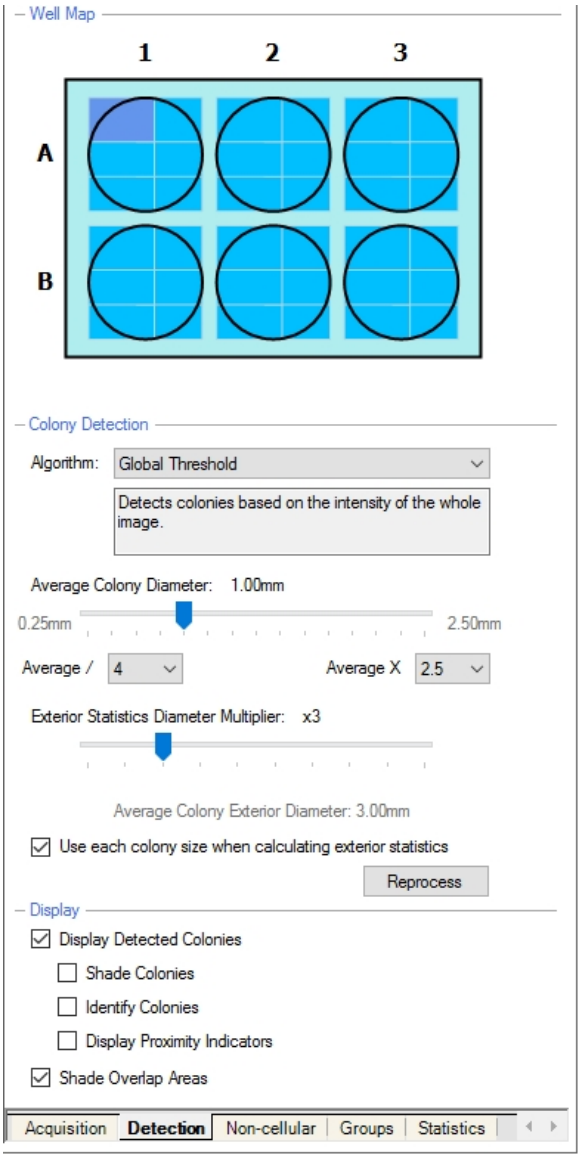
   CAUTION! Do not delete the **Default** option. It is required for other functions.

5. For **Prime Configuration**, select **TransWL**.
6. To capture the images:
   - For the area currently highlighted in the Well Map, click **Grab Image**.
   - For another area on the Well Map, click elsewhere on the Well Map and then click **Grab Image**.
7. When you see three thumbnails on the left named **Composite**, **TransWL** and **FITC 1000ms**, toggle between the thumbnails to inspect the images.
8. When you see red pixels on the image, there is overexposure and you must lower either the **Exp. Time (ms)** or **LED Intensity**, and then click **Grab Image** again. Repeat until the red pixels are gone.



Set Up Detection Settings

1. Click the **Detection** tab.



2. For **Algorithm**, select **Local Threshold**.
3. Select to view the **TransWL**, set **Average Colony Diameter** to a size that best detects the colonies, likely between 0.1 mm and 0.5 mm, depending on the size of the colonies, and then click **Reprocess** to apply the changes.
4. For **Exterior Statistics Diameter Multiplier**, leave the **x3** default setting.
5. For **Use each colony size when calculating exterior statistics**, verify that the check box is selected.

6. For **Display**, leave the default settings.
7. To continue, click **Next**.

Select Wells

The red wells are the source plate wells that will be imaged and used for picking.

Tip: If needed, you can change the well selections here.

To continue, click **Next**.

Summary

In the **Summary** tab, view all of the imaging parameters that you have selected for this run, as well as your colony detection settings.

To continue, click **Next**.

