CloneSelect

Imager FL

User Guide

5090618 B
December 2023

MOLECULAR DEVICES

Product manufactured by Molecular Devices, LLC.
3860 N. First Street, San Jose, California, 95134, United States of America.
Molecular Devices, LLC is ISO 9001 registered.

Contents

Safety Information

Information about the safe use of the instrument includes an understanding of the user attention statements in this guide, the safety labels on the instrument, precautions to follow before you operate the instrument, and precautions to follow while you operate the instrument.

Make sure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Read and understood all Safety Data Sheets (SDS) for all materials being used.

Read and observe all warnings, cautions, and instructions. The most important key to safety is to operate the instrument with care.

WARNING! If the instrument is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.

Warnings, Cautions, Notes, and Tips

All warning symbols are framed within a yellow triangle. An exclamation mark is used for most warnings. Other symbols can warn of other types of hazards such as biohazard, electrical, or laser safety warnings as are described in the text of the warning. Follow the related safety information.

The following user attention statements can display in the text of Molecular Devices user documentation. Each statement implies the amount of observation or recommended procedure.

WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed.

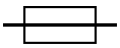
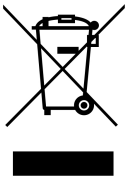
CAUTION! A caution indicates a situation or operation that could cause damage to the instrument or loss of data if correct procedures are not followed.

Note: A note calls attention to significant information.

Tip: A tip provides useful information or a shortcut, but is not essential to the completion of a procedure.

Symbols on the Instrument

Each safety label on the instrument contains an alert symbol that indicates a type of potential safety hazard.

Symbol	Indication
	Consult the product documentation.
	Potential electrical-shock hazard from a high-voltage source. All safety instructions must be read and understood before proceeding with the installation, maintenance, and servicing of all modules.
	Potential pinch hazard.
	Location of a fuse.
	The instrument manufacture date.
	CSA certification.
	European technology conformity.
	United Kingdom technology conformity.
	Compliance with Australian radio communication requirements.
	Compliance with Chinese RoHS Pollution Control Requirements.
	This symbol is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. You must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system. For products under the requirement of the WEEE directive, contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.
	There is an authorized representative in the European community.
	The instrument manufacturer.
	California Proposition 65 requires businesses to warn Californians about significant exposures to chemicals that cause cancer, birth defects, or other reproductive harm.

Light Engine Safety

WARNING! DO NOT stare into the output of the light engine. The brightness of this light engine is higher than most commercial lighting fixtures and is intended to couple directly into a microscope or other bio-analytical instrument.

WARNING! Possibly hazardous optical radiation emitted from this product. Do not look at operating lamp. Eye injury may result.

WARNING! Infrared (IR) emitted from this product. Do not look at operating lamp.

WARNING! UV emitted from this product. Avoid eye and skin exposure to unshielded product.

Electrical Safety

To prevent electrically related injuries and property damage, inspect all electrical equipment before use and immediately report all electrical deficiencies. Contact Molecular Devices Technical Support to service equipment that requires the removal of covers or panels.

The instrument must be connected to a properly grounded power outlet to protect from the risk of electric shock. The main chassis of the instrument is grounded together with all related electrical components.

In the event of a liquid spillage into the main cavity of the instrument, disconnect the mains power supply before trying to clean up.

Do not remove the fixed covers, as there are no user-serviceable parts inside. All electrical work must be referred to Molecular Devices approved service personnel.

If the external covers on the instrument are removed, the power supply does not automatically stop.

WARNING! HIGH VOLTAGE Power off the instrument and disconnect the power cord before you do maintenance procedures that require removal of a panel or cover or disassembly of an interior instrument component.

Do not try to use the instrument until all covers are replaced.

To provide access for disconnecting power from the instrument, maintain a 66 cm (26 in.) minimum clearance area on the right side of the instrument.

To protect against fire hazard, replace the fuses only with the same type and rating as the original factory-installed fuses.

Chemical and Biological Safety

Normal operation of the instrument can involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When you use such materials, observe the following precautions:

- Handle infectious samples based on good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original containers of solutions before their use.
- Dispose of all waste solutions based on the waste disposal procedures of your facility.
- Operate the instrument in accordance with the instructions outlined in this guide, and take all the required precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids can occur. Take applicable safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Observe the applicable cautionary procedures as defined by your safety officer when using hazardous materials, flammable solvents, toxic, pathological, or radioactive materials in or near a powered-up instrument.

WARNING! Never use the instrument in an environment where potentially damaging liquids or gases are present.

Moving Parts Safety

The instrument contains moving parts that can cause injury. Under normal conditions, the instrument is designed to protect you from these moving parts.

To prevent injury:

- Never try to exchange labware, reagents, or tools while the instrument is operating.
- Never try to physically restrict the moving components of the instrument.

WARNING! Do not attempt to access the interior of the instrument unless specifically instructed to do so. The moving parts inside the instrument can cause injury. Do not operate the instrument with any covers or panels removed.

Note: Observe all warnings and cautions listed for all external devices attached to or in use during the operation of the instrument. See the applicable user guide for the operating and safety procedures of that device.

Health and Safety

Transport and Storage

Store and transport the instrument in temperatures within the range –25°C to +55°C. Transport the instrument with the original packing foam inserts in place to protect the objective and objective holder. The instrument is intended for bench top operation. Take care when you move the instrument. You should have two people lift it.

External Covers

WARNING! If you remove any of the external covers on the instrument, the power supply is not automatically interrupted. If you need to remove an external cover you must ensure that the power is switched off first and do not attempt to use the robot until you replace the covers.

Electrical Safety

WARNING! HIGH VOLTAGE. You must connect the instrument to a properly earthed power outlet to protect users from the risk of electric shock. The main chassis of the machine is earthed together with all associated electrical components. Do not remove any of the fixed covers, as there are no user serviceable parts inside. You should refer all internal work to Molecular Devices approved service personnel.

Drive Safety

WARNING! PINCH HAZARD. There is a potential pinch hazard with the drive mechanism. Please ensure that you do not attempt to load the plate until the drive has fully extended.

Noise levels

During normal operation the level of airborne noise emitted by the robot will not exceed 70 db measured at 1 meter.

Chapter 1: Introduction

The CloneSelect® Imager FL is an automated CMOS camera-based imaging system, for imaging in white light and multi-channel fluorescence. A 5W Xenon flash lamp provides illumination for white light imaging, while an external solid state light engine in combination with red and green filter sets allows multichannel fluorescence imaging. CloneSelect Imager FL processes include single cell fluorescent detection, for monoclonality workflows, and confluence determination (both white light and fluorescent).

Note: The CloneSelect Imager FL is for research use only and is not intended or recommended for the diagnosis of disease in humans or animals.

WARNING! If the instrument is used in a manner not specified in this guide, the protections provided by the equipment can be impaired.

Workflows

Imaging in White Light and Fluorescent Multi-Channel

The imaging strategy is a two step process to identify single wells with one fluorescent cell on Day 0 and provide temporal images of the colony formation from one cell in white light imaging.

1. Use the Image Microplate Multi Channel process on day 0 to image with both white light and fluorescent channel to identify hits that have a single cell in a well.

   Day 0 Image multichannel

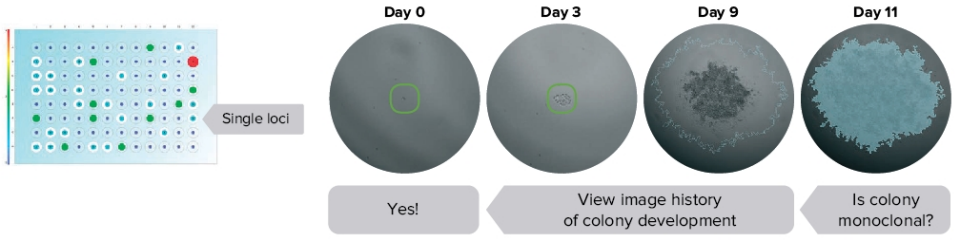
2. Use the Image Microplate process to use white light to image over time.

   Day 0 → Day 1 → Additional scans from D7-14

96-well Plate and White Light Image

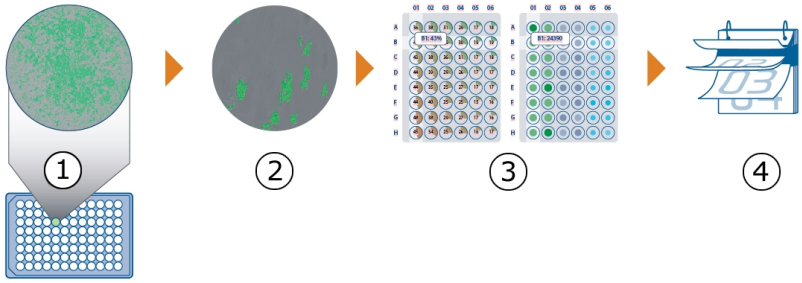
Verifying Monoclonality

After initial seeding, image every well, at any time point, using the loci of growth functionality to highlight the wells that contain a single colony. See Loci Count on page 80 and Monoclonality Workflow on page 82.



Analysis in White Light Mode

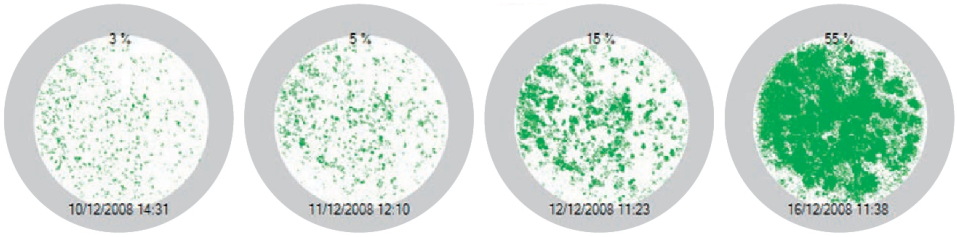
Display cell confluence and cell number estimation for each well and calculate growth curves.



Item	Description
1	Well selection
2	Cell distribution highlighted by software overlay
3	Cell confluence and cell number estimation for each well
4	Repeat over several days

Reporting

Track and view growth of every cell line, and store plate data, including cell confluence, cell number estimation, and growth curve.



3% Day 1 **5% Day 2** **15% Day 3** **55% Day 7**

Chapter 2: Instrument Overview

2

Use the CloneSelect Imager FL system to image every well to count colony number, estimate colony area, track colony growth and monoclonality.

Before you operate the instrument or do maintenance operations, make sure that you are familiar with the safety information in this guide. See Safety Information on page 7.

CloneSelect Imager FL Overview

Item	Description
1	Cable Connection Ports and Power Switch
2	Status Indicator Lights
3	Plate Holder
4	External Light Engine

For technical specifications, see Technical Specifications on page 129.

Setting Up The Instrument

Place the CloneSelect Imager FL, the external light engine, and the computer peripherals (monitor, keyboard, and mouse) on a stable, level surface. Place the computer in a position where the connecting cables can reach the instrument, the external light engine, and the peripherals. Level the instrument by loosening the locking nuts and turning the leveling feet to ensure that the instrument is stable and not able to move. After adjustment, tighten the locking nuts.

Leg Leveling Locking Nuts

Computer

Note: Your Molecular Devices representative installs the CloneSelect Imager FL software during the initial instrument installation and configuration.

The CloneSelect Imager FL ships with a computer that meets the hardware and software requirements to operate the instrument.

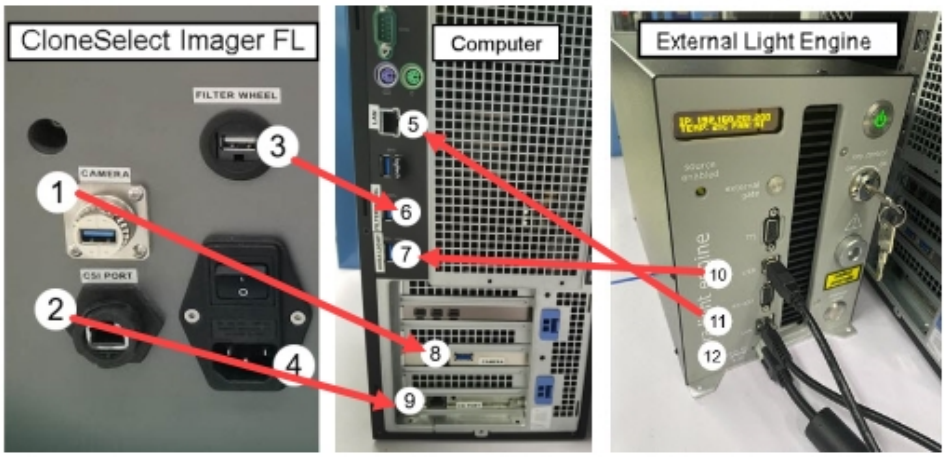
Note: Do not attempt to use any other computer or change the operating system.

Security configuration is required to connect the computer to your network. Ensure that no further changes are made to the configuration of the network connection.

Note: Do not change the standard English settings. If you change these settings the performance of the system can be impacted.

Connecting Instrument Cables

The cable connections panel on the CloneSelect Imager FL is on the left side of the instrument, the cable connections for the computer are on the rear of the computer, and the cable connections for the external light engine are on the front of the light engine.



Cable Connection Ports

Item	Description	Connects To	Item
1	CloneSelect Imager FL USB 3.0 CMOS Camera Port	Computer	8
2	CloneSelect Imager FL Ethernet CSI Port	Computer	9
3	CloneSelect Imager FL USB Filter Wheel Port	Computer	6
4	CloneSelect Imager FL Electrical Port	Wall Outlet	n/a
5	Computer LAN Port or Network/Internet	Light Engine or Internet	11
6	Computer USB Filter Wheel Port	Instrument	3
7	Computer USB Light Engine Port	Light Engine	10
8	Computer USB 3.0 CMOS Camera Port	Instrument	1
9	Computer CSI LAN Port	Instrument	2
10	Light Engine USB 3.0 CMOS Camera Port	Computer	7
11	Light Engine LAN Port	Computer	5
12	Light Engine Electrical Port	Wall Outlet	n/a

Connect the computer to the CloneSelect Imager FL, the external light engine, and the computer peripherals. Plug in all four power supplies (leave powered off) before you make the network connections.

The network connection to the CloneSelect Imager FL is a private Gigabit Ethernet link and the connection must be made to the computer add-on card Gigabit Ethernet port.

Powering On the System

CAUTION! Before you use the instrument, it is very important that you read this guide and understand all the safety instructions.

To power on the instrument:

1. On the front of the external light engine, press the power button (#1).

2. Wait for the screen (#2) to display information and then turn the key (#3) to the On position.
3. On the left side of the CloneSelect Imager FL, toggle the power switch to on.

4. Wait for the Ready light to illuminate.
5. Leave the instrument in the Ready state for more than 5 minutes to warm up the instrument. When the unit is cold to the touch, wait for 30 minutes.
6. If the computer and monitor are powered off, power on the monitor and computer.
7. Double-click the desktop icon to start the CloneSelect Imager FL software.

Note: Do not start the software before the instrument is powered on and warmed up.

Rebooting the CloneSelect Imager FL

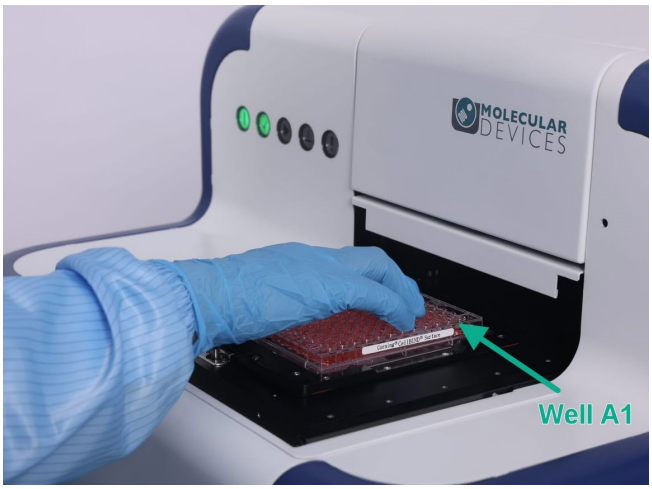
To reboot the instrument:

1. On the left side of the CloneSelect Imager FL, toggle the power switch to off.
2. Wait for 30 seconds.
3. Toggle the power switch to on.

When the Ready light illuminates, the instrument is ready to use.

Plate Holder

The plate holder consists of a glass plate surrounded by a vacuum bed seal. Place the plate on the plate holder so that the skirt is over the vacuum bed seal and well A1 is to the back right. When the plate holder retracts, the instrument pushes the plate into the back right corner of the plate holder for imaging.



Before imaging, the instrument applies a vacuum under the plate to hold it flat on the glass plate so that all the wells are in the same focal plane. This eliminates the need to autofocus on every well.

Note: The plate skirt of the plate needs to be intact for the vacuum seal to work.

Compatible Plate Types

Costar 96-well (3300) and Greiner 384-well (781182/781185) microplates are validated for multichannel processes.

For a list of recommended plates and the files you can use to create plate types in the software, see the Molecular Devices Knowledge Base for plate compatibility information.
https://support.moleculardevices.com/s/article/CloneSelect-Imager-Plates

Using Barcodes

If you use barcodes, the following barcode parameters are required:

- Use a legible barcode of the following types: 1D (linear) barcodes with code 11, 39, 93 and 128.

- Do not use special characters, such as hyphens, in the barcode. Special characters can cause missed reads and other errors downstream.
- Place the barcode centrally on one of the short sides of the plate.

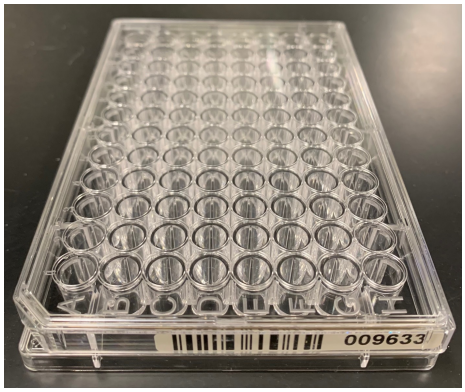


Plate With Barcode

For troubleshooting, see Troubleshooting Barcodes on page 128.

Powering Off the System

To power off the instrument:

1. In the computer software, on the Navigation screen, click **File** > **Exit** to exit the CloneSelect Imager FL software.
2. Click **Start** > **Shut Down** to power off the computer.
3. On the front of the external light engine, turn the key (#1) to the Off position and then press the power button (#2) to power the light engine off.

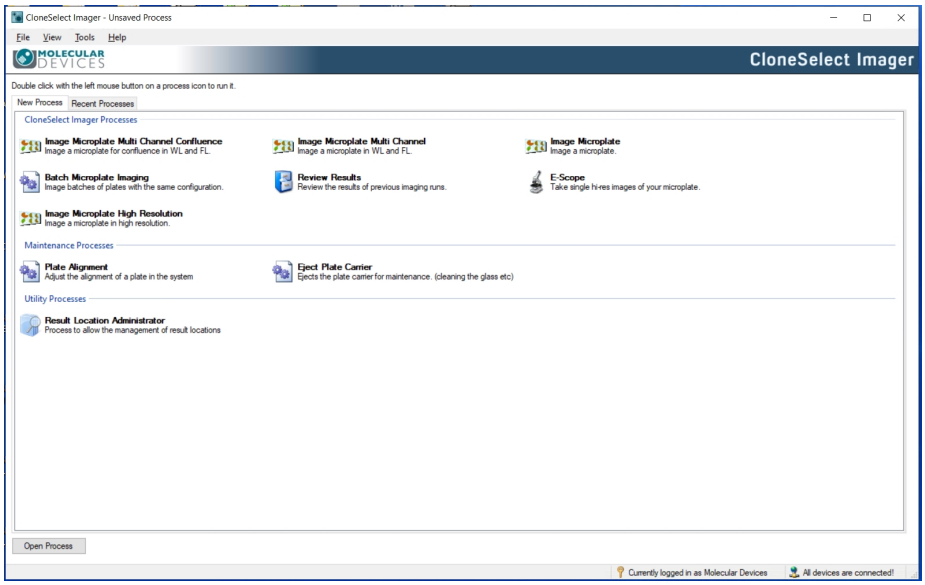
4. On the left side of the CloneSelect Imager FL, toggle the power switch to off.

Chapter 3: Software Overview

The CloneSelect Imager FL Software controls the CloneSelect Imager FL and is based on the concept of the instrument running processes.

When the software first starts, the Navigation screen displays.

- The New Processes tab lists the default processes that you use to create processes.
- The Recent Processes tab lists the processes that were recently opened.
- The Open Process button allows you to select a process file to open.



Navigation Screen

Note: Available options depend on your system configuration.

The device connection status displays at the bottom right of the screen.

Menu Options

The options in the menus at the top of the Navigation screen change depending on the screen view and selections.

File Menu

- **Open Process:** Allows you to open a saved process.
- **Save Process:** Saves the process.
- **Save Process As:** Allows you to save the process with a different name or location.
- **Close Process:** Closes the process.
- **Save As Template:** Saves the process as a template that you can use to create new processes.
- **Recent Processes:** Allows you to open a recent process.
- **Exit:** Shuts down the software.

View Menu

- **Properties:** Displays the process properties before you start the process.
- **Progress:** Displays the progress of a running process.
- **Administrate Properties:** Allows you to change the display and workflow on the Properties screen.

Tools Menu

- **Configuration:** Allows you to edit the instrument configuration settings.

  CAUTION! Changes to the instrument configuration settings can cause the instrument to be inoperable. Proceed with caution and create a restore point before you make changes.

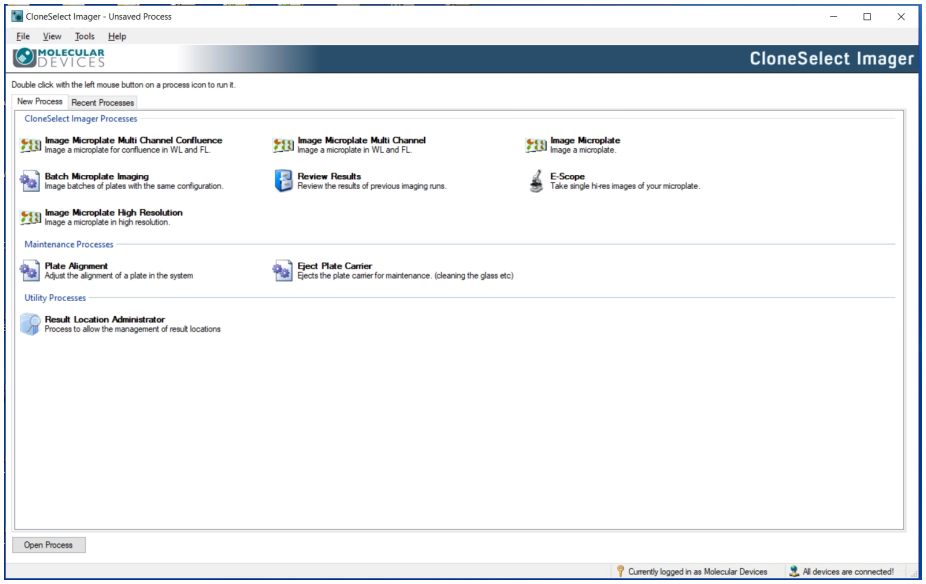
- **Prepare Error Report:** Starts the Error Reporting wizard to create a data file that contains the configuration and recent log files to help troubleshoot problems.

Help

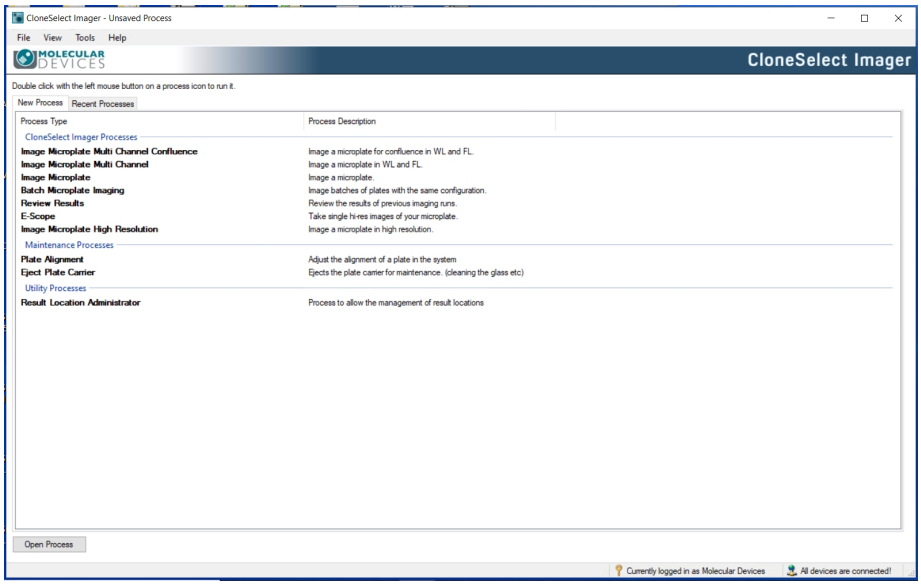
- **About:** Displays the software modules version numbers.
- **Online Support:** Displays the Molecular Devices support web site if the computer has an Internet connection.
- **Help View:** Displays the application help.

Navigation Screen

Right-click to change the display of the processes on the Navigation screen to either icons or text.



Navigation Screen - Process Icons



Navigation Screen - Process List

New Process Options

The New Process tab on the Navigation screen lists the default processes that allow you to run and manage processes.

Processes

Processes include the following:

- **Image Microplate:** Does standard white light imaging. See Image Microplate Process on page 27.
- **Batch Microplate Imaging:** Does white light imaging of batches of plates that have the same configuration without reviewing the focus and alignment between plates. See Batch Microplate Imaging Process on page 39.
- **Image Microplate High Resolution:** Does high-resolution imaging. See Image Microplate High Resolution Process on page 49.
- **Image Microplate Multi Channel:** Does standard white light imaging and fluorescence imaging. See Image Microplate Multi Channel Process on page 57.
- **Image Microplate Multi Channel Confluence:** Does white light and fluorescence imaging for confluence. See Image Microplate Multi Channel Confluence Process on page 65.
- **Review Results:** Displays results from previous runs including confluence, cell number estimation, and monoclonality analysis for review. See Review Results Process on page 73.
- **E-Scope:** Does a single high-resolution image. Images are not saved. See E-Scope Process on page 107.

Maintenance Processes

Maintenance Processes include the following:

- **Plate Alignment:** Runs the procedures to calibrate the plate alignment within the instrument. See Plate Alignment Process on page 113.
- **Eject Plate Carrier:** Opens the plate carrier for plate removal and cleaning. See Eject Plate Carrier Process on page 119.

Utility Processes

Utility Processes include the following:

- **Result Location Administrator:** Allows you to manage where you save the results. See Result Location Administrator on page 121.

Running Processes

On the Navigation screen, double-click a process to display the Properties screen where you start processes.

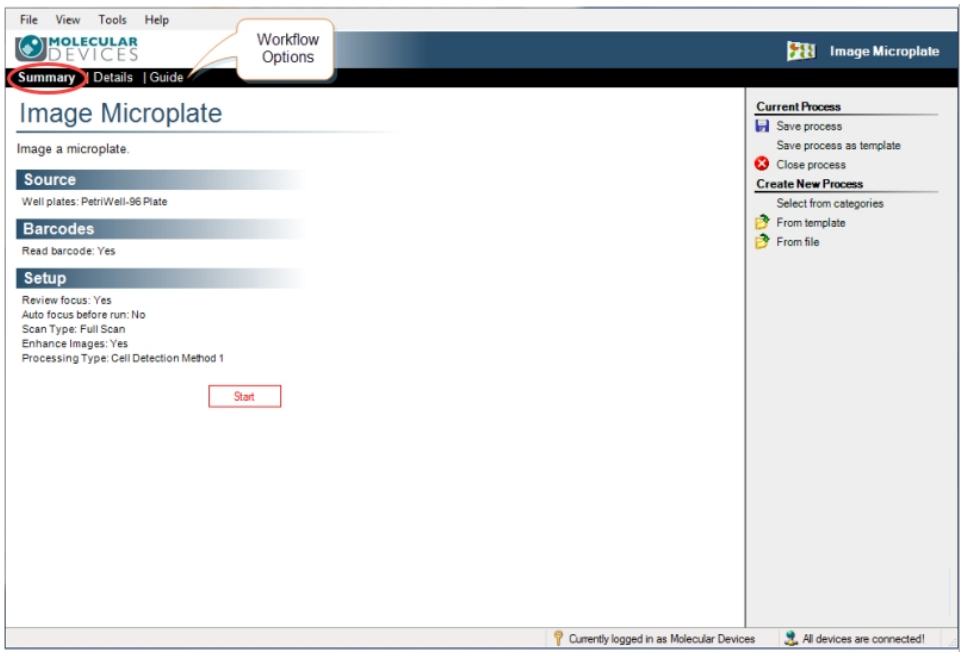
Setting Process Properties

The process starts and ends on the Properties screen. The Properties screen has three workflow options that allow you to change the way you define process properties. Each workflow has the same fields but the field presentation varies with the workflows.

Note: The following screen captures are for the Image Microplate process. The properties are different for each process.

Summary Workflow

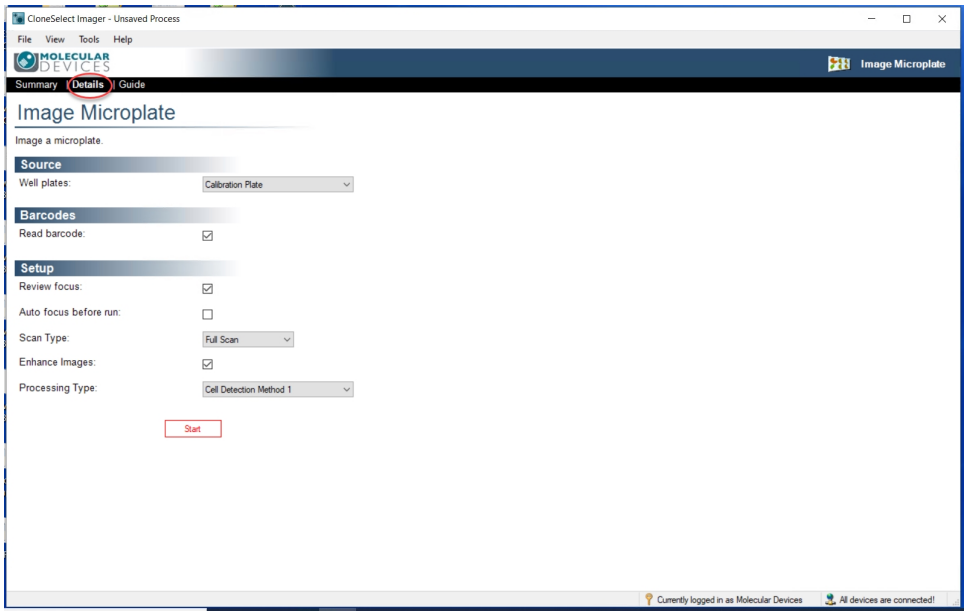
The Summary workflow displays property settings grouped in categories. Click a category heading to display the properties for that heading group. The right side of the Summary workflow provides links to save the process, close the process, and to create processes from templates or files.



Properties Screen - Summary Workflow

Details Workflow

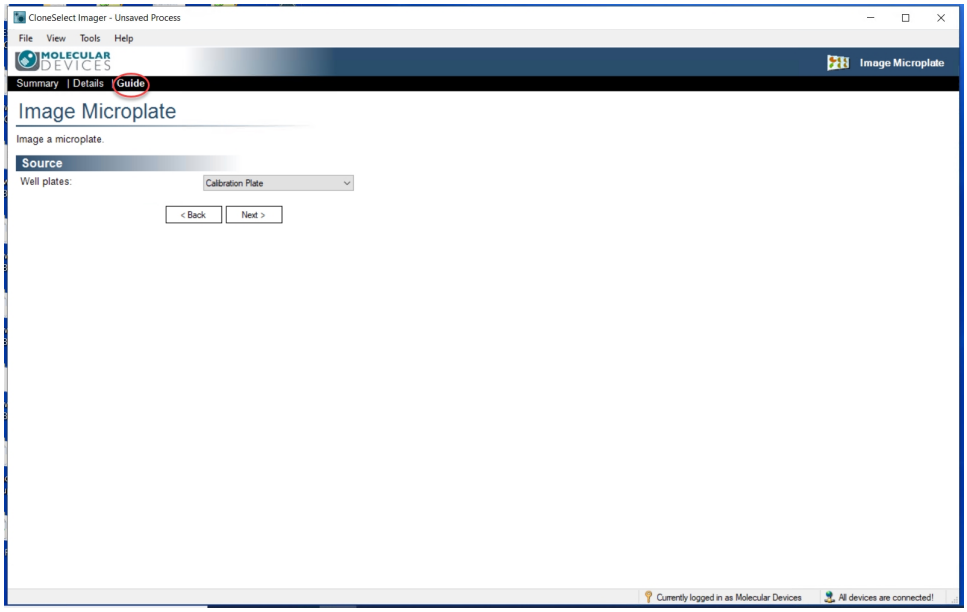
The Details workflow displays process properties as a list on a single page.



Properties Screen - Details Workflow

Guide Workflow

The Guide workflow presents process properties in a wizard with Back and Next buttons.



Properties Screen - Guide Workflow

Chapter 4: Image Microplate Process

Use the Image Microplate process to image cells in plates to determine the confluence and carry out further analysis, such as cell number estimation and loci counts. Image resolution in this process is 3.5 µm per pixel.

To start the Image Microplate process, on the Navigation screen, double-click **Image Microplate** to display the Properties screen with the Summary workflow selected.

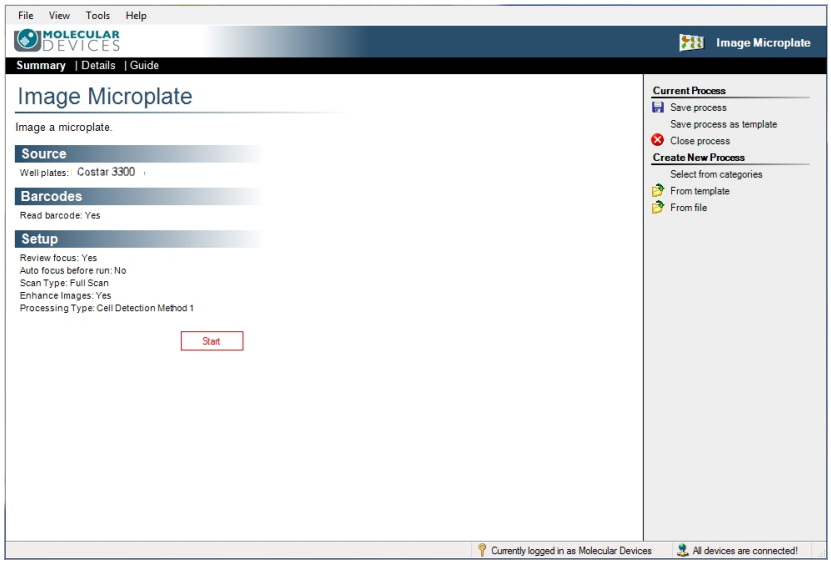
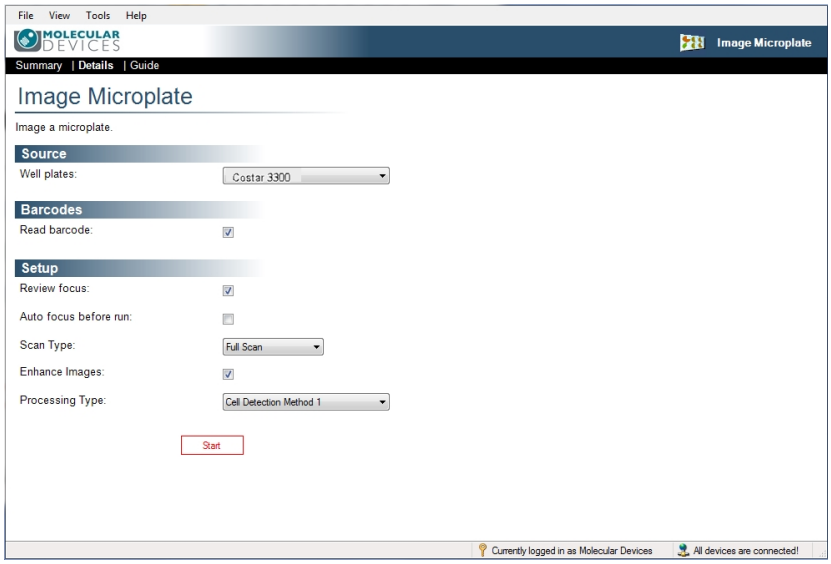


Image Microplate Process - Properties Screen - Summary Workflow

Setting Image Microplate Process Properties

Start the Image Microplate process from the Details workflow on the Properties screen.



