CloneSelect™

Imager

Version 1.5

User Guide

MOLECULAR DEVICES

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Product manufactured by Molecular Devices, LLC.
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

Contents

Chapter 1: Introduction

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CloneSelect Imager

The CloneSelect™ Imager is an automated CCD camera-based system for imaging and confluence determination of cells growing in microplates.

Note: The CloneSelect Imager is strictly 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.

System Features

- Automated, rapid confluence determination of cells growing in microplates
- Automatically plots time course of cell growth
- Cell number estimation
- Cell colony loci counting
- Fast scan and full scan modes
- Standard and high-resolution imaging
- Generates "hit lists" of wells
- Exports data and images for further analysis

Before You Start

CAUTION! Before using the instrument, it is very important that you read this guide and understand all the safety instructions. Then follow the procedures in the Power On Procedure on page 10.

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 possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you seek, follow the links to the Technical Support Service Request Form to send an email to our technical support representatives.

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

To find regional support contact information, visit www.moleculardevices.com/contact.

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

Chapter 2: Hardware Guide

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CloneSelect Imager Layout and Dimensions

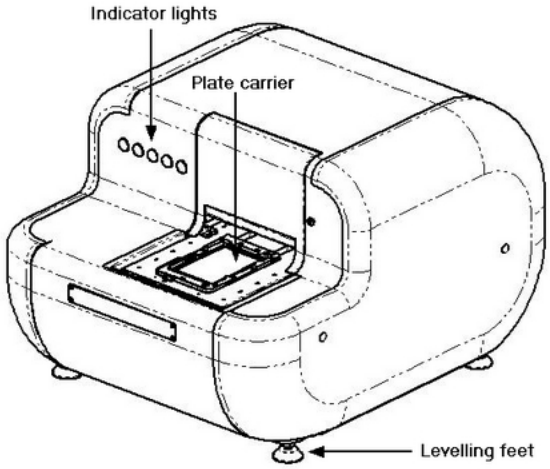

Figure 2-1: The overall layout of the instrument.

Indicator Lights

The indicator lights show the status of the instrument.

Figure 2-2: The functions of the indicator lights.

Plate Carrier

The plate carrier consists of a glass plate surrounded by a vacuum bed seal. The microplate is placed on the plate carrier so that the skirt is over the vacuum bed seal. When the plate carrier retracts into the instrument for imaging, the microplate is pushed into the back right corner of the plate carrier into the correct position for imaging.

Before imaging, a vacuum is applied under the plate that holds it down 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.

CAUTION! The plate skirt of the plate needs to be intact for the vacuum seal to work.

Levelling Feet

If needed, adjust the levelling feet by loosening the locking nuts and turning the feet until the instrument is level and stable. After you make adjustments, tighten the locking nuts.

Instrument Dimensions

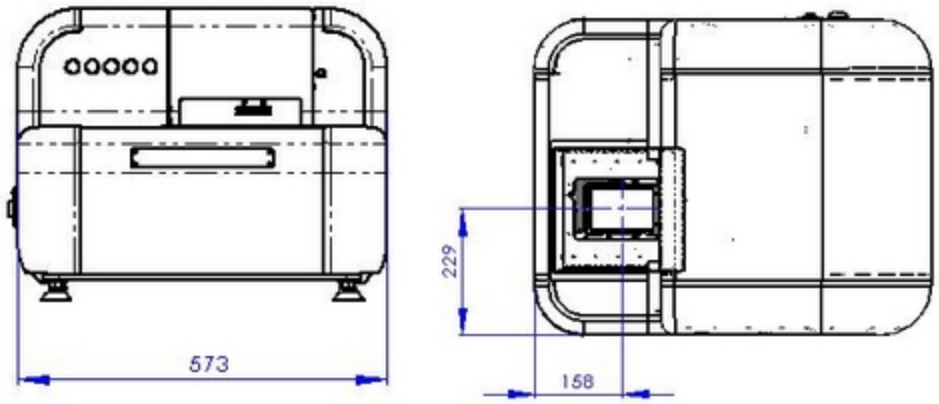

Figure 2-3: The front and top views of the instrument.

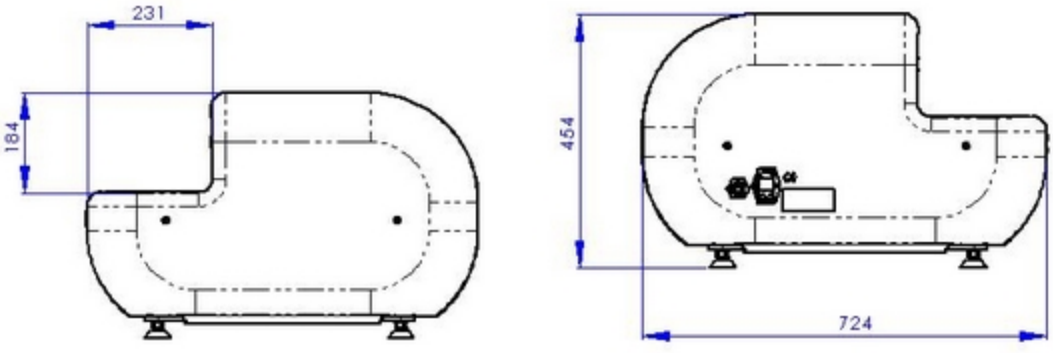

Figure 2-4: The right side* and left side views of the instrument.

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

Note: All dimensions are in millimeters.

Setup

Locating the CloneSelect Imager

Place the CloneSelect Imager on a stable, level surface. Level the instrument by loosening the locking nuts and turning the levelling feet to ensure that the CloneSelect Imager is stable and not able to move. After adjustment, tighten the locking nuts.

Computer

The CloneSelect Imager ships with a computer with special hardware and software to support the imaging function.

CAUTION! Do not attempt to use any other computer to operate the CloneSelect Imager.

The computer includes Windows 7 or Windows 10. To connect to your network, security configuration is required. When that is done, ensure that no changes are made to the configuration of the private network connection to the CloneSelect Imager because that stops it operating.

CAUTION! Do not change the standard English settings. If these settings change, the performance of the system can be impacted.

Connecting the CloneSelect Imager

The connections to the CloneSelect Imager are on the connections panel on the left side of the instrument.

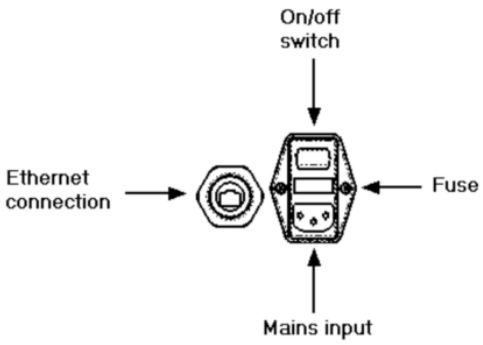

Figure 2-5: The connections panel

Connect the mains cables to the CloneSelect Imager, the computer and to the monitor before making the network connections.

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

Power On Procedure

To power on the CloneSelect Imager:

1. Turn on the CloneSelect Imager.
2. Wait for the Ready light to illuminate.
3. Before use, to warm up the instrument, leave the CloneSelect Imager in the **Ready** state for more than 5 minutes. When the unit is cold to the touch, before use, wait for 30 minutes.
4. Turn on the computer and start the CloneSelect Imager Software by double-clicking the desktop icon shortcut.

The instrument is now ready for use.

Rebooting the CloneSelect Imager

If you have to reboot the instrument, power it off. Wait for 30 seconds, and then power it on again. After the **Ready** light illuminates, the instrument is ready to use. See Indicator Lights on page 7.

Calibration and Alignment

A Molecular Devices engineer does calibration and alignment before the CloneSelect Imager ships. If the instrument requires recalibration and realignment do the following:

1. On the Navigation screen, select the **E-Scope** process.
2. Select the **Calibration** plate to be imaged.
3. When prompted, start the run and insert the Calibration plate (shipped with instrument).
4. Align onto well A1 of the Calibration plate.
5. On the image selection screen, select the lower left quadrant image of well D1.
6. Adjust the focus until the cells on the Calibration plate are in focus.
7. Image wells A12 and H12, then check that the focus is correct in each image. Do not change focus, only review.

If the focus is not correct, to schedule an alignment, contact Molecular Devices. See Obtaining Support on page 6.

Chapter 3: Software Overview

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The CloneSelect Imager Software controls the CloneSelect Imager instrument and is based around the concept of the instrument running processes. Using the default set of processes, you can modify and save them.

To start the software, from the Windows desktop, double-click the **CloneSelect Imager** icon.

When the software first starts, the Navigation screen displays the **New Process** tab (Figure 3-1). Processes are managed from the Navigation screen. Selecting a process takes you to the chosen process start-screen from where you can set the properties of the process and run it. See Process Properties Screen on page 16.

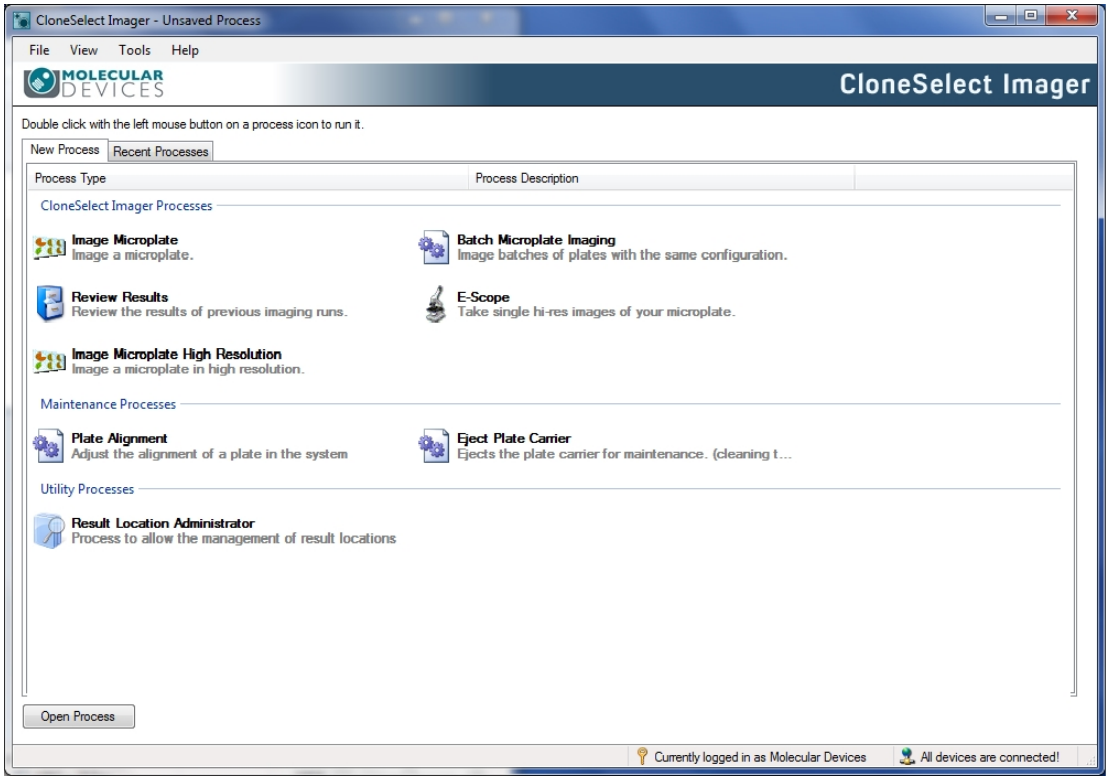


Figure 3-1: CloneSelect Imager Software Navigation Screen

Menu Options

The available 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 previously saved process.
- **Save Process** saves the current process.
- **Save Process As** allows you to save the current process with a different name or location.
- **Close Process** closes the current process and returns you to the Process Selection Window.
- **Save As Template** saves the current process as a template that can be used for creating new processes.
- **Recent Processes** allows you to select and open a recent process.
- **Switch User** can be used in a multiple user environment to log off the current user so that a different user can log on.
- **Exit** closes the Navigation window and shuts down the software.