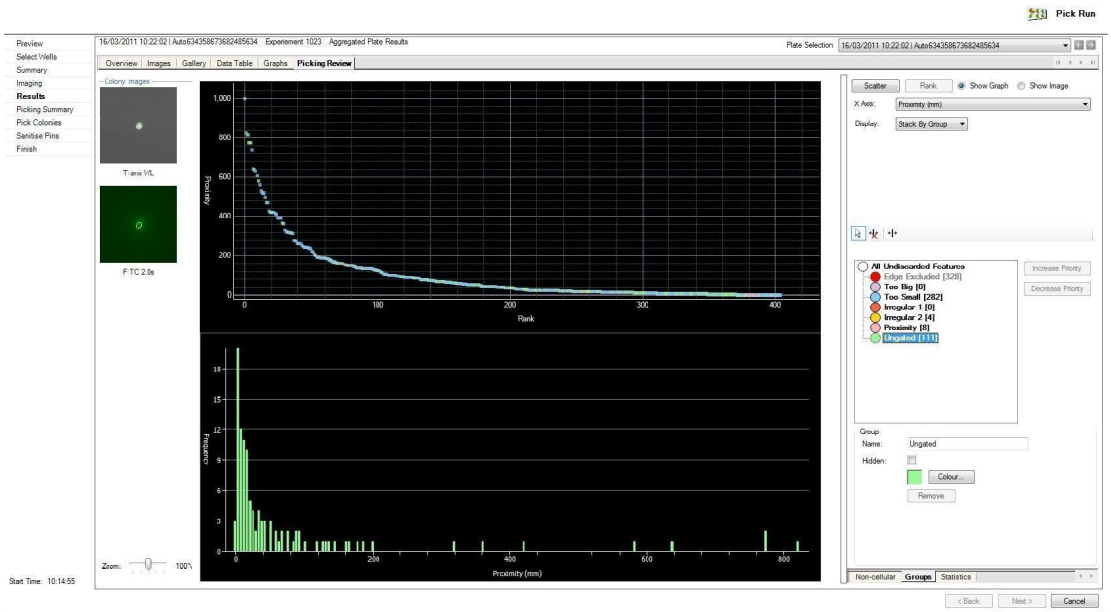
Imaging

Imaging, colony detection, and data calculation, automatically begin for all source plates.

While the imaging step is in progress, pre-fill the number of needed 96-well destination plates with liquid medium (150 µl) and place in a tissue culture incubator to equilibrate.

Results

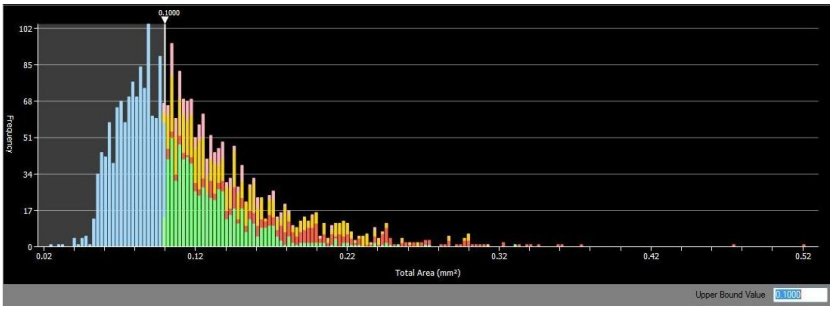
Imaging results appear on the **Results** screen.

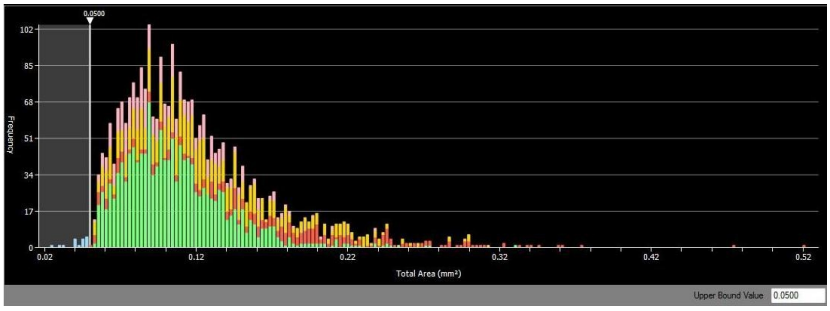


1. Click on the **Groups** tab.
2. When possible, leave the Groups default values.

> **Tip:** Any Group can be changed, however clonality and viability might be compromised.
>
> Molecular Devices recommends keeping the default values for the pre-defined Groups, with the exception of the **Too Small** group, set the lower threshold to 0.03.

3. To edit **Groups**, double-click on a group and move the default gate. For example:
   - To reduce a **Too Small** group cut-off point, double-click on the group and then drag the slide bar on the histogram to 0.05, or the needed value.