Properties Screen - Details Workflow

To set process properties:

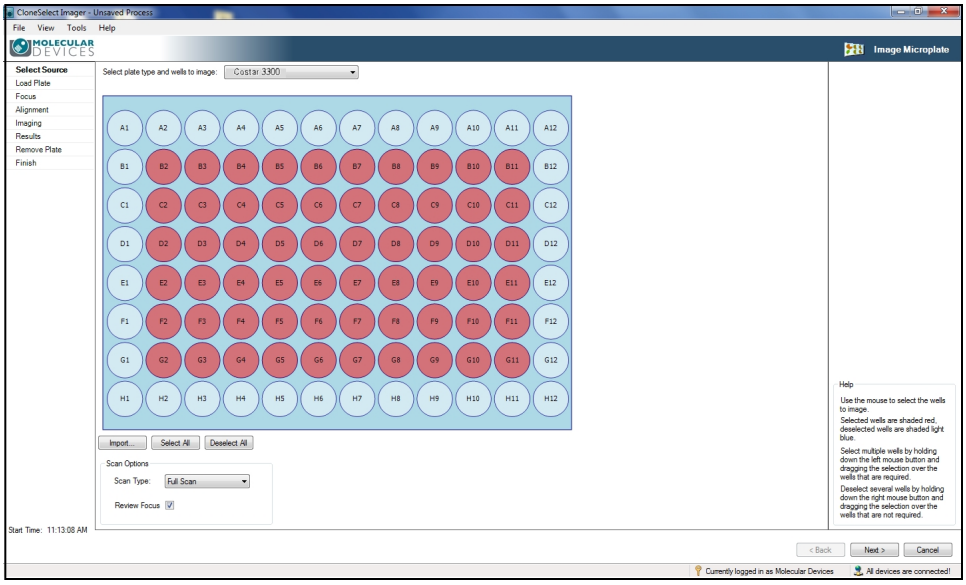
1. Select the **Details** workflow at the top of the screen.
2. Click the **Well Plates** drop-down and select a plate type.

   Note: If your plate type does not appear in the list, contact Molecular Devices. See Obtaining Support on page 132.

3. Select the **Read Barcode** check box if you use barcodes.
   Clear the **Read Barcode** check box if you do not use barcodes.
   If you select this check box and the instrument fails to read the barcode, a message displays to allow you to manually enter the barcode. You can use the Review Results process to edit the barcode or name. See Review Results Process on page 73.
4. Select the **Review Focus** check box to have the software prompt you to check the focus and brightness settings on the Focus screen before you image the plate. See Focus on page 30.
   Clear the **Review Focus** check box to not receive a prompt to check the focus.
5. Select the **Auto Focus Before Run** check box to have the software run an autofocus procedure before imaging the plate.
   Clear the **Auto Focus Before Run** check box if you work with a sample that does not include wells with high contrast cells.
6. Click the **Scan Type** drop-down:
   - Select **Full Scan** to image the entire area of each well. This is recommended for monoclonality applications.
   - Select **Partial Scan** to image an area from the center of each well and speed up imaging. This is suitable when the data from the center of each well is representative of the whole well.
7. Select the **Enhance Images** check box to enhance images for display by flattening the background. This is recommended for monoclonality applications.
   Clear the **Enhance Images** check box to not use the enhance images feature.
8. Click the **Processing Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
9. Click **Start**.

Select Source

Use the Select Source page to select the plate wells to image. The software marks the wells you select to image red and marks unselected wells light blue.



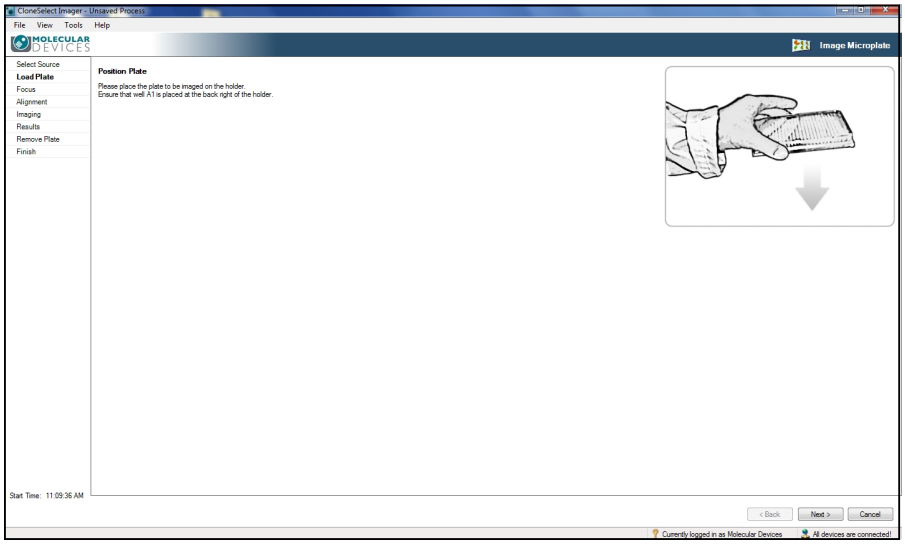
Select Source Screen

To select source wells:

1. Above the plate map, click the **Select Plate Type and Well to Image** drop-down and select a different plate type, if needed.
2. Select the wells to image.
   - Click on a well to select or deselect individual wells.
   - Right-click and drag to select or deselect groups of wells.
   - Click **Import** to use a well list file to select wells. See Well List File Format on page 133.
   - Click **Select All** to select all wells.
   - Click **Deselect All** to deselect all wells.
3. Click the **Scan Type** drop-down:
   - Select **Full Scan** to image the entire area of each well. This is recommended for monoclonality applications.
   - Select **Partial Scan** to image an area from the center of each well and speed up imaging. This is suitable when the data from the center of each well is representative of the whole well.
4. Select the **Review Focus** check box to have the software prompt you to check the focus and brightness settings on the Focus screen before you image the plate. See Focus on page 30.
   Clear the **Review Focus** check box to not receive a prompt to check the focus.
5. Click **Next**.

Load Plate

The plate carrier opens and the software prompts you to load the plate.



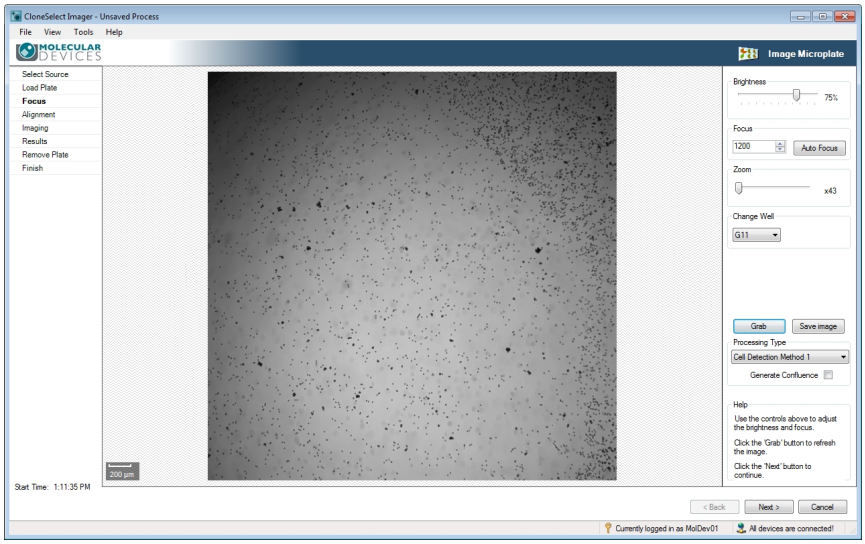
Load Plate Screen

To load a plate:

1. When the plate carrier opens, place the plate on the carrier with the A1 well in the back right corner.
2. Click **Next**.
3. If prompted, enter the barcode name and click **Next**.

Focus

Use the Focus screen to optimize the image view before the scan. The software stores the last focus setting you used for each plate type. After you image a plate type for the first time, additional focus adjustments should be minimal.



Focus Screen

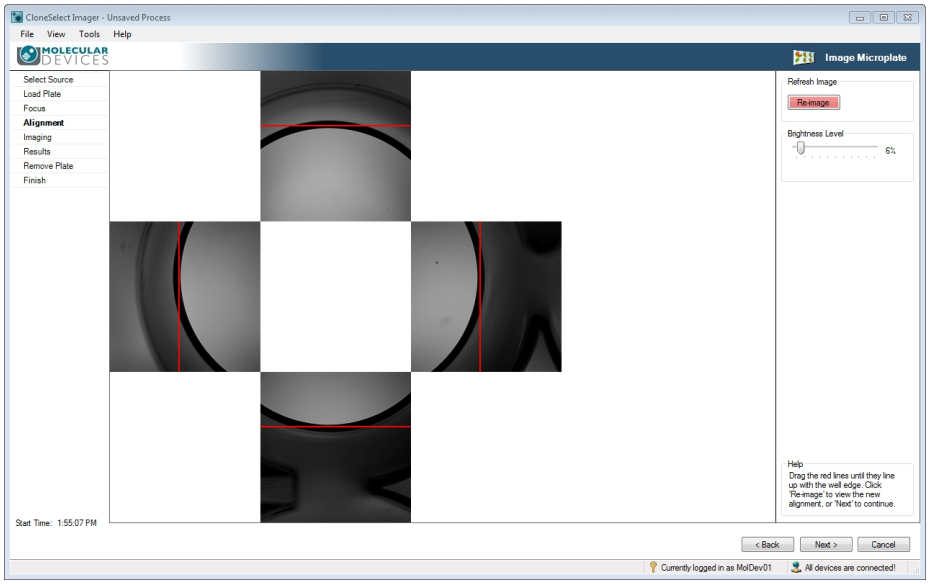
The software determines the optimal brightness setting for the image, but you can adjust the Brightness setting. You can use the Auto Focus feature or adjust the focus manually. By default the focus is done on well A1. If A1 is not being imaged, the focus is done on the first well of the plate to image.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus.
5. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
6. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
7. Click the **Processing Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
8. Select the **Generate Confluence** check box to generate confluence.
9. Click **Next**.

Alignment

Use the Alignment screen to confirm that the camera aligns with the wells. The software takes four images over the north, south, east, and west edges of well A1. The well edges should align with the middle of the box formed by the four red lines.



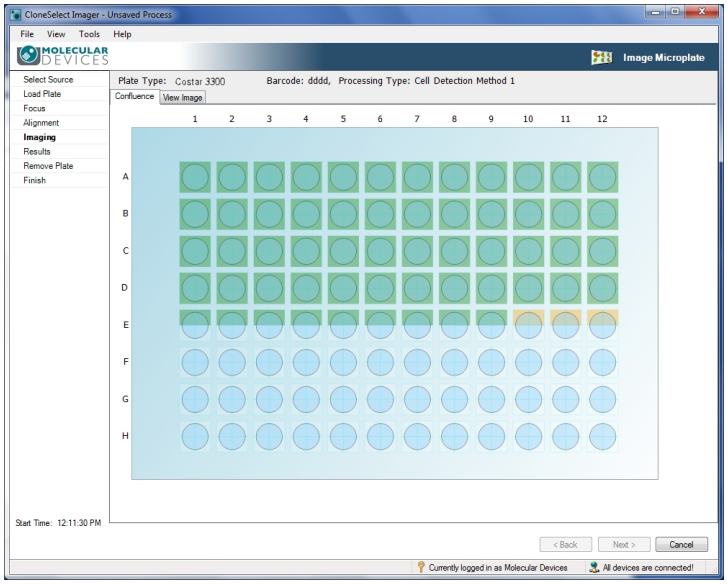
Alignment Screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines, click and drag on the lines to correct the alignment.
2. When the **Re-image** button is pink, click to recheck the alignment.
3. Move the **Brightness** slider to adjust brightness of the alignment image only.
4. Click **Next**.

Imaging

Imaging starts automatically. When the Imaging process finishes, the Results screen displays.



Imaging Screen - Confluence Tab

The Imaging screen has two tabs.

Confluence Tab

Imaging runs on the Confluence tab and you watch the process progress in the plate schematic. The plate schematic displays with the image frames superimposed in light blue. As the software captures and processes the images, the well frame changes color, light brown then to green. After a frame changes to green, click on the frame to view the image on the View Image tab.

View Image Tab

As the imaging runs on the Confluence tab, you watch the progress in the plate schematic. If you wait until the imaging completes, the Results page automatically displays.

On the Confluence tab, after a frame changes to green, you can click on the frame to display the Results screen with the View Image tab selected for that frame. See View Image on page 77.

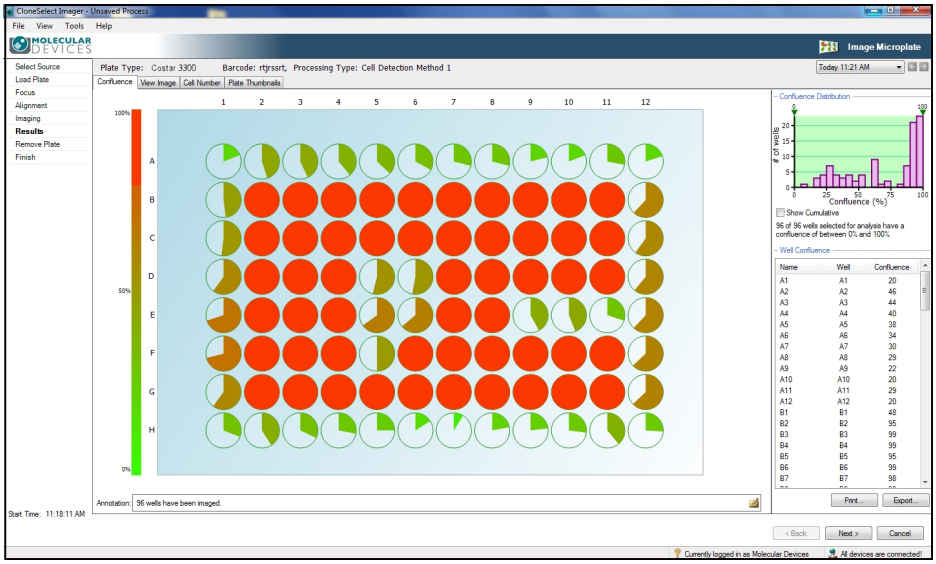
When imaging completes, the Results screen displays.

Results

After the process completes, the Results screen displays with the Confluence tab selected. The plate type, barcode, and processing type display for reference. Use the tabs to review and analyze the results. See Review Results Process on page 73.

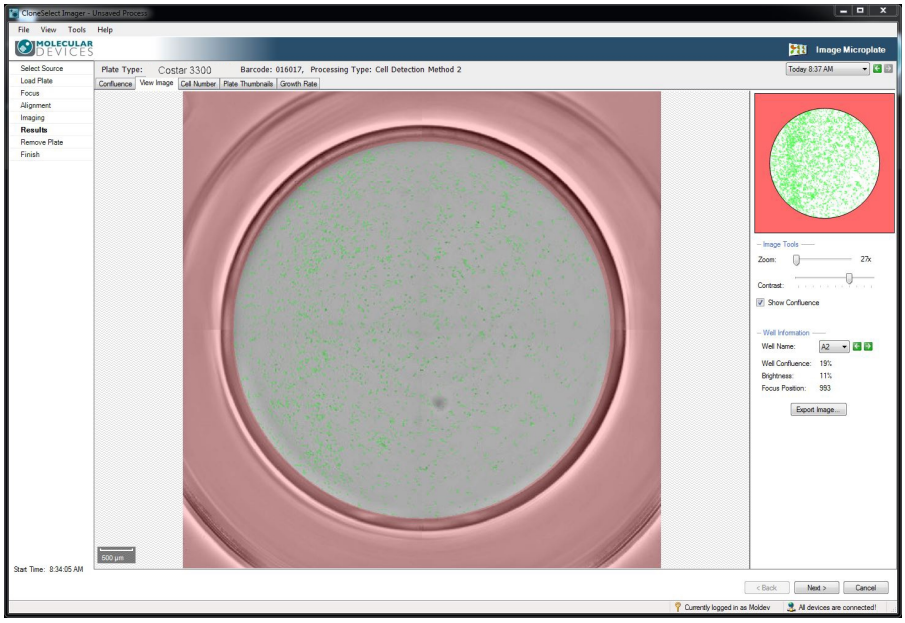
The Results screen includes the following tabs:

- **Confluence:** Displays the confluence level for each well. See Confluence on page 76.



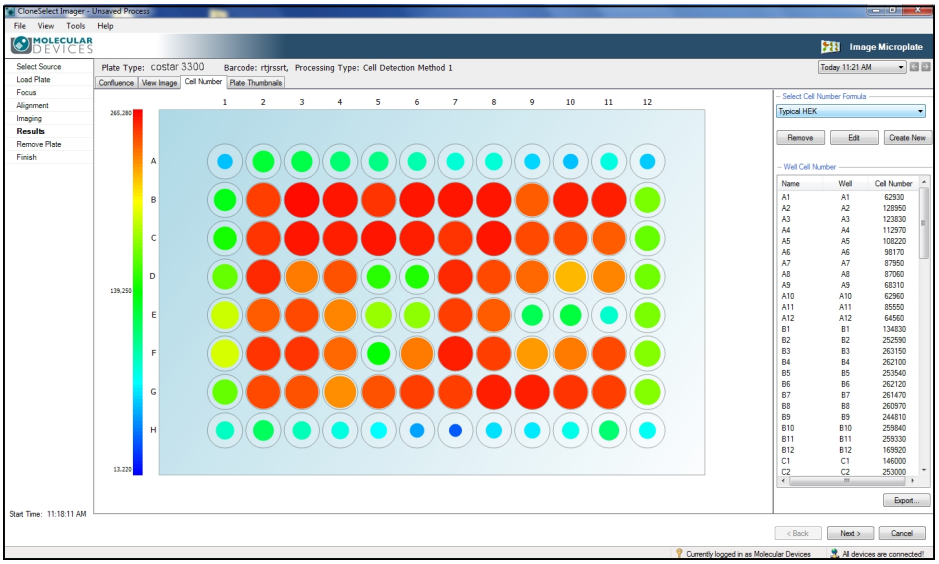
Results Screen - Confluence Tab

- **View Image:** Displays the image for the well you select. See View Image on page 77.



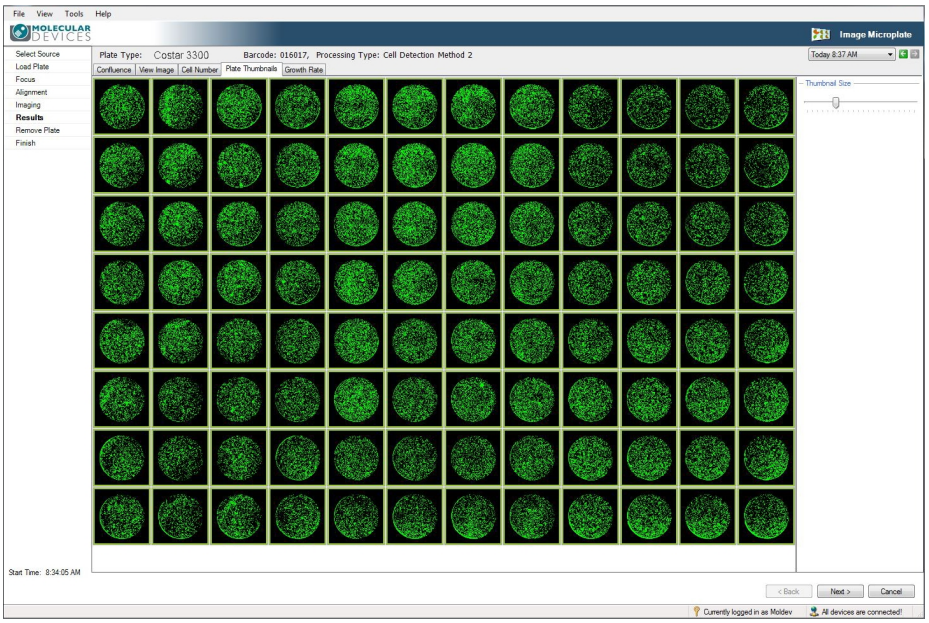
Results Screen - View Image Tab

- **Cell Number:** Displays the estimated number of cells in each well. See Cell Number on page 78.



Results Screen - Cell Number Tab

- **Plate Thumbnails:** Displays the confluence area for each well. See Plate Thumbnails on page 91.

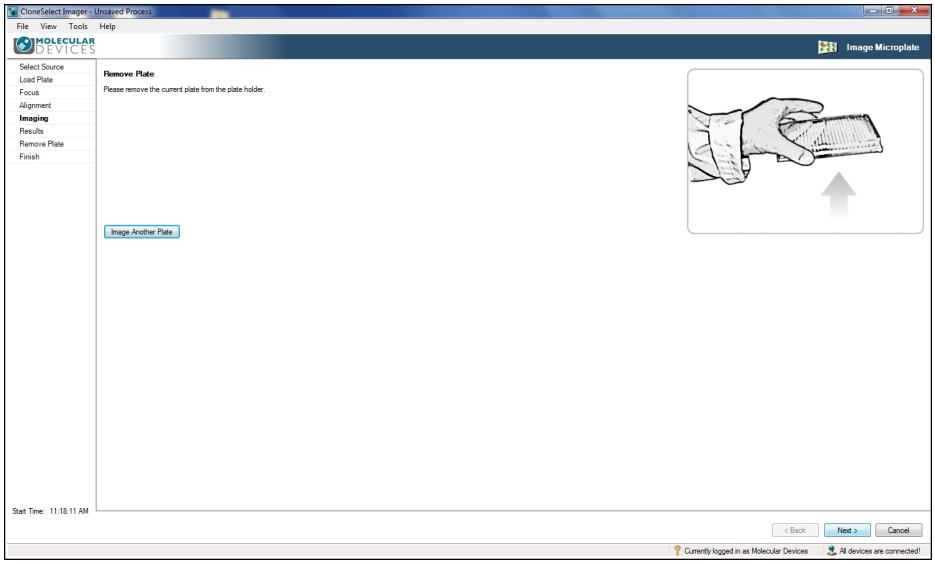


Results Screen - Plate Thumbnails Tab

Click **Next**.

Remove Plate

In the Remove Plate step, the plate carrier opens and the software prompts you to remove the plate from the plate carrier. You can repeat the process with another plate or continue to the end of the process.



Remove Plate Screen

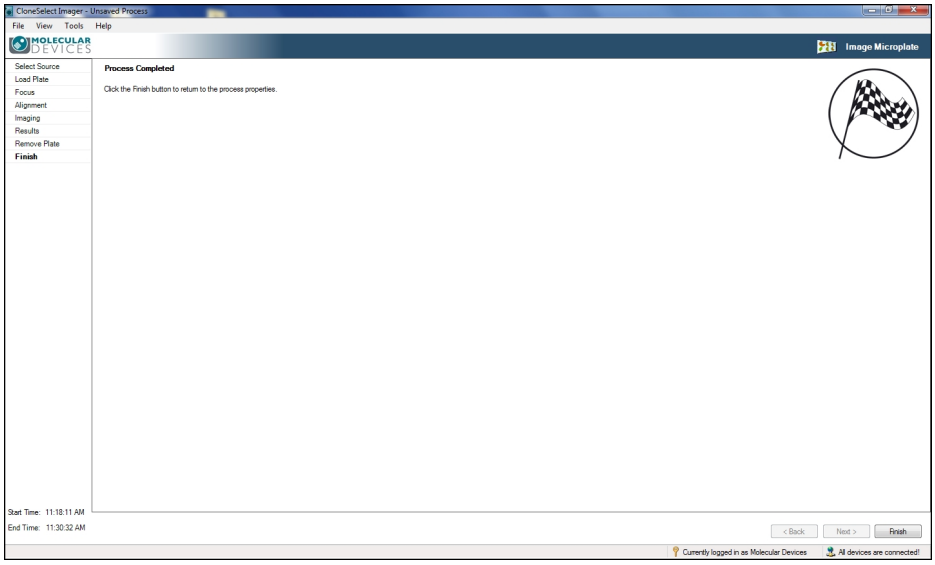
To image another plate click **Image Another Plate**. The plate carrier remains in the open position and the Select Source screen displays. See Select Source on page 29.

Click **Next**.

The plate carrier closes and the **Finish** screen displays.

Finish

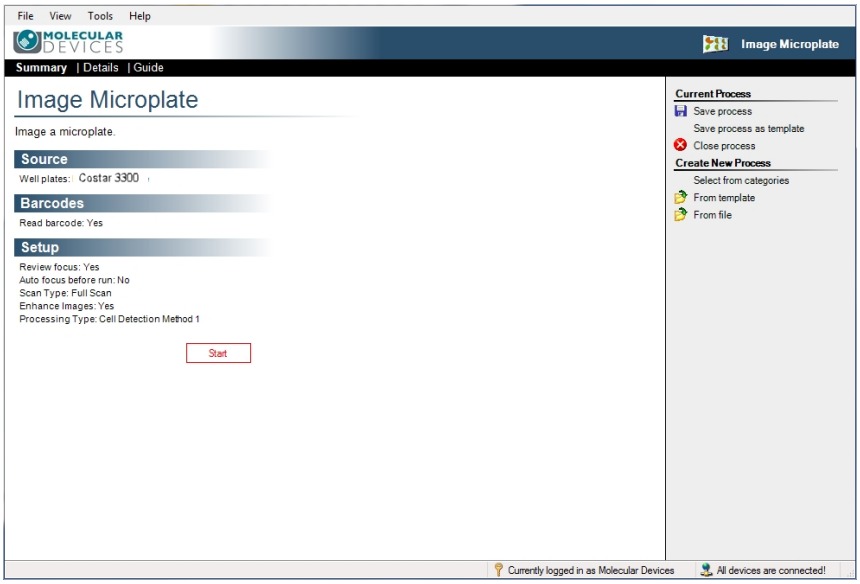
When the process ends, the **Finish** screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

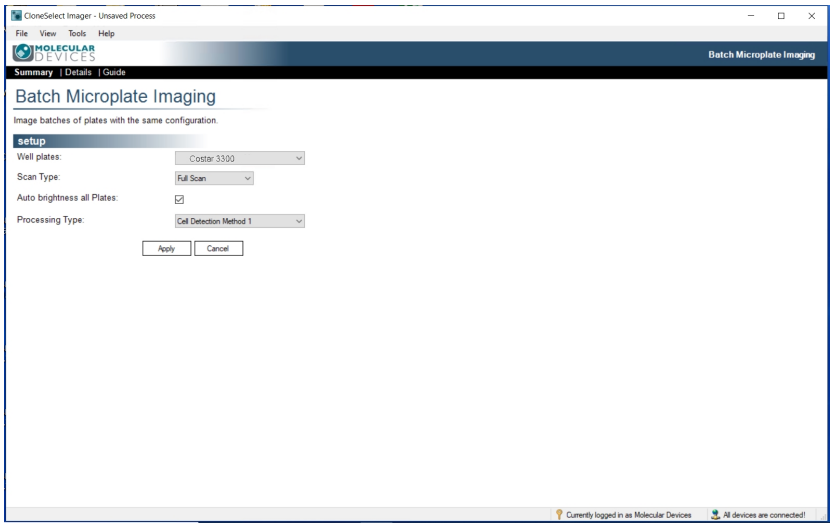
Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 5: Batch Microplate Imaging Process

Use the Batch Microplate Imaging process to image multiple plates with the same configuration without having to review the focus and alignment between plates. This process is for white light standard resolution imaging only, similar to the Image Microplate process. It cannot be used for high resolution or fluorescent imaging.

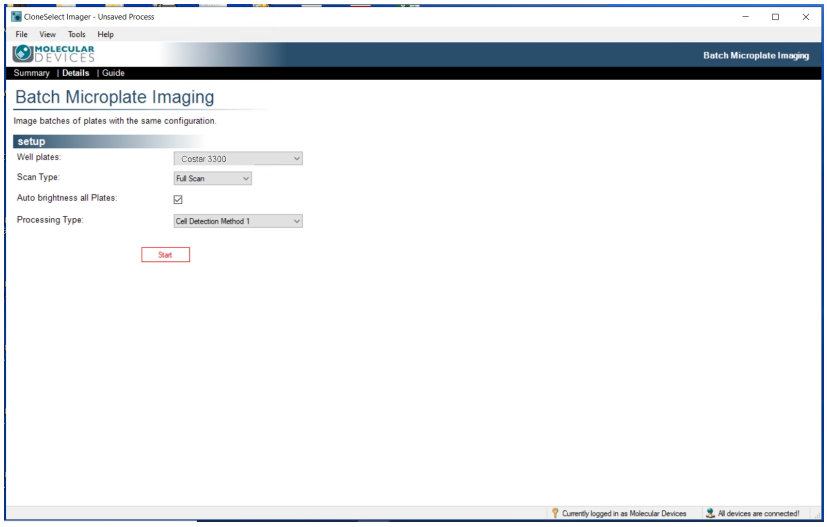
To start the Batch Microplate Imaging process, on the Navigation screen, double-click **Batch Microplate Imaging** to display the Properties screen with the Summary workflow selected.



Batch Microplate Imaging Process - Properties Screen - Summary Workflow

Setting Batch Microplate Imaging Process Properties

Start the Batch Microplate Imaging properties from the Details workflow on the Properties screen.



Properties Screen - Details Workflow

To set process properties:

1. Select the **Details** workflow at the top of the screen.
2. Click the **Well Plates** drop-down and select a plate type.

   Note: If your plate type does not appear in the list, contact Molecular Devices. See Obtaining Support on page 132.

3. Click the **Scan Type** drop-down:
   - Select **Full Scan** to image the entire area of each well. This is recommended for monoclonality applications.
   - Select **Partial Scan** to image an area from the center of each well and speed up imaging. This is suitable when the data from the center of each well is representative of the whole well.
4. Select the **Auto Brightness** check box to have the software select the appropriate brightness for the image.
   Clear the **Auto Brightness** check box to manually set the brightness.
5. Click the **Processing Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
6. Click **Start**

Select Source

Use the Select Source page to select the plate wells to image. The software marks the wells you select to image red and marks unselected wells light blue.

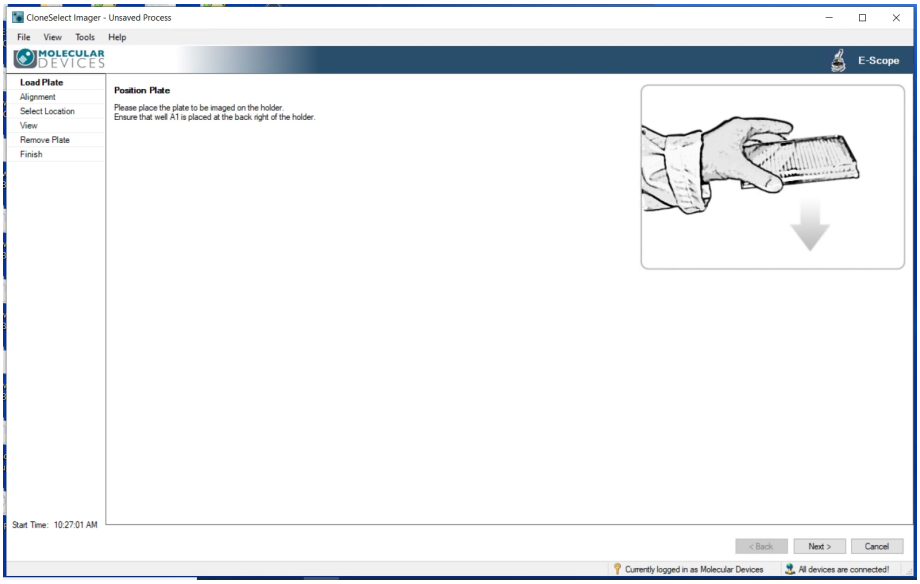
Select Source Screen

To select source wells:

1. Above the plate map, click the **Select Plate Type and Well to Image** drop-down and select a different plate type, if needed.
2. Select the wells to image.
   - Click on a well to select or deselect individual wells.
   - Right-click and drag to select or deselect groups of wells.
   - Click **Import** to use a well list file to select wells. See Well List File Format on page 133.
   - Click **Select All** to select all wells.
   - Click **Deselect All** to deselect all wells.
3. Click **Next**.

Load Plate

The plate carrier opens and the software prompts you to load the plate.



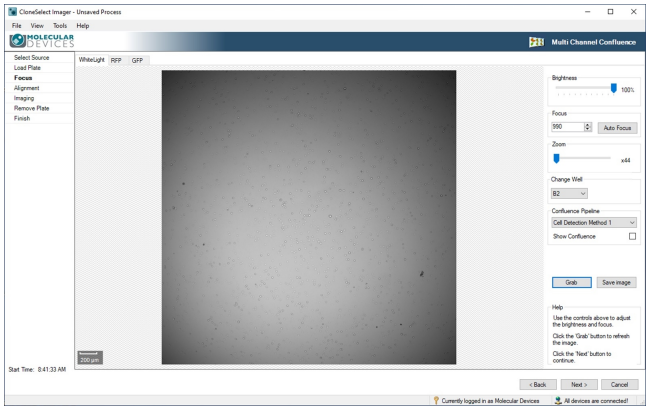
Load Plate Screen

To load a plate:

1. When the plate carrier opens, place the plate on the carrier with the A1 well in the back right corner.
2. Click **Next**.
3. If prompted, enter the barcode name and click **Next**.

Focus

Use the Focus screen to optimize the image view before the scan. The software stores the last focus setting you used for each plate type. After you image a plate type for the first time, additional focus adjustments should be minimal.



Focus Screen

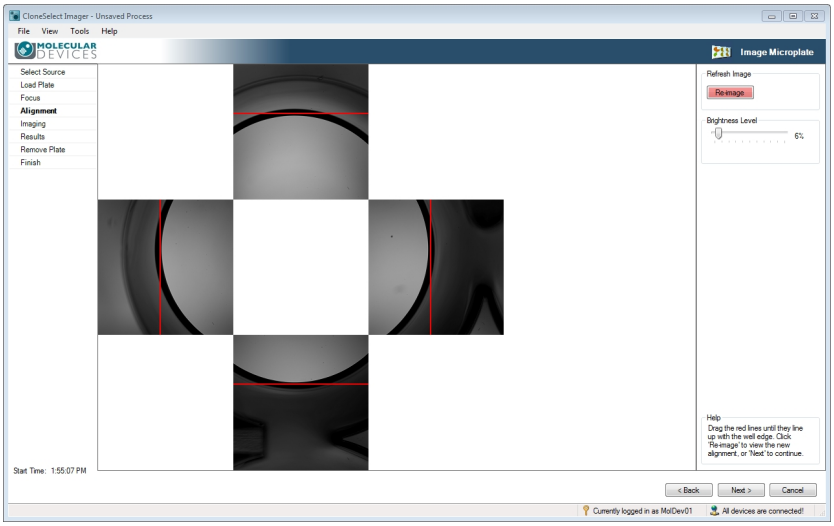
The software determines the optimal brightness setting for the image, but you can adjust the Brightness setting. You can use the Auto Focus feature or adjust the focus manually. By default the focus is done on well A1. If A1 is not being imaged, the focus is done on the first well of the plate to image.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus.
5. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
6. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
7. Click the **Processing Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
8. Select the **Generate Confluence** check box to generate confluence.
9. Click **Next**.

Alignment

Use the Alignment screen to confirm that the camera aligns with the wells. The software takes four images over the north, south, east, and west edges of well A1. The well edges should align with the middle of the box formed by the four red lines.



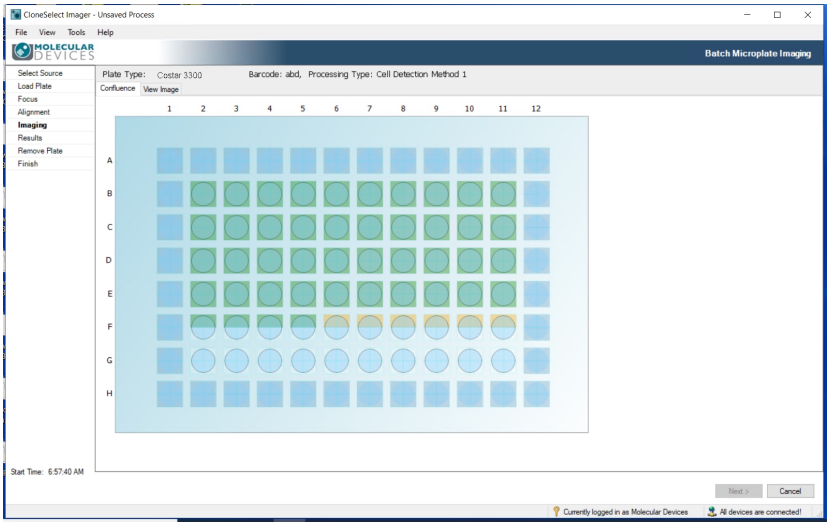
Alignment Screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines, click and drag on the lines to correct the alignment.
2. When the **Re-image** button is pink, click to recheck the alignment.
3. Move the **Brightness** slider to adjust brightness of the alignment image only.
4. Click **Next**.