View Menu

- **Properties** displays the properties of the process that is being set before starting the process.
- **Progress** displays the progress of a running process.
- **Administrate Properties** lets you change the Properties view and default values.

Tools Menu

- **Configuration** displays the **Edit Configuration** dialog.

  CAUTION! Molecular Devices recommends that only trained personnel configure these settings.

- **Prepare Error Report** starts the **Error Reporting Wizard** to create a data file containing the configuration and recent log files to help troubleshoot your problem.

Help

- **About** displays the version numbers of the software modules.
- **Online Support** displays the support web page. This option requires an active internet connection.
- **Help View** displays the online help file.

Navigation Screen

The Navigation screen is the CloneSelect Imager Software start-screen from which you select to run processes. Using the right-click option menu, you can view the process options as icons (Figure 3-2) or as a text list (Figure 3-3).

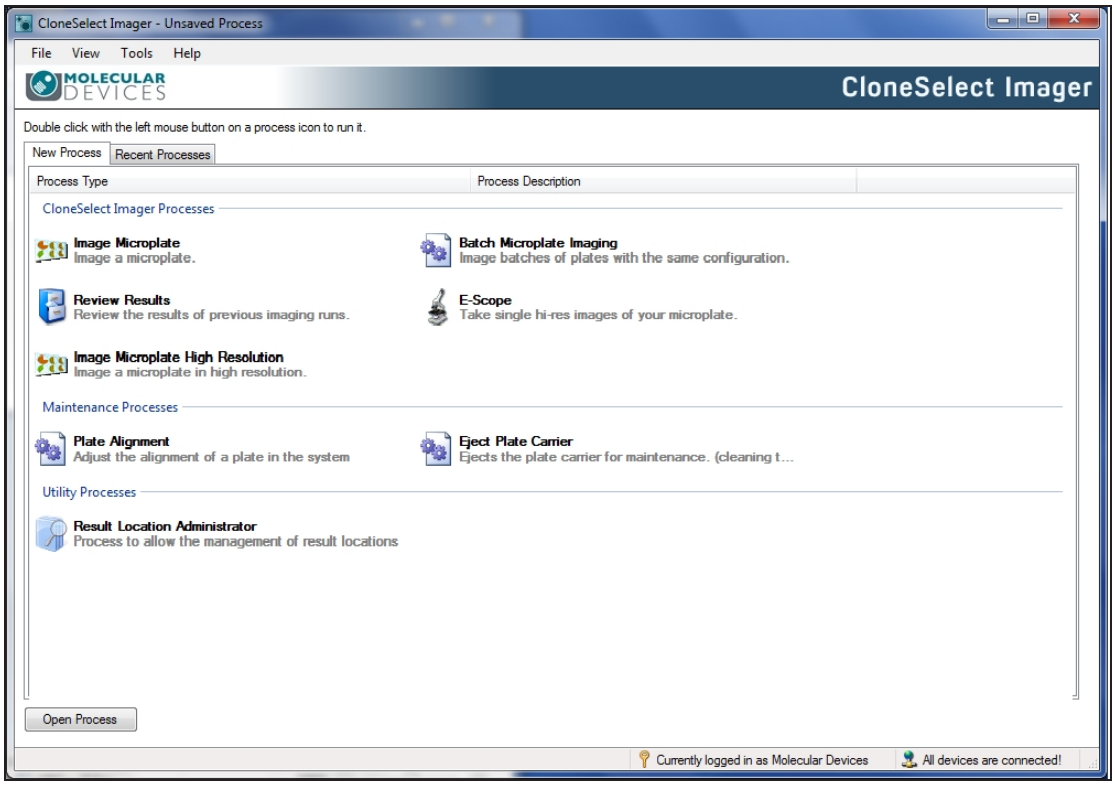


Figure 3-2: The Navigation screen with process options as icon view

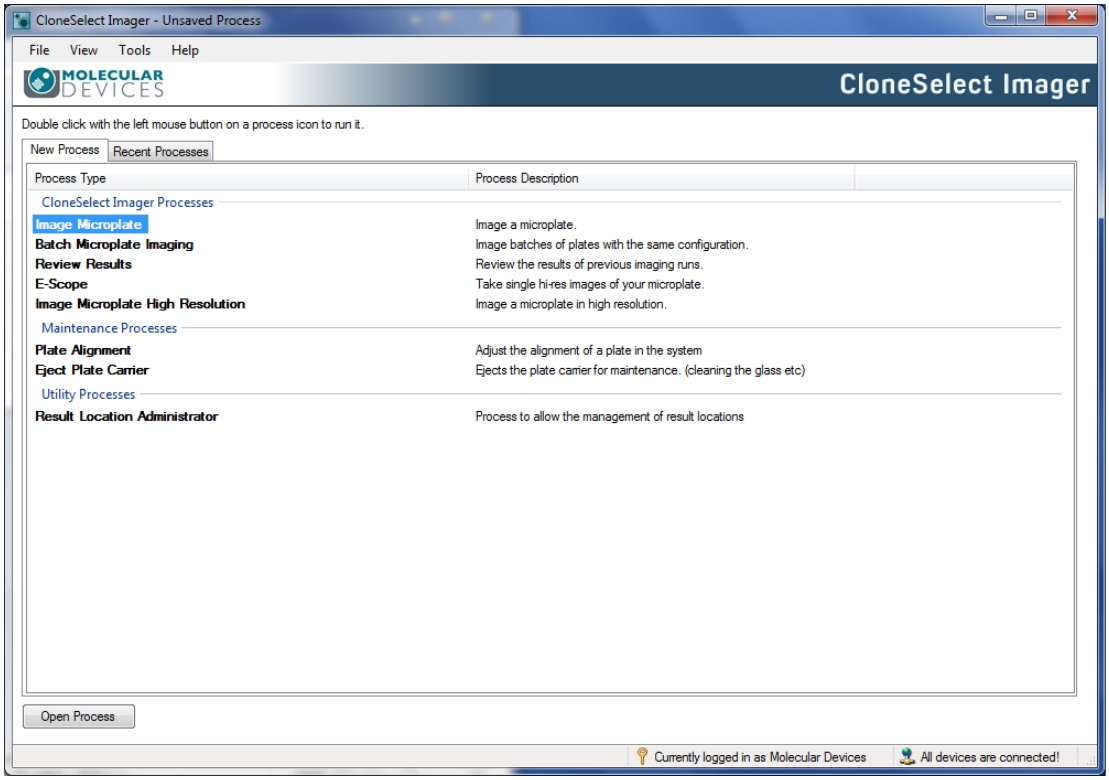


Figure 3-3: The Navigation screen with process options as text list view

The Navigation screen includes the following functionality:

- **Open Process** button displays the **Open** dialog where you can select and open a saved process file. Can also be opened using the **File > Open Process** menu option.
- **Connection Status** appears in the bottom right corner of the Navigation screen and displays the status of the Ethernet connection between the computer and the CloneSelect Imager.
- **New Process Tab** displays the available processes, which are grouped into the following categories:
  - **CloneSelect Imager Processes**
  - **Maintenance Processes**
  - **Utility Processes**
  - (Optional) **Automation Process**—Available only if optionally purchased to use to integrate the CloneSelect Imager with other devices, such as automated incubators or robotic arms.

  See New Process Options on page 15.
- **Recent Processes Tab** displays a list of recently opened processes. This is the same list of processes available from the **File > Recent Processes** menu option.

New Process Options

The **New Process** tab of the Navigation screen gives you a full set of options for creating and managing processes and system maintenance.

CloneSelect Imager Processes

CloneSelect Imager Processes include the following:

- **Image Microplate** does standard imaging of microplates, saving the images and results for later review.
- **Batch Microplate Imaging** does imaging of batches of plates with the same configuration without reviewing the focus and alignment between plates.
- **Review Results** displays results from previous runs including confluence, cell number estimation, and monoclonality analysis for reviewing.
- **E-Scope** does single high-resolution image of microplates. Images are not saved.
- **Image Microplate High Resolution** does high-resolution imaging of microplates, saving the images and results for later review.

Maintenance Processes

Maintenance Processes include the following:

- **Plate Alignment** runs the procedures for calibrating the instrument.
- **Eject Plate Carrier** ejects the plate carrier for cleaning and removal of microplates.