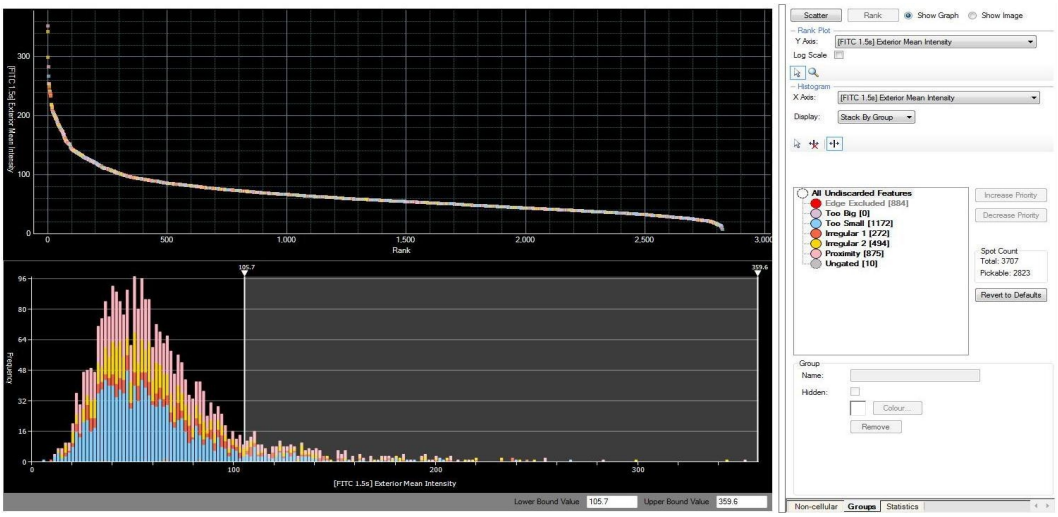


   - To further isolate only the colonies in the **Ungated** group that have the highest associated FITC fluorescence, to display all the colonies on the histogram, click **All Undiscarded Features**.

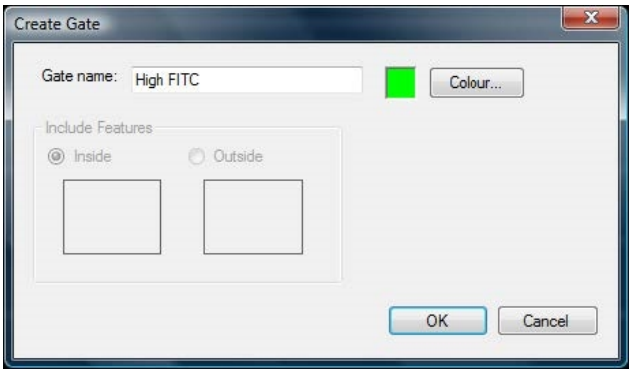
> **Tip:** Colonies in the **Ungated** group have good size, shape and distance from other colonies.

4. For **Histogram**, select **[FITC] Exterior Mean Intensity**.

5. To draw a gate on the histogram, click on the histogram where the cut-off point should be and then drag the gate off the right end of the histogram.



6. When the **Create Gate** dialog appears, name the group **HIGH FITC**, select a different color, and then click **OK**.

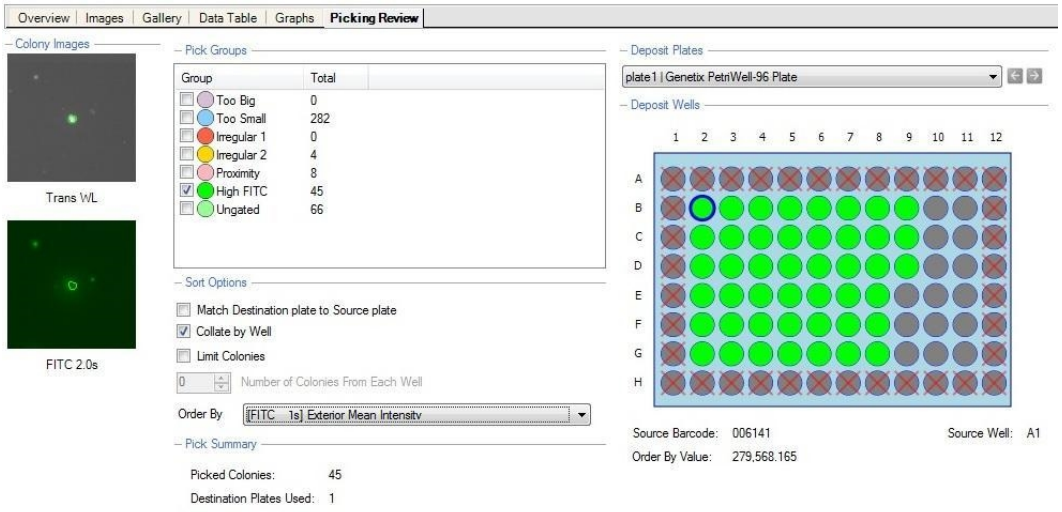


7. To select the newly-created group, click on it, then click **Decrease Priority** multiple times so that it is positioned just above the **Ungated** group.
8. To continue, click **Next**.