Imaging

Imaging starts automatically. When the Imaging process finishes, the Results screen displays.



Imaging Screen - Confluence Tab

Imaging runs on the Confluence tab and you watch the process progress in the plate schematic. The plate schematic displays with the image frames superimposed in light blue. As the software captures and processes the images, the well frame changes color, light brown then to green. After a frame changes to green, click on the frame to view the image on the View Image tab. See View Image on page 77.

To see image thumbnails, select the Plate Thumbnails tab. See Thumbnails on page 96.

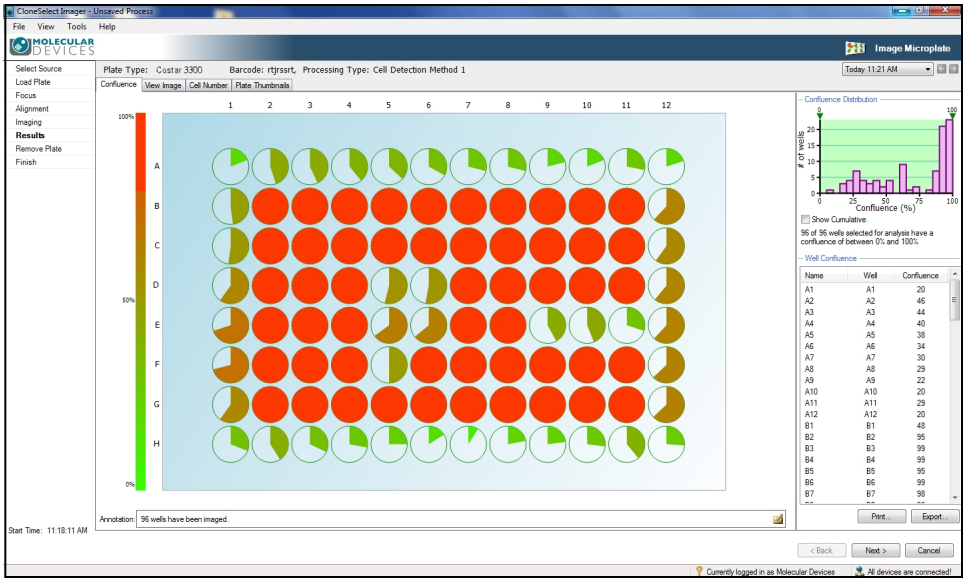
When imaging completes, the Results screen displays.

Results

After the process completes, the Results screen displays with the Confluence tab selected. The plate type, barcode, and processing type display for reference. Use the tabs to review and analyze the results. See Review Results Process on page 73.

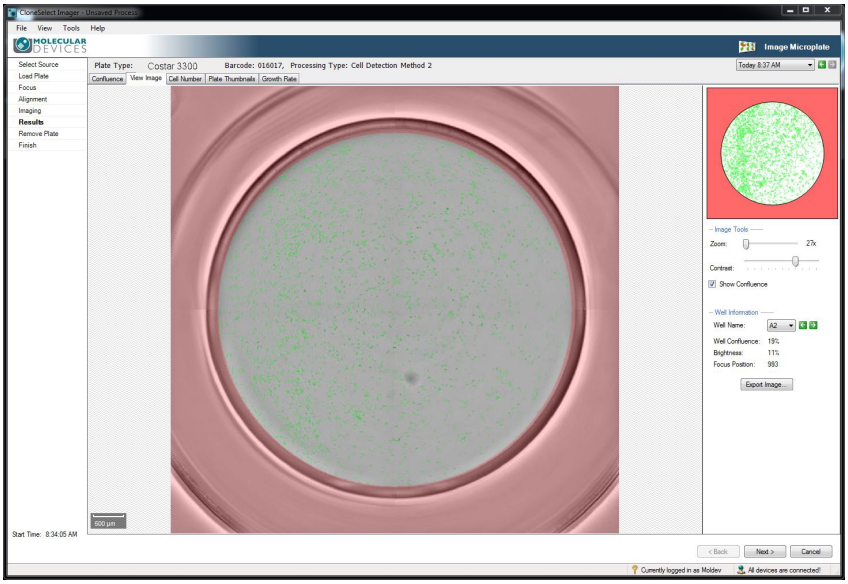
The Results screen includes the following tabs:

- **Confluence:** Displays the confluence level for each well. See Confluence on page 76.



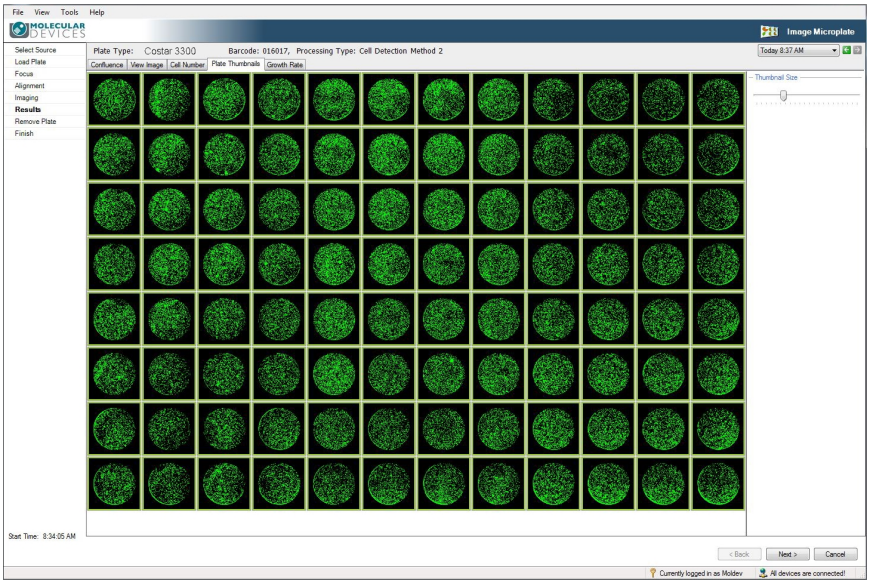
Results Screen - Confluence Tab

- **View Image:** Displays the image for the well you select. See View Image on page 77.



Results Screen - View Image Tab

- **Plate Thumbnails:** Displays the confluence area for each well. See Plate Thumbnails on page 91.

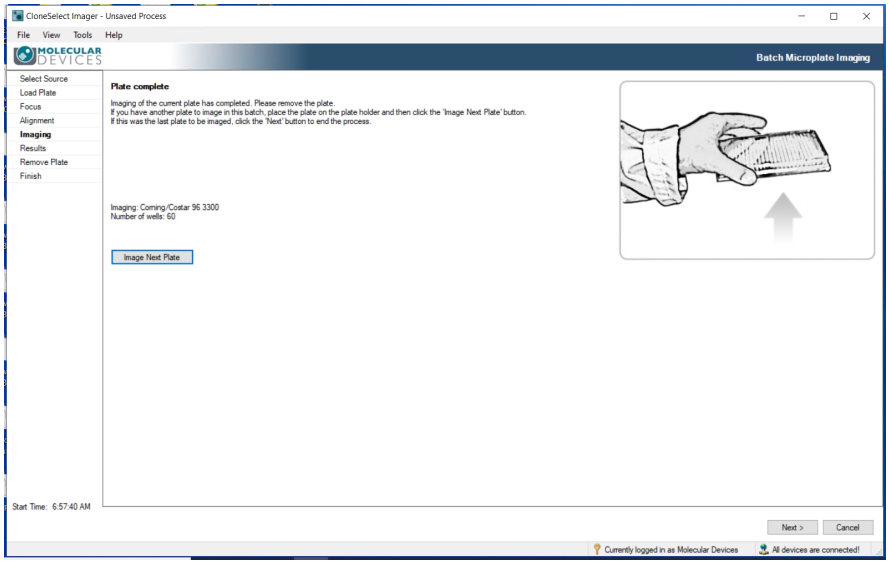


Results Screen - Plate Thumbnails Tab

Click **Next**.

Remove Plate

In the Remove Plate step, the plate carrier opens and the software prompts you to remove the plate from the plate carrier. You can repeat the process with another plate or continue to the end of the process.



Remove Plate Screen

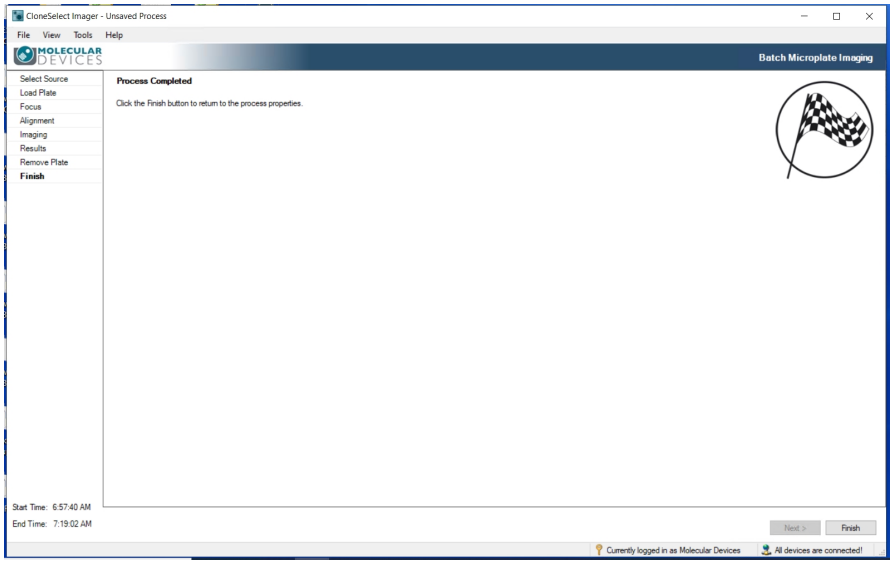
To image another plate click **Image Another Plate**. The plate carrier remains in the open position and the Select Source screen displays. See Select Source on page 29.

Click **Next**.

The plate carrier closes and the **Finish** screen displays.

Finish

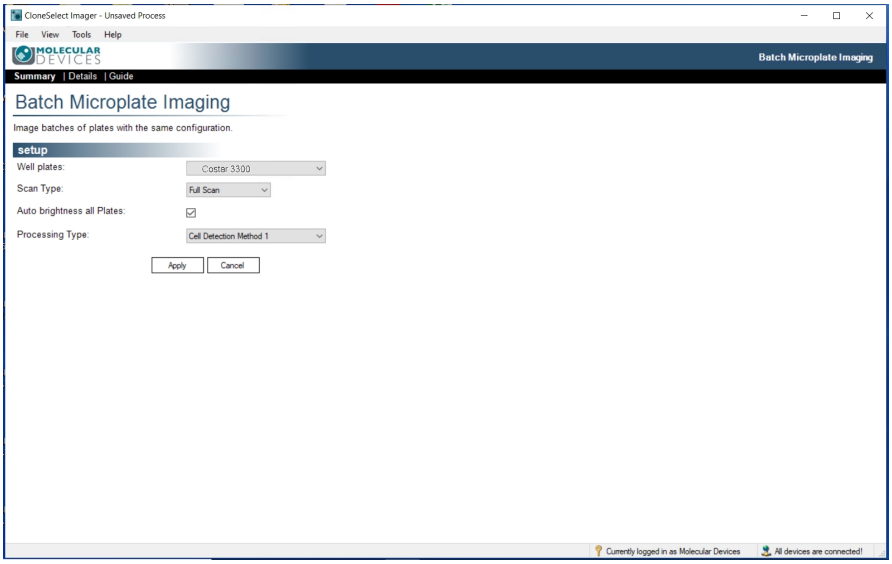
When the process ends, the **Finish** screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 6: Image Microplate High Resolution Process

Use the Image Microplate High Resolution process to image cells in plates to determine the confluence and carry out further analysis, such as cell number estimation and loci counts. Image resolution in this process is 1.725 µm per pixel.

To start the Image Microplate High Resolution process, on the Navigation screen, double-click **Image Microplate High Resolution** to display the Properties screen with the Summary workflow selected.

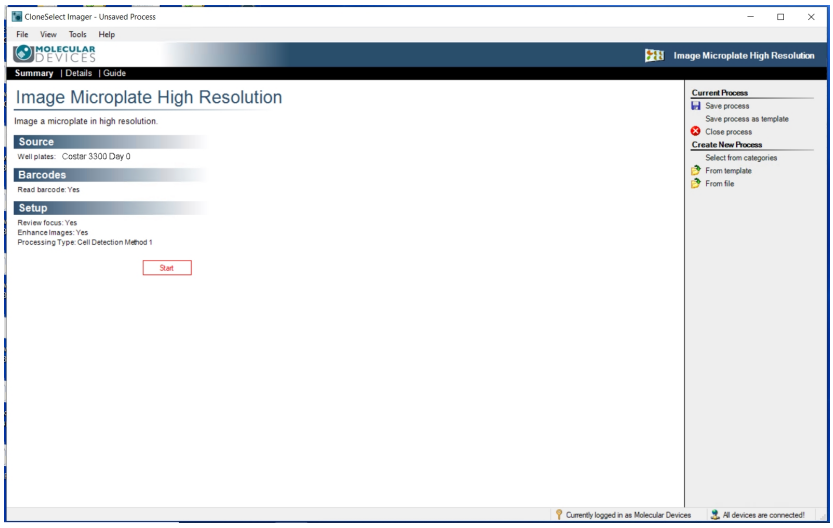
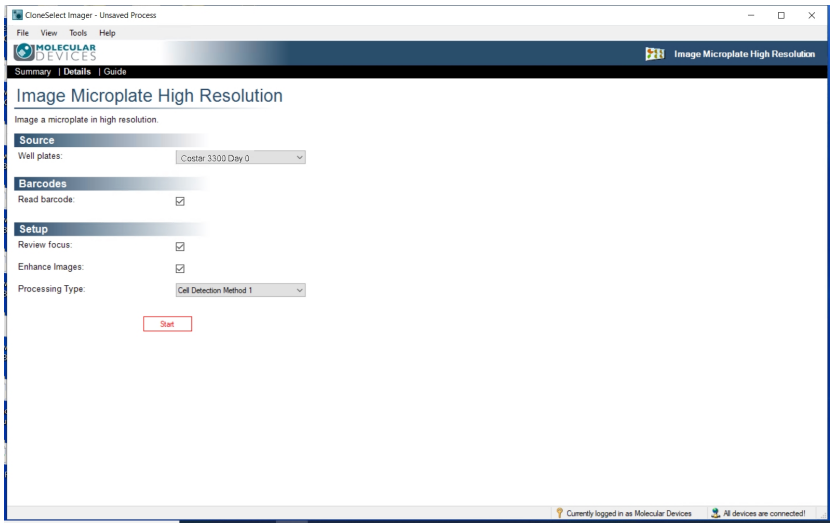


Image Microplate High Resolution Process - Properties Screen - Summary Workflow

Setting Image Microplate High Resolution Process Properties

Start the Image Microplate High Resolution process from the Details workflow on the Properties screen.



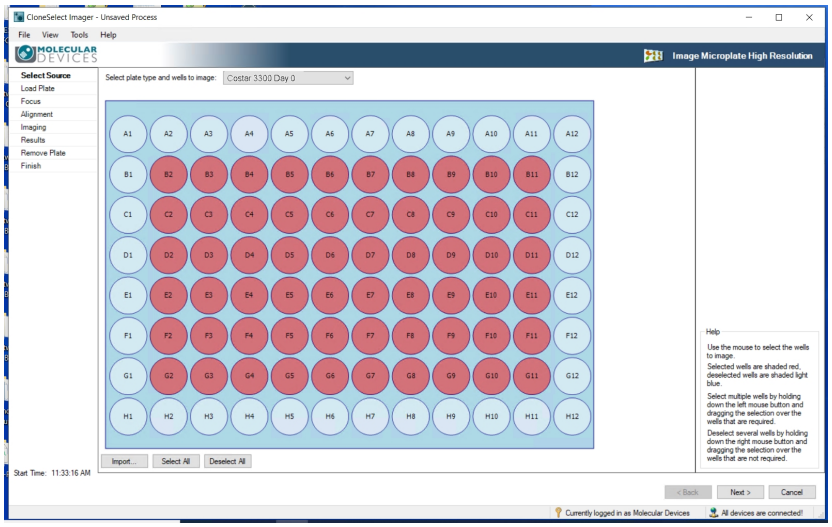
Properties Screen - Details Workflow

To set process properties:

1. Select the **Details** workflow at the top of the screen.
2. Click the **Well Plates** drop-down and select a plate type.
3. Select the **Read Barcode** check box if you use barcodes.
   Clear the **Read Barcode** check box if you do not use barcodes.
   If you select this check box and the instrument fails to read the barcode, a message displays to allow you to manually enter the barcode. You can use the Review Results process to edit the barcode or name. See Review Results Process on page 73.
4. Select the **Review Focus** check box to have the software prompt you to check the focus and brightness settings on the Focus screen before you image the plate. See Focus on page 30.
   Clear the **Review Focus** check box to not receive a prompt to check the focus.
5. Select the **Enhance Images** check box to enhance images for display by flattening the background. This is recommended for monoclonality applications.
   Clear the **Enhance Images** check box to not use the enhance images feature.
6. Click the **Processing Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
7. Click **Start**.

Select Source

Use the Select Source page to select the plate wells to image. The software marks the wells you select to image red and marks unselected wells light blue.



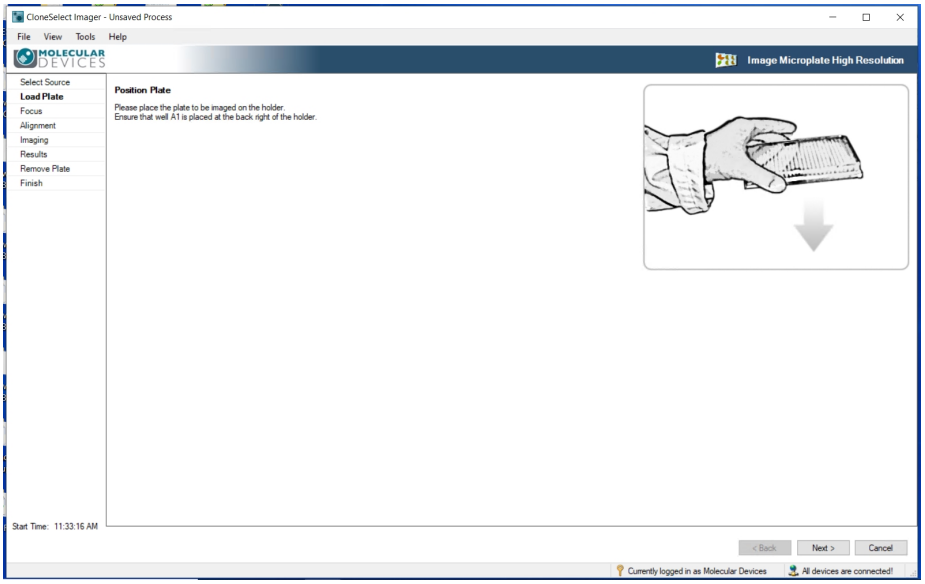
Select Source Screen

To select source wells:

1. Above the plate map, click the **Select Plate Type and Well to Image** drop-down and select a different plate type, if needed.
2. Select the wells to image.
   - Click on a well to select or deselect individual wells.
   - Right-click and drag to select or deselect groups of wells.
   - Click **Import** to use a well list file to select wells. See Well List File Format on page 133.
   - Click **Select All** to select all wells.
   - Click **Deselect All** to deselect all wells.
3. Click **Next**.

Load Plate

The plate carrier opens and the software prompts you to load the plate.



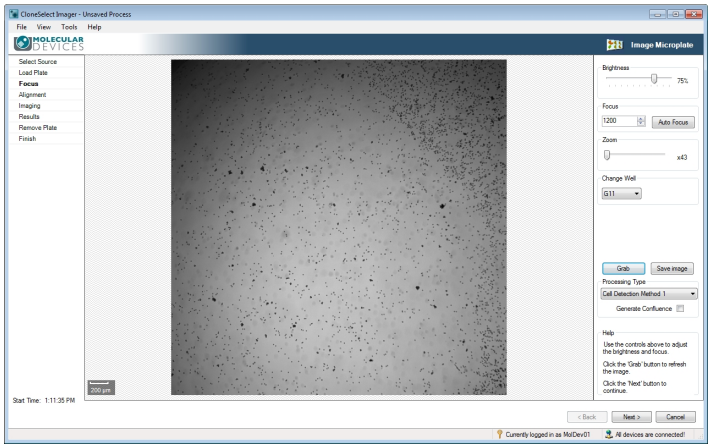
Load Plate Screen

To load a plate:

1. When the plate carrier opens, place the plate on the carrier with the A1 well in the back right corner.
2. Click **Next**.
3. If prompted, enter the barcode name and click **Next**.

Focus

Use the Focus screen to optimize the image view before the scan. The software stores the last focus setting you used for each plate type. After you image a plate type for the first time, additional focus adjustments should be minimal.



Focus Screen

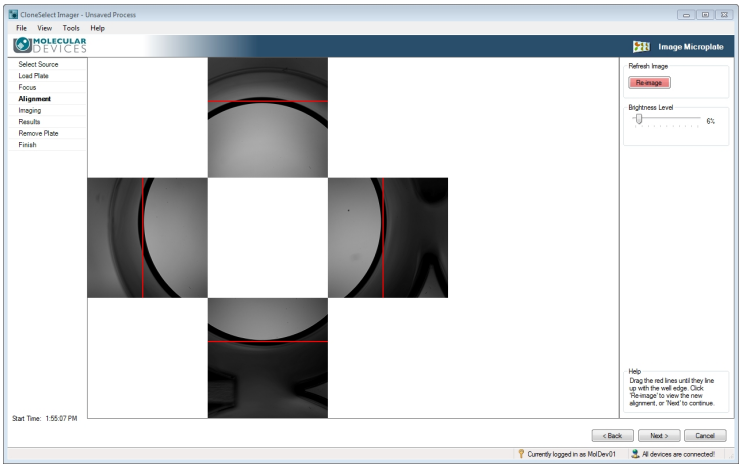
By default the focus is done on well A1. If A1 is not being imaged, the focus is done on the first well of the plate to image.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus.
5. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
6. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
7. Click the **Processing Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
8. Select the **Generate Confluence** check box to generate confluence.
   Clear the **Generate Confluence** to not generate confluence.
9. Click **Next**.

Alignment

Use the Alignment screen to confirm that the camera aligns with the wells. The software takes four images over the north, south, east, and west edges of well A1. The well edges should align with the middle of the box formed by the four red lines.



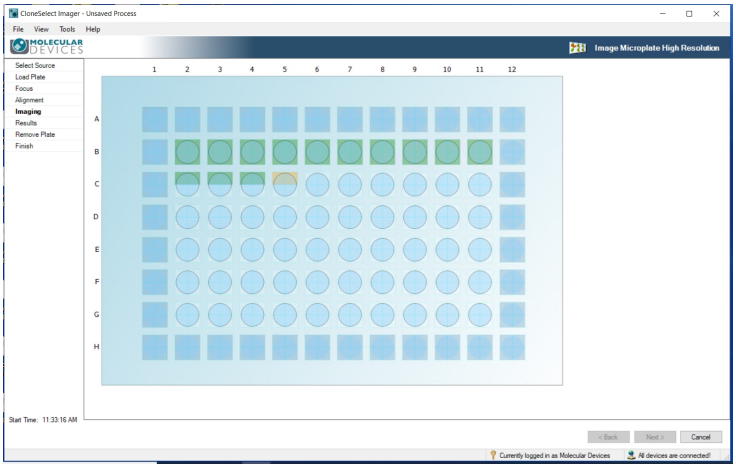
Alignment Screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines, click and drag on the lines to correct the alignment.
2. When the **Re-image** button is pink, click to recheck the alignment.
3. Move the **Brightness** slider to adjust brightness of the alignment image only.
4. Click **Next**.

Imaging

Imaging starts automatically. When the process finishes, the Results screen displays.



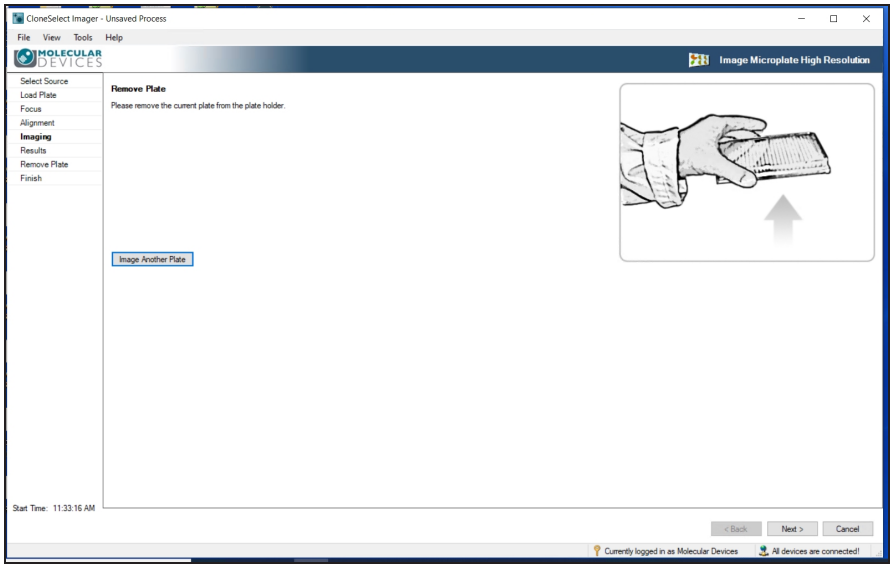
Imaging Screen

Watch the process progress in the plate schematic. The plate schematic displays with the image frames superimposed in light blue. As the software captures and processes the images, the well frame changes color, light brown then to green.

When imaging completes, the Remove Plate screen displays.

Remove Plate

In the Remove Plate step, the plate carrier opens and the software prompts you to remove the plate from the plate carrier. You can repeat the process with another plate or continue to the end of the process.



Remove Plate Screen

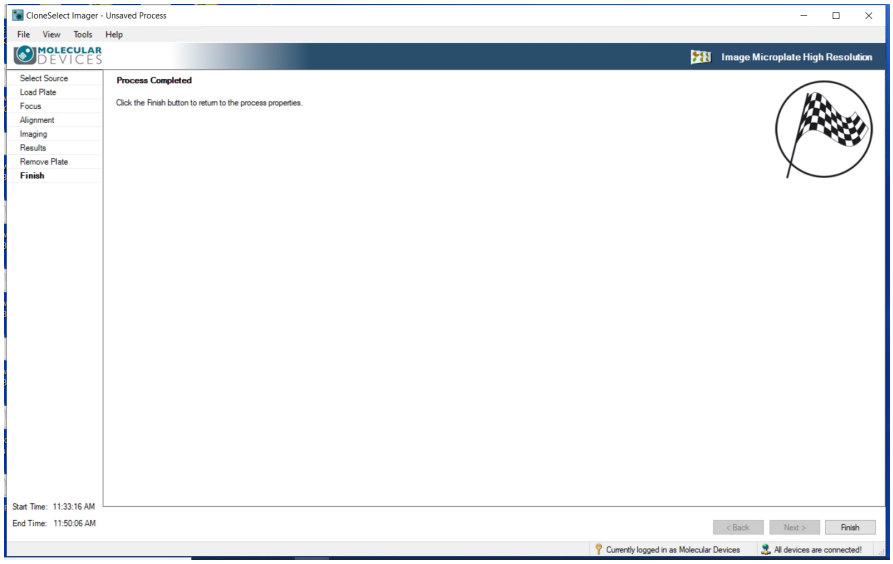
To image another plate click **Image Another Plate**. The plate carrier remains in the open position and the Select Source screen displays. See Select Source on page 29.

Click **Next**.

The plate carrier closes and the **Finish** screen displays.

Finish

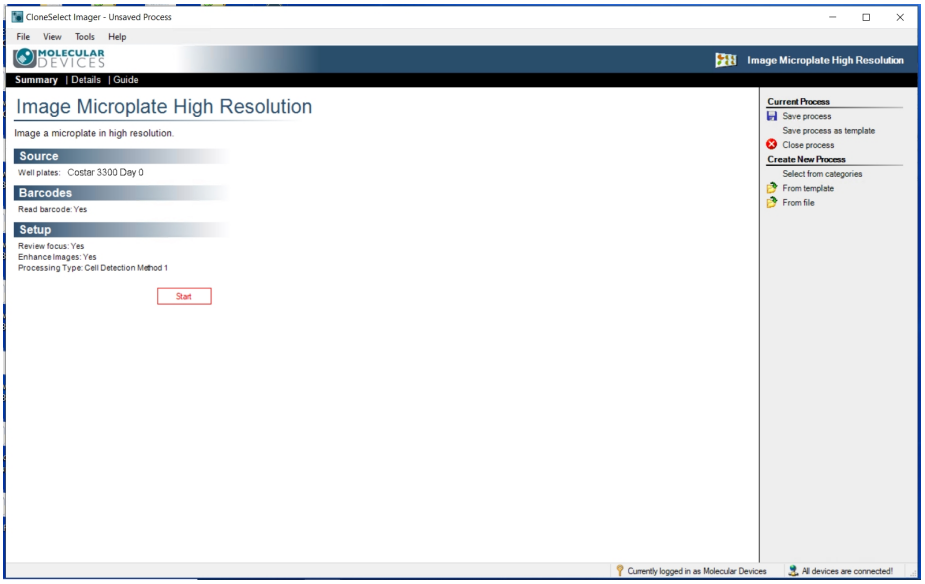
When the process ends, the **Finish** screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 7: Image Microplate Multi Channel Process

Use the Image Microplate Multi Channel process to do white light imaging and fluorescence imaging of cells in plates for monoclonality application.

To start the Image Microplate Multi Channel process, on the Navigation screen, double-click **Image Microplate Multi Channel** to display the Properties screen with the Summary workflow selected.

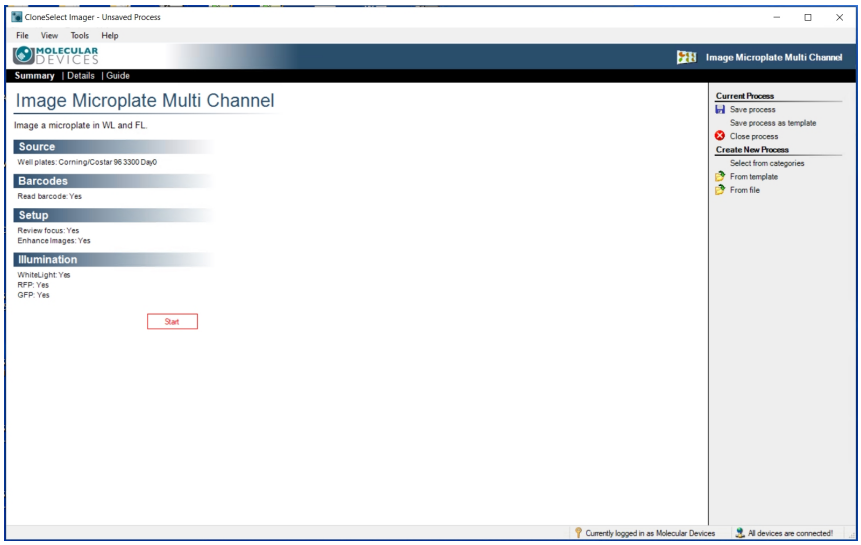
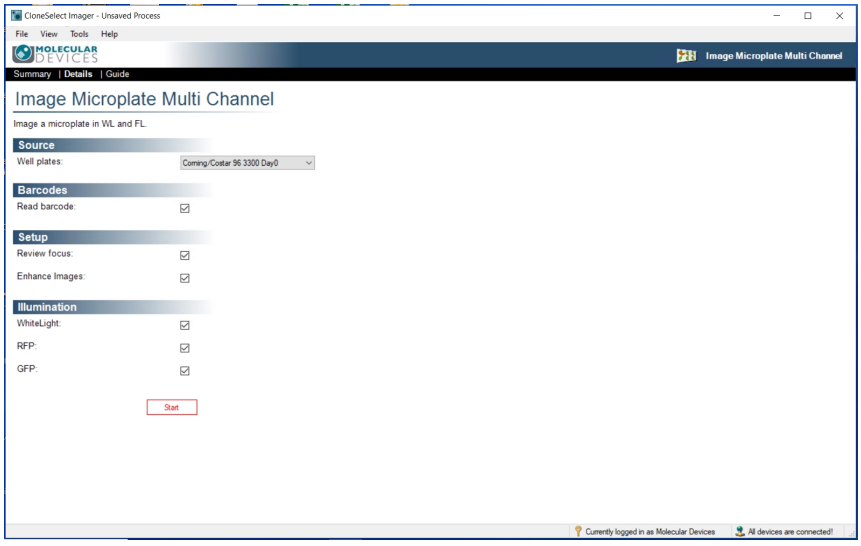


Image Microplate Multi Channel Process - Properties Screen - Summary Workflow

Setting Image Microplate Multi Channel Process Properties

Start the Image Microplate Multi Channel process from the Details workflow on the Properties screen.



Properties Screen - Details Workflow

To set process properties:

1. Select the **Details** workflow at the top of the screen.
2. Click the **Well Plates** drop-down and select a day zero plate type such as Corning/Costar 96-well 3300 Day 0 to ensure the entire well is imaged.

   Note: Images that use a standard configuration plate type can cut off the well edge.

3. Select the **Read Barcode** check box if you use barcodes.
   Clear the **Read Barcode** check box if you do not use barcodes.
   If you select this check box and the instrument fails to read the barcode, a message displays to allow you to manually enter the barcode. You can use the Review Results process to edit the barcode or name. See Review Results Process on page 73.
4. Select the **Review Focus** check box to have the software prompt you to check the focus and brightness settings on the Focus screen. See Focus on page 60.
   Clear the **Review Focus** check box to not receive a prompt to check the focus.
5. Select the **Enhance Images** check box to enhance images for display by flattening the background. This is recommended for monoclonality applications.
   Clear the **Enhance Images** check box to not use the enhance images feature.
6. Select the **White Light** check box to use white light.
   Clear the **White Light** check box to not use white light.
7. Select the **RFP** check box to use RFP.
   Clear the **RFP** check box to not use RFP.
8. Select the **GFP** check box to use GFP.
   Clear the **GFP** check box to not use GFP.

   Note: You must select the RFP check box and/or the GFP check box for the Image Microplate Multi Channel process. For White Light only detection, use the Image Microplate process or the Image Microplate High Resolution process.

9. Click **Start**.

Select Source

Use the Select Source page to select the plate wells to image. The software marks the wells you select to image red and marks unselected wells light blue.

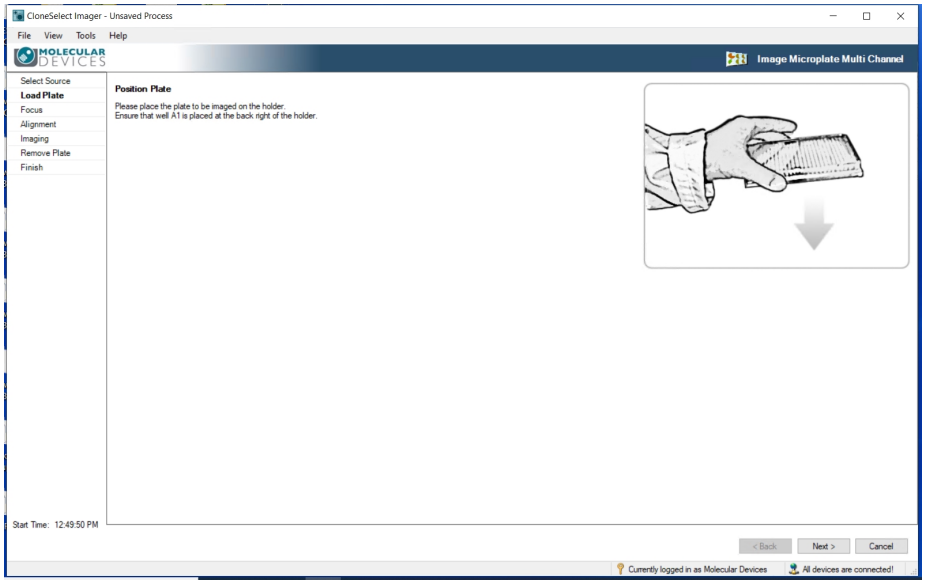
Select Source Screen

To select source wells:

1. Above the plate map, click the **Select Plate Type and Well to Image** drop-down and select a different plate type, if needed.
2. Select the wells to image.
   - Click on a well to select or deselect individual wells.
   - Right-click and drag to select or deselect groups of wells.
   - Click **Import** to use a well list file to select wells. See Well List File Format on page 133.
   - Click **Select All** to select all wells.
   - Click **Deselect All** to deselect all wells.
3. Click **Next**.