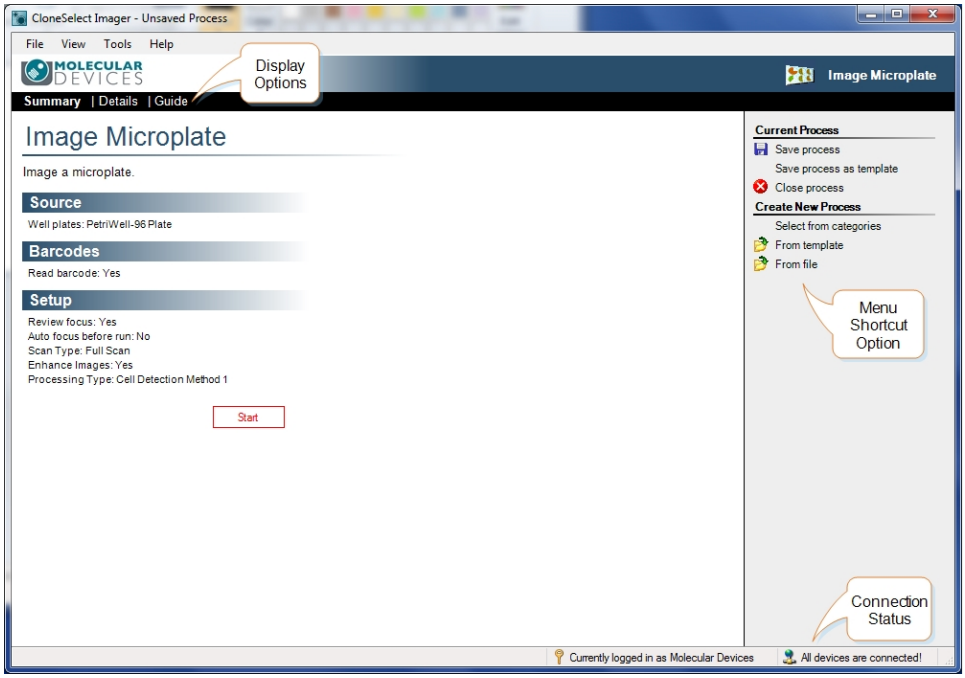
Utility Processes

Utility Processes include the following:

- **Result Location Administrator** lets you manage where the results get located.

Process Properties Screen

The process starts and ends on this **Summary** screen. Process properties can be edited using either of the three display options.



Display Options

There are three options for selecting the way in which the process settings can appear for editing:

- **Summary** is the default display option. The current settings for the process appear grouped in categories. Click on the category heading to open a screen where the relevant settings can be changed using drop-down menus.
- **Details** offers the current process settings displayed in drop-down menus from which they can be edited directly.
- **Guide** steps you through the setup process like a wizard with **Back** and **Next** buttons to scroll through the settings. When all the settings are done, you return to the **Summary** view.

Shortcuts

On the right side of the Process Properties screen there are shortcuts to frequently used menu options.

Connection Status

The status of the Ethernet connection between the computer and the CloneSelect Imager appears in the bottom right corner of the Process screen.

Chapter 4: Running Processes

This section explains in detail how to run the following processes on the CloneSelect Imager.

Image Microplate

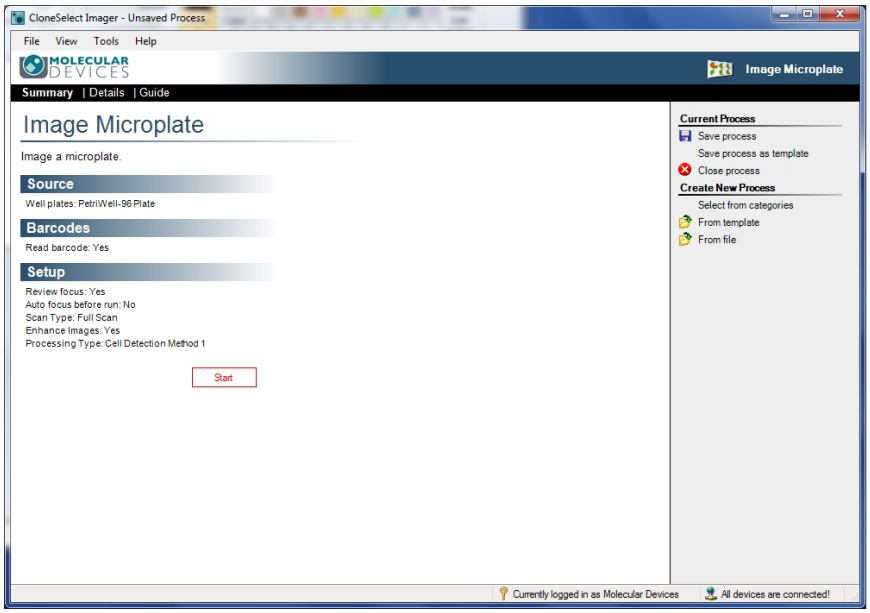
Image Microplate is the default process for imaging cells in microplates to determine their confluence and carry out further analysis, such as cell number estimation and loci counting. Image resolution in this process is 3.5 µm.

To enter an Image Microplate process, in the Navigation screen, click **Image Microplate**. See Navigation Screen on page 13. Alternatively, you can open a previously saved Image Microplate-based process from the **Recent Processes** tab.

To set the properties for the Image Microplate process, the process screen opens in the Process Properties screen in the **Summary** view. See Process Properties Screen on page 16. Change the screen view, as needed, and change properties as needed.

Alternatively, to create your new process, you can use the shortcuts menu on the right side of the screen to do any of the following **Create New Process** start tasks:

- **From template**—Starts the new process from a template file.
- **From file**—Starts the new process from a previously saved file.



The Image Microplate process involves the following tasks:

1. Set Properties, see page 18
2. Select Source, see page 20
3. Load Plate, see page 21
4. Focus, see page 22
5. Alignment, see page 24
6. Imaging, see page 25
7. Results, see page 28
8. Remove Plate, see page 31
9. Finish, see page 32

Set Properties

Set the properties for the Image Microplate process you want to run.

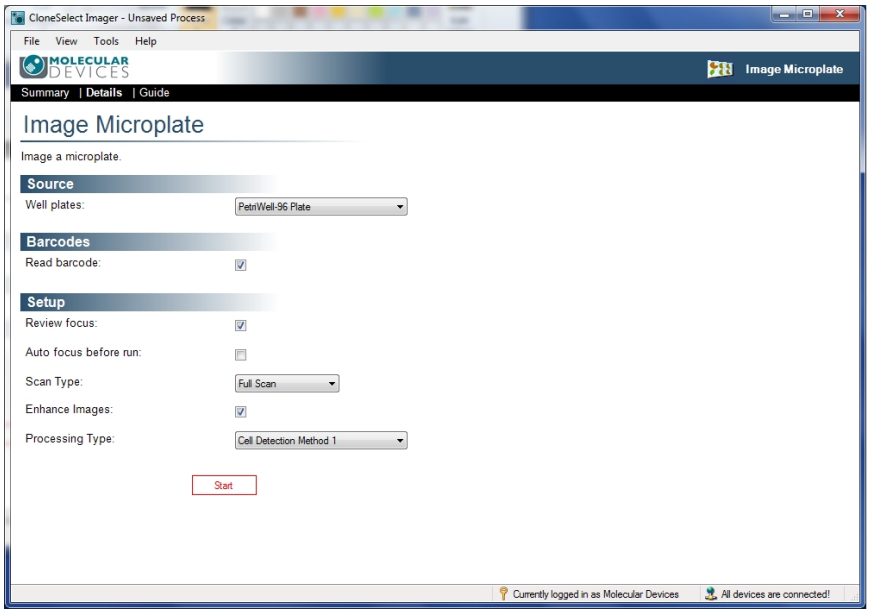


Figure 4-1: Process properties Details view

To set process properties, do the following:

1. In the **Source** section, for **Well plates**, select your microplate from the list menu.

> **Note:** To image a plate type that does not appear in the list, contact Molecular Devices. See Obtaining Support on page 6.

2. In the **Barcodes** section, if you are using barcodes, click the **Read barcode** check box.

   Tip: If barcode reading is enabled and the instrument fails to read the barcode, a dialog appears to let you manually enter the barcode. Also, if needed, you can edit the barcode or name within **Review Results**. See Review Results on page 33.

3. In the **Setup** section, specify the following:

   - **Review focus**—When enabled, before imaging a microplate, allows you to check the focus and brightness settings. Opens the **Focus** dialog. See Focus on page 22.

     Tip: You can also save individual images from the **Focus** dialog.

   - **Auto focus before run**—When enabled, before imaging the microplate, an auto-focus procedure runs on the instrument.
   - **Scan type**—Select one of the following scan methods from the list menu:
     - **Full Scan**—Images the entire area of each well in the microplate.
     - **Partial Scan**—Images an area from the center of each well.

       Tip: This speeds up imaging. Suitable when the data from the center of each well is representative of the whole well.

   - **Enhance Images**—When enabled, to enhance images for display, the background is flattened.
   - **Processing Type**—Select one of the following cell and colony detection processing types from the list menu:
     - **Cell Detection Method 1**—Formally know as *Typical Adherent Cell Confluence*, this algorithm is ideal for settled suspension cells and adherent cells with good contrast.
     - **Cell Detection Method 2**—This algorithm is ideal for detecting cell samples when contrast is low, cells are clumped, or cells are at the edge of the well.
     - **Cell Detection Method 3**—This algorithm is ideal for detecting colonies and distinguishing them from any artifacts around the edges of the colonies.
     - **Very Low Contrast**

4. After you set the process properties, to enter the process, click **Start**. See Select Source on page 20.

Select Source

The first step of the Image Microplate process is called **Select Source**. The sources you select are microplate wells. Wells selected for imaging are marked red, and those not selected for imaging are marked light blue.

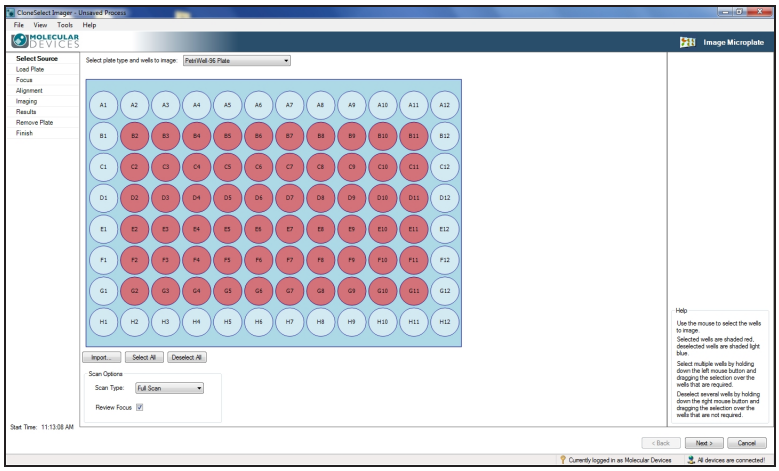


Figure 4-2: Select Source screen for selecting wells

Figure 4-3: The Select Wells screen

To select source wells:

1. If you want to change the plate type, select your new microplate type from the list menu.
2. Select wells. By default, all of the wells are selected.
   - To select or deselect individual wells, left click on a well.
   - To select or deselect groups of wells, right-click and drag over the wells.
   - To deselect all wells, click **Deselect All**.
   - To select all wells, click **Select All**.
   - To select wells using a well list file, click **Import**, and then select your file containing your list of wells to image.