Picking Review

On the **Picking Review** tab screen, a summary of the colonies to be picked appear.

1. In the **Picking Review** tab, select only the High FITC group.



2. Under **Sort Options**, for **Order By**, select **[FITC 1s] Exterior Mean Intensity**.
3. To continue, click **Next**.

Picking

On the **Picking Summary** screen, the pick information appears before the picking starts.

1. When prompted, load the required number of destination plates into the destination stacker.

> **Tip:** The number of required destination plates appears in the **Picking Review** tab in the **Pick Summary**.

2. To continue, click **Next**.

The picking step proceeds automatically until all colonies are picked by the instrument for the selected group.

Finish

When the picking step concludes, the finish screen appears.

Click **Finish** to return to the Pick Run process Start screen.

To return to the **Main Navigation** screen click **Close Process**. If the process has not been saved, a window appears prompting for the settings to be saved.

To view the results of the picking run, go to **Review Results**.

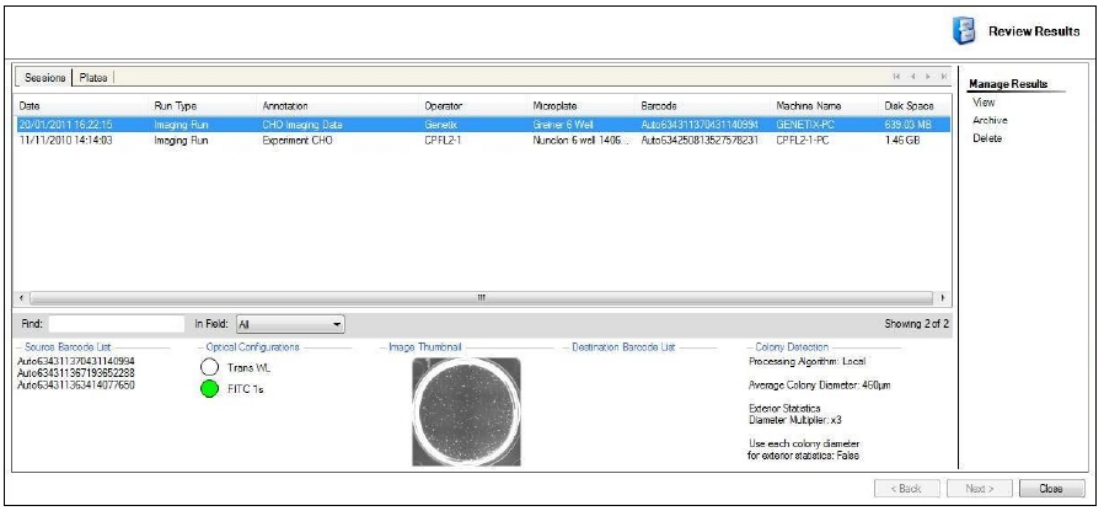
Review Results Process

Use the **Review Results** process screen for reviewing a completed **Imaging Run** and **Picking Run**.

By default, data is stored on the C: drive in a folder called **Image Archive**.

To open the **Image Archive**, click **Next** or to access data remotely, click **Browse**.

The following **Sessions** tab screen appears showing a list of completed runs:



The runs appear in two tabs:

- **Sessions** tab—Results appear as one row even if the run consisted of several plates.
- **Plates** tab—Results appear as one row per plate processed so if there were three plates in the run, three individual rows appear.

The results list can be re-ordered by a single click on the table headers, or a double-click to invert the selection.

To view the data, do one of the following:

- On the **Sessions** tab, either double-click on the required row or click once on the required row and the click **View** to open the data set.
- On the **Plates** tab, do the same as in the Sessions tab, or to view multiple plates, use Shift-Click or Control-Click to highlight the required rows, and then click **View**. You can also combine the data from multiple plates and view and process the plates as a batch.
- Search the data sets using **Find**. The fields available to search in are the various information points: **Date**, **Run Type**, **Annotation**, **Operator**, **Microplate**, **Barcode**, and **Machine Name**.

When a data set is selected, the following information appears:

- **Source Barcode List**—Lists all the barcodes on the source plates used.
- **Optical Configurations**—Lists all the optical configurations used in the selected run and the colors associated with them.
- **Image Thumbnail**—Displays the prime configuration well image.
- **Destination Barcode List**—Lists all the barcodes on the destination plates used.
- **Colony Detection**—A small summary of the parameters used to detect the colonies is displayed here.