Load Plate

The plate carrier opens and the software prompts you to load the plate.



Load Plate Screen

To load a plate:

1. When the plate carrier opens, place the plate on the carrier with the A1 well in the back right corner.
2. Click **Next**.
3. If prompted, enter the barcode name and click **Next**.

Focus

Use the Focus screen to optimize the image view before the scan. The software stores the last focus setting you used for each plate type. After you image a plate type for the first time, additional focus adjustments should be minimal.

The Focus screen has up to three tabs.

White Light

Focus Screen - White Light Tab

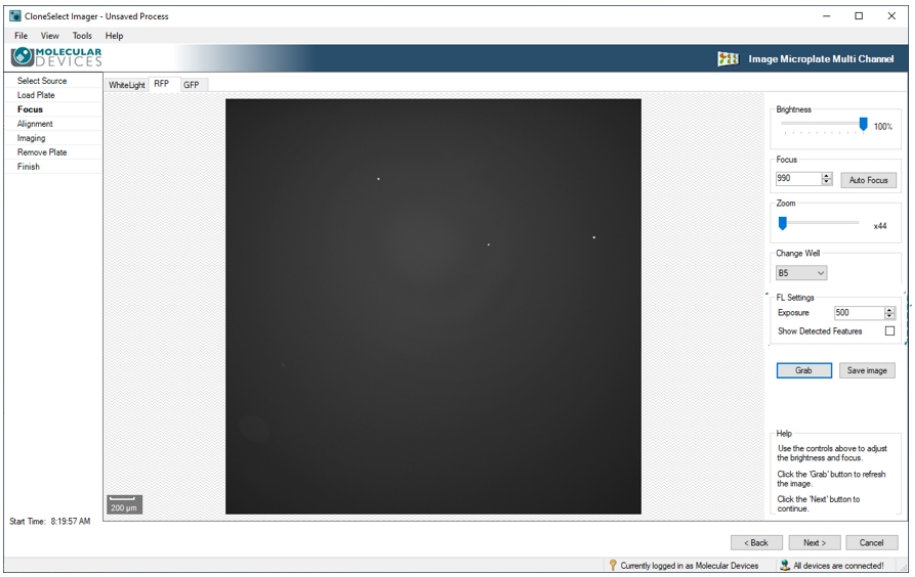
Note: Do not use an edge or corner well for focus determination.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness. Recommended setting is 70-100% brightness, depending on plate and media type you use.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus. We recommend using well B2.
5. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
6. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
7. Click **Next**.

RFP Tab and GFP Tab

The RFP tab and the GFP tab have the same workflow and settings.



Focus Screen - RFP Tab and GFP Tab

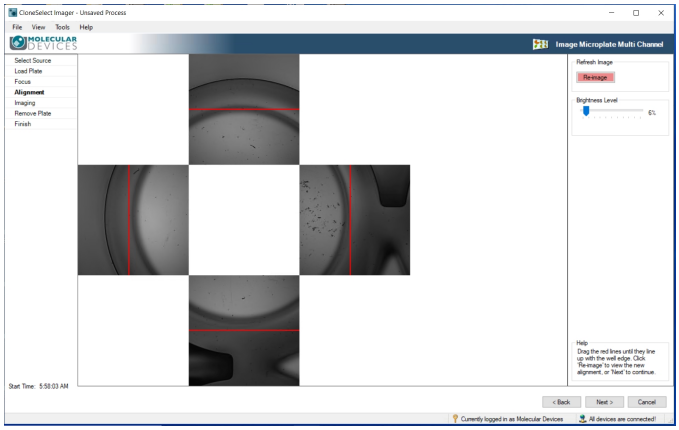
Note: Do not use an edge or corner well for focus determination.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus. We recommend using well B2.
5. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
6. In the **FL Settings Exposure** field, enter the exposure.
7. Select the **Show Detected Features** check box to show detected features.
   Clear the **Show Detected Features** check box to not show detected features.
8. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
9. Click **Next**.

Alignment

Use the Alignment screen to confirm that the camera aligns with the wells. The software takes four images over the north, south, east, and west edges of well A1. The well edges should align with the middle of the box formed by the four red lines.



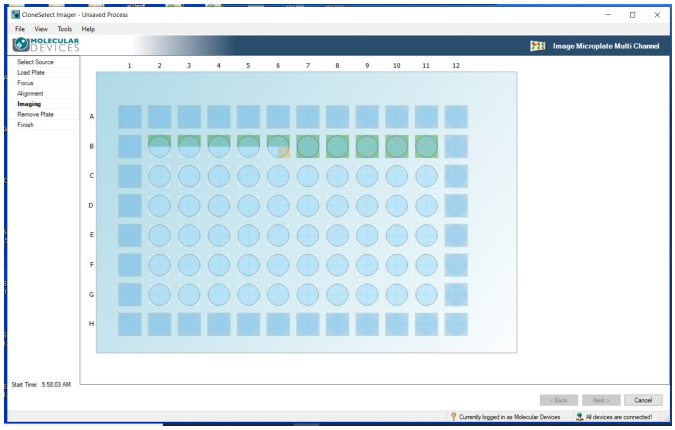
Alignment Screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines, click and drag on the lines to correct the alignment. For day zero plates, the well diameter is slightly smaller than the box formed by the red lines. Center the well within the red lines.
2. When the **Re-image** button is pink, click to recheck the alignment.
3. Move the **Brightness** slider to adjust brightness of the alignment image only.
4. Click **Next**.

Imaging

Imaging starts automatically. When the process finishes, the Remove Plate screen displays.

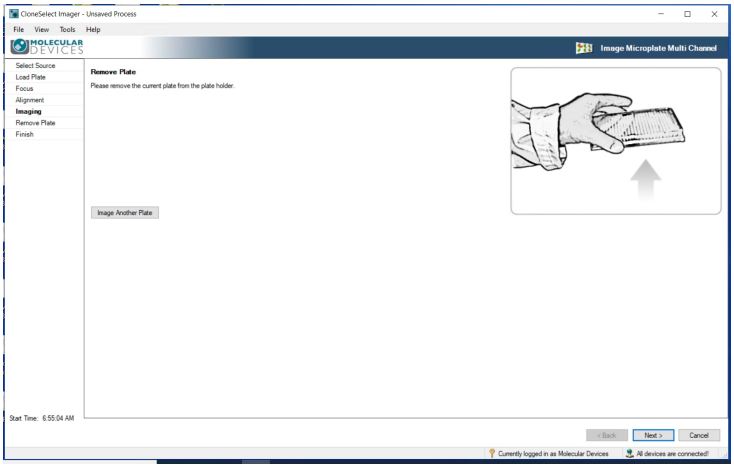


Imaging Screen

Watch the process progress in the plate schematic. The plate schematic displays with the image frames superimposed in light blue. As the software captures and processes the images, the well frame changes color, light brown then to green. When the imaging process finishes the Remove Plate screen displays.

Remove Plate

In the Remove Plate step, the plate carrier opens and the software prompts you to remove the plate from the plate carrier. You can repeat the process with another plate or continue to the end of the process.



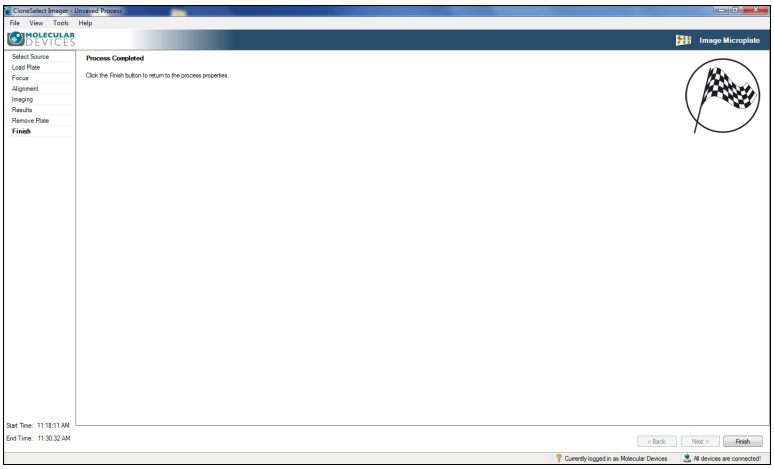
Remove Plate Screen

To image another plate click **Image Another Plate**. The plate carrier remains in the open position and the Select Source screen displays. See Select Source on page 58.

To continue to the next step, click **Next**. The plate carrier closes and the **Finish** screen displays.

Finish

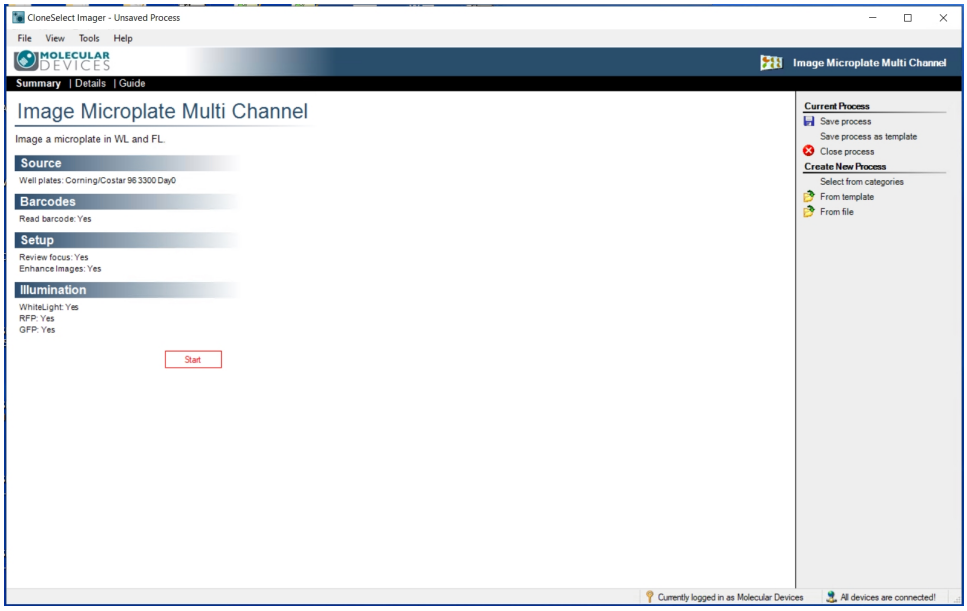
When the process ends, the **Finish** screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.
- Run the Review Results process to review the results. See Review Results Process on page 73.

Chapter 8: Image Microplate Multi Channel Confluence Process

Use the Image Microplate Multi Channel Confluence process to do white light and fluorescence imaging for confluence.

To start the Image Microplate Multi Channel Confluence process, on the Navigation screen, double-click **Image Microplate Multi Channel Confluence** to display the Properties screen with the Summary workflow selected.

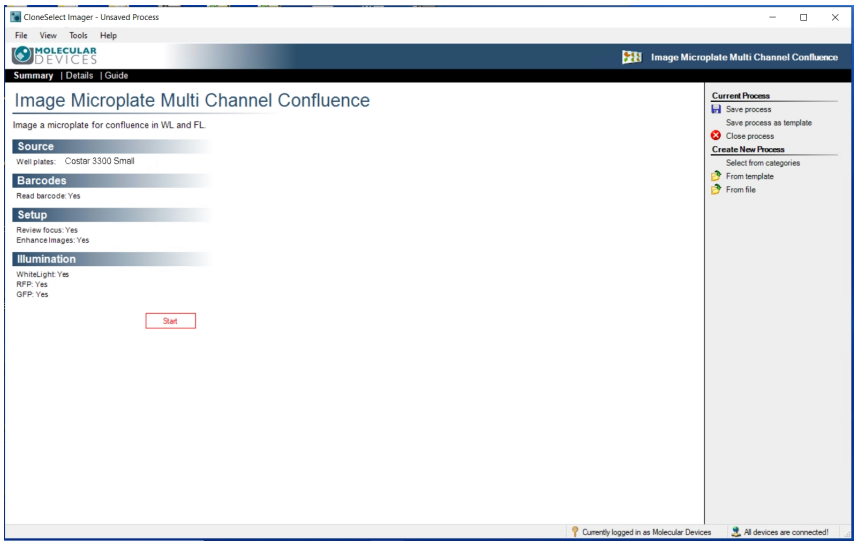
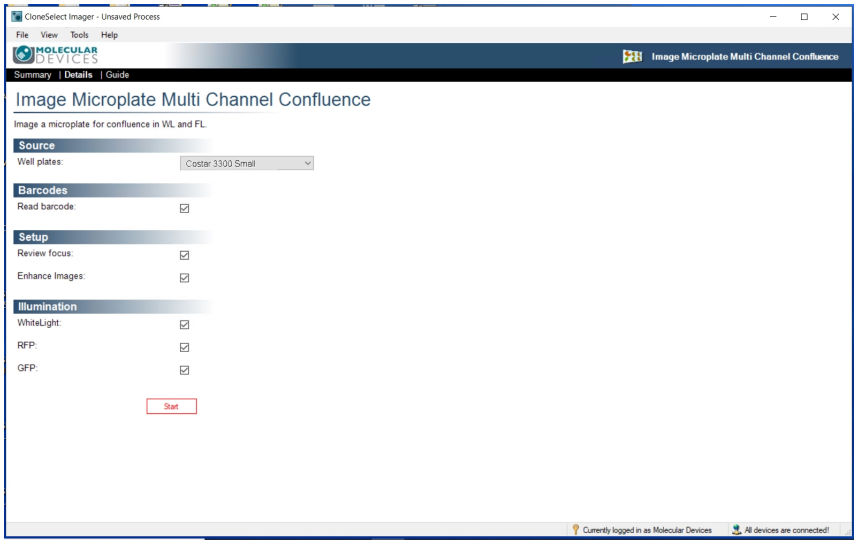


Image Microplate Multi Channel Confluence Process - Properties Screen - Summary Workflow

Setting Multi Channel Confluence Process Properties

Start the Multi Channel Confluence process from the Details workflow on the Properties screen.



Properties Screen - Details Workflow

To set process properties:

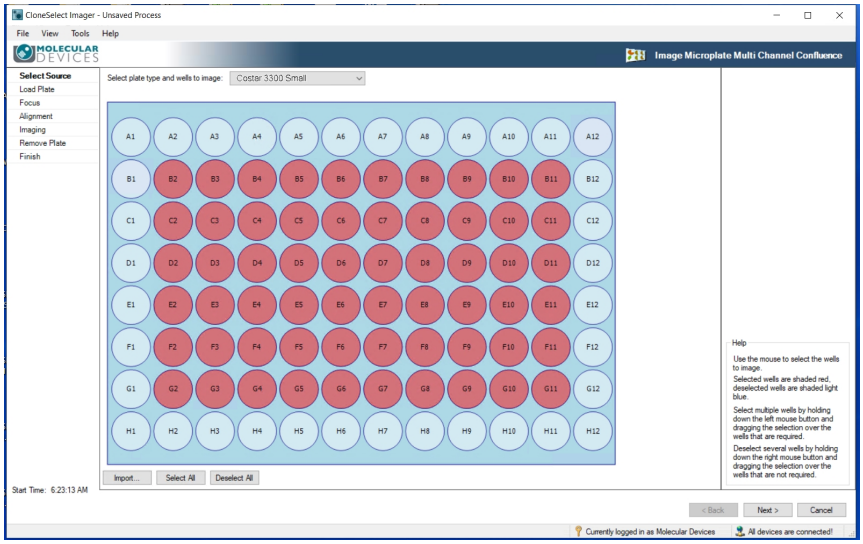
1. Select the **Details** workflow at the top of the screen.
2. Click the **Well Plates** drop-down and select a plate type that has a configuration that is smaller than the well diameter such as Corning/Costar 96-well 3300 Small, otherwise the instrument detects the well edge as confluence which can greatly skew results.
3. Select the **Read Barcode** check box if you use barcodes.
   Clear the **Read Barcode** check box if you do not use barcodes.
   If you select this check box and the instrument fails to read the barcode, a message displays to allow you to manually enter the barcode. You can use the Review Results process to edit the barcode or name. See Review Results Process on page 73.
4. Select the **Review Focus** check box to have the software prompt you to check the focus and brightness settings on the Focus screen before you image the plate. See Focus on page 60.
   Clear the **Review Focus** check box to not receive a prompt to check the focus.
5. Select the **Enhance Images** check box to enhance images for display by flattening the background. This is recommended for monoclonality applications.
   Clear the **Enhance Images** check box to not use the enhance images feature.
6. Select the **White Light** check box to use white light.
   Clear the **White Light** check box to not use white light.
7. Select the **RFP** check box to use RFP.
   Clear the **RFP** check box to not use RFP.
8. Select the **GFP** check box to use GFP.
   Clear the **GFP** check box to not use GFP.

   Note: You must select the RFP check box and/or the GFP check box for the Image Microplate Multi Channel Confluence process. For White Light only detection, use the Image Microplate process or the Image Microplate High Resolution process.

9. Click **Start**.

Select Source

Use the Select Source page to select the plate wells to image. The software marks the wells you select to image red and marks unselected wells light blue.



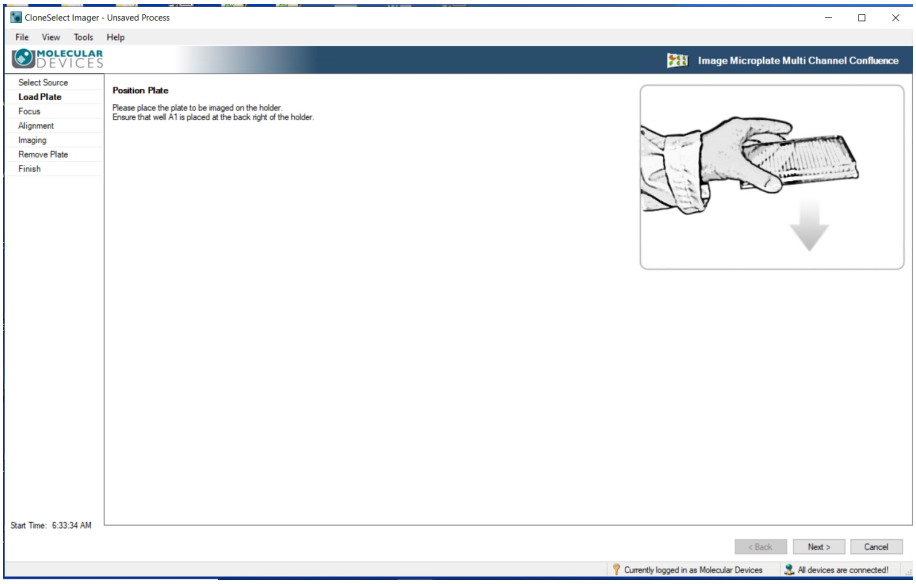
Select Source Screen

To select source wells:

1. Above the plate map, click the **Select Plate Type and Well to Image** drop-down and select a different plate type, if needed.
2. Select the wells to image.
   - Click on a well to select or deselect individual wells.
   - Right-click and drag to select or deselect groups of wells.
   - Click **Import** to use a well list file to select wells. See Well List File Format on page 133.
   - Click **Select All** to select all wells.
   - Click **Deselect All** to deselect all wells.
3. Click **Next**.

Load Plate

The plate carrier opens and the software prompts you to load the plate.



Load Plate Screen

To load a plate:

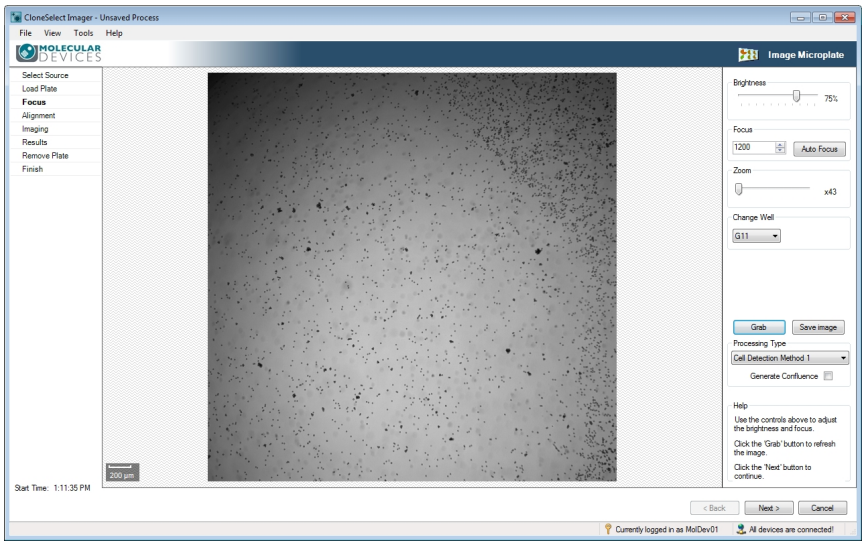
1. When the plate carrier opens, place the plate on the carrier with the A1 well in the back right corner.
2. Click **Next**.
3. If prompted, enter the barcode name and click **Next**.

Focus

Use the Focus screen to optimize the image view before the scan. The software stores the last focus setting you used for each plate type. After you image a plate type for the first time, additional focus adjustments should be minimal.

The Focus screen has up to three tabs.

White Light



Focus Screen - White Light Tab

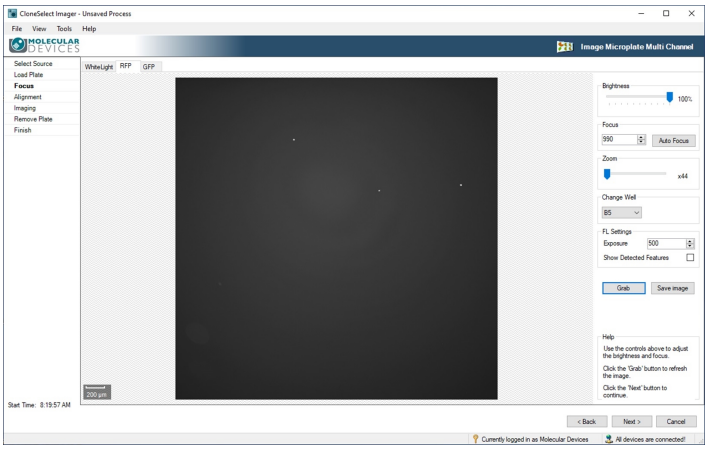
Note: Do not use an edge or corner well for focus determination.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness. Recommended setting is 70-100% brightness, depending on plate and media type you use.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus. We recommend using well B2.
5. Click the **Confluence Pipeline Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
6. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
7. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
8. Click **Next**.

RFP Tab and GFP Tab

The RFP tab and the GFP tab have the same workflow and settings.



Focus Screen - RFP Tab and GFP Tab

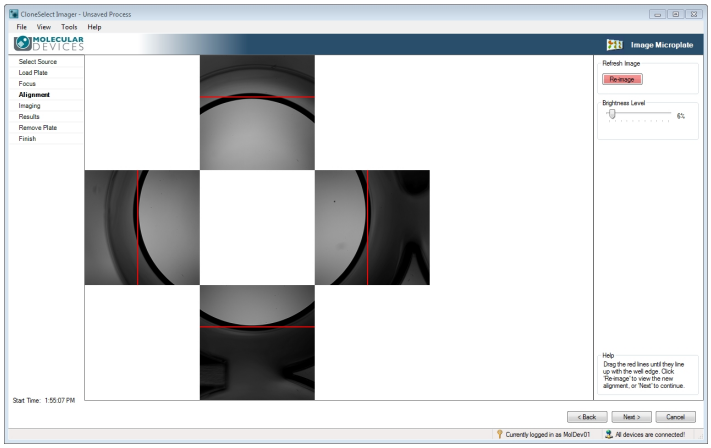
Note: Do not use an edge or corner well for focus determination.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus. We recommend using well B2.
5. Click the **Confluence Pipeline** drop-down:
   - Select **Confluence High Contrast** for brightly fluorescent cells which have high contrast against the image background.
   - Select **Confluence Medium Contrast** for fluorescent cells which have medium contrast against the image background.
   - Select **Confluence Low Contrast** for dimly fluorescent cells which have low contrast against the image background.
6. Select the **Show Confluence** check box to overlay the area of the image outside the well in red and the regions of the well where colony growth is detected in green.
   Clear the **Show Confluence** check box to not overlay the area of the image.
7. In the **FL Settings Exposure** field, enter the exposure.
8. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
9. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
10. Click **Next**.

Alignment

Use the Alignment screen to confirm that the camera aligns with the wells. The software takes four images over the north, south, east, and west edges of well A1. The well edges should align with the middle of the box formed by the four red lines.



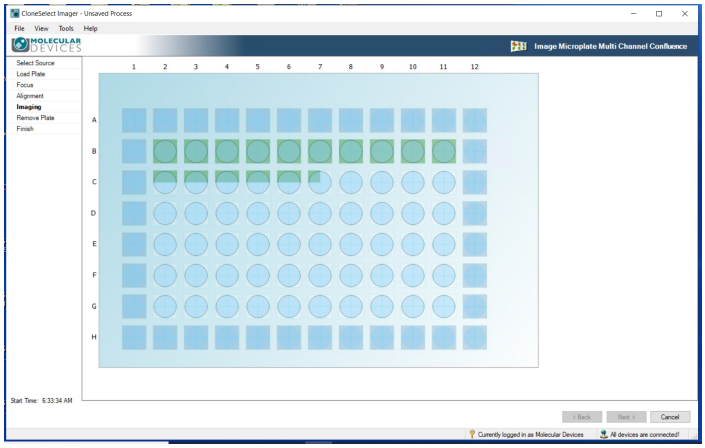
Alignment Screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines, click and drag on the lines to correct the alignment. For day zero plates, the well diameter is slightly smaller than the box formed by the red lines. Center the well within the red lines.
2. When the **Re-image** button is pink, click to recheck the alignment.
3. Move the **Brightness** slider to adjust brightness of the alignment image only.
4. Click **Next**.

Imaging

Imaging starts automatically. When the process finishes, the Remove Plate screen displays.

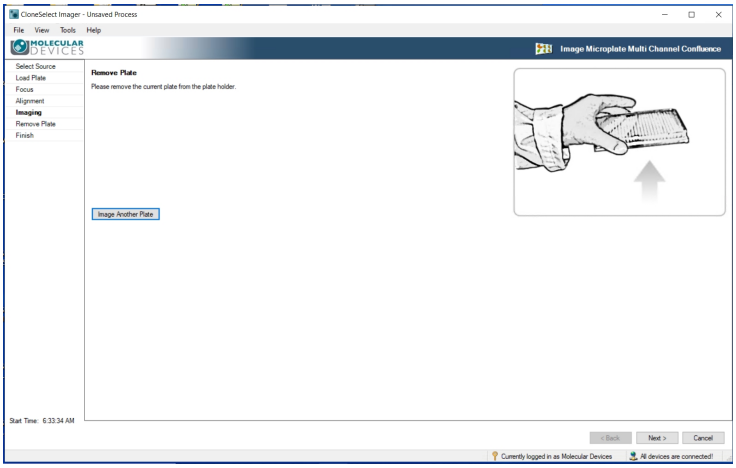


Imaging Screen

Watch the process progress in the plate schematic. The plate schematic displays with the image frames superimposed in light blue. As the software captures and processes the images, the well frame changes color, light brown then to green. When the imaging process finishes the Remove Plate screen displays.

Remove Plate

In the Remove Plate step, the plate carrier opens and the software prompts you to remove the plate from the plate carrier. You can repeat the process with another plate or continue to the end of the process.



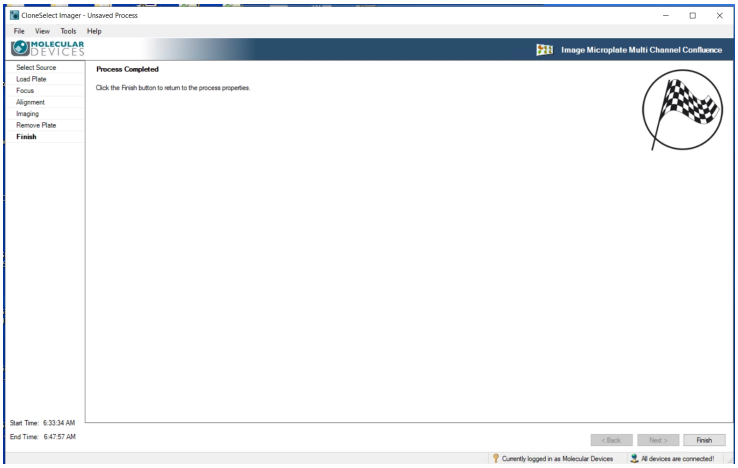
Remove Plate Screen

To image another plate click **Image Another Plate**. The plate carrier remains in the open position and the Select Source screen displays. See Select Source on page 66.

To continue to the next step, click **Next**. The plate carrier closes and the **Finish** screen displays.

Finish

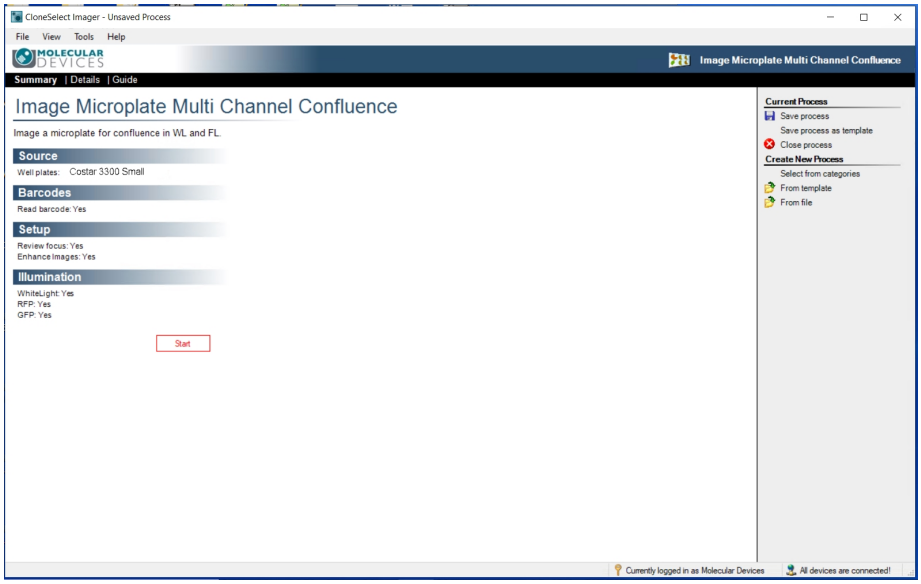
When the process ends, the **Finish** screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

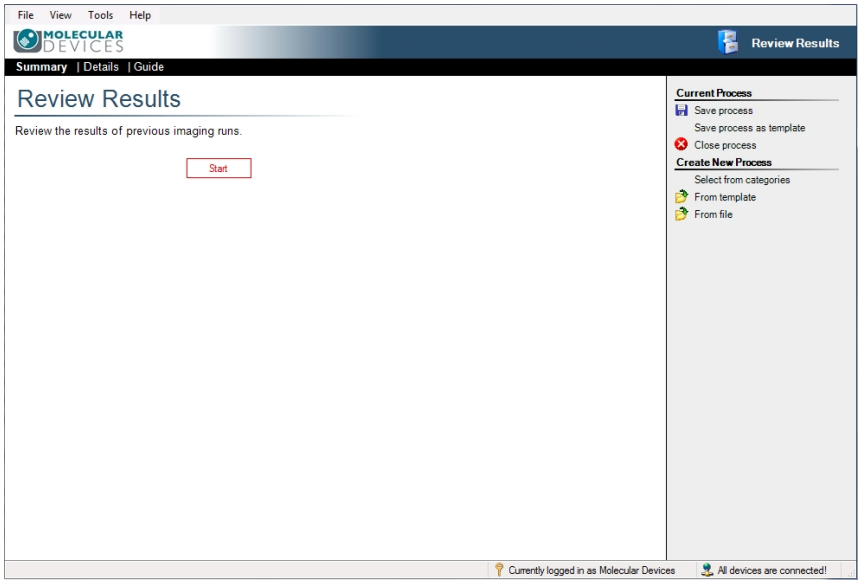
Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.
- Run the Review Results process to review the results. See Review Results Process on page 73.

Chapter 9: Review Results Process

Use the Review Results process to review previous image runs and to do further data processing, such as confluence, monitor cell growth, cell number estimation, monoclonality analysis, and migration assay analysis.

To start the Review Results process, on the navigation screen, double-click Review Results to display the Properties screen with the Summary workflow selected.



Review Results Process - Properties Screen - Summary Workflow

To get started:

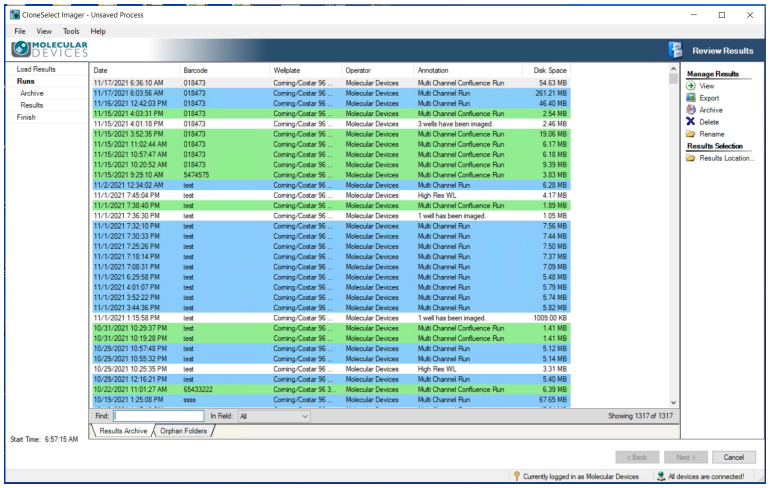
- Click **Start** to start the process.

Load Results

Use the Runs screen to load results and to manage the list of imaged plate results.

The software stores image and data files in the following location by default: `C:\Image Archive\<yyyy-mm>\<image and data files>`. The folders below the Image Archive folder organize the image and data files for each individual run by year and month, for example `2021-10`. The software names the image and data files with the date and time stamp for the run, for example `2021-10-01T11-52-10`.

If free space on the disk runs low, an orange bar displays at the top of the window with a message. When disk space is critically low the bar turns red.



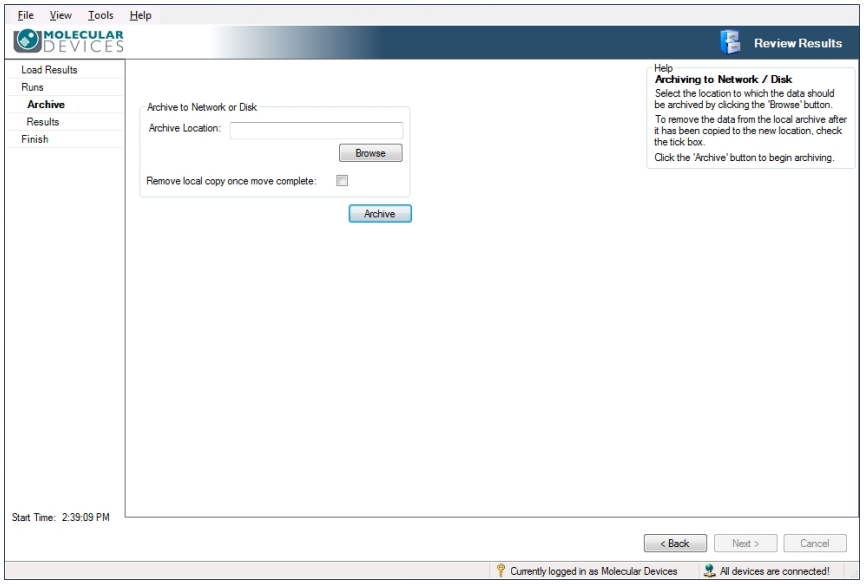
Runs Screen

To load and manage results:

- In the results list, double-click on a run date to analyze the results on the Results screen.
  - Results with a white background are white light images. See Analyze White Light Image Results on page 76.
  - Results with a blue background are multi channel runs. See Analyze Multi Channel Run Results on page 99.
  - Results with a green background are multi channel confluence runs. See Analyze Multi Channel Confluence Run Results on page 101.
- Use Ctrl+Click or Shift+Click to select multiple plates in the list.
- Select a result and click **View** on the right to analyze the results on the Results screen.
- Select results in the list and click **Export** to display the Data Export wizard. See Data Export Wizard on page 123.
- Select results in the list and click **Archive** to archive the results you select to the location you define on the Archive screen. See Archive Results on page 75.
- Select results in the list and click **Delete** to delete the result.
- Select a result in the list and click **Rename** to rename the barcode identification information. To do Growth Rate analysis, this information must be the same for multiple runs.
- Click **Results Location** to load result files to a location other than the default location.
- Click **Migration** to view the results for a migration assay. This requires an additional software license. See Migration Analysis on page 95.
- Click **Cancel** to finish the Review Results process. See Finish on page 106.