     Tip: For the format of the file, see Well List File Format on page 87.
3. If you want to change the **Scan Options**:
   - In the **Scan Type** list menu, change your selection using the list menu.
   - If needed, change the **Review Focus** check box setting.

4. Do one of the following to continue:
   - To go to the next step, click **Next**. See Load Plate on page 21.
   - To exit the process and return to the Image Microplate process properties screen, click **Cancel**. See Set Properties on page 18.

Load Plate

The plate carrier automatically ejects and you are prompted to load the microplate.

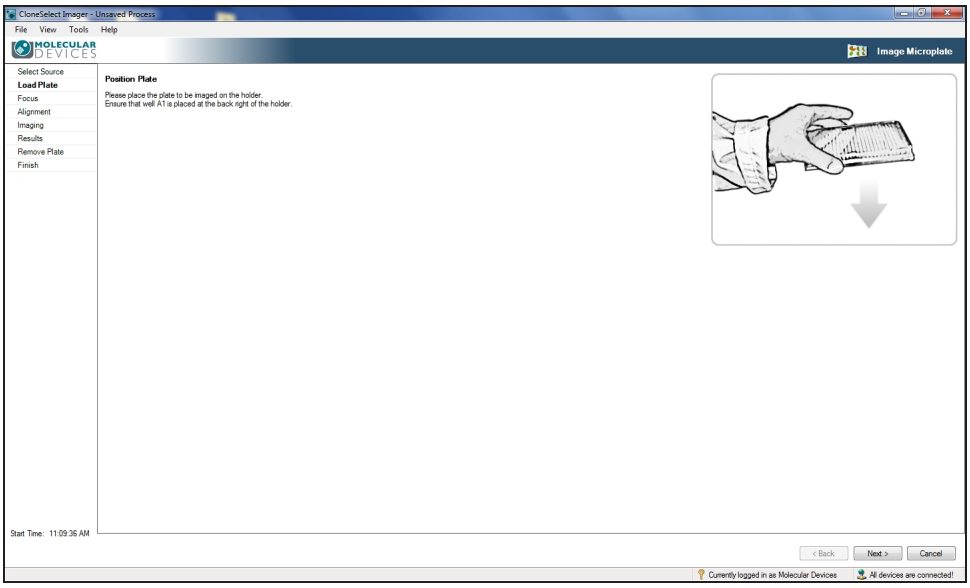


Figure 4-4: Load Plate screen

To load a microplate:

1. When the plate carrier ejects, place your microplate on the carrier with the A1 well in the back right corner.
2. Do one of the following to continue:
   - To go to the next step, click **Next**. See Focus on page 22.
   - To exit the process and return to the Image Microplate process properties screen, click **Cancel**. See Set Properties on page 18.

Focus

Use the **Focus** step to optimize the image view before the scan.

On this screen, you can do the following:

- Adjust Image Brightness on page 22
- Adjust Image Focus on page 23
- Save Image on page 23
- Change Processing Type on page 24
- Generate Confluence on page 24
- Continue to the Next Step on page 24

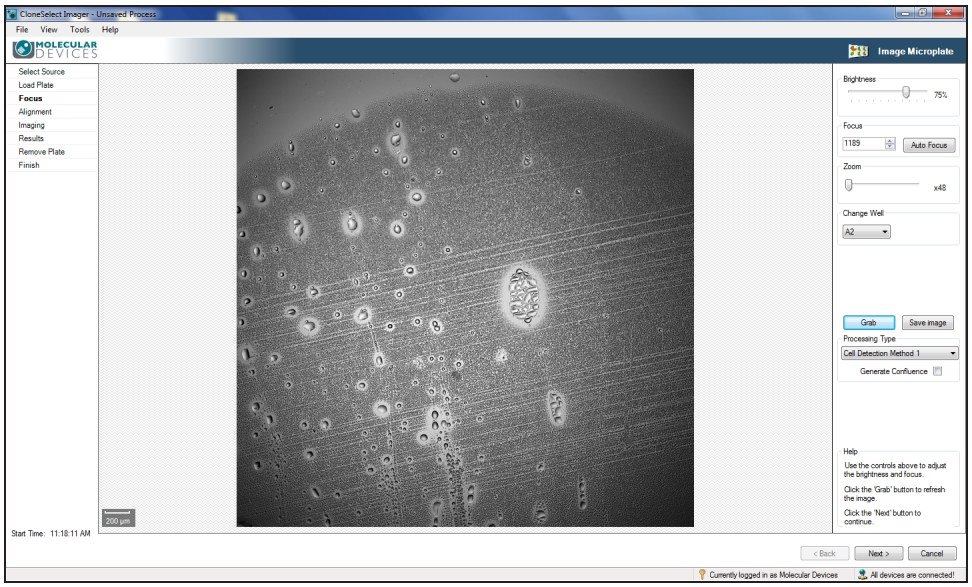


Figure 4-5: Focus screen

Adjust Image Brightness

The software automatically determines the optimal brightness setting for imaging, but you can manually adjust the **Brightness** setting.

To adjust image brightness:

1. Move the **Brightness** slider to the percentage value you want to use.
2. To update the image view using your new setting, click **Grab**.

   Tip: Saturated pixels appear in red. Avoid making your image too bright.

3. Repeat steps 1 and 2 as needed.

Adjust Image Focus

The system stores the last focus setting used for each plate type. After imaging a plate type for the first time, the need for additional focus adjustments should be minimal.

Auto Focus

To adjust image focus:

1. Click **Auto Focus**.
2. To update the image view using your new focus setting, click **Grab**.

   The changed focus setting becomes the default focus setting for the plate type in this process run.

Tip: 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, which requires manually focusing.

Manual Focus

To manually focus on the image:

1. In the **Focus** section of controls on the right side of the screen, click the up or down arrows to change the focus value, or type a new value in the field.
2. If needed, 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 of the image window automatically adjusts.

   Tip: When the zoom setting figure on the right of the slider is black, the system is zooming optically. When the figure turns red, the system is zooming digitally and you might see some pixelation of the image.

3. If needed, select a different well to focus on from the **Change Well** list menu.

   Tip: By default focusing is done on well A1, or if A1 is not being imaged, focusing is done on the first well of the plate to be imaged.

4. To update the image view using your new focus settings, click **Grab**.
   The changed focus setting becomes the default focus setting for the plate type in this process run.

Save Image

Images can be saved in bmp, jpg or png format.

To save the current image used for focusing:

1. Click **Save Image**.
2. In the **Save File** dialog, type and name and click **Save**.

Change Processing Type

If needed, you can change the Processing Type you set in properties. See Set Properties on page 18.

To change, in the **Processing Type** list menu, select a new cell and colony detection processing type.

Generate Confluence

When enabled, all of the objects within the well get identified and highlighted in green.

To enable, select the **Generate Confluence** check box.

Continue to the Next Step

To go to the next step, click **Next**. See Alignment on page 24.

To exit the process and return to the Image Microplate process properties screen, click **Cancel**. See Set Properties on page 18.

Alignment

Use the **Alignment** screen to check that the camera aligns properly with the wells of the microplate. Four images are taken 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.

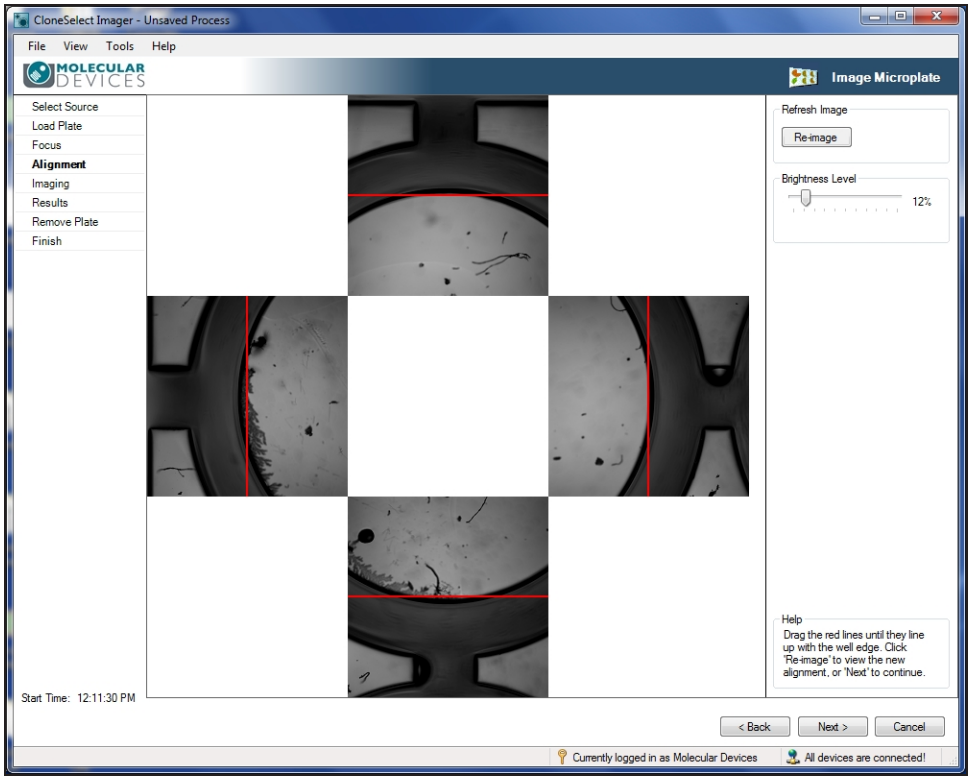


Figure 4-6: Alignment screen

To align the camera:

1. If the well edges do not fall in the middle of the box formed by the four red lines on screen, click and drag on the lines to correct the alignment.
2. If needed, you can also adjust the image brightness by moving the **Brightness** slider to the percentage value you want to use.

   Note: Brightness changes only for the alignment image.

3. When the button is pink, click **Re-image** to recheck the alignment.

Continue to the Next Step

To go to the next step, click **Next**. See Imaging on page 25.

To exit the process and return to the Image Microplate process properties screen, click **Cancel**. See Set Properties on page 18.

Imaging

Imaging starts automatically.

Note: When the **Imaging** process finishes, you automatically go to the **Results** screen step.