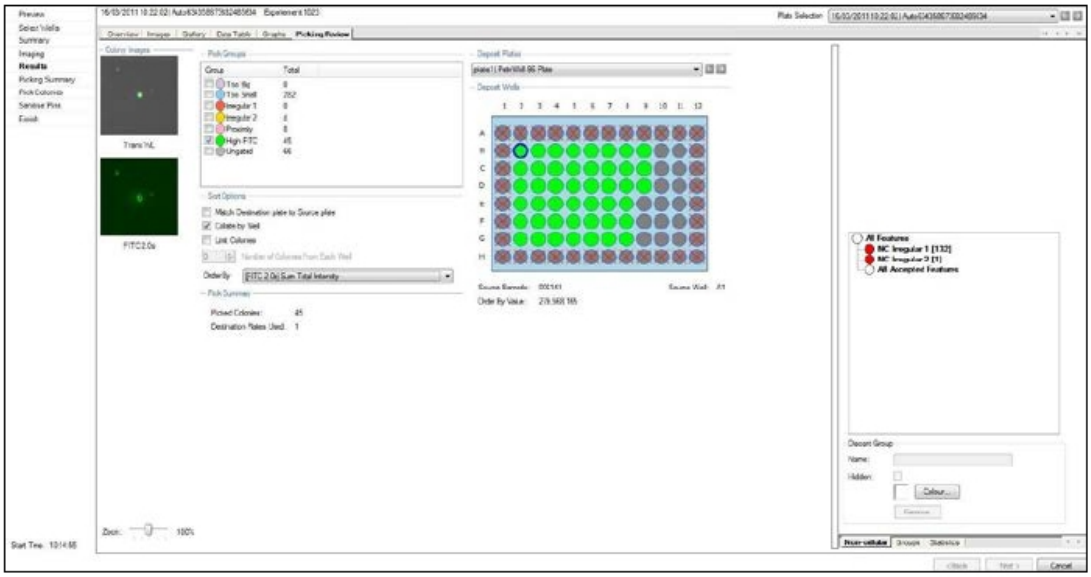
After data has been opened for viewing, depending on whether imaging or picking data is being opened, all the tabs available are the same as seen throughout the **Pick Run** and **Imaging Run** stages. In a picking run data set, the **Picking Review** tab is visible. See Picking Review Tab on page 29.

Note: For data integrity, **Pick Run** data is fully accessible in Review Results but the data are locked and cannot be changed. **Imaging Run** data can be changed on-screen but cannot be saved.

- **Archive**—Stores data to **Image Archive** or transfers data to CD or DVD. Shift-click or Control-click to highlight the required rows and then click **Archive**.
- **Delete**—Permanently removes the data from the hard drive in **Image Archive**. Select a data set, and then click **Delete**.

Picking Review Tab

On the **Picking Review** tab screen, a summary of the colonies to be picked appear. Any number of groups can be selected to be picked here, and to be ordered using various criteria.



A summary of the picking review tab appears to the right of the main screen, including the following:

- **Colony Images**—When a well in Deposit Wells is selected, the corresponding colony image is displayed to the left. It is possible to zoom in and out of these colony images using the Zoom slider below the images.
- **Picking Groups**—All the groups available to be picked are listed here, including exclusion groups such as Too Small and new groups that have been created with specific characteristics.
- **Sort Options**—Offers several ways colonies can be sorted.
  - **Order by**—Allows colonies within a checked group to be picked in order of any of the parameters in the drop-down list, for example **Fluorescent Exterior Mean Intensity**. If several groups have been selected, it is advisable to order by **Group**.
- **Pick Summary**—Displays the number of colonies to be picked and the number of destination plates into which colonies are picked.
- **Deposit Plates**—Lists the number and type of destination plates (as previously defined in Modify Settings, Picking Settings) that are required for picking.
- **Deposit Wells**—Displays the wells that the colonies are going to be picked into. The wells are colored according to the corresponding colony that is picked into them. Any wells that have been excluded from picking are displayed with red crosses over them. Underneath the well map is the following information which is displayed when hovering the mouse pointer over the colony assigned wells:
  - **Source Barcode**—Displays the barcode of the source plate where the picked colony comes from.
  - **Source Well**—Provides the source well number of the picked colony.
  - **Order By Value**—Provides the order value (assigned from the sort options drop-down list) of the colony to be picked.

Chapter 7: Powering Off the System and the Software

To power off the ClonePix 2 Mammalian Colony Picker:

1. To exit the ClonePix 2 Software, on the **Main Navigation** screen, select **File > Exit**.
2. To shutdown your computer, click **Start > Shut down**, and then wait for computer to power off completely.
3. To power off the instrument, on the front of the instrument, press the **Stop** button.
4. Turn the power off at the mains.

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