Archive Results

Use the Archive screen to archive results to a network location other than the default location.



Archive Screen

To archive results:

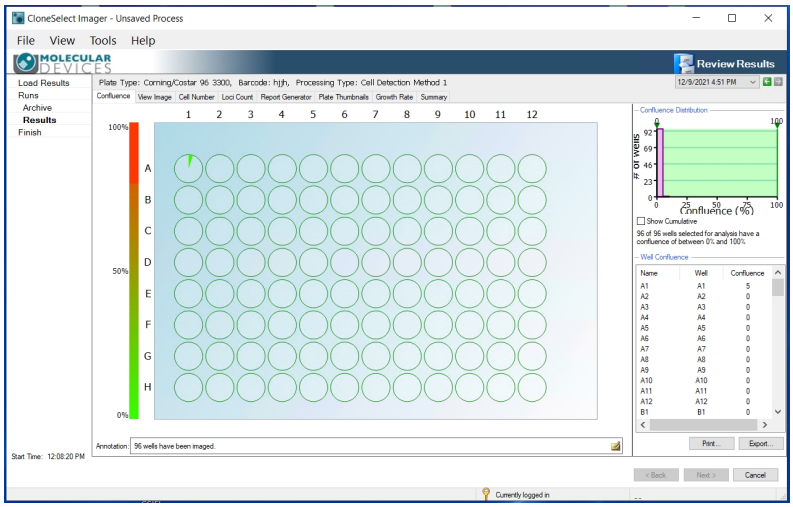
1. Below the Archive Location field, click **Browse** to select the location where results are to be archived.
2. Select the **Remove Local Copy Once Move Complete** check box to perform a cut and paste action instead of a copy paste action so that the results are moved not duplicated.
3. Click **Archive** to archive the results.
4. Click **Next**.

Analyze White Light Image Results

The Results screen contains relevant tabs that allow you to analyze the results. The tabs that display depend upon the result images. The plate type, barcode, and processing type display at the top of the screen for reference.

Confluence

The Confluence tab displays the confluence level for each well, both graphically and as a list. Use the Plate Thumbnails tab to filter the list of wells. See Plate Thumbnails on page 91.



Results Screen - Confluence Tab

The Plate Schematic displays each well as a pie chart of the confluence level. The pie charts are color-coded such that low confluence is green and high confluence is red with shades in between that represent the intermediate levels.

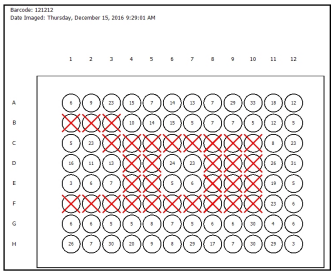
- Hover the cursor over the plate schematic to see the percentage of confluence for all the wells in the plate. Confluence lower than 5% displays <5 and confluence above 80% displays >80.
- Hover the cursor over a single well to view coordinates and confluence for the well.
- Click a well to see an image of the well on the View Image tab. See View Image on page 77.
- To copy the overview to the clipboard, hover the cursor over a well, right-click and select **Copy to Clipboard**.
- The **Annotation** field below the plate schematic displays the number of imaged wells. You can edit the annotation to enter more meaningful information. You cannot edit the annotation when you view the data through a remote data viewer.

The Confluence Distribution graph displays the number of wells with a given confluence level.

- Drag the handles across the graph to display the corresponding confluence data. The software grays out any wells that do not fall inside the lower and upper gates. The text below the graph displays the number of wells that fall within the confluence. For example, 10 out of 36 wells selected for analysis have a confluence of between xx % and xx %.
- Select the **Show Cumulative** check box to merge the handles and display the confluence as continuous data.

The Well Confluence table displays the confluence for each well.

Click **Print** to print a schematic presentation of the confluence in the plate format. The confluence values appear within the wells and the plate details display at the top of the overview.

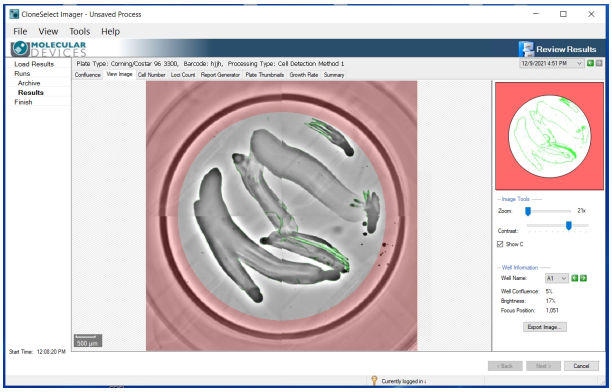


Print Schematic Presentation of the Confluence Overview

Click **Export** to display the Data Export wizard where you save the well confluence list in a .csv, .xls, or .xml file format. See Data Export Wizard on page 123.

View Image

The View Image tab displays the images for the well you select.



Results Screen - View Image Tab

The well schematic displays the entire cell. The confluence levels, brightness, and focus position for the whole well display on the right.

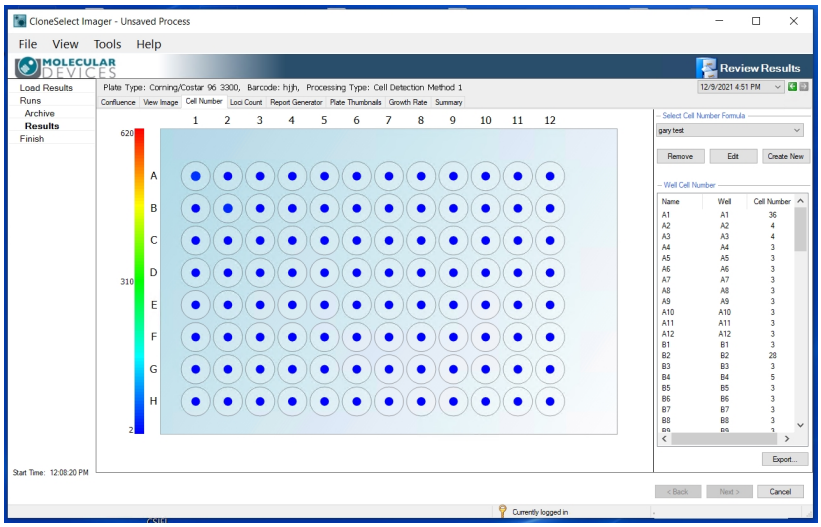
To analyze the cells in the well:

- For plates that are imaged multiple times, click the **Date** drop-down or the  to select a different time point.
- Click on areas of interest within the well to analyze specific areas of the well. The well map on the right displays the section of the well on which you set your focus.
- Move the **Zoom** slider to zoom in or out. The lowest magnification of the image is 18x and the highest is 144x. The figure turns red when there is distorting image pixelation.
- Move the **Contrast** slider to adjust the image contrast.
- Select the **Show Confluence** check box to overlay the area of the image outside the well in red and the regions of the well where colony growth is detected in green.
  Clear the **Show Confluence** check box to not overlay the area of the image.
- Click the **Well Name** drop-down or the  and select a different well.
- Click **Export Image** to save the image in a .bmp, .jpg or .png file format. If you select the Show Confluence check box, the confluence overlay displays with the image. The zoom position saves within the image. See Data Export Wizard on page 123.

Cell Number

The Cell Number tab displays the estimated number of cells in each well, both graphically and as a list. Use the Plate Thumbnails tab to filter the list of wells. See Plate Thumbnails on page 91.

The software uses a formula that you create to estimates the number of cells. You create the formula for each cell type from the confluence readings of a standard plate that contains a known numbers of cells.



Results Screen - Cell Number Tab

In the plate schematic, each well displays varying sized colors to indicate the estimated cell number in the well. A large red fill area represents a high cell number and a small blue fill area represents a low cell number. The color scale displays on the left.

To analyze the cell number information:

- Hover the cursor over a well to display the well coordinate and the estimated cell number.
- Click the **Cell Number Formula** drop-down and select a different cell number formula.
- Click **Remove** to delete the cell number formula you select.
- Click **Edit** to edit the cell number formula you select.
- Click **Create New** to create a new cell number formula.
- In the Well Cell Number section, click in the **Name** column, pause, and then click the name again. An edit field displays for you to enter a new name.
- Click **Export** to save the well cell number list in .csv, .xls, or .xml file format. See Data Export Wizard on page 123.

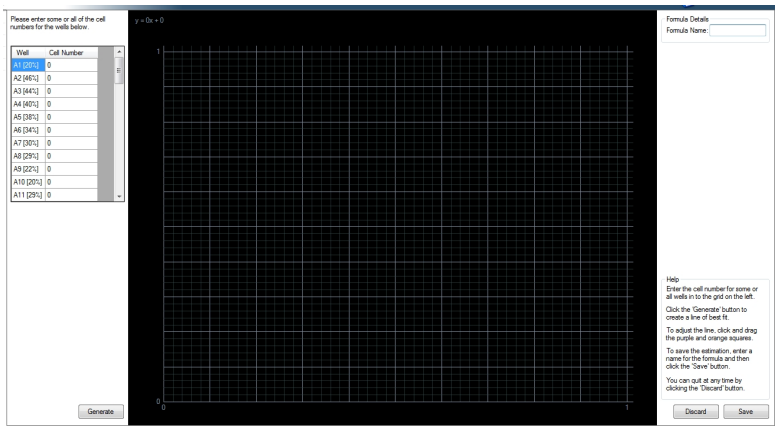
Cell Number Formula Management

When you create a new cell number estimation formula you need to set up a standard plate that has a known number of cells in a range of wells. Make a dilution series of a cell suspension on which a cell count has been performed (e.g. using trypan blue). Dispense an aliquot of each dilution into a well and incubate for a few hours to allow the cells to attach. Then image the plate and follow the instructions to create a new cell number estimation formula.

Before you create or edit a cell number formula, ensure that the confluence results that display on the Confluence tab are those for a standard plate that contains a known numbers of cells. The software selects up to twelve wells with confluence values that range between 10% and 80% and displays them at the bottom of the graph.

Create New Cell Number Formulas

Use the Create New Cell Number Formula screen to create a new cell number estimation formula based on the information from the Confluence tab.



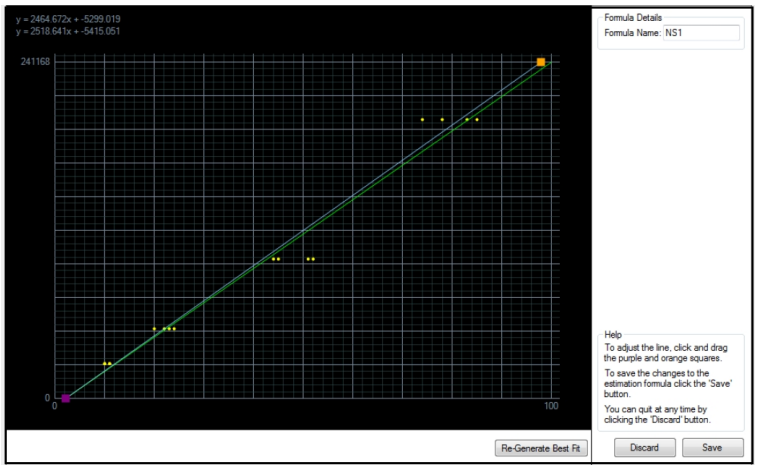
Create New Cell Number Formula Screen

To create a new cell formula:

1. In the **Formula Name** field, enter the formula name.
2. In each **Cell Number** field to the left of the graph, enter the cell number for each well.
3. Click **Generate**.
4. Click **Save**.

Edit Cell Number Formulas

The Edit Cell Number Formula screen displays a graph of the cell number against confluence for the formula.



Edit Cell Number Formula Screen

To edit a cell number formula:

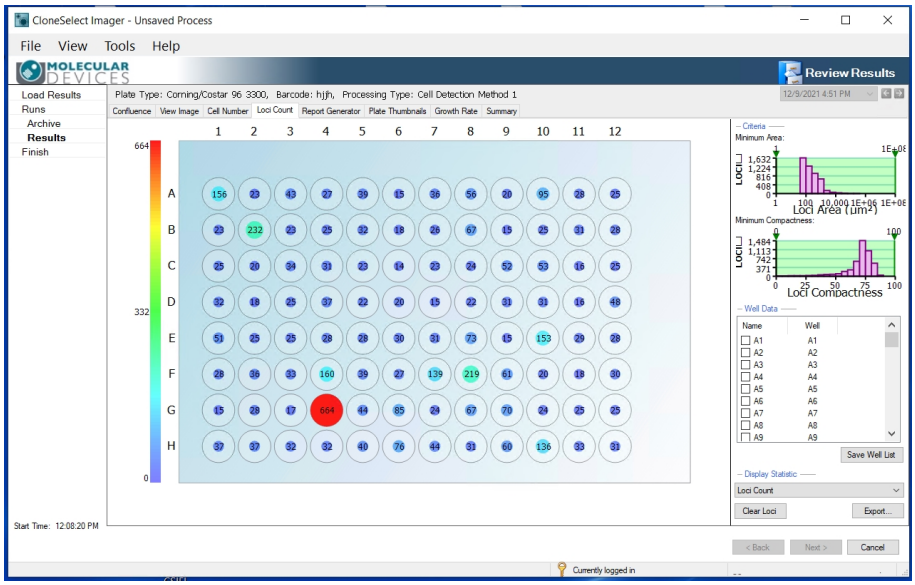
- In the **Formula Name** field, edit the formula name.
- Drag the handles at each end of the graph line. The formula displays at the top left and the edited version displays immediately below it.
- Click **Regenerate Best Fit** to generate a best fit line.
- Click **Discard** to cancel the formula edits.
- Click **Save** to save the formula edits.

Loci Count

For colony formation assays, use the Loci Count screen to count the number and size (area) of colonies in each well and to process images. Use the Plate Thumbnails tab to filter the list of wells. See Plate Thumbnails on page 91.

Loci count detects and counts the number of loci of growth, for example, cell colonies for applications including monoclonality verification and colony forming assays. See Monoclonality Workflow on page 82 and Colony Forming Assay Data Workflow on page 84.

If you image a plate multiple times during colony growth, you can view the history of each well with one identified colony for visual proof that the colony is derived from a single cell progenitor.



Results Screen - Loci Count Tab - Plate Schematic View

In the Plate Schematic view, the Loci Count tab displays a plate schematic, two graphs, and a Well Data table.

On the bottom right, click the **Display Statistic** drop-down:

- Select **Loci Count** to display the number of cell colonies found in each well.
- Select **Mean Loci Area** to display the mean loci area within each well.

The **Minimum Area** graph displays the loci count (frequency) plotted against the loci area (μm^2) in a log scale. Slide the handles to eliminate the unwanted objects or debris within the well that artificially inflate the number of loci.

The **Minimum Compactness** graph displays the loci count (frequency) plotted against the loci compactness. Slide the handles to eliminate irregularly shaped objects from the loci count. Compactness is the relation between the area and the perimeter, expressed as a ratio of the actual area and that of a perfect circle with the same perimeter.

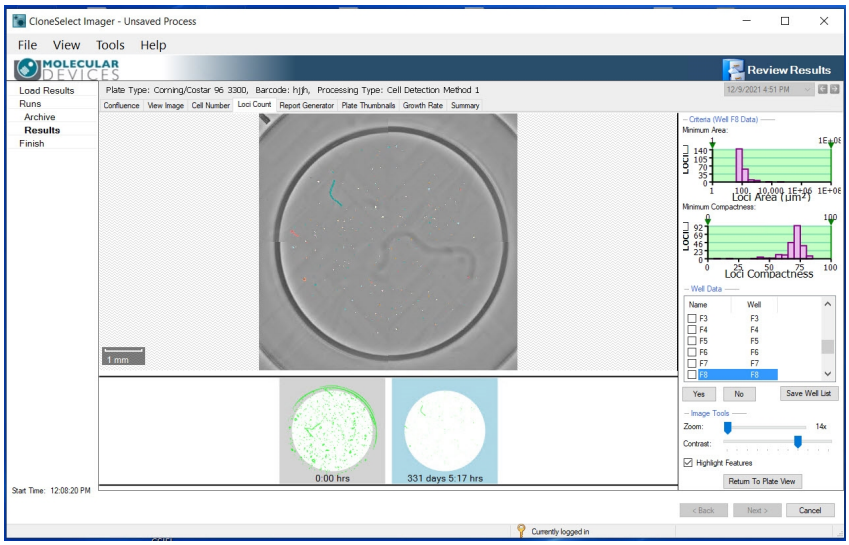
The Well Data table allows you to select the check box next to each well to save. Click **Save Well List** to save the wells in a .csv file format. The file includes the plate barcode, run date, operator, and the selected wells.

Click **Clear Loci** to clear all current loci data processing.

Click **Export** to display the Data Export Wizard. See Data Export Wizard on page 123.

Loci Count Tab - Well View

Click on a well in the plate schematic to display the image for the well with an overlay over areas of growth according to the Minimum Area and Minimum Compactness criteria.



Loci Count Tab - Well View

If you image the plate at several time points, a film strip of the well images displays for each time point across the bottom of the tab with loci colored in green. This allows you to check if a colony results from a single cell or from more than one cell. Click on each thumbnail to view the well image at each time point with the loci from the latest image outlined.

Right-click on the sequence and select **Export Image Sequence** to save the sequence in a .bmp, .jpg or .png file format. The file includes the confluence percentage and time and date the image was acquired.

Well View - Well Data

When the well image displays, the Well Data section allows you to analyze the image and mark each well as monoclonal.

Use the imaging tools to enhance the image:

- Move the **Zoom** slider to look closer or farther at the image. The lowest magnification of the image is 18x and the highest is 144x. When the figure turns red, the system zooms digitally and can cause some pixelation of the image. When you zoom into an image, the zoom area displays on the image thumbnail to the right.
- Move the **Contrast** slider to improve the image contrast.
- Select the **Highlight Features** check box to mark features with different overlay colors.
- Click **Return To Plate View** to return to the plate view on the Loci Count tab to view other wells.

In the Well Data list:

- Click **Yes** to mark the current well monoclonal and scroll to the image for the next well. Click **No** to scroll to the image of the next well without marking the image in the current well monoclonal.
- Click on a well to view its image and click on the check box to mark it as monoclonal.

Click **Save Well List** to save the wells you select in a .csv file format. The file includes the plate barcode, run date, operator, and the selected wells.

Monoclonality Workflow

Use the Plate Thumbnails tab to filter the list of wells.

To run the Loci Count process for a monoclonal dataset:

1. After you image the plate at several time points, select the **Plate Thumbnails** tab and exclude wells without at least one colony within the well. See Plate Thumbnails on page 91.

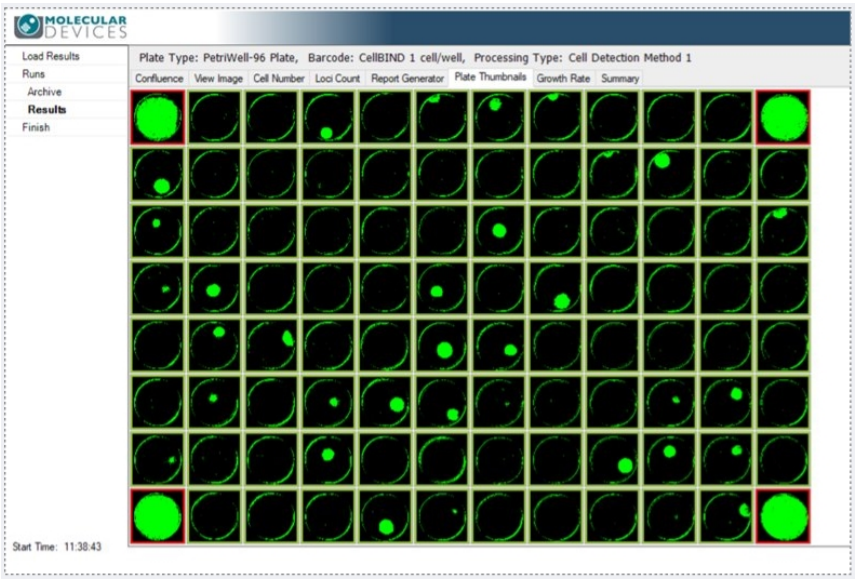
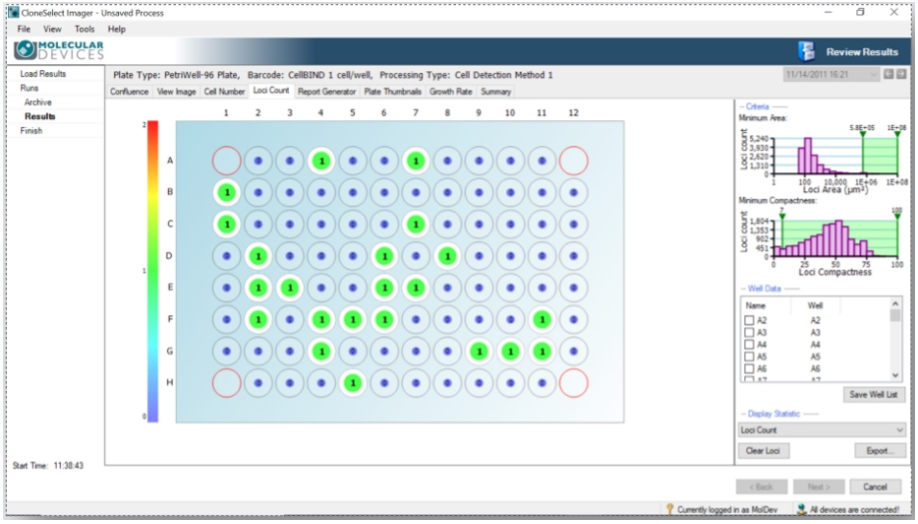


Plate Thumbnails Tab

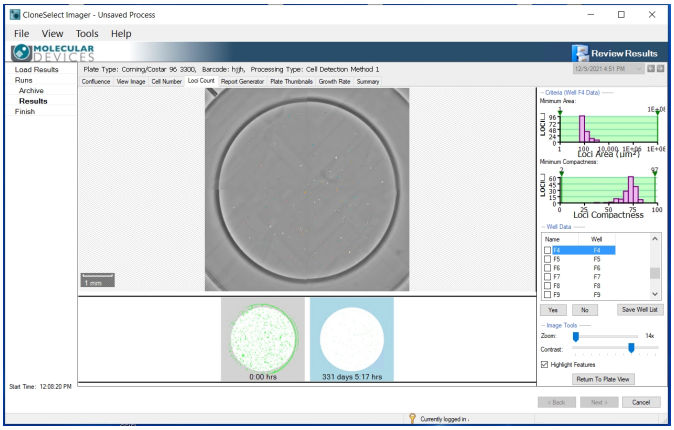
2. Select the **Loci Count** tab and adjust the well diameter as needed. See Loci Count on page 80.



Loci Count Tab - Plate Schematic View

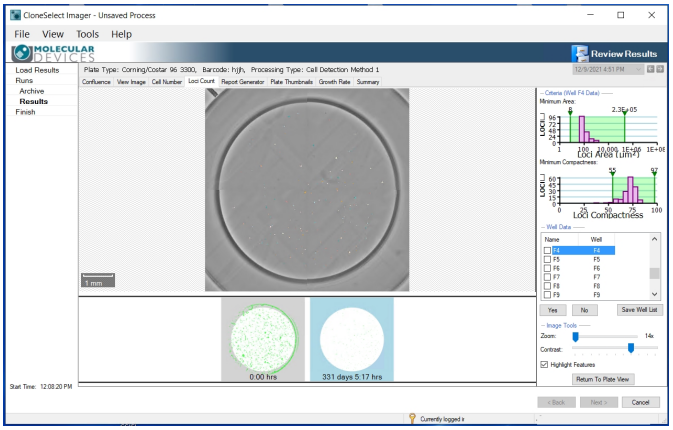
3. Click **Process** to produce the loci counts for the wells you select. The Loci Count tab displays an overview of all the wells you select and the corresponding loci count.

4. Click on a well to display the well image with the loci processing.



Loci Count Tab - Well View

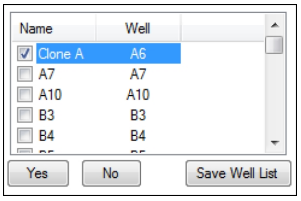
5. In the Minimum Area graph and the Minimum Compactness graph, move the handles to gate out any unwanted debris and misshaped colonies within the wells. Adjust to show only wells with 1 or 0 colonies.



Loci Count Tab - Well View

6. Click **Return To Plate View** and check through the other wells to exclude debris and misshapen colonies. The plate overview reflects the processing and displays the new loci count number in each well.
7. Click through each well in the **Well Data** list. As the well image appears, determine monoclonality by looking back through the series of well images and identifying if the colony originated from a single cell.
8. If the colony originates from a single cell, either select the check box or click **Yes**.

Note: Do not change the well Name. This can cause report errors.



After you select all applicable wells, you can save and print the list of wells that contain a monoclonal colony. See Report Generator on page 87.

Colony Forming Assay Data Workflow

Analyzing colony forming assay data sets are very similar to gating monoclonality data, however the data export can be more detailed.

1. After you image the plate at several time points, use the Review Results process to open the latest data point on the Results screen.
2. Select the **Plate Thumbnails** tab and exclude wells without at least one colony within the well. See Plate Thumbnails on page 91.

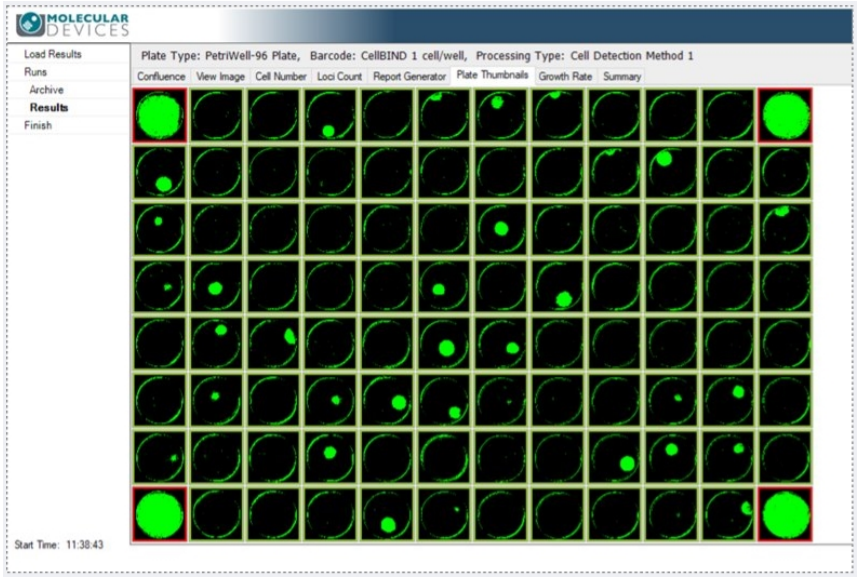
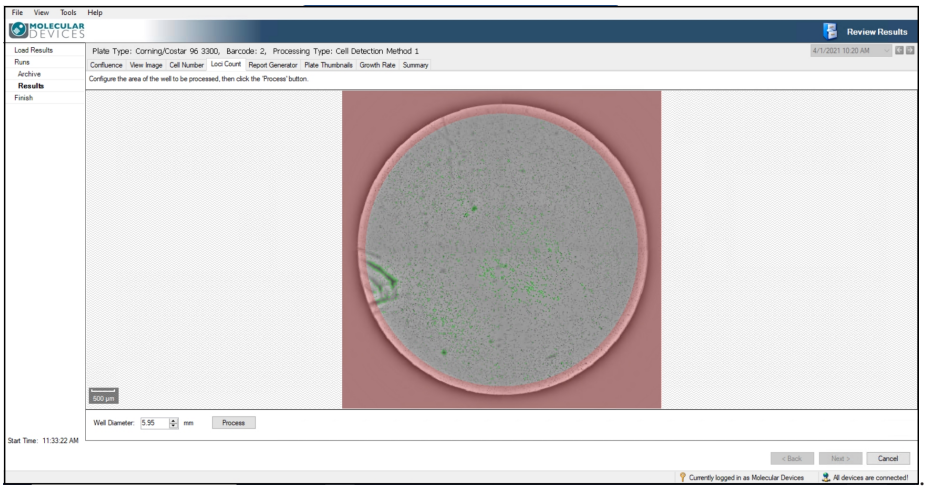


Plate Thumbnails Tab

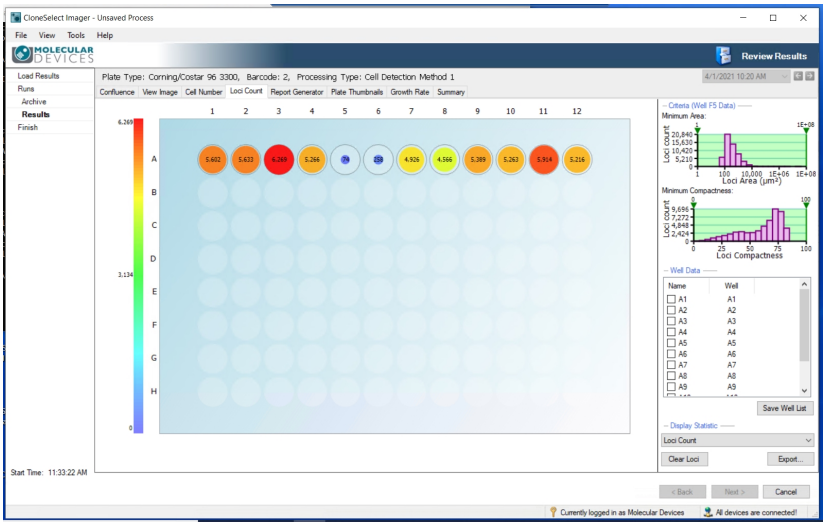
3. Select the **Loci Count** tab. The Well View displays.



Loci Count Tab - Well View

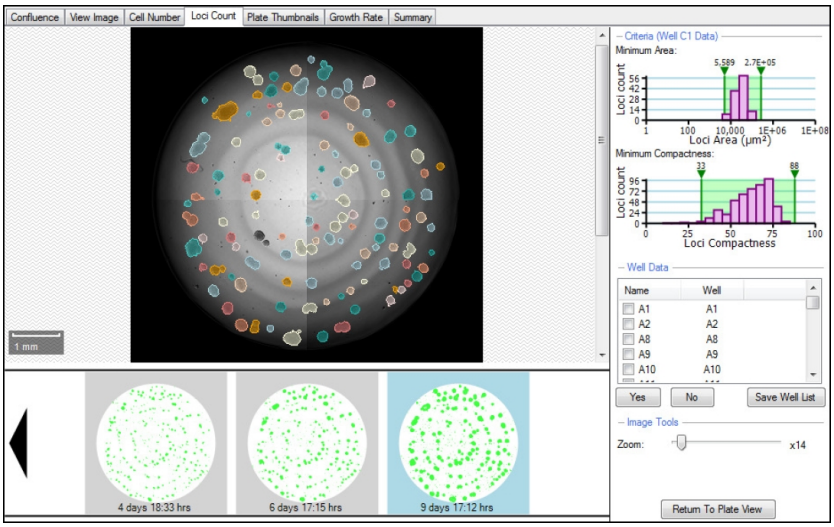
4. In the **Well Diameter** field, adjust the well diameter, as needed.

5. Click **Process** to produce the loci counts for the wells you select. The Loci Count tab displays an overview of all the wells you select and the corresponding loci count.



Loci Count Tab - Plate Schematic View

6. Adjust the **Minimum Area** and **Minimum Compactness** to gate any unwanted debris and misshaped colonies within the wells. Adjust to display only wells with one or zero colonies.
7. Click on a well to display the well image with the loci processing.

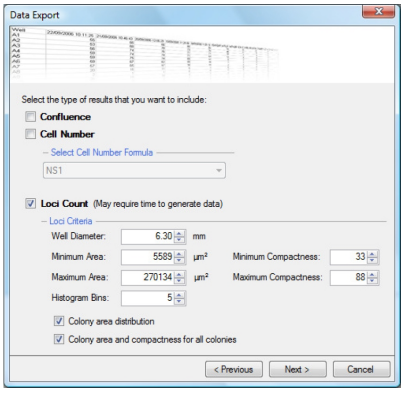


Loci Count Tab - Well View

8. In the Minimum Area graph and the Minimum Compactness graph, move the handles to gate out any unwanted debris and misshaped colonies within the wells.

Note: The Well Data list is used to accept wells based on monoclonality. You do not need to select the check boxes for the wells for this assay.

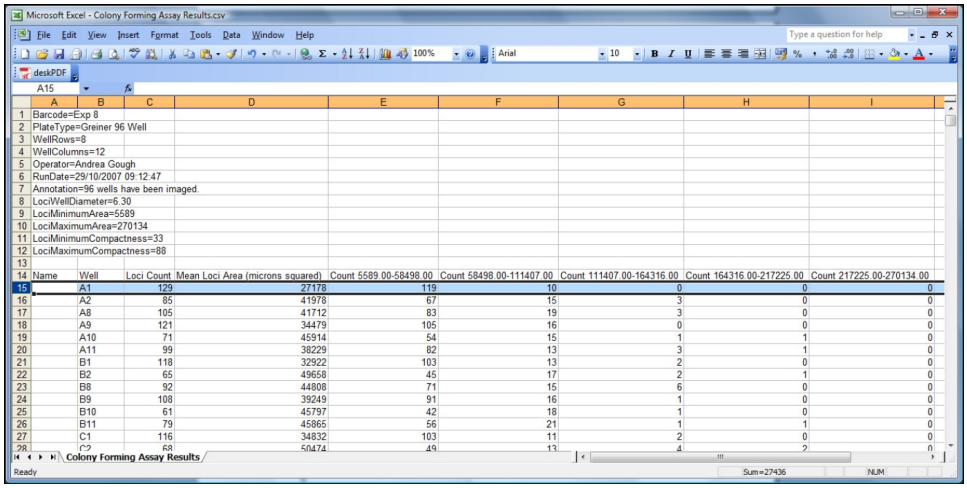
9. After you are satisfied with the gating of the data and the loci count, use the Data Export wizard to export the data. The data exports for all wells.



Data Export Wizard

10. In the Data Export wizard, select the **Loci Count** check box. The criteria automatically populate. The default number of bins is 20, but in the example it has been changed to 5 bins.
11. Select the **Colony Area Distribution** check box.
12. Select the **Colony Area And Compactness For All Colonies** check box.
13. Click **Next** to finish exporting the data.

A summary of the experiment criteria appears in the first rows the spreadsheet (top left).

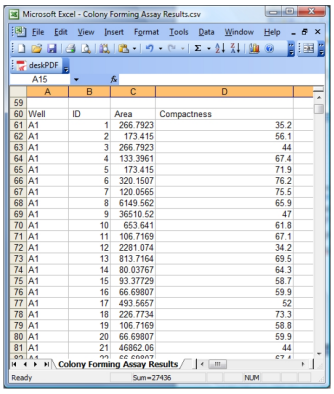


Colony Forming Assay Export Data

When you select the **Colony Area Distribution** check box, the following information is produced for each well:

- Name (if changed within the software)
- Well number (e.g. A1)
- Loci Count (this is the total)
- Mean Loci Area
- Bins equally splitting the loci area range into equal groups so that each identified loci will fall into one of the corresponding groups based on its size. For example, out of the 129 identified loci within well A1, 119 of these fall into the first bin with a loci area range of 5589µm^2 to 58498µm^2.

When you select the **Colony Area And Compactness For All Colonies** check box, the area and compactness of each identified loci displays.