The **Imaging** screen step includes the following tabs:

- Confluence Tab on page 26
- View Image Tab on page 27

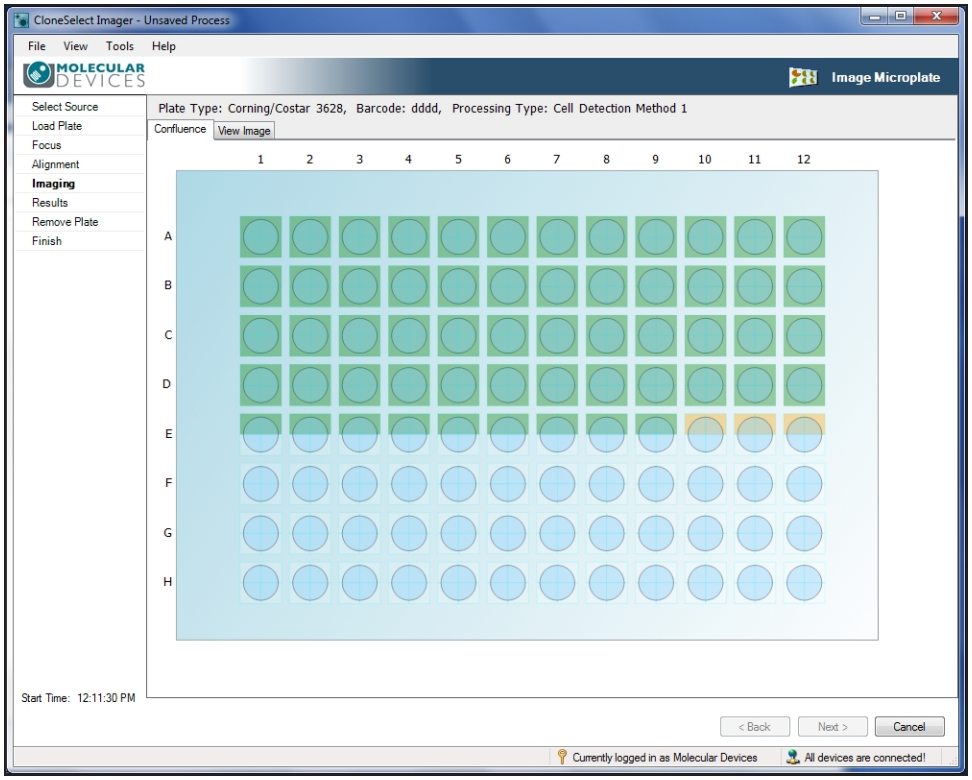


Figure 4-7: Imaging progress screen

Confluence Tab

Imaging runs in the **Confluence** tab and you can watch the process progress in a plate view. A schematic of the plate appears with the image frames superimposed in light blue. As images are captured and processed, the well frame changes color, light brown then to green. After a frame changes to green, you can click on it, and view the image in the **View Image** tab.

View Image Tab

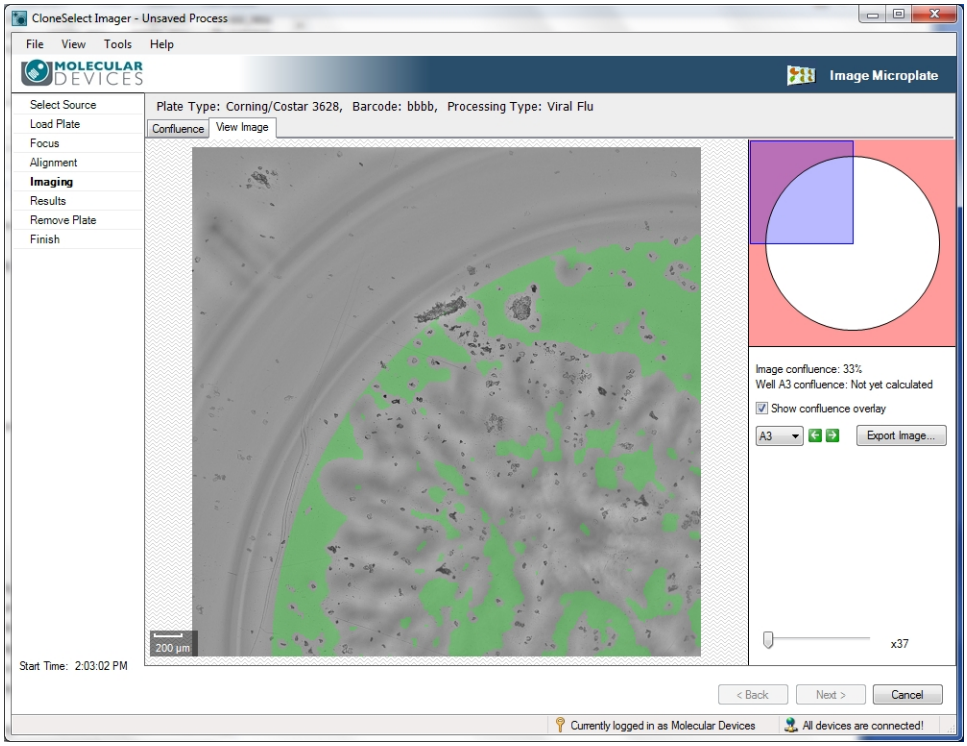


Figure 4-8: View Image screen

To view a frame image:

1. In the **Confluence** tab, after a frame changes to green, click on it.
2. In the **View Image** tab screen (Figure 4-8), you can use the following controls:
   - Well Schematic—Shows you which part of the well you are viewing.
   - Show confluence overlay—Click the check box to toggle off or on the green overlay.
   - Change well view—Click on the well name list menu to select a different well to view.
   - Export Image—Click to save the current image in bmp, jpg or png format. The overlay is not saved with the image.
   - Zoom—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 of the image window automatically adjusts.

     Tip: When the zoom setting figure on the right of the slider is black, the system is zooming optically. When the figure turns red, the system is zooming digitally and you might see some pixelation of the image.
3. To return to the plate view, click the **Confluence** tab.

Continue to the Next Step

When the **Imaging** process finishes, you automatically go to the **Results** screen step. See Results on page 28.

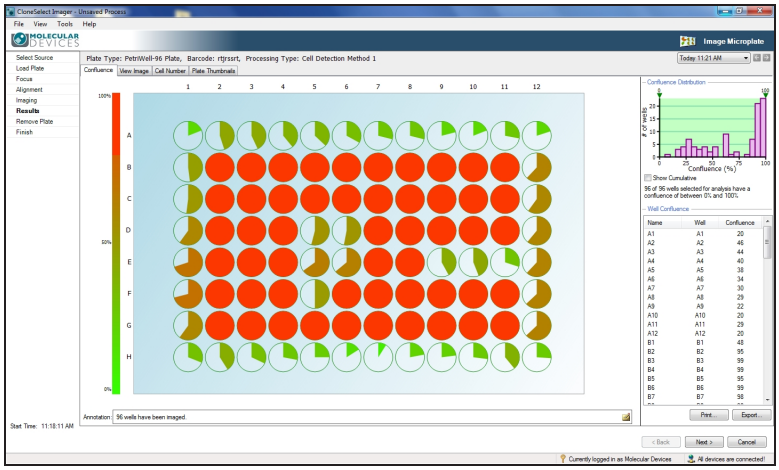
To exit the process and return to the **Image Microplate** process properties screen, click **Cancel**. See Set Properties on page 18.

Results

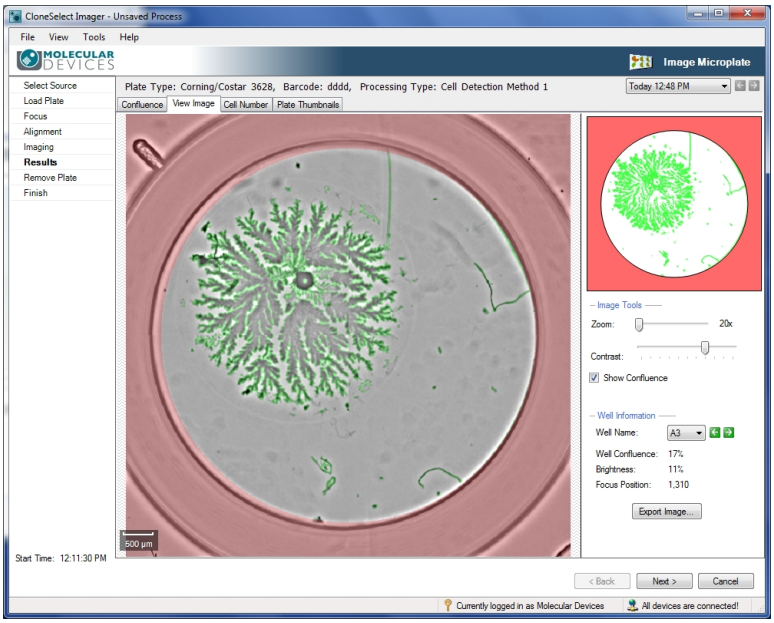
After an **Imaging** process completes, by default the **Results** screen for the **Image Microplate** process opens in the **Confluence** tab. The **Plate Type**, **Barcode** and **Processing Type** appear at the top of the screen for reference.

The **Results** screen for the **Image Microplate** process includes the following tabs:

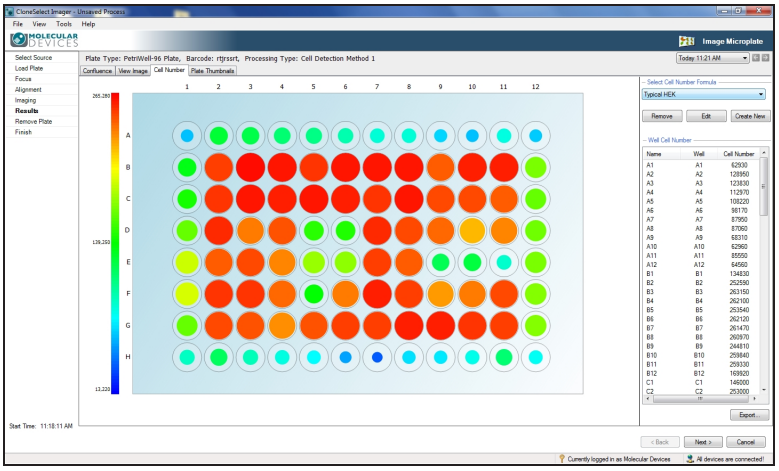
- **Confluence**—Shows the confluence level for each well, both graphically and as a list. Also see Confluence on page 38.



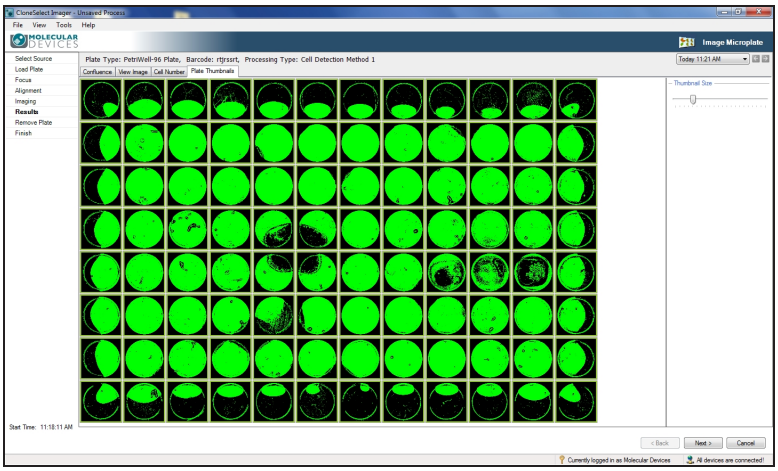
- **View Image**—Shows the image for a selected well. Also see View Image on page 41.



- **Cell Number**—Shows the estimated number of cells in each well, both graphically and as a list. Also see Cell Number on page 42.



- **Plate Thumbnails**—Shows the confluence area for each well. Also see Plate Thumbnails on page 50.



Tip: You can use these tabs and more for reviewing and analyzing the results in the **Review Results** process. See Review Results on page 33.

Continue to the Next Step

To continue to the next step, click **Next**. See Remove Plate on page 31.

To exit the process and return to the Image Microplate process properties screen, click **Cancel**. See Set Properties on page 18.

Remove Plate

In the **Remove Plate** screen step, the plate carrier automatically ejects, and you are prompted to remove the microplate from the plate carrier. You can choose to either repeat the imaging process with another plate, or continue to the end of the process.

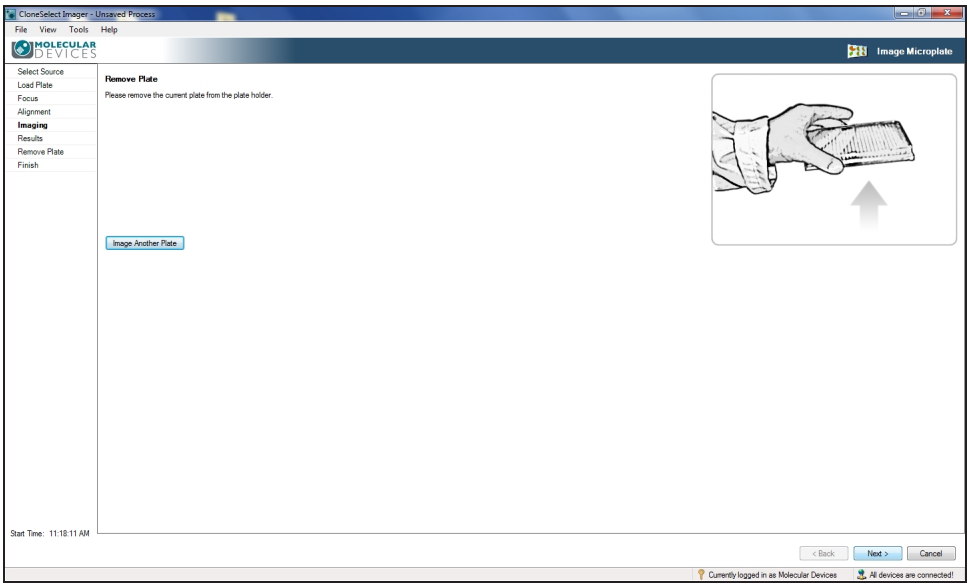


Figure 4-9: Remove Plate prompt screen

Image Another Plate

To image another plate:

- If you want to image another plate, click **Image Another Plate**.

  The plate carrier remains in the ejected position, and the **Select Source** screen appears for you to continue. See Select Source on page 20.

Continue to the Next Step

To continue to the next step, click **Next**. The plate carrier retracts, and the **Finish** screen appears. See Finish on page 32.

To exit the process and return to the Image Microplate process properties screen, click **Cancel**. See Set Properties on page 18.

Finish

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

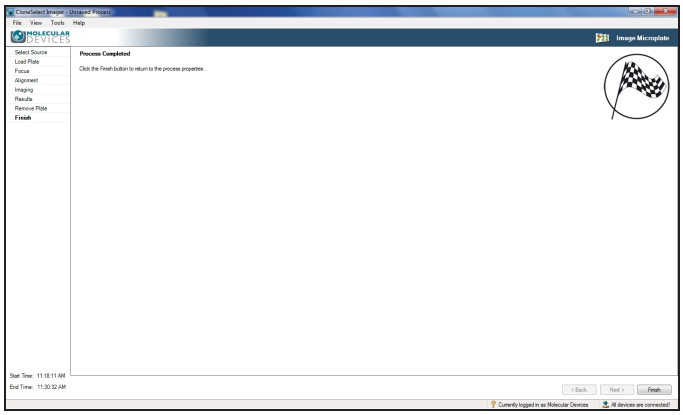


Figure 4-10: Image Microplate process Finish screen

To close the screen, click **Finish**. You return to the **Image Microplate** process **Summary** screen.

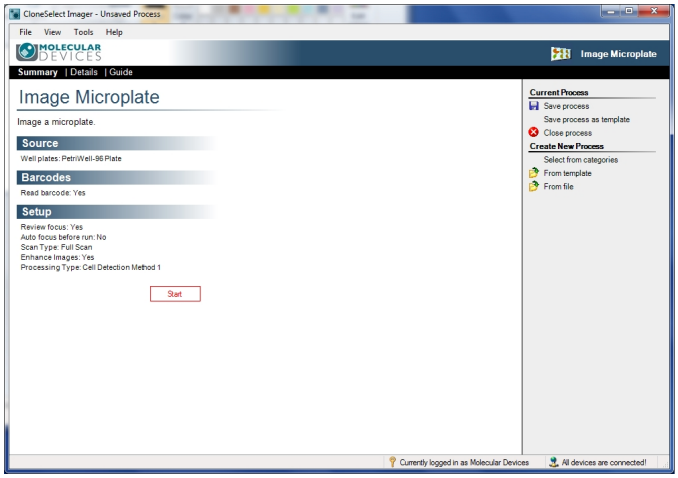


Figure 4-11: Image Microplate process properties Summary screen

In the **Image Microplate** process **Summary** screen, you can use the shortcut menu on the right side of the screen to do any of the following **Current Process** tasks:

- **Save process**—Saves the current process to the **Recent Processes** tab for future access.
- **Save process as template**—Saves the current process to the **Template** tab for future access.

  Note: A saved template cannot be changed.

- **Close Process**—Closes the process and returns you to the **Navigation** screen. See Navigation Screen on page 13.

Review Results

The **Review Results** process lets you review previous imaging runs to be reviewed and also to do further data processing, such as confluence, monitor cell growth, cell number estimation, monoclonality analysis and migration assay analysis.

Tip: After an **Image Microplate** process completes, the results automatically appear in a **Results** process screen. See Results on page 28. That screen includes a subset of the functionality that can be done using the **Review Results** process.

To review results:

1. From the **Navigation** screen, click on **Review Results** to start the process.
2. From the **Review Results** properties screen, click **Start**.

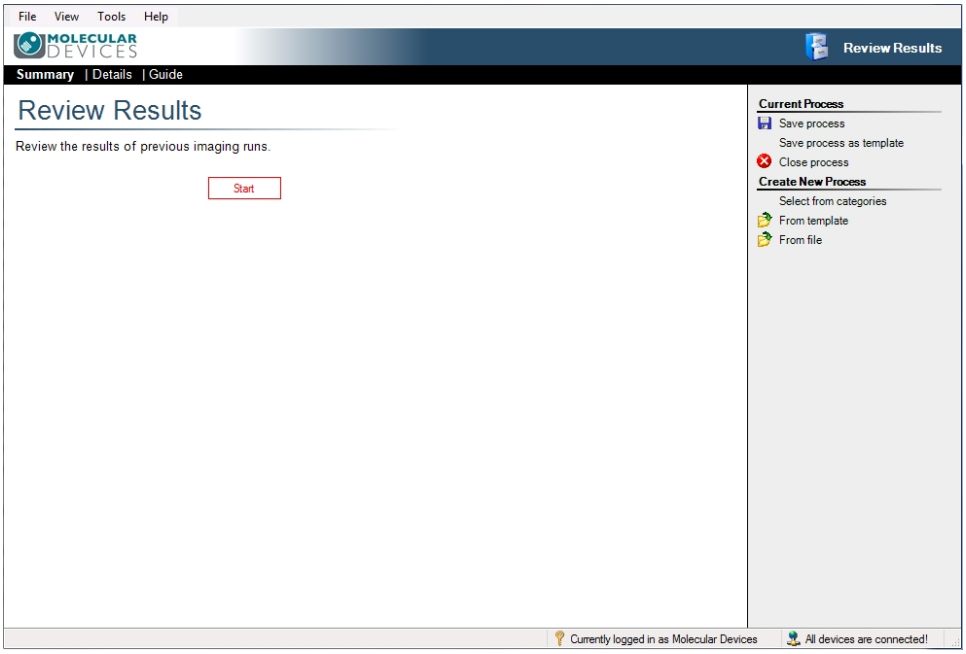


Figure 4-12: Review Results start screen

3. Continue in the **Load Results** screen. See Load Results on page 34.

Load Results

The default location where imaging results are stored is the *C:\Image Archive* folder. All the image and data files for each individual run are stored in a folder named with the date and time stamp for the run, for example `2016-11-01T11-52-10`. Within the *Image Archive* folder, the run folders are automatically organized into separate folders for each month, for example `2016-10`.

The **Review Results** process, starts in the **Results Archive** tab, in the **Runs** list. Plates imaged on the system appear in a list.

Note: If free space on the disk is running low, the bar at the top of the window appears orange and a warning is displayed in the top right corner. If disk space is getting critically low the bar is colored red.