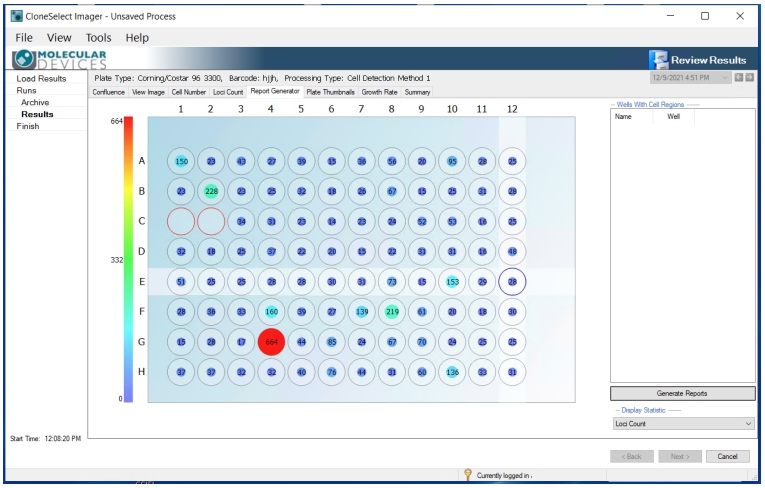


Colony Forming Assay Export Data

Report Generator

The Report Generator tab displays between the Loci Count tab and the Plate Thumbnail tab. Reports use information from both of these tabs.

Report generation is only for white light results and you must image all time points with the same process. For example, all Image Microplate process or all Image Microplate High Resolution process.



Note: If a message: *Report generation failed. Please contact technical support for assistance.* Make sure that the DocumentAssemblerServiceSetup.exe is installed. Refer to the installation instructions in the *CloneSelect Imager software Release Notes.*

Results Screen - Report Generator Tab

To generate a monoclonality report:

1. Follow the procedure in the Monoclonality Workflow section to confirm the presence of a single cell. See Monoclonality Workflow on page 82.
2. When the plate schematic view of the Loci Count tab displays a colony number that matches the Plate Thumbnail tab, (preferably all ones and zeros,) select the **Report Generator** tab.
3. Select a monoclonal well of interest.
4. Click **Return To Plate View** to return to the plate view at any time.
5. In the timeline at the bottom of the tab, select the **0.00 hrs** time point.

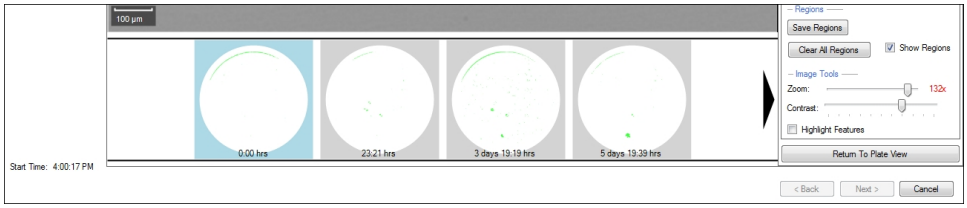
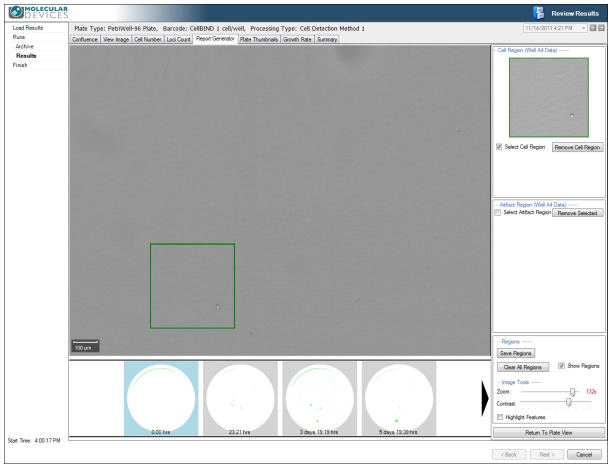


Image Timeline and Imaging Tools

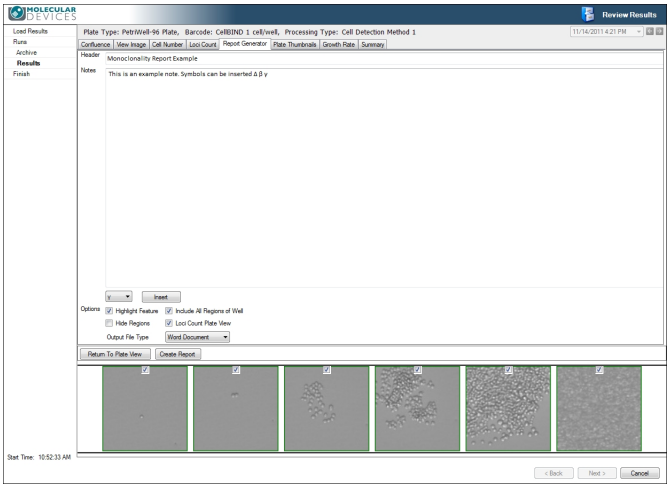
6. Use the Imaging Tools to enhance the image:
   - Move the **Zoom** slider to look closer or farther at the image. The lowest magnification of the image is 18x and the highest is 144x.
     When the figure turns red, the system zooms digitally and can cause some pixelation of the image. When you zoom into an image, the zoom area displays on the image thumbnail to the right.
   - Move the **Contrast** slider to improve the image contrast.
     The Contrast setting is included in the report. If you batch generate reports, the software uses the last image Contrast setting in all of the reports.
   - Select the **Highlight Features** check box to mark features with different overlay colors.
7. Below Cell Region (Well <xx> Data), select the **Select Cell Region** check box to mark cell regions.
   a. Move the green region selector box to the region to mark and then click to apply the mark. You can mark one cell region per time point image and you cannot adjust the size of the green selector box. The area that displays in the Cell Region appears in the report.
   b. By default, the location of the cell region in the first image remains fixed for all other time points. To adjust the location of the cell region for other time points, click on a different time point thumbnail, select the **Select Cell Region** check box, re-position the green box, and click. Repeat the region adjustment process as needed.
      - Clear the **Select Cell Region** check box to end the marking process.
      - Cell region marking automatically ends when you proceed to mark artifact regions.
      - Click **Remove Cell Region** to remove a cell region mark.

8. Below Artifact Region (Well <xx> Data), select the **Select Artifact Region** check box to mark artifact regions in the well. The images that display in the Artifact Region area appear in the report.



Cell Regions

   a. Move the red selector-box over an artifact and click to apply the mark. You cannot adjust the size of the selector box.
   b. Repeat to mark up to ten artifacts in the well.
      - If you zoom in close and want to scroll around the well image, you must clear the **Select Artifact Region** check box, click and drag to move within the cell view, and then select the **Select Artifact Region** check box again.
      - To remove an artifact region, on the right in the Artifact Region pane, select the artifact thumbnail to remove (Ctrl+Click to multi-select) and click **Remove Selected**.
9. When you finish marking regions, click **Save Regions**.
10. Click **Return To Plate View** to continue.
11. In the Wells With Cell Regions list on the right, select the check box for each well of interest. Only wells that you mark with cell regions appear in the list. If you select multiple check boxes the software generates multiple reports with one well detailed per report.
12. Click **Generate Report** to display the Report Preparation screen.



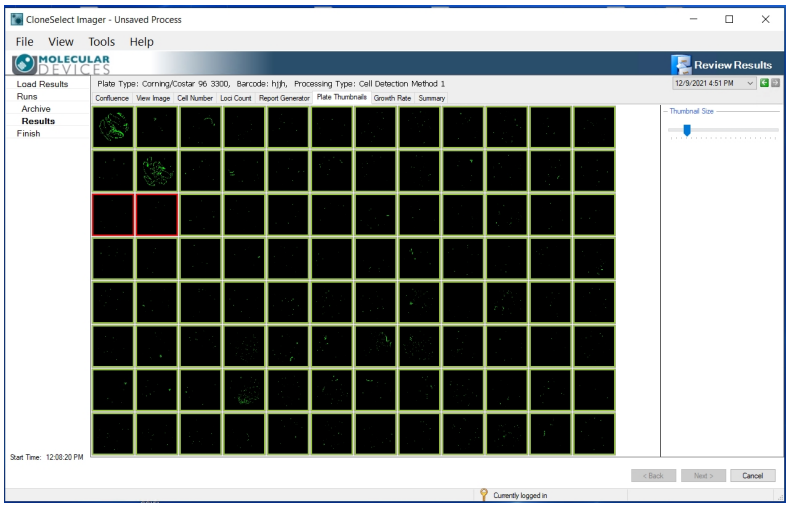
Report Preparation Screen

13. In the **Header** field, enter the report title.

14. In the **Notes** field, enter any additional information. If you generate multiple reports, this appears in all the reports.
15. Click the symbol drop-down and select a symbol and then click **Insert** to include a symbol.
16. Select the **Highlight Feature** check box to highlight the colony in the report.
17. Select the **Hide Region** check box to exclude the cell and artifact region markings on the whole well image fin the report.
18. Select the **Include All Regions of Well** check box to include all images from the well at single cell resolution. This generates a much longer report because all images are exported.
19. **Select the Loci Count Plate View** to include the Loci Count Plate View including well details and criteria settings in the report.
20. Click the **Output File Type** drop-down:
    - Select **Word Document** to generate the report in Word format.
    - Select **PDF Document** to generate the report in Adobe .pdf file format.
21. At the bottom of the screen, by default all time point thumbnails are selected to be included in the report. Clear the check box to exclude a time point thumbnail from the report.
22. Click **Create Report**.
23. Specify where the report should be saved and click **OK**.
24. When the *Report Generation Success* message appears, click **OK**.
25. When finished, click **Return to Plate View**.

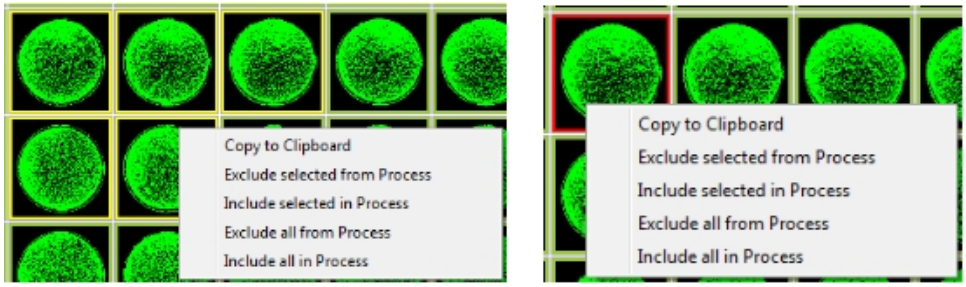
Plate Thumbnails

The Plate Thumbnails tab displays the entire plate and the wells you image as thumbnails. The thumbnails include the confluence overlay of each well you image. Use this tab to select the wells to include or exclude from analysis. The software includes or excludes the wells from the Plate Thumbnails tab on the Confluence tab, Cell Number tab, Loci Count tab, and Growth Rate tabs.



Results Screen - Plate Thumbnails Tab

- Yellow: Indicates a group well selection.
- Red: Indicates excluded wells.
- Green: Indicates included wells.



Group Selected Wells and Excluded Well With Selection Options Menu

To specify what to do with each well, do the following:

1. Hover the cursor over a well of interest.

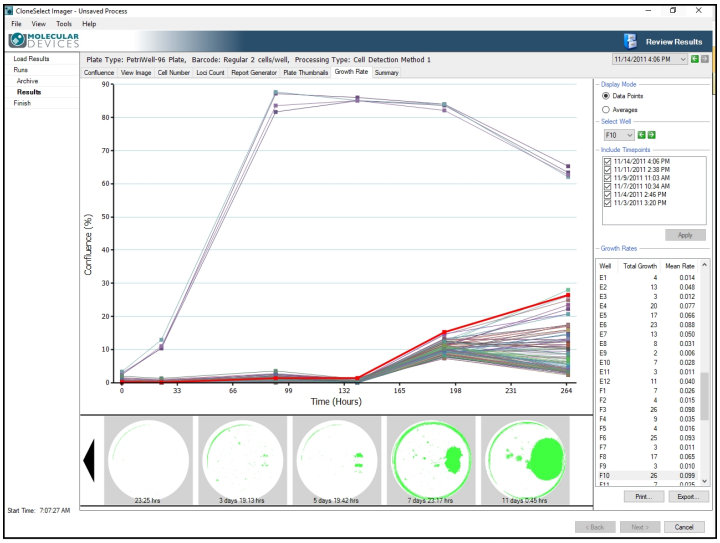
   Tip: To select a group of wells, press and hold **CTRL** and then select multiple wells. Release **CTRL** when you finish selecting.

2. Right-click on a well of interest:
   - Select **Copy to Clipboard** to copy the well to the computer clipboard.
   - Select **Exclude Selected From Process** to exclude the well from the selected process.
   - Select **Include Selected In Process** to include the well in the selected process.
   - Select **Exclude All From Process** to exclude the well from all processes.
   - Select **Include All In Process** to include the well in all processes.

Growth Rate

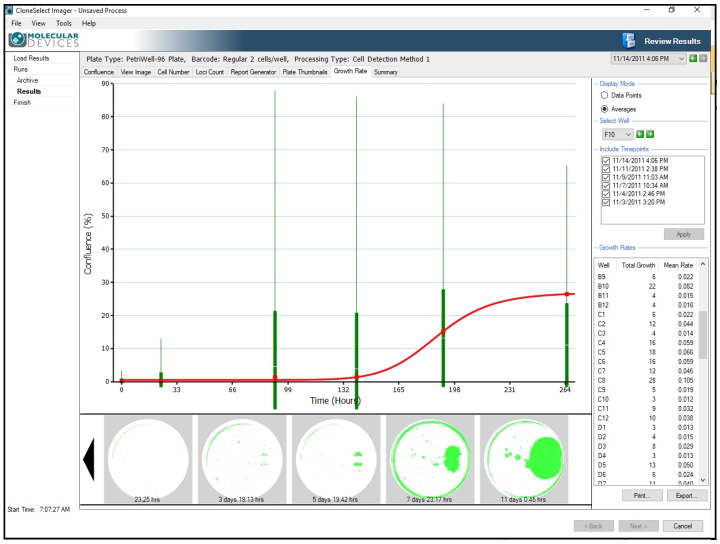
The Growth Rate tab displays the graph of the growth rate of the wells. Growth curves appear for all wells in the plate. The software uses the barcode in the stored result to determine if the plate has been imaged. If you do not use barcodes, you can use named plates, but you must enter the name exactly the same way each time you image the plate. You should not rename plates to reduce the risk of including mis-named plates in the growth curve data. Use the Plate Thumbnails tab to filter the list of wells. See Plate Thumbnails on page 91.

1. Select a well to display the captured images for the well in the well schematic below the graph.
2. Hover the cursor over a data point to display the well coordinate for the point. The thick red line within the graph displays the growth rate for the well.
3. Select a **Display Mode** option:
   - Select **Data Points** to display a graph of all well data points.



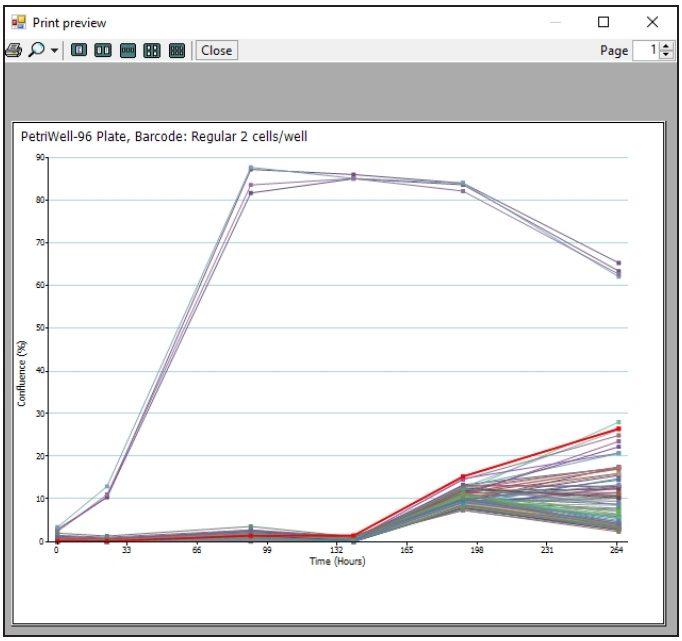
Data Points Graph

   - Select **Averages** to display a graph of the averages of the well data points.



Averages Graph

4. Click the **Select Well** drop-down or arrows and select a different well.
5. In the **Include Time Points** list, select the check box for each time point to include in the growth rate output. Clear the check box for each time point to exclude. Click **Apply**.
6. View the Growth Rates list information that includes the Well, Total Growth, and Mean Rate for each well.
7. Click **Print** to print the graph.



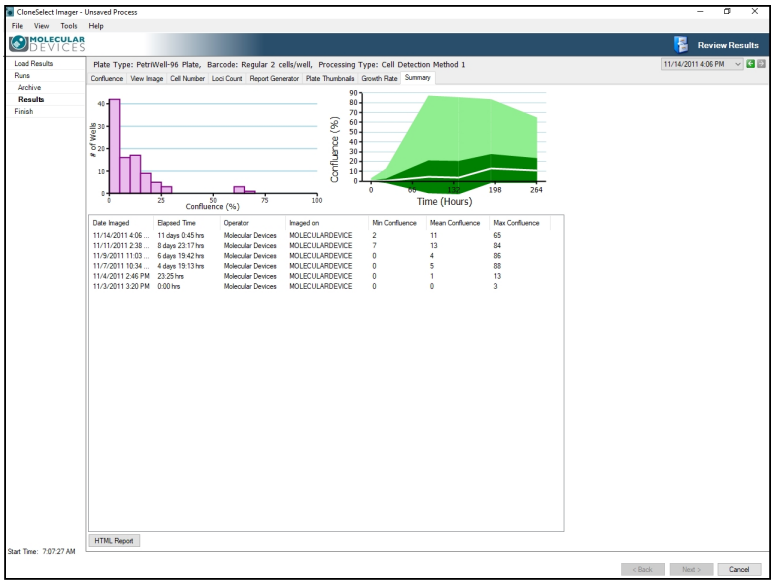
Growth Curve Graph Print Preview

8. Click Export to display the Data Export Wizard where you can export the data in a .csv, .xls, or .xml file format. See Data Export Wizard on page 123.

Summary

The Summary tab displays a summary of the confluence data, growth curve data, and imaging credentials.

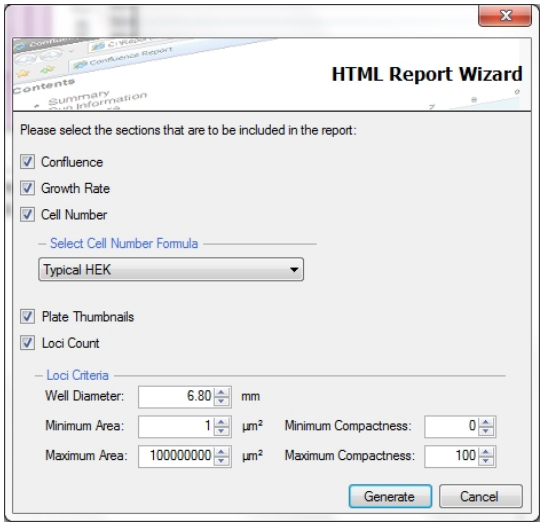
The table lists the Date Imaged, Elapsed Time, Operator, Imaged On, Minimum Confluence, Mean Confluence, and Maximum Confluence for each time the plate was imaged.



Results Screen - Summary Tab

To create an HTML report:

1. Click **HTML Report** to display the HTML Report wizard.



HTML Report Wizard

2. Select the check box for each type of information to include in the report.

> **Note:** The loci data uses the minimum area, maximum area, minimum compactness, and maximum compactness from the Loci Count tab. See Loci Count on page 80.

3. Click **Generate** to save the report in an .html file format.

Migration Analysis

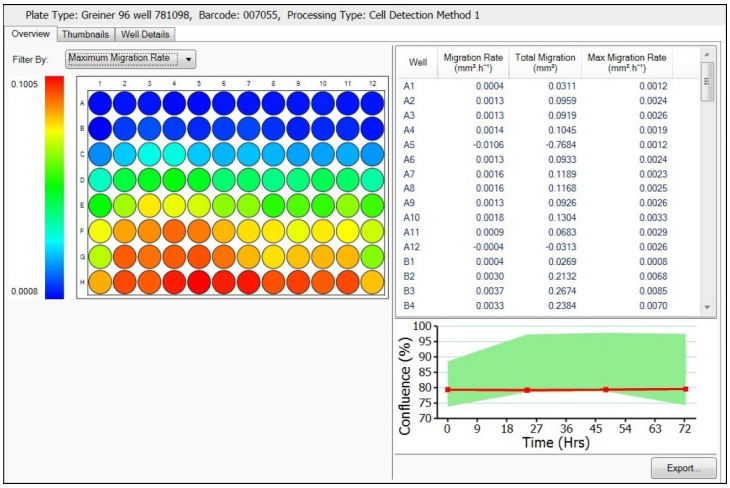
Note: Migration analysis requires a separate software license.

Use the Migration Data Analysis screen to do migration analysis on data that you image over a number of time points. For each data set you analyze, the plate type, barcode, and processing type displays at the top of the screen.

To access the Migration Data Analysis screen, in the Results Archive list on the computer hard drive, double-click the run date to use and then click Migration.

Overview

The Overview tab provides an overview of the migration assay data.



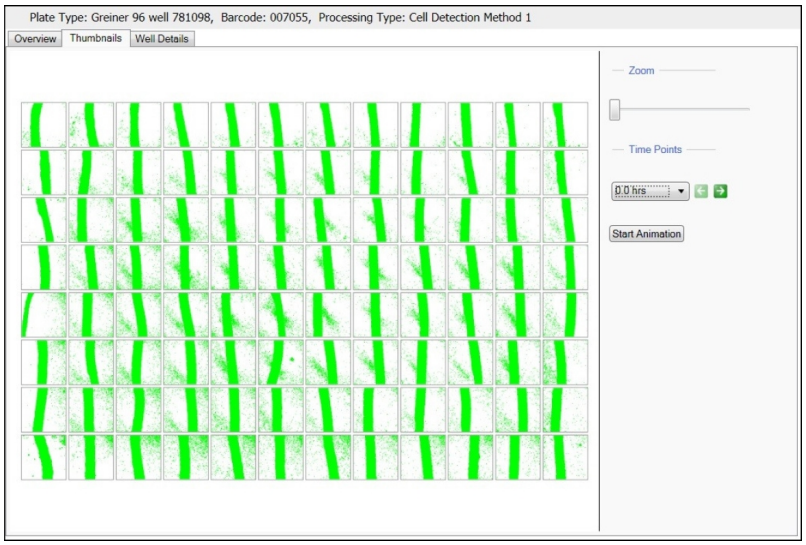
Migration Data Analysis Screen - Overview Tab

To manage the display of the data migration analysis overview information:

- Click the **Filter By** drop-down:
  - Select **Migration Rate** to measure the rate of increase in cell movement (mm^2 per hour) over the entire time period.
  - Select **Total Migration** to measure the increase in area of cell movement (mm^2) over the entire time period.
  - Select **Maximum Migration Rate** to measure the largest rate of increase in cell movement (mm^2 per hour) between any two adjacent time points.
- Right-click on the heat map and select **Copy to Clipboard** to copy the heat map to the computer clipboard.
- Click a well to highlight the well in the information table and on the Confluence (%) graph.
- Double-click a well to view the well on the Well Details tab. See Well Details on page 97.
- In the table, click on a column header to re-sequence the statistics for each well.
- Click **Export** to display the Data Export wizard that allows you to save data for either Selected Time Points or for All Time Points in a .csv, .xls, or .xml file format. See Data Export Wizard on page 123.

Thumbnails

The Thumbnails tab provides an overview of each well image with wound detection, where no cells are detected.



Migration Data Analysis Screen - Thumbnails Tab

Note: Time points that display are restricted by the End Time Point you define in the Graph view of the Well Details tab. See Well Details on page 97.

To manage the display of the data migration analysis thumbnail information:

- Right-click on the thumbnail map and select Copy to Clipboard to copy the thumbnails to the computer clipboard.
- Move the **Zoom** slider to look closer or farther at the whole thumbnail map.
- Click the **Time Points** drop-down or arrows to select a different time point.
- Click **Start Animation** to observe the migration that takes place in the wells. This animates the thumbnails from the start time point to the final time point.

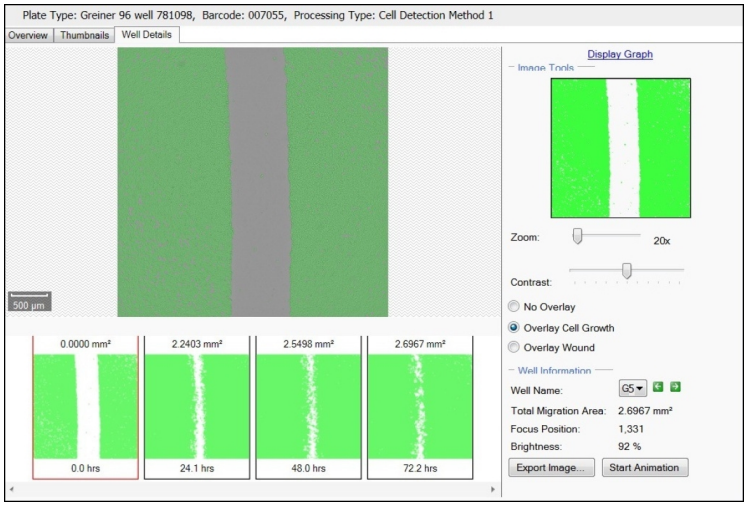
Well Details

The Well Details tab displays details the software generates from individual wells.

The Well Details tab has two views:

- **Image:** Displays a whole well image with time point thumbnails in a film strip below.
- **Graph:** Displays the information gathered from the individual wells in graph format.

Image View



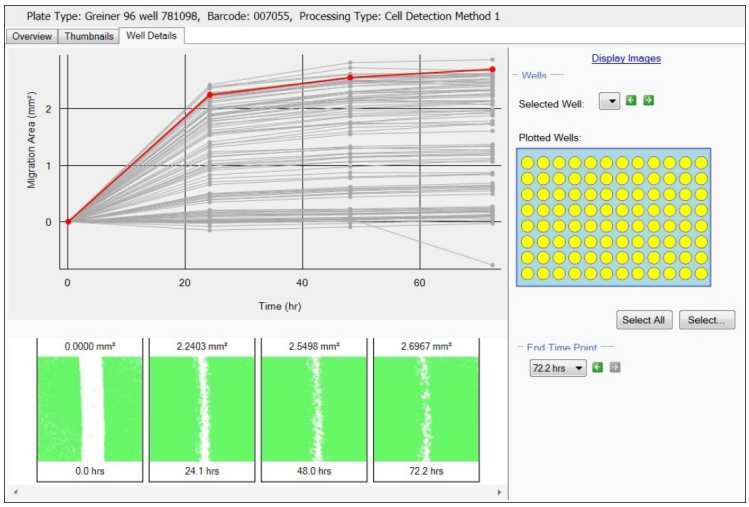
Migration Data Analysis Screen - Well Details Tab - Display Image View

To manage the display of the data migration analysis well details information in the Image view:

- The Image view displays by default. To display the Image view from the Graph view, click **Display Image**.
- Click a thumbnail in the film strip below to display the enlarged image for a time point.
- Move the **Zoom** slider to zoom out or in. The lowest magnification of the image is 18x and the highest is 144x. When the figure turns red, the system zooms digitally and can cause some pixelation of the image. The zoom area displays on the image thumbnail to the right.
- Move the **Contrast** slider to improve the image contrast.
- Select an option:
  - Select **No Overlay** to hide the overlay highlights.
  - Select **Overlay Cell Growth** to highlight all areas with cells in green.
  - Select **Overlay Wound** to highlight all areas without cells in green.
- Click the **Well Name** drop-down or arrows to select a different well.
- The **Total Migration Area** displays the measurement of the total cell growth in mm^2 over the entire time period for the well.
- The **Focus Position** displays the focus position used for the microplate.
- The **Brightness** displays the brightness value used for imaging.
- Click **Export Image** to save the image in a .bmp, .jpg, or .png file format. See Data Export Wizard on page 123.
- Click **Start Animation** to observe the migration that takes place in the wells. This animates the thumbnails from the starting time point to the final time point.
  Time points that display are restricted by the End Time Point you define in the Graph view.
- Click **Display Graph** to change the tab display to the Graph view.

Graph View

The Graph view displays information the software gathers from the individual wells in graph format. The graph displays Migration Area (mm^2) plotted against Time (hours).

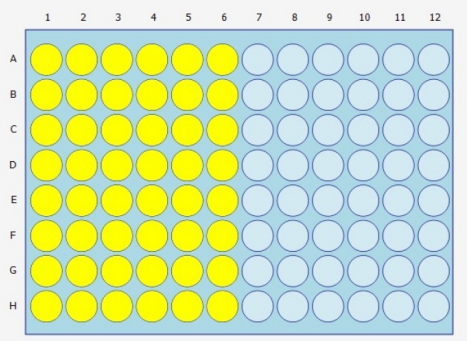


Migration Data Analysis Screen - Well Details Tab - Graph Display

The red line in the graph represents the time point thumbnail of the well you select. The film strip below the graph displays the total migration at that time point and time passed since the first time point in the series.

To manage the display of the data migration analysis well details information in the Graph view:

- Hover the cursor over a data point on the red line in the graph to highlight the corresponding thumbnail in the film strip.
- Click the **Selected Wells** drop-down or arrows and select a different well.
- In the **Plotted Wells** plate schematic, select the wells to plot in the graph. The selection and deselection of these wells only apply to the graph and not the overview. Yellow indicates selected wells. Blue indicates deselected wells.



Plotted Wells Plate Schematic

- Click the **End Time Point** drop-down or arrows and select the end time point. The graph shows the time points before and including the end point you select.

> **Note:** This End Time Point also limits the time points that appear on the Thumbnails tab and for the Image View on the Well Details tab. The software recalculates the data on the Overview tab to include the data for the time points you include.

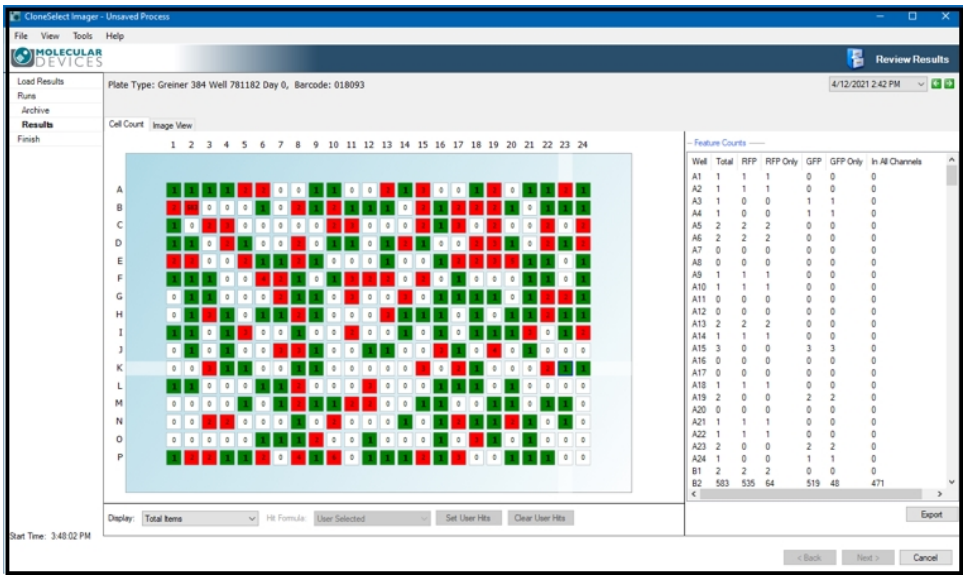
- Click **Display Image** to change the tab display to the Image view.

Analyze Multi Channel Run Results

The Results screen contains relevant tabs that allow you to analyze the results. The tabs that display depend upon the result images. The plate type, barcode, and processing type display at the top of the screen for reference.

Cell Count

The Cell Count tab displays the estimated number of cells in each well, both graphically and in a data table that displays the cell counts in each channel.



Results Screen - Cell Count Tab

In the plate schematic, each well displays the estimated cell count in the well.

- Green = Single cell detected
- Red = > 1 cell detected
- White = 0 cell detected

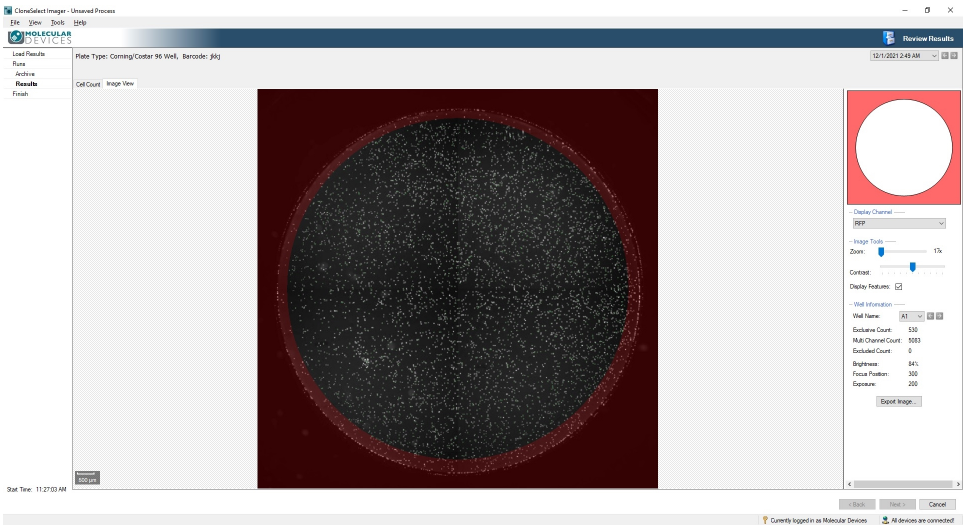
To analyze the cell number information:

1. For plates that are imaged multiple times, click the **Date** drop-down or the  to select a different time point.
2. Click on a well to display the image on the Image View tab. See Image View on page 100.

3. Click the **Display** drop-down:
   - Select **Total Items** to display the number of cells detected in any channel.
   - Select **RFP** to display the number of cells detected in the RFP channel.
   - Select **RFP Only** to display the number of cells detected only in the RFP channel.
   - Select **GFP** to display the number of cells detected in the GFP channel.
   - Select **GFP Only** to display the number of cells detected only in the GFP channel.
   - Select **In All Channels** to display the number of cells detected in all channels.
   - Select **Hits** display only wells of interest. If you select this option:
     - Click the **Hit Formula** drop-down:
       - Select **Single Multichannel Item** to display hits for wells with a single cell detected in both FL channels.
       - Select **Single Item Channel 1** to display hits for wells with a single cell detected in channel 1 (RFP) only.
       - Select **Single Item Channel 2** to display hits for wells with a single cell detected in channel 2 (GFP) only.
       - Select **User Selected** to allow the selection of hits and then click either **Set User Hits** and select the wells of interest then **End Edit** to save the wells you select or click **Clear User Hits** to delete all wells you select.
4. Click **Export** to save the well cell number list in .csv file format. See Data Export Wizard on page 123.

Image View

The Image View tab displays the image for the well you select, a thumbnail that indicates the location of the detected objects by a red dot, and other analysis tools.



Results Screen - Image View Tab

The well schematic displays the entire cell and analysis tools display on the right.

To analyze the cells in the well:

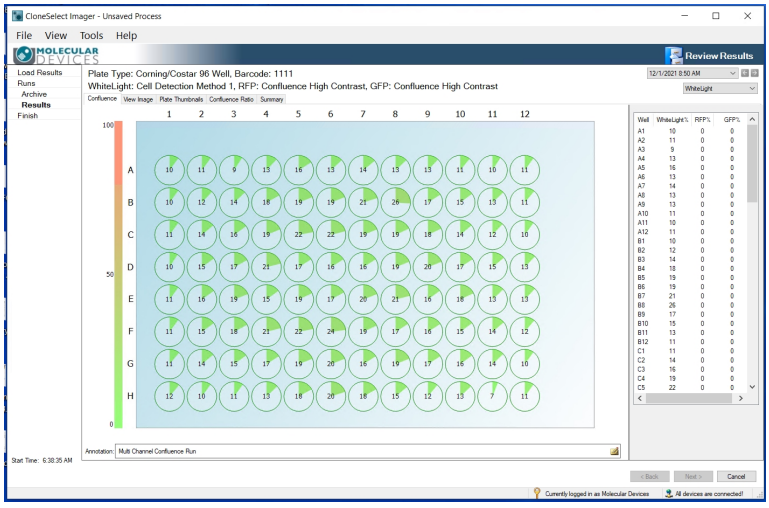
- For wells that are imaged multiple times, click the **Date** drop-down or the  to select a different time point.
- Click the **Display Channel** drop-down:
  - Select **White Light** to display White Light channel images.
  - Select **RFP** to display RFP channel images.
  - Select **GFP** to display GFP channel images.
- Move the **Zoom** slider to zoom in or out. The lowest magnification of the image is 18x and the highest is 144x. The figure turns red when there is distorting image pixelation.
- Move the **Contrast** slider to adjust the image contrast.
- Select the **Display Features** check box to display detected features on the image with a green dot or a red dot.
  Clear the **Display Features** to not display a green dot or red dot for detected features.
- Click the **Well Name** drop-down or  and select a different well to display a different image and related information on the right.
- Right-click the image to save the image to the computer clipboard or to exclude/include a feature.
- Right-click a green dot or red dot:
  - Select **Exclude Feature** to exclude a feature.
  - Select **Include Feature** to include a feature.
- Click **Export Image** to save the image in a .bmp, .jpg or .png file format. See Data Export Wizard on page 123.
- Click **Cancel** to return to the Runs page. See Load Results on page 74.

Analyze Multi Channel Confluence Run Results

The Results screen contains relevant tabs that allow you to analyze the results. The tabs that display depend upon the result images. The plate type, barcode, and processing type display at the top of the screen for reference.

Confluence

The Confluence tab displays the confluence level for each well, both graphically and as a list. Use the Plate Thumbnails tab to filter the list of wells. See Plate Thumbnails on page 103.



Results Screen - Confluence Tab

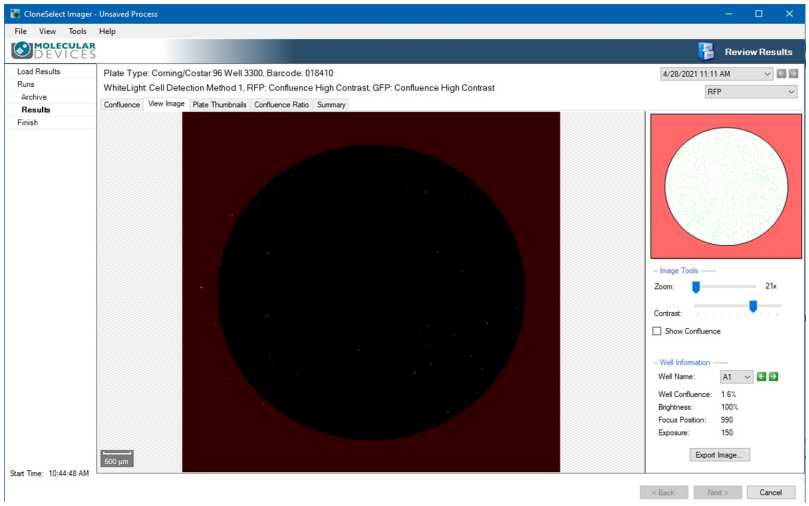
The Plate Schematic displays each well as a pie chart of the confluence level. The pie charts are color-coded such that low confluence is green and high confluence is red with shades in between that represent the intermediate levels.

- For plates that are imaged multiple times, click the **Date** drop-down at the upper right corner or the  to select a different time point.
- Click the drop-down:
  - Select **White Light** to display white light confluence values.
  - Select **RFP** to display RFP confluence values.
  - Select **GFP** to display GFP confluence values.
- Hover the cursor over the plate schematic to see the percentage of confluence for all the wells in the plate. Confluence lower than 5% displays <5 and confluence above 80% displays >80.
- Hover the cursor over a single well to view coordinates and confluence for the well.
- Click a well to see an image of the well on the View Image tab. See View Image on page 102.
- To copy the overview to the clipboard, hover the cursor over a well, right-click and select **Copy to Clipboard**.
- The **Annotation** field below the plate schematic displays the number of imaged wells. You can edit the annotation to enter more meaningful information.

The Well Confluence table displays the confluence for each well.

View Image

The View Image tab displays the images for the well you select.



Results Screen - View Image Tab

The well schematic displays the entire well. The confluence levels, brightness, and focus position for the whole well display on the right.

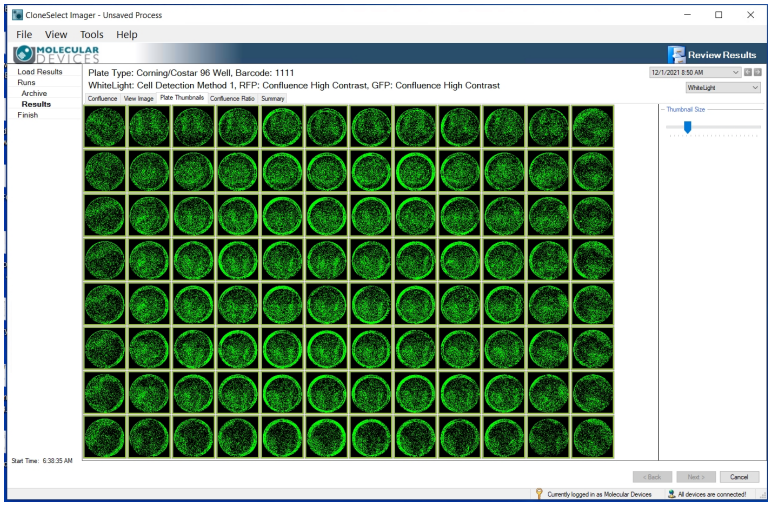
To analyze the cells in the well:

- For plates that are imaged multiple times, click the **Date** drop-down or the  to select a different time point.

- Click the drop-down:
  - Select **White Light** to display white light images.
  - Select **RFP** to display RFP images.
  - Select **GFP** to display GFP images.
- Move the **Zoom** slider to zoom in or out. The lowest magnification of the image is 18x and the highest is 144x. The figure turns red when there is distorting image pixelation.
- Move the **Contrast** slider to adjust the image contrast.
- Select the **Show Confluence** check box to overlay the area of the image outside the well in red and the regions of the well where colony growth is detected in green.
  Clear the **Show Confluence** check box to not overlay the area of the image.
- Click the **Well Name** drop-down or the  and select a different well.
- Click **Export Image** to save the image in a .bmp, .jpg or .png file format. If you select the Show Confluence check box, the confluence overlay displays with the image. The zoom position saves within the image. See Data Export Wizard on page 123.

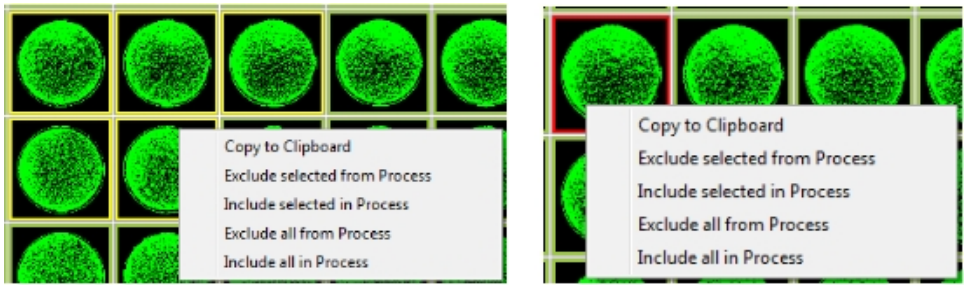
Plate Thumbnails

The Plate Thumbnails tab displays the entire plate and the wells you image as thumbnails. The thumbnails include the confluence overlay of each well you image. Use this tab to select the wells to include or exclude from analysis. The software includes or excludes the wells from the Plate Thumbnails tab on the Confluence tab and Confluence Ratio tab.



Results Screen - Plate Thumbnails Tab

- Yellow: Indicates a group well selection.
- Red: Indicates excluded wells.
- Green: Indicates included wells.



Group Selected Wells and Excluded Well With Selection Options Menu

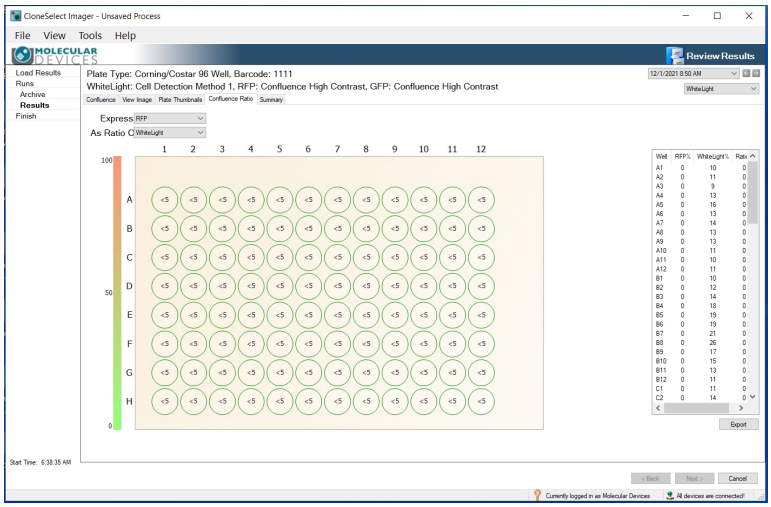
To specify what to do with each well, do the following:

1. For plates that are imaged multiple times, click the **Date** drop-down or the  to select a different time point.
2. Click the drop-down:
   - Select **White Light** to display white light images.
   - Select **RFP** to display RFP images.
   - Select **GFP** to display GFP images.
3. Hover the cursor over a well of interest.
4. Right-click on a well of interest:
   - Select **Copy to Clipboard** to copy the well to the computer clipboard.
   - Select **Exclude Selected From Process** to exclude the well from the selected process.
   - Select **Include Selected In Process** to include the well in the selected process.
   - Select **Exclude All From Process** to exclude the well from all processes.
   - Select **Include All In Process** to include the well in all processes.

   Tip: To select a group of wells, press and hold **CTRL** and then select multiple wells. Release **CTRL** when you finish selecting.
5. Move the **Thumbnail Size** slide to adjust the size of the thumbnails.

Confluence Ratio

The Confluence Ratio tab displays the confluence ratio for each well, both graphically and as a list. Use the Plate Thumbnails tab to filter the list of wells. See Plate Thumbnails on page 103.



Results Screen - Confluence Ratio Tab

The Plate Schematic displays each well as a pie chart of the confluence ratio. The pie charts are color-coded such that low confluence is green and high confluence is red with shades in between that represent the intermediate levels.

- For plates that are imaged multiple times, click the **Date** drop-down or the  to select a different time point.
- Use the **Express** drop-down in conjunction with the **As Ratio Of** drop-down to set the confluence ratio to display. For example, to display RFP confluence as a percentage of White Light confluence, click the Express drop-down and select RFP and then click the As Ratio Of drop-down and select White Light.

- Hover the cursor over the plate schematic to see the confluence ratio for all the wells in the plate. Confluence lower than 5% displays <5 and confluence above 80% displays >80.
- Hover the cursor over a single well to view coordinates and confluence ratio for the well.
- Click a well to see an image of the well on the View Image tab. See View Image on page 102.
- To copy the overview to the clipboard, hover the cursor over a well, right-click and select **Copy to Clipboard**.

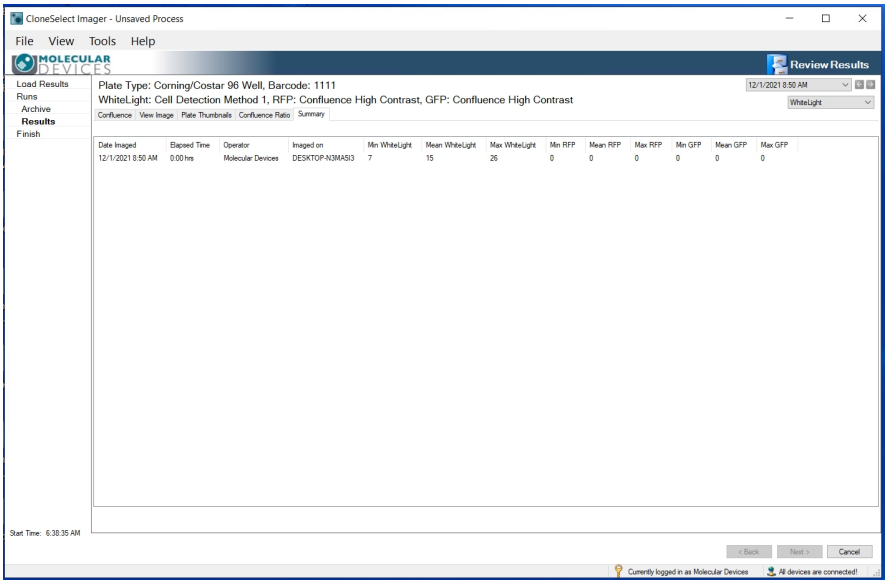
The Well Confluence table displays the confluence ratio for each well.

Click **Export** to display the Data Export wizard where you save the well confluence list in a .csv, .xls, or .xml file format. See Data Export Wizard on page 123.

Summary

The Summary tab displays a summary of the data.

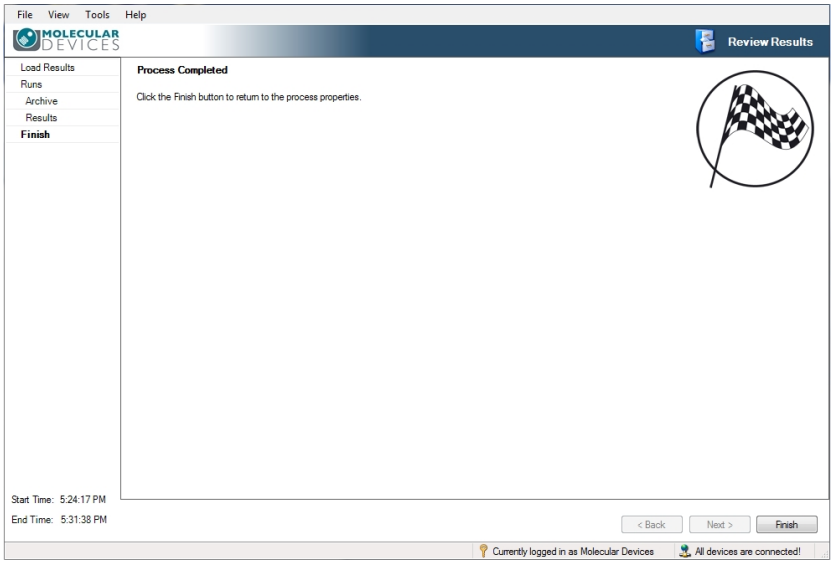
The table lists the Date Imaged, Elapsed Time, Operator, Imaged On, Minimum White Light, Mean White Light, Maximum White Light, Minimum RFP, Mean RFP, Maximum RFP, Minimum GFP, Mean GFP, and Maximum GFP.



Results Screen - Summary Tab

Finish

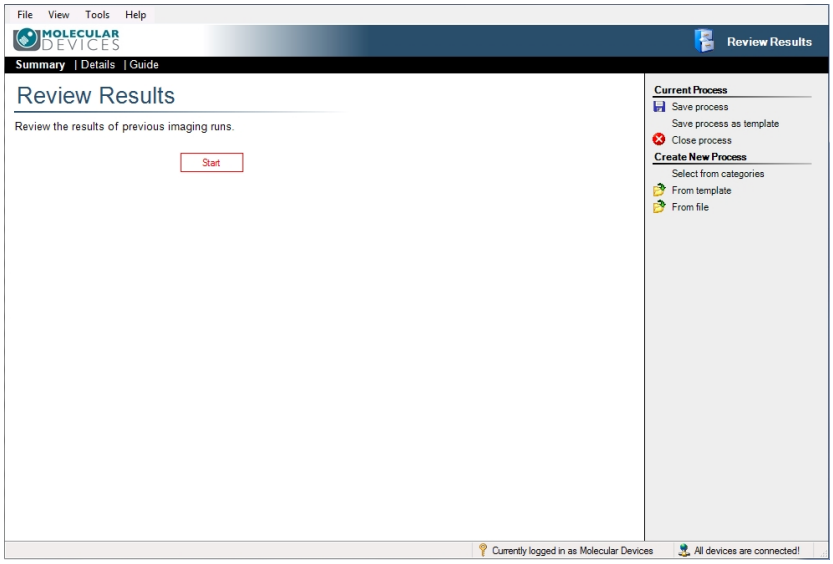
When the Review Results process ends, the Finish screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

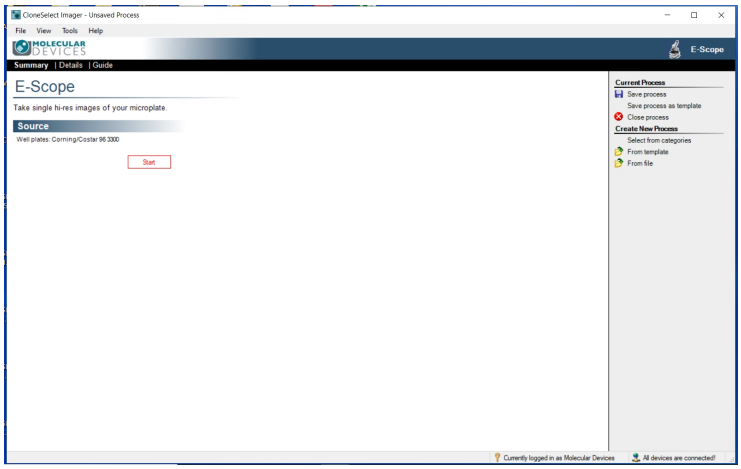
Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 10: E-Scope Process

Use the E-Scope process to view cells at a high resolution (1.85 µm) without performing any image analysis. This is for white light imaging only and images are not saved.

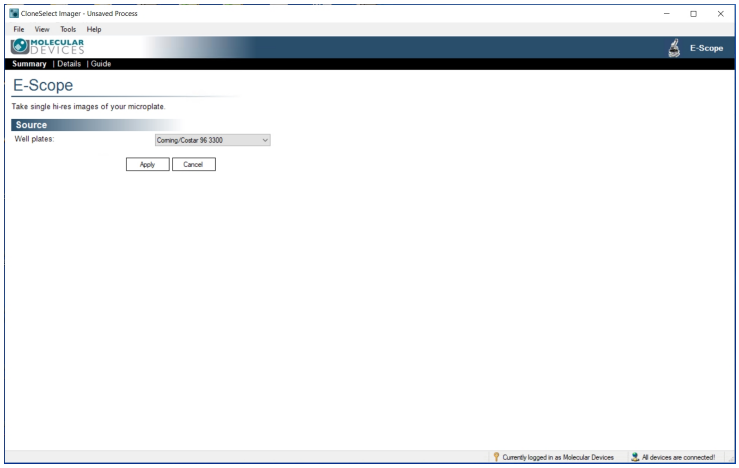
To start the E-Scope process, on the Navigation screen, double-click **E-Scope** to display the Properties screen with the Summary workflow selected.



E-Scope Process - Properties Screen - Summary Workflow

Setting E-Scope Process Properties

Start the E-Scope process from the Details workflow on the Properties screen.



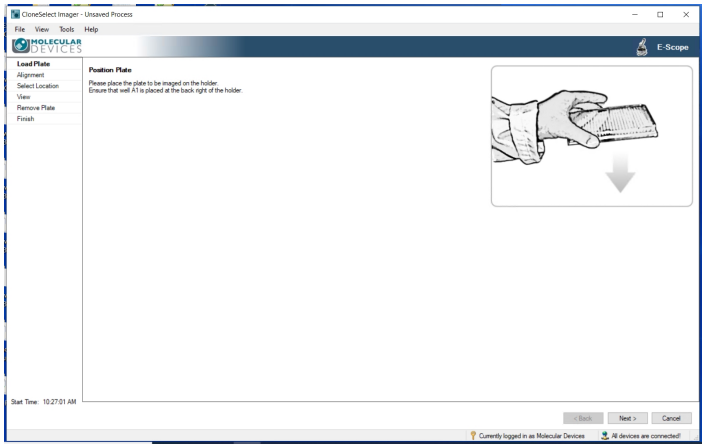
Properties Screen - Details Workflow

To set process properties:

1. Select the **Details** workflow at the top of the screen.
2. Click the **Well Plates** drop-down and select a plate type.
3. Click **Start**.

Load Plate

The plate carrier opens and the software prompts you to load the plate.



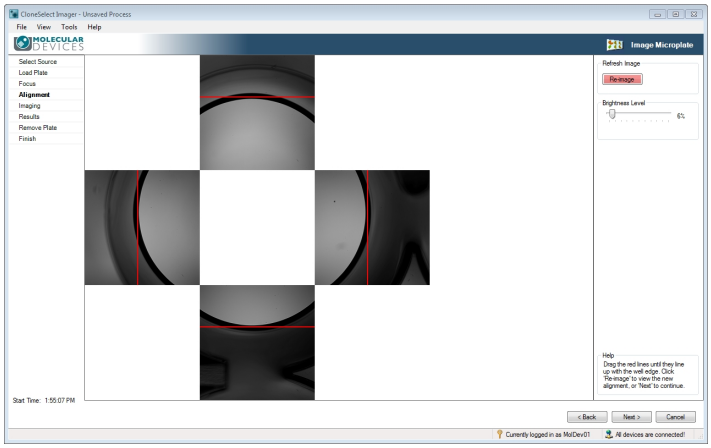
Load Plate Screen

To load a plate:

1. Place the plate on the carrier with the A1 well in the back right corner.
2. Click **Next**.
3. If prompted, enter the barcode name and click **Next**.

Alignment

Use the Alignment screen to confirm that the camera aligns with the wells. The software takes four images over the north, south, east, and west edges of well A1. The well edges should align with the middle of the box formed by the four red lines.



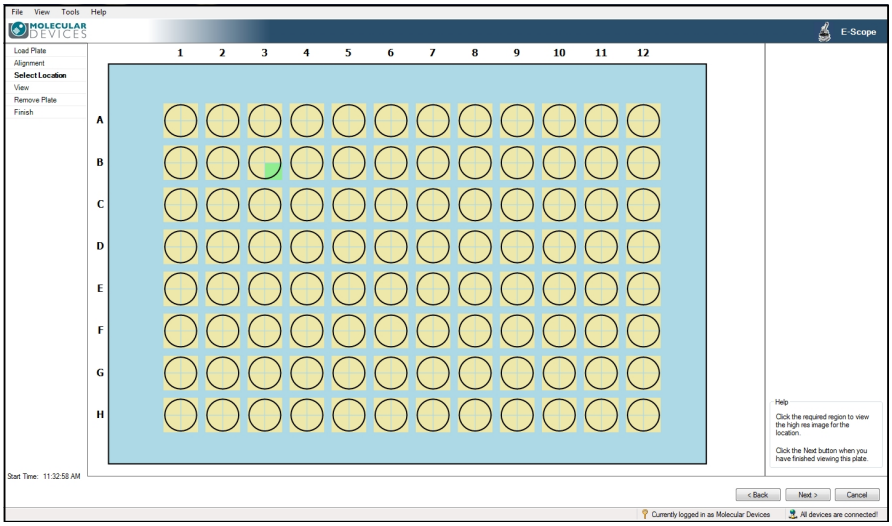
Alignment Screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines, click and drag on the lines to correct the alignment.
2. When the **Re-image** button is pink, click to recheck the alignment.
3. Move the **Brightness** slider to adjust brightness of the alignment image only.
4. Click **Next**.

Select Location

Use the Select Location screen to select a location.



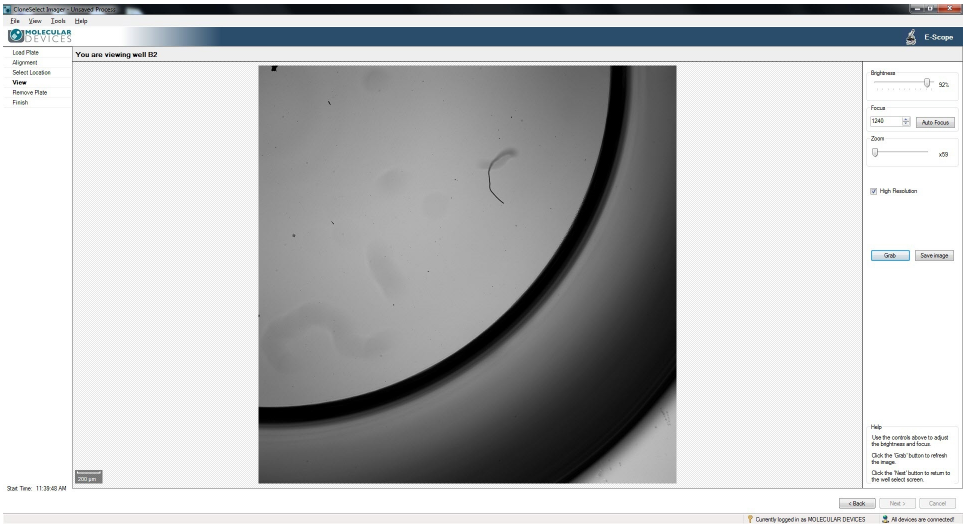
Select Location Screen

To select a location:

- In the plate schematic, click on an image frame to display the image on the View screen.
- To proceed without viewing an image, click **Next** to display the Remove Plate screen.

View

The View screen displays with the image frame.



View Screen

You can do any of the following on the View screen:

- **Brightness**—Move the slider either left or right to improve the image brightness.
- **Focus**—Click the up or down arrows to change the focus value or enter a new value in the field.
- **Auto Focus**—Click to automatically adjust the image focus.
- **Zoom**—Move the slider to look closer or farther at the image.
  As you change the zoom level, the Scale Bar in the lower left of the image window adjusts.

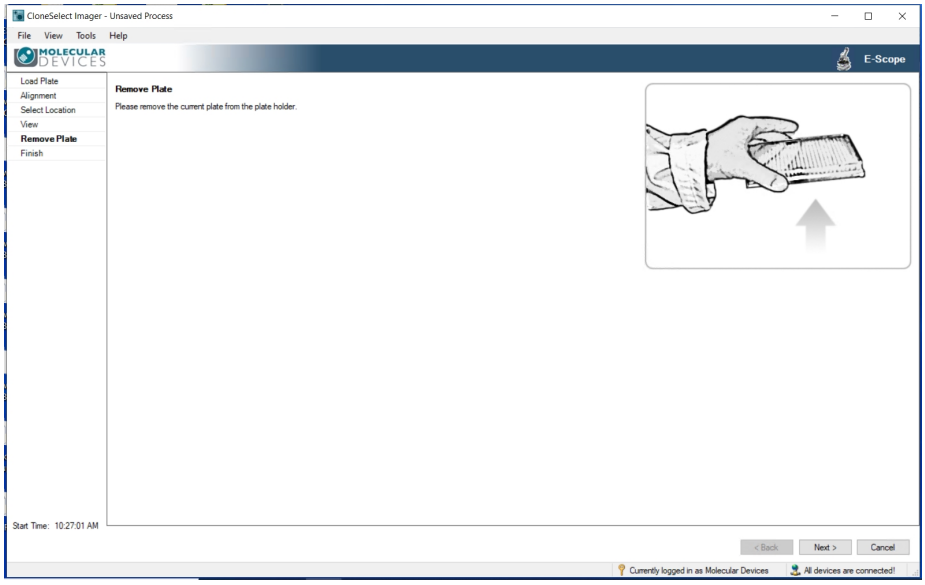
  Tip: The figure turns red when there is distorting image pixelation.

- **High Resolution**—Select to use high resolution imaging (1.85 µm), deselect to use standard resolution imaging (3.7 µm).
- **Grab**—Click to apply the changes you made while viewing the image.
- **Save Image**—Click to save the current image in bmp, jpg or png format.

Click **Back** to display the Select Location screen where you can select a different image or you can proceed with the E-Scope process.

Remove Plate

In the Remove Plate step, the plate carrier opens and the software prompts you to remove the plate from the plate carrier. You can repeat the process with another plate or continue to the end of the process.



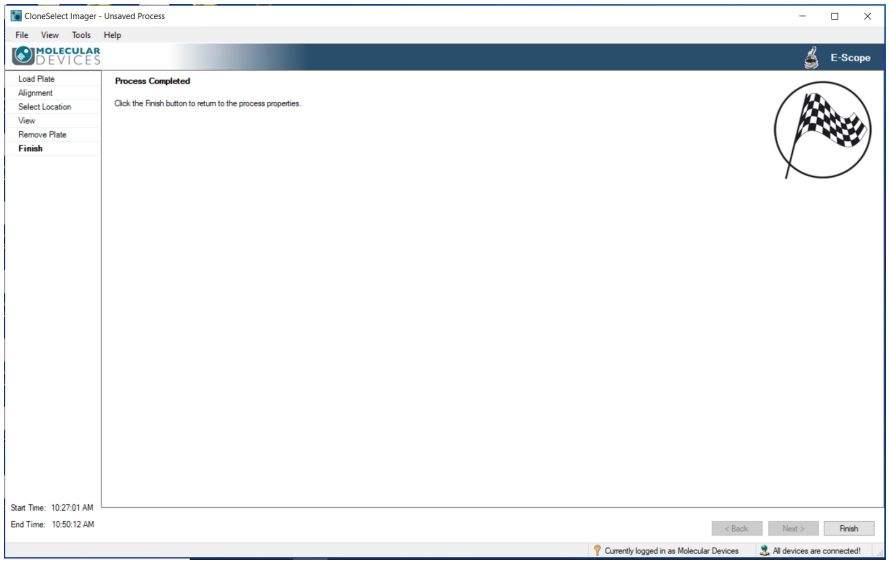
Remove Plate Screen

Click **Next**.

The plate carrier closes and the **Finish** screen displays.

Finish

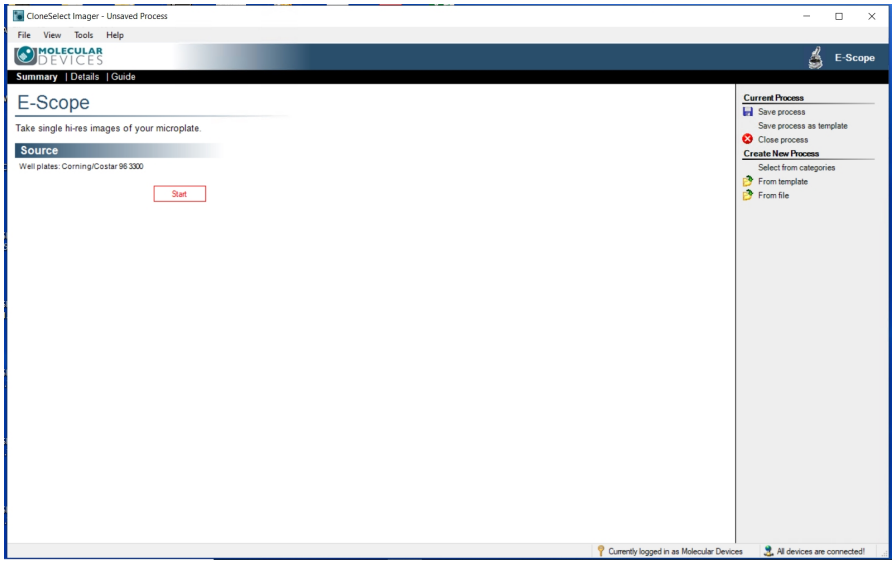
When the Image Microplate process ends, the **Finish** screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

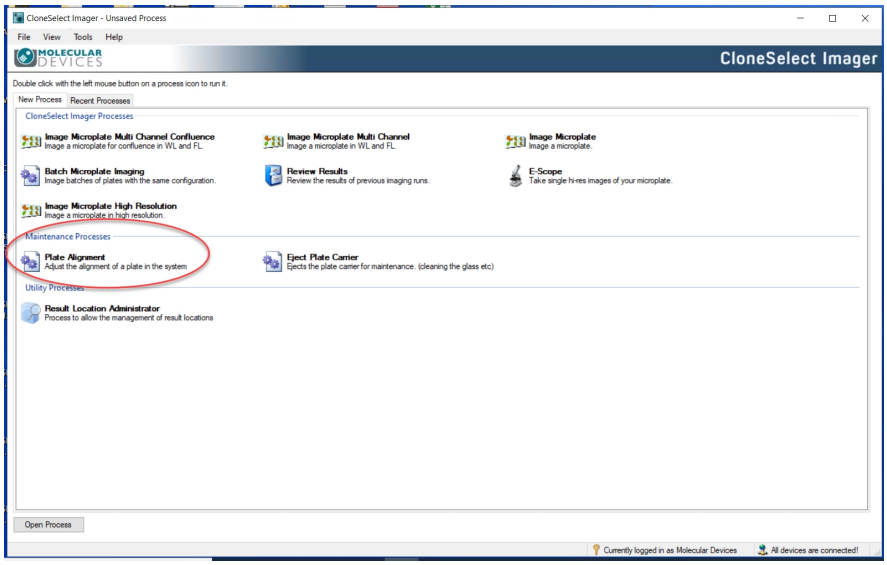
Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 11: Plate Alignment Process

Use the Plate Alignment process to align the plate.

To start the Plate Alignment process, on the Navigation screen, in the Maintenance Processes area, double-click **Plate Alignment**.



Navigation Screen

The Properties screen with the Summary workflow selected displays.

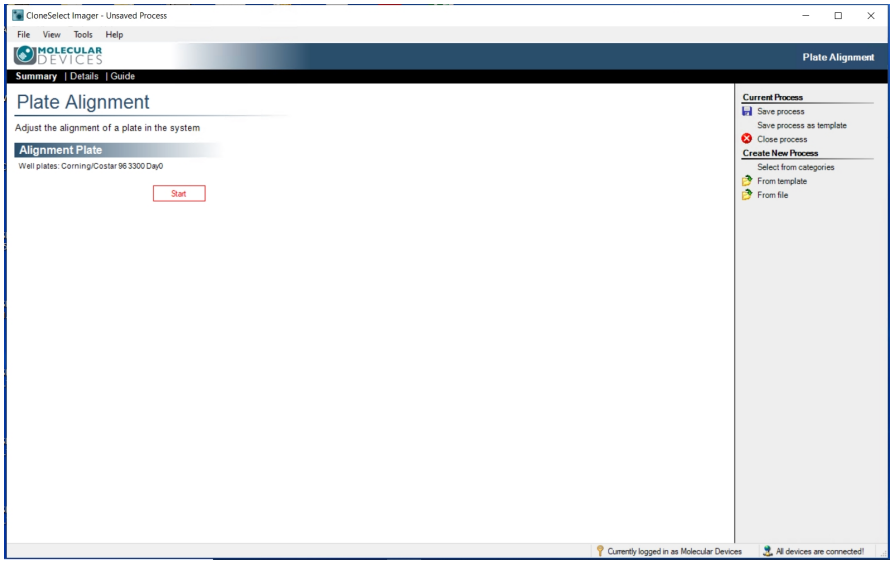
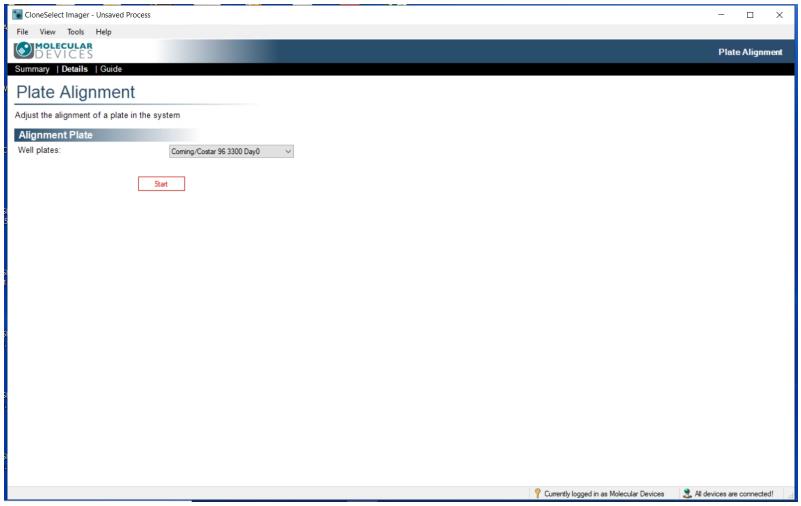


Plate Alignment Process - Properties Screen - Summary Workflow

Setting Plate Alignment Process Properties

Start the Plate Alignment process from the Details workflow on the Properties screen.



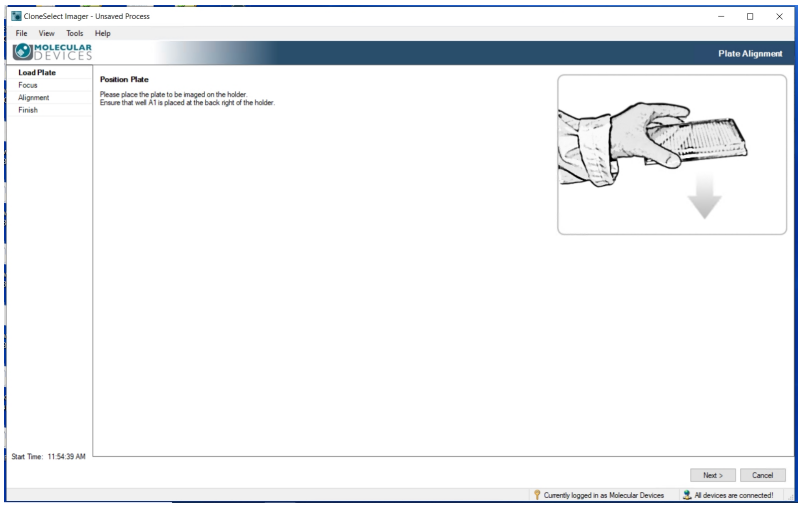
Properties Screen - Details Workflow

To set process properties:

1. Select the **Details** workflow at the top of the screen.
2. Click the **Well Plates** drop-down and select a plate type.
3. Click **Start**.