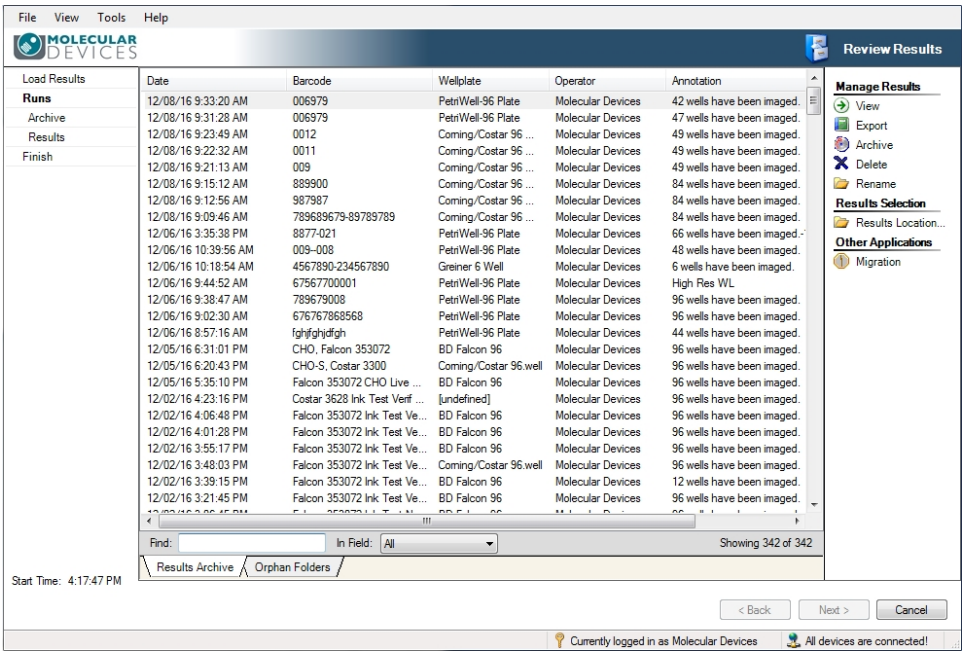


Figure 4-13: Review Results Runs screen

To load results:

- In the **Results Archive** list, double-click on the run date you want to view.
- Alternatively, you can use the following menu options on the right side of the screen:
  - **Manage Results**—See Manage Results on page 35.
  - **Results Selection > Results Location**—When you save results data to a location other than the default *C:\Image Archive* folder location, click to select from that alternate location.
  - **Other Applications > Migration**—(Optional) You must purchase a license for this software module. See Migration Analysis on page 58.

Manage Results

The right side of the **Review Results** process screen includes the **Manage Results** menu. In this menu you can do the following tasks:

- View on page 35
- Export on page 36
- Archive on page 36
- Delete on page 36
- Rename on page 37

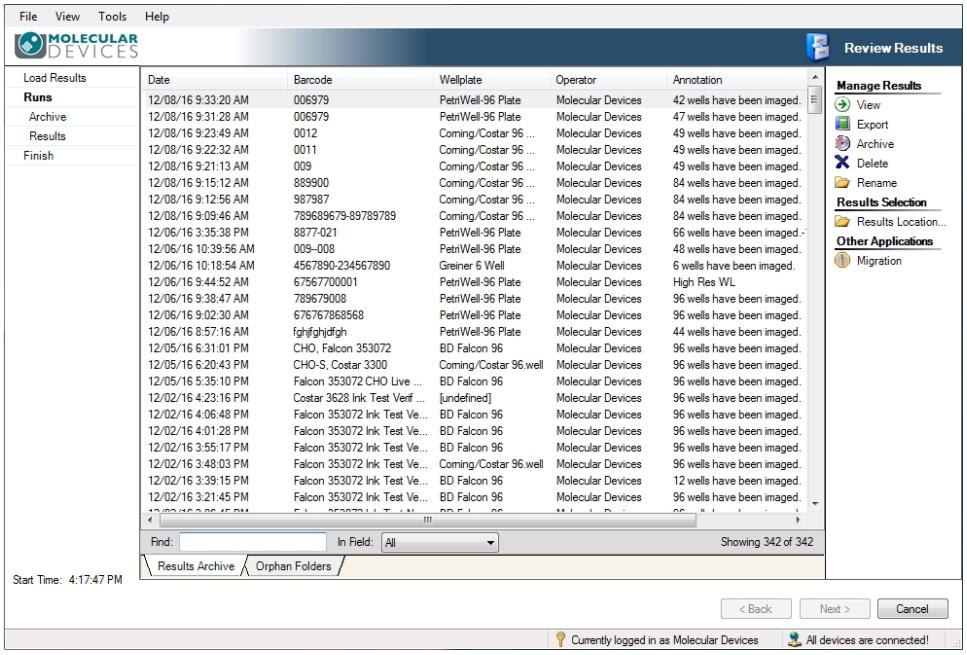


Figure 4-14: Review Results screen

View

This is a another way to view from the **Results Archive** list.

- Select a run date from the **Results Archive** list, and then click **View**.

Export

Opens an export wizard dialog. Using the wizard you can select the results data you want to export.

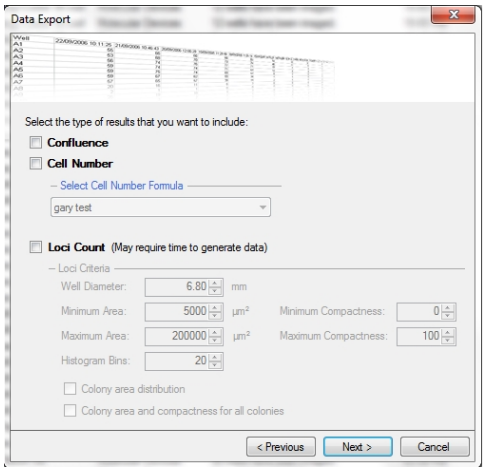


Figure 4-15: Data Export wizard dialog

Archive

Lets you archive to a network drive or disk of your choice the results data stored in the default *C:\Image Archive* folder location.

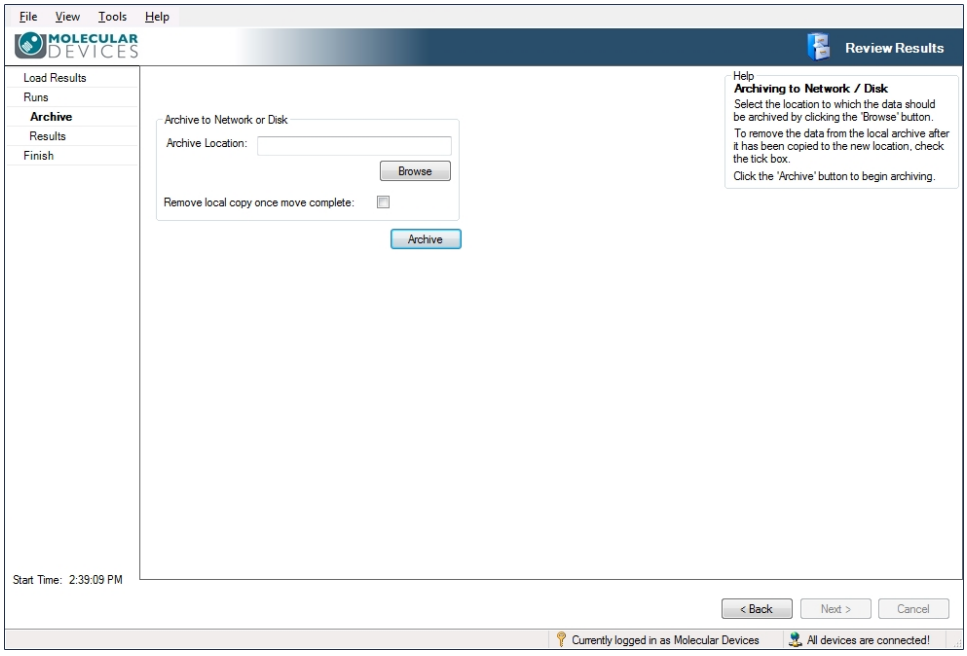


Figure 4-16: Archive screen

Delete

Deletes all the images and results for the selected data set.

Rename

Lets you rename the **Barcode** identification information.

Tip: To do **Growth Rate** analysis, this information must be the same for multiple runs.

To rename a run in the list:

1. Select a run date from the **Results Archive** list, and then click **Rename**.
2. In the **Barcode** field, type new identification information, and then press **ENTER**.

Analyzing Results

When you load results, by default the **Results** screen for the **Review Results** process opens in the **Confluence** tab. The **Plate Type**, **Barcode** and **Processing Type** appear at the top of the screen for reference.

The **Results** screen for the **Review Results** process includes the following tabs:

- Confluence on page 38
- View Image on page 41
- Cell Number on page 42
- Loci Count on page 46
- Plate Thumbnails on page 50
- Growth Rate on page 52
- Summary on page 56

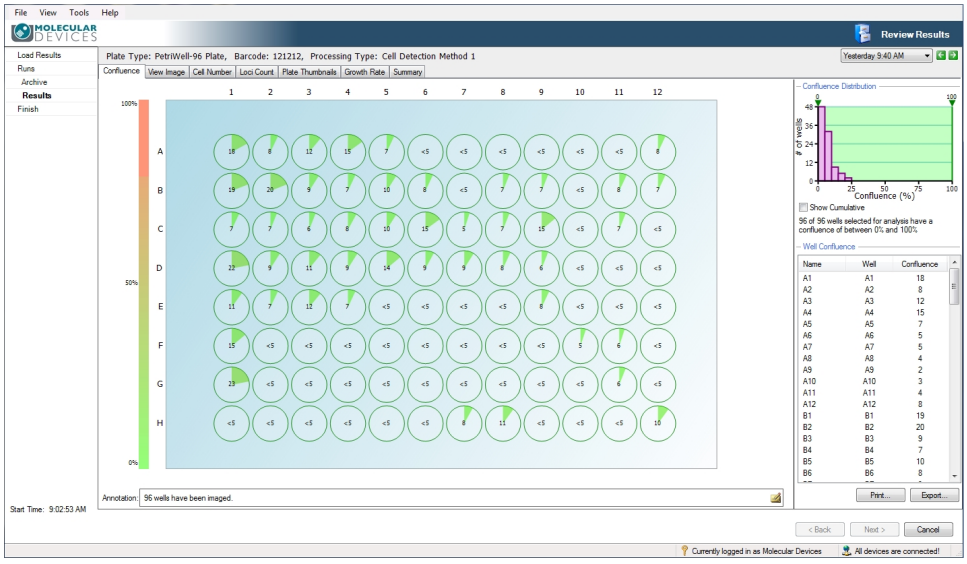


Figure 4-17: Results screen for the Review Results process

Confluence

The **Confluence** tab displays the confluence level for each well, both graphically and as a list.