Load Plate

The plate carrier opens and prompts you to load the plate.



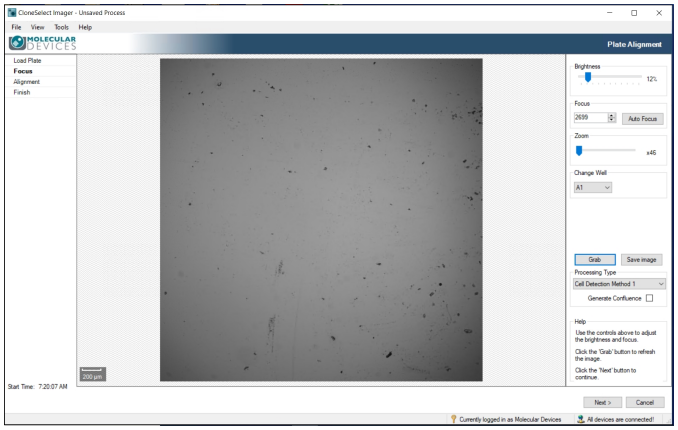
Load Plate Screen

To load a plate:

1. When the plate carrier opens, place the plate on the carrier with the A1 well in the back right corner.
2. Click **Next**.
3. If prompted, enter the barcode name and click **Next**.

Focus

Use the Focus screen to optimize the image view before the scan. The software stores the last focus setting you used for each plate type. After you image a plate type for the first time, additional focus adjustments should be minimal.



Focus Screen

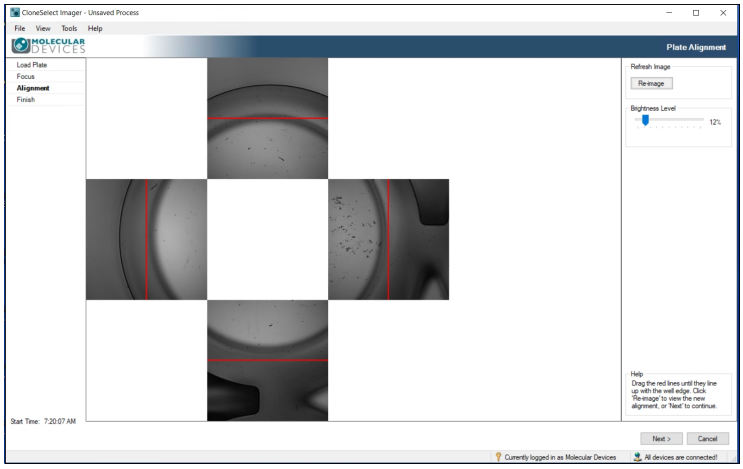
The software determines the optimal brightness setting but you can adjust the Brightness setting. By default the focus is done on well A1. If A1 is not being imaged, the focus is done on the first well of the plate to image.

To adjust focus:

1. Move the **Brightness** slider to adjust the image brightness.
2. To use Auto Focus, click **Auto Focus**. If there is debris on the bottom of the plate or on the glass plate carrier, the auto focus might focus on these instead of the cells. In this scenario use the manual focus steps.
3. To manually focus:
   a. In the **Focus** field, enter a new focus value.
   b. Move the **Zoom** slider to look closer or farther at the image. As you change the zoom level, the scale bar in the lower left adjusts. When the zoom figure on the right of the slider is black, the system zooms optically. When the figure turns red, the system zooms digitally and you might see some pixelation of the image.
4. Click the **Change Well** drop-down and select a different well on which to focus.
5. Click **Grab** to update the image with the new focus settings. This becomes the default focus setting for the plate type in this process run.
6. Click **Save Image** to display the Save File dialog where you enter the name and click **Save**.
7. Click the **Processing Type** drop-down:
   - Select **Cell Detection Method 1** for settled suspension cells and adherent cells with good contrast.
   - Select **Cell Detection Method 2** to detect cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
   - Select **Cell Detection Method 3** to detect colonies and distinguish them from the artifacts around the edges of the colonies.
   - Select **Very Low Contrast** for low contrast cells.
8. Select the **Generate Confluence** check box to generate confluence.
   Clear the **Generate Confluence** to not generate confluence.
9. Click **Next**.

Alignment

Use the Alignment screen to confirm that the camera aligns with the wells. The software takes four images over the north, south, east, and west edges of well A1. The well edges should align with the middle of the box formed by the four red lines.



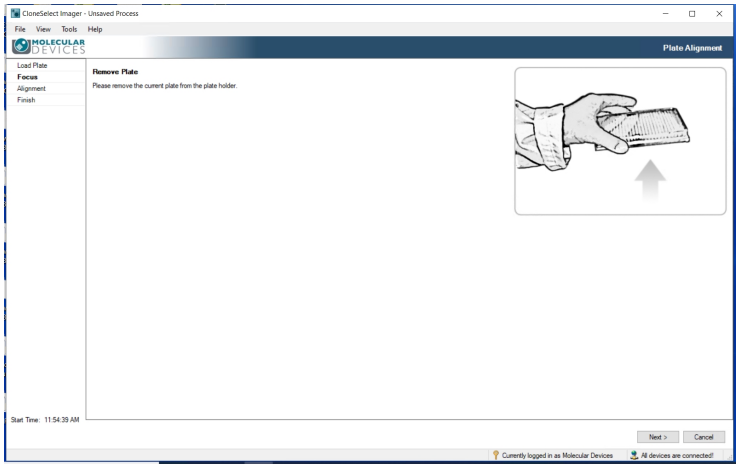
Alignment Screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines, click and drag on the lines to correct the alignment.
2. When the **Re-image** button is pink, click to recheck the alignment.
3. Move the **Brightness** slider to adjust brightness of the alignment image only.
4. Click **Next**.

Remove Plate

In the Remove Plate step, the plate carrier opens and the software prompts you to remove the plate from the plate carrier. You can repeat the process with another plate or continue to the end of the process.



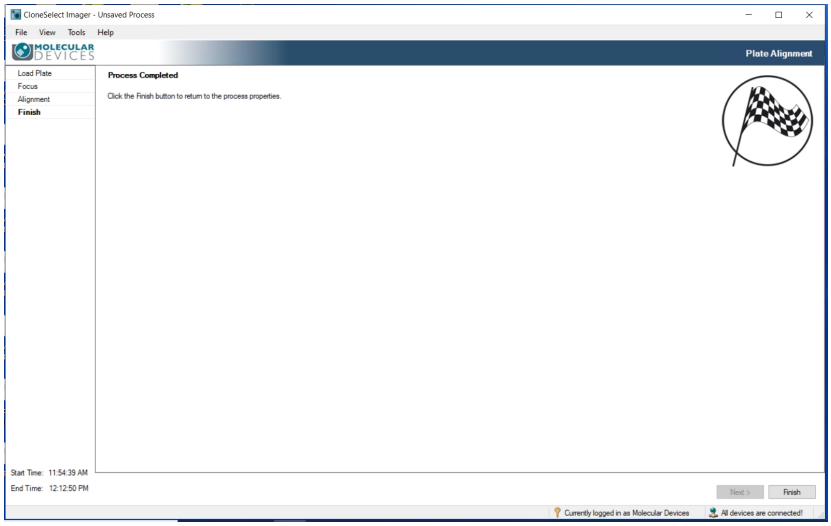
Remove Plate Screen

Click **Next**.

The plate carrier closes and the **Finish** screen displays.

Finish

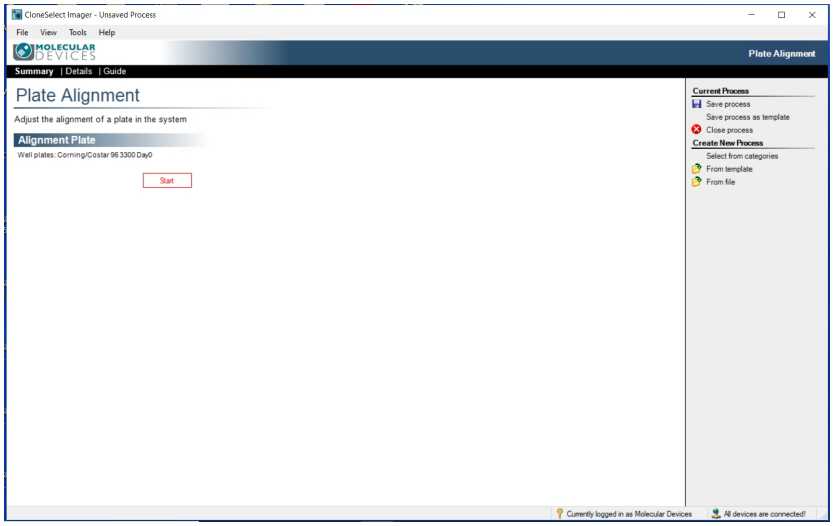
When the Image Microplate process ends, the **Finish** screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



Properties Screen - Summary Workflow

Select the Summary workflow at the top of the screen and then click the following on the right:

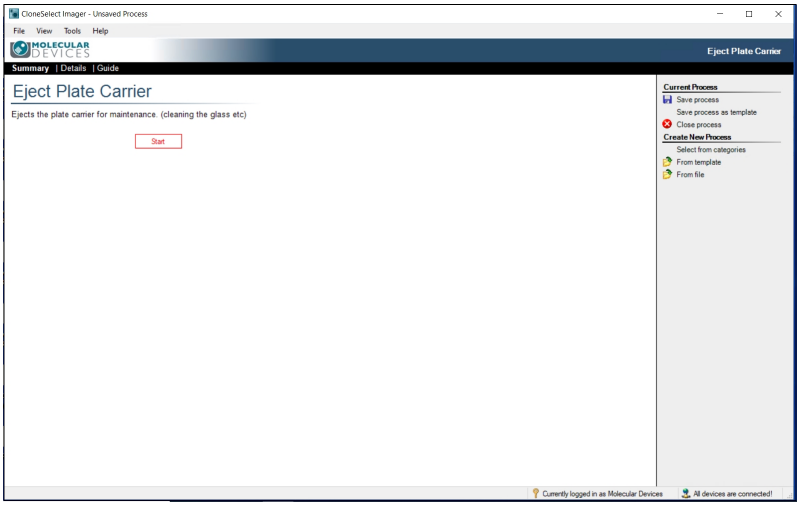
- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 12: Eject Plate Carrier Process

Use the Eject Plate Carrier process to open the plate carrier for cleaning or to remove a plate left in the instrument.

To open the plate carrier:

1. On the Navigation screen, double-click **Eject Plate Carrier** to display the Properties screen with the Summary workflow selected.

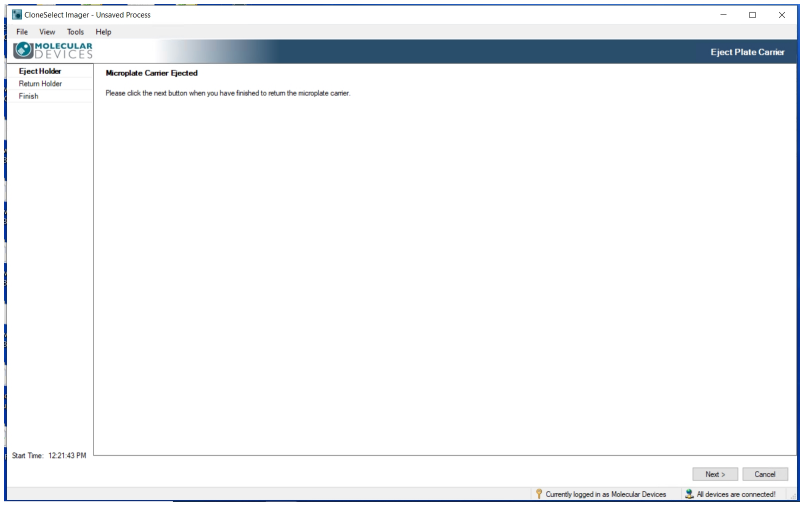


Eject Plate Carrier Process Properties Screen - Summary Workflow

2. Click **Start**.

Eject Holder

In the Eject Holder step, the plate carrier opens and the software prompts you to remove the plate holder from the plate carrier.

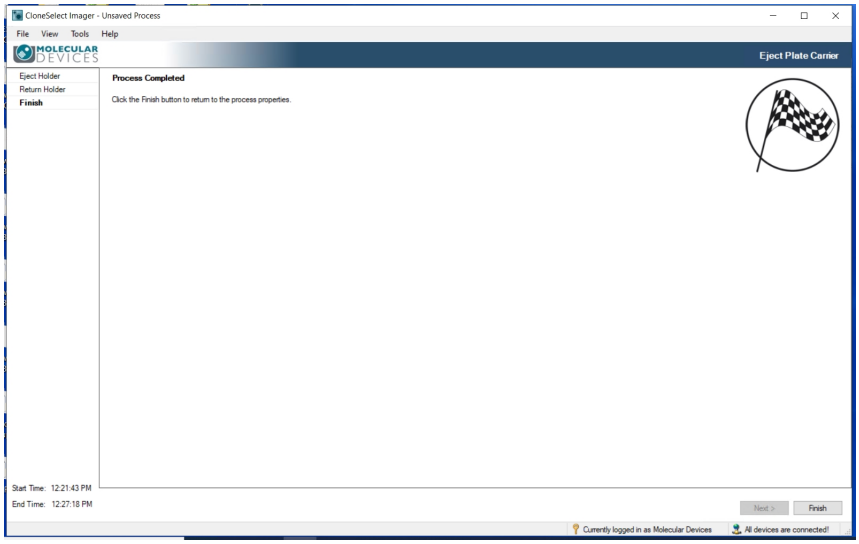


Eject Holder Screen

Click **Next**.

Return Holder

In the Return Holder step, the plate carrier closes and the software prompts you to finish the process.

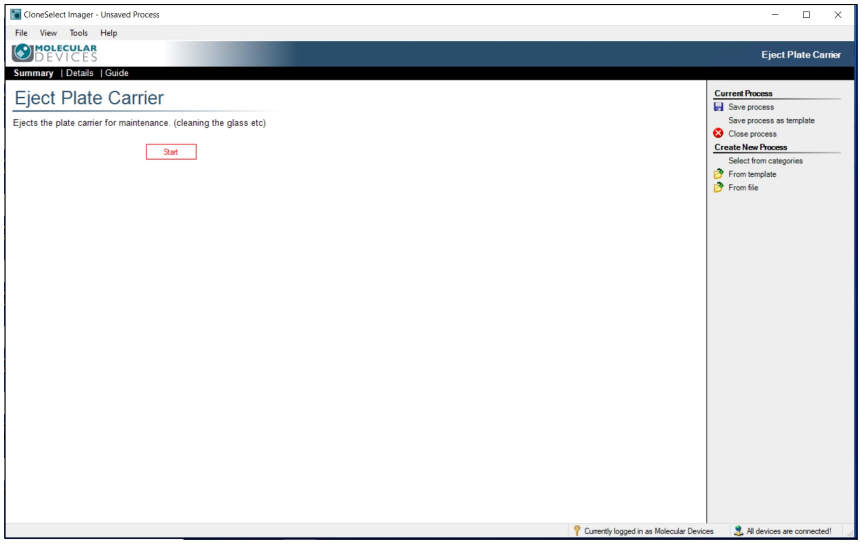


Return Holder Screen

Click **Finish**.

Finish

When the plate carrier closes, the Properties screen displays.



Properties Screen - Summary Workflow

Select the Summary workflow at the top of the screen and then click the following on the right:

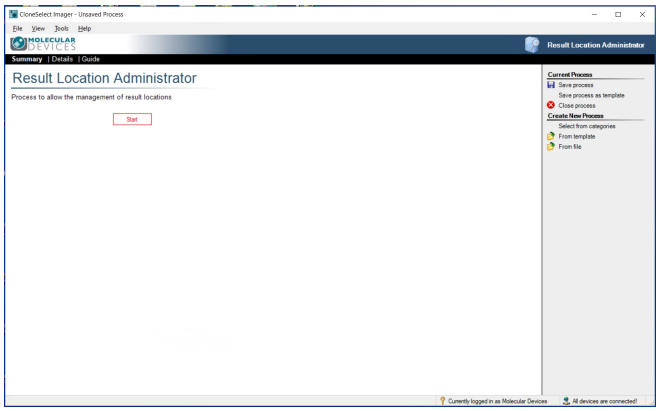
- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 13: Result Location Administrator

Use the Result Location Administrator to set the default location to save results.

To define the default result location:

1. On the Navigation screen, double-click **Result Location Administrator** to display the Properties screen with the Summary workflow selected.

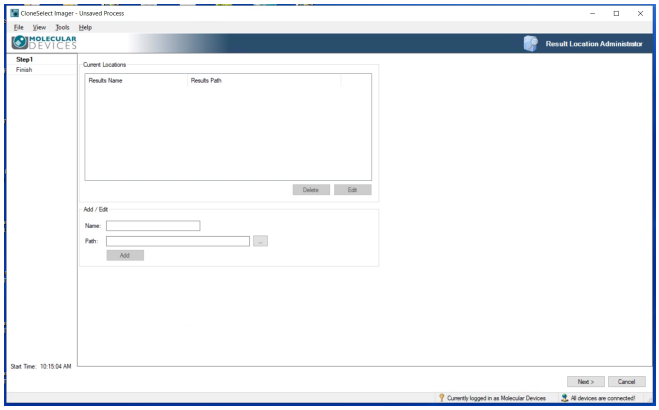


Result Location Administrator - Properties Screen - Summary Workflow

2. Click **Start**.

Result Location Administrator Step 1

Use the Step 1 page to define the location where you want the software to save results by default.



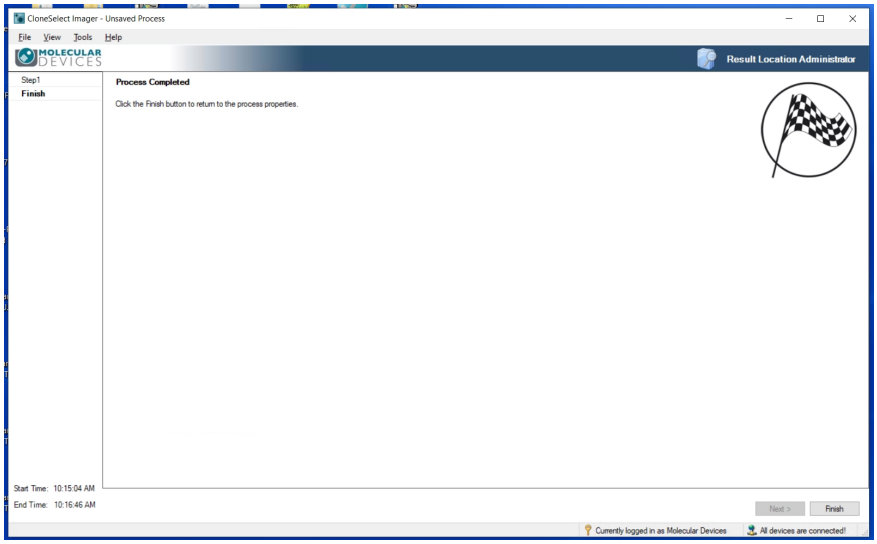
Step 1 Screen

To define the default result location:

1. In the **Name** field, enter the name for default result folder.
2. To the right of the **Path** field, click the **...** and navigate the folder hierarchy to where you want to set the default results folder.
3. Click **Add**.
4. Repeat to add more default result locations or select a result in the list and click **Edit** to edit a location or click **Delete** to delete a location.
5. Click **Next**.

Finish

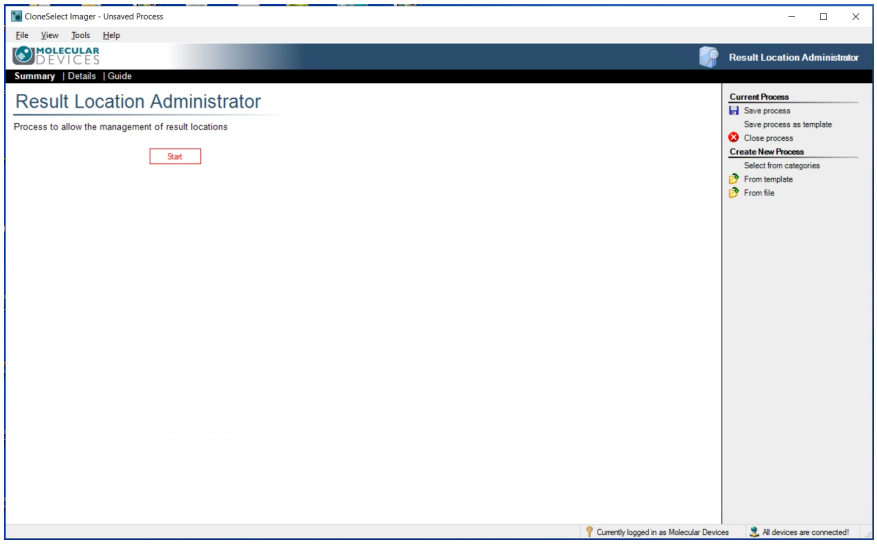
After you define the default result location, the Finish screen displays.



Finish Screen

Click **Finish** to display the Properties screen.

Properties Summary



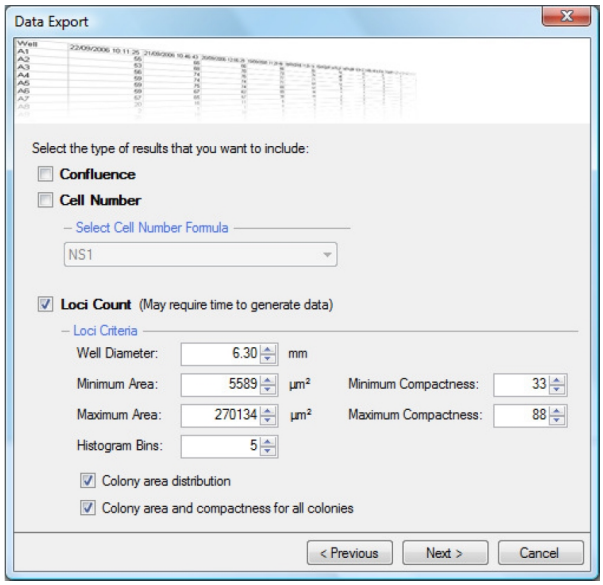
Properties Screen - Summary Workflow

Select the Summary workflow at the top of the screen and then click the following on the right:

- Click **Save Process** to save the process on the Recent Processes tab for future access.
- Click **Save Process as Template** to save the process on the Template tab for future access. You cannot change a saved template.
- Click **Close Process** to close the process and display the Navigation screen. See Navigation Screen on page 23.

Chapter 14: Data Export Wizard

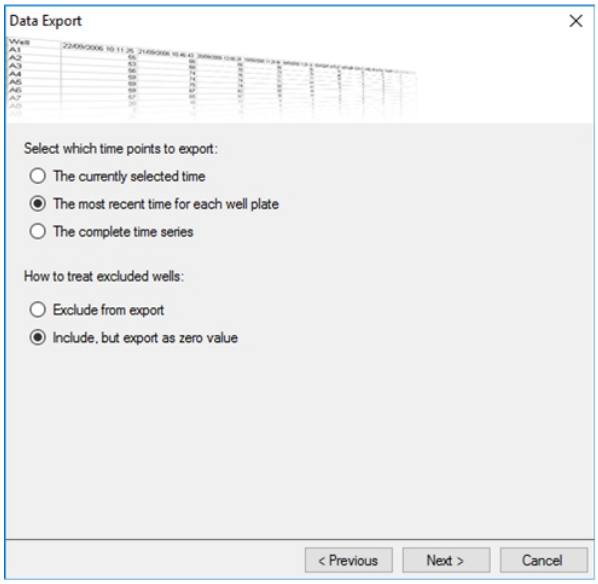
Use the Data Export wizard to select the result data to export. Several workflows contain an Export button that displays the Data Export wizard. Fields that are available depend upon the way you access the wizard. You can select to export the Confluence data, Cell Number data, and Loci Count data. Depending on the data, you can export to a .csv, .xls, or .xml file format.



Data Export Wizard

The wizard provides the option to export the data for the following time points:

- The Currently Selected Time
- The Most Recent Time For Each Well Plate
- The Complete Time Series



Data Export Wizard With Excluded Wells Options

When you select the **Include, But Export As Zero** option, the excluded wells are listed in the exported data with a zero value in the Cell Number column.

Well	Confluence	Cell Number	Loci Count	
A1	0	0	0	
A2	0	4	0	
A3	0	0	0	
A4	1	8	0	
A5	1	9	0	
A6	1	16	0	
B1	1	9	0	
B2	1	13	0	
B3	1	9	0	
B4	0	5	0	
B5	2	19	0	
B6	1	16	0	

Example Export Data With Well A1 and Well A3 Excluded

Chapter 15: Maintenance

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only. See Obtaining Support on page 132.

WARNING! Service or maintenance procedures other than those specified in this guide can be done only by Molecular Devices qualified personnel. When service is required, contact Molecular Devices Technical Support.

Preventive Maintenance

You are responsible for doing daily and weekly maintenance. You should have Molecular Devices do a complete instrument preventative maintenance service annually.

General Precautions

- All waste must be disposed of according to local regulations.

WARNING! Do not use in explosive environments.

Daily Maintenance

- Keep the instrument dirt-free and dust-free.
- Clean the top and bottom of the removable glass plate holder using a lint-free cloth sprayed with 80% ethanol.

Weekly Maintenance

- Check operation of equipment.
- Check for damage.

Annual Maintenance

A complete instrument maintenance should be done annually by an approved service engineer. To obtain a maintenance contract or schedule a service visit, contact Molecular Devices technical support. See Obtaining Support on page 132.

Cleaning the Instrument

Clean the instrument using 80% ethanol or dilute detergent and a lint-free cloth.

CAUTION! Do not use organic solvents and abrasive cleaners because they can damage the cover.

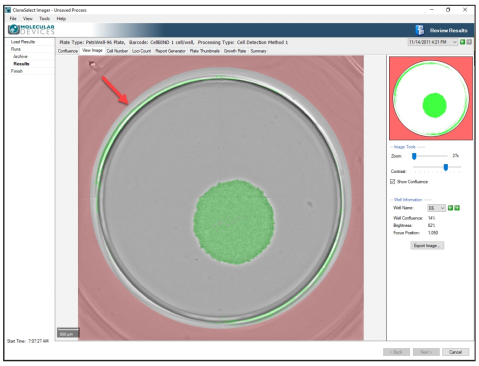
Apply cleaning solution using a suitable lint-free cloth.

CAUTION! Do not pour cleaning solution directly on to the instrument or other objects.

Troubleshooting Well Detection Area

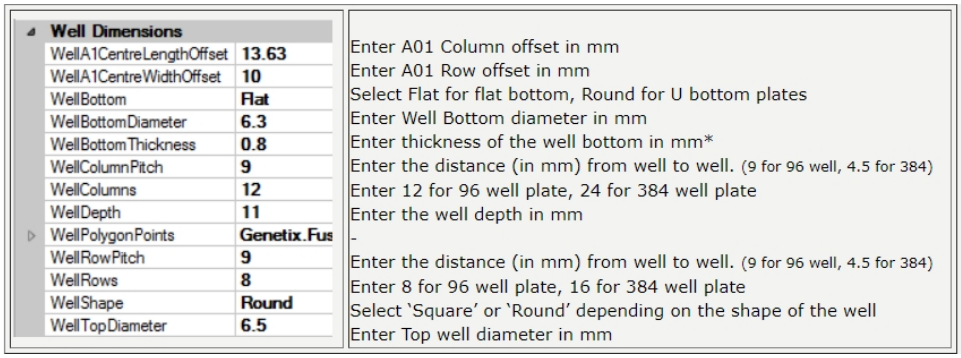
If the detection area is outside the well, change the detection area by changing the plate parameters.

- Do not use Day 0 plates for the Image Microplate Multichannel process. The well diameter of this plate configuration is larger than the actual well size to ensure the capture of the entire well and not miss any cells
- Do not use Day 0 plates for the Image Microplate Multi Channel Confluence process. The well diameter of this plate configuration is smaller than the actual well size to avoid detection of the well edge as confluence, which greatly skews any results obtained.



To change the well detection area plate parameters:

1. In the software select **Tools > Configuration**.
2. In the confirmation message, click **Yes** to display the Edit Configuration dialog.
3. Select **Consumables**.
4. Expand the **Microplates** list.
5. Select a plate to display the well detection information in the gray area.



Well Dimensions	
WellA1CentreLengthOffset	13.63
WellA1CentreWidthOffset	10
WellBottom	Flat
WellBottomDiameter	6.3
WellBottomThickness	0.8
WellColumnPitch	9
WellColumns	12
WellDepth	11
WellPolygonPoints	Genetix.Fus
WellRowPitch	9
WellRows	8
WellShape	Round
WellTopDiameter	6.5

Enter A01 Column offset in mm
Enter A01 Row offset in mm
Select Flat for flat bottom, Round for U bottom plates
Enter Well Bottom diameter in mm
Enter thickness of the well bottom in mm*
Enter the distance (in mm) from well to well. (9 for 96 well, 4.5 for 384)
Enter 12 for 96 well plate, 24 for 384 well plate
Enter the well depth in mm
-
Enter the distance (in mm) from well to well. (9 for 96 well, 4.5 for 384)
Enter 8 for 96 well plate, 16 for 384 well plate
Select 'Square' or 'Round' depending on the shape of the well
Enter Top well diameter in mm

6. Adjust the **Well Bottom Diameter** to change the size of the red overlay. Decrease this number make the outcut in the red overlay smaller.
7. Adjust the **A01 Offsets** to shift the hole up, down, left, or right.
8. Adjust the **Pitch** to change the distance between one well and the next, center to center.
9. Click **Close**.

If detection area is still a problem, contact Molecular Devices Technical Support. See Obtaining Support on page 132.

Troubleshooting Barcodes

If you use barcodes and the instrument does not detect them correctly, try the following:

- Rotate the plate 180 degrees. The instrument scans from either the front or the back, but only one sensor is active. Contact Molecular Devices Technical Support if you need to change the scan direction on your instrument.
- If one out of 10 plates fails to be read, check the placement of the barcode label on the plate. This might be fixed with a small change in the plate configuration.
- Add more white space at beginning or end.
- Restrict numbers to a maximum of six (6) digits.
- Verify that the codes meet the requirements. See Using Barcodes on page 20.

If detection is still a problem, contact Molecular Devices Technical Support. See Obtaining Support on page 132.

Generating Error Reports

When the instrument is in an error state, you can generate an error report. This report is required when you contact Molecular Devices Technical Support.

To generate an error report:

1. Select **Tools > Configuration**.
2. Select **System Log**.
3. In the **System Log** list, select the most recent Log.
4. In the **Event Severity** section, select the **Error** check box and the **Fatal** check box.
5. To review the details, highlight the results lines.
6. To generate a report of the results listed, select **Tools > Prepare Error** report to display the Error Reporting wizard.
7. Click **Next**.
8. Specify the location to save the error logs.
9. Send the report file to Molecular Devices Technical Support.

Appendix A: Technical Specifications

The following list the technical specifications for the CloneSelect Imager FL.

Dimensions (Total Assembled)

Item	Description
Instrument Size	57.5 cm (width) x 72.0 cm (depth) x 43.8 cm (height) Excluding ancillary equipment
Instrument Weight	45 kg
Light Engine Size	12.5 cm (width) x 26.3 cm (length) x 16.3 cm (height)
Light Engine Weight	3.6 kg

Operating Environment (Indoor Use Only)

Item	Description
Temperature	10°C to 40°C
Humidity	20% to 80% non-condensing
Altitude	Up to 2000 M
Mains Supply	± 10% Rated Voltage
Transient Overvoltage	Installation Category (Overvoltage category) II
Rated Pollution	Pollution degree 2

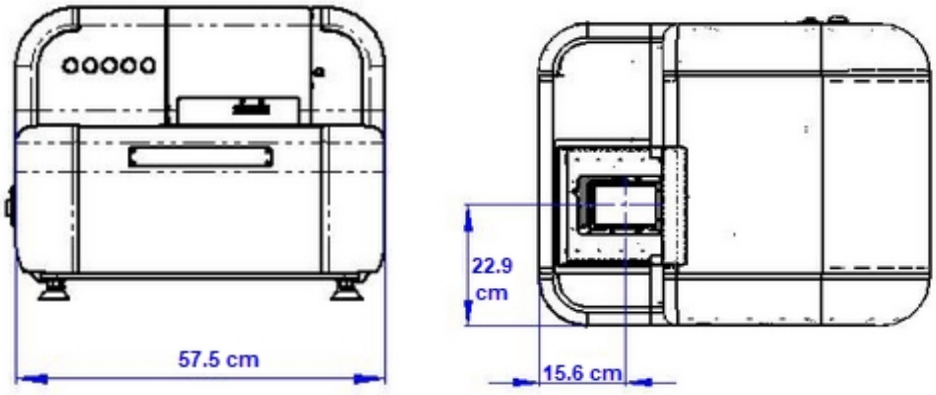
Electrical Supply

Item	Description
Voltage	100 to 240 VAC, 50 to 60 Hz, single phase
Power	100 VA
Connections	IEC input
Fuses	F1 - F3A

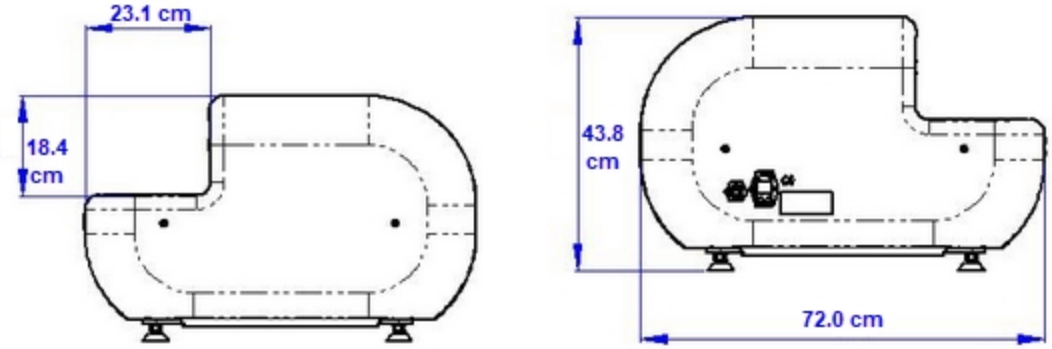
Fluorescent Specifications

Item	Description
Microplates	96-well and 384 well plates (see Compatible Plate Types on page 19
White Light Source	Xenon Flash Lamp (5 watts)
Green Channel Filters	Excitation 470 nm with bandwidth of 24 nm Emission 520 nm with bandwidth of 54 nm
Red Channel Filters	Excitation 555 nm with bandwidth of 28 nm Emission 609 nm with bandwidth of 54 nm
Dichrotic Filter	493/574-D01 dual band dichrotic
Image Speed (fluorescent exposure time of 50 ms)	≤2 minute white light standard read mode ≤6 minute white light maximum read mode ≤8 minutes single color fluorescent maximum read mode ≤15 minutes white light and single color fluorescent maximum read mode ≤25 minutes white light and dual color fluorescent maximum read mode
Fluorescent Light Source	Aura III Light Engine 100-700 mW solid state light (LED) engine for each color channel
Camera	High resolution CMOS camera

Instrument Dimensions



Front and Top Views



Right Side* and Left Side Views

* The vertical measurement is from the top of the glass in the plate carrier to the top of the instrument

Light Engine Dimensions

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—support.moleculardevices.com/—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

To speed up the process, please generate and submit an error report if your instrument is in an error state. 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.

Appendix B: Well List File Format

Note: You can only have one Well List File per plate type.

Use the following format for the Well List File:

```
Molecular Devices 96 well
A6
B5
E8
```

and so on.

To create a Well List File:

1. In the first line, enter the Plate Type name.
2. On a new line, enter the name of a well to image.
3. Repeat step 3 until all the wells to image are listed.
4. Save as a text file (.txt).
5. Use this file as your Import file during an Image Microplate process from the Select Source screen. See Select Source on page 29.

Contact Us

Phone: +1-800-635-5577
Web: moleculardevices.com
Email: info@moldev.com

Visit our website for a current listing of worldwide distributors.

5090618 B

MOLECULAR DEVICES