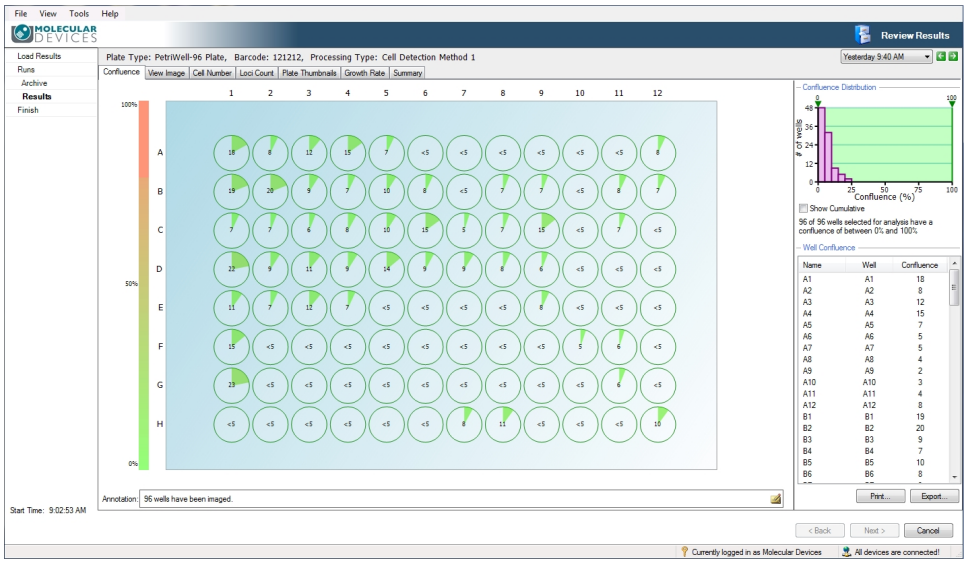


Figure 4-18: Confluence tab screen

Plate Confluence Overview

Each well is represented by a pie chart of the confluence level for that well. The pie charts are color-coded such that low confluence is green and high confluence is red with shades in between representing the intermediate levels.

To see the percentage of confluence for all the wells in the plate, hover the cursor over the pie chart area. Confluence lower than 5% appears as **<5** and confluence above 80% appears as **>80**.

To see coordinates and confluence for an individual well, hover the cursor over a single well.

To see an image of a well in the **View Image** tab, click on a well. See View Image on page 41.

To copy the copy the overview to the clipboard, hover the cursor over a well, right-click, and then click **Copy to clipboard**.

Confluence Distribution

The bar chart at the top right of the window shows the number of wells with a given confluence level.

Dual Gates

The arrows pointing down at the top of the bar chart can be dragged across the chart. As both lower and upper arrows are dragged, the corresponding confluence data appears. Any wells that do not fall inside these lower and upper gates will be grayed out. The text shown below the chart updates to give the number of wells that fall within the selected confluence. For example, 10 out of 36 wells selected for analysis have a confluence of between xx % and xx %.

Show Cumulative

When you select **Show Cumulative**, the individual bars in the chart merge to show the confluence as continuous data.

Well Confluence

The confluence for each well appears in the **Well Confluence** list on the right.

Print

The **Print** option prints a schematic presentation of the confluence overview in the plate format. The confluence values appear within the wells and the plate details appear at the top of the overview.

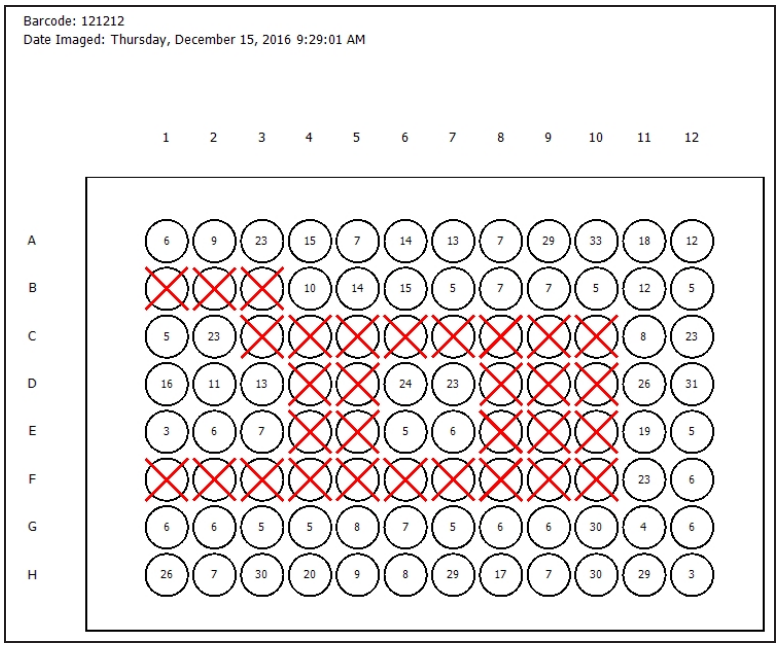


Figure 4-19: Print schematic presentation of the confluence overview

To print the list, click **Print**.

Export

The **Export** option lets you save the well confluence list as a **Comma Separated Values (CSV)**, **Excel**, or **XML** file.

The **Data Export** wizard guides you through the process of exporting the **Confluence** data. **Cell Number** and **Loci Count** data can also be exported.

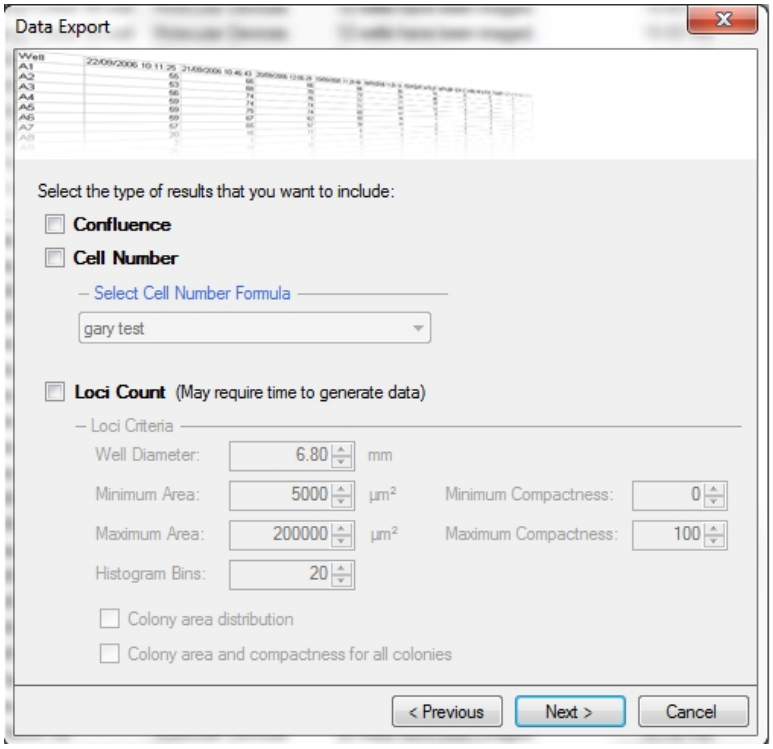


Figure 4-20: Data Export wizard dialog

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.

Annotation

By default the **Annotation** field shows the number of wells that have been imaged. You can edit this to enter some more meaningful annotation.

Note: This cannot be changed when viewing the data through a remote data viewer.

Cancel

If you click **Cancel**, you are prompted to confirm you want to end the process. Clicking **Yes** at the prompt takes you to the **Load Results** screen. See Load Results on page 34.

To finish, see Finish on page 65.

View Image

The **View Image** tab displays the images for a selected well. The whole well is displayed.

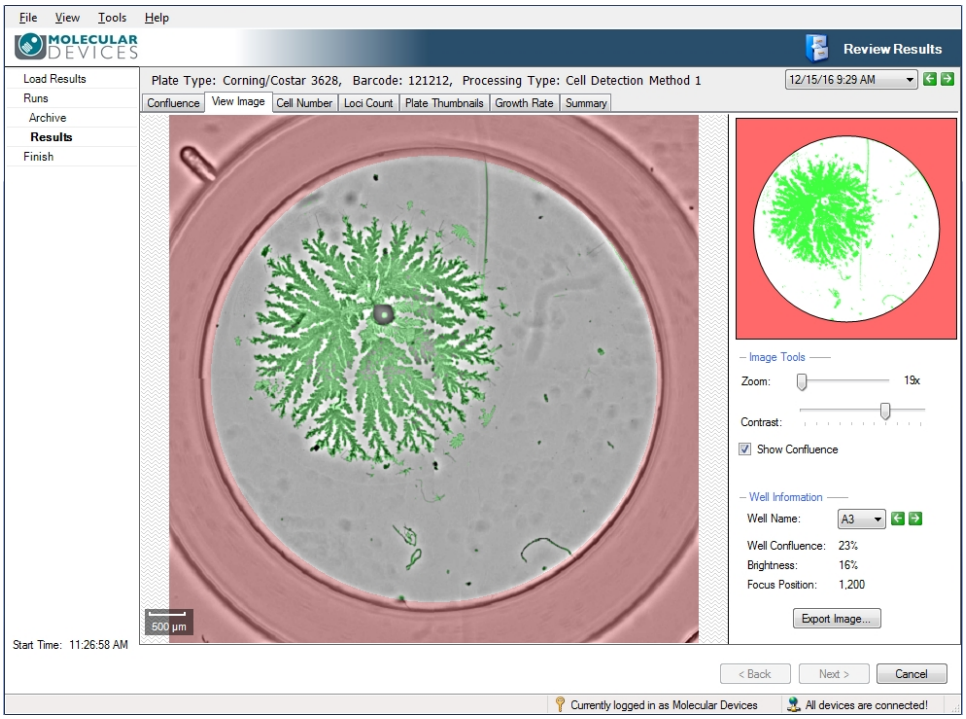


Figure 4-21: View Image tab screen

Well Schematic

By clicking on the image of the well currently being displayed, you can navigate to different areas of the well and see where you are in the well in the image schematic on the right. The confluence levels of the currently selected whole well are displayed below the schematic.

Imaging Tools

The following controls are available in the **Imaging Tools** section:

- **Zoom**—Move the slide either left or right 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 is zooming digitally and can cause some pixelation of the image. When zoomed into an image, the zoomed area will be displayed on the image thumbnail to the right.
- **Contrast**—Move the slider either left or right to improve the image contrast.
- **Show Confluence**—Select to turn the overlay on or off. The area of the image outside the well is overlaid in red and the regions of the well where colony growth is detected are overlaid in green.

Well Information

Well Information includes the following:

- **Well Name**—Use the drop down menu or the arrows to select a different well.
- **Well Confluence**—Shows the well confluence percentage for the selected well.
- **Focus Position**—Shows the focus point of the image when captured.
- **Brightness**—Shows as a percentage the brightness level used to capture the imaged.

Export Image

The **Export Image** option lets you save the currently displayed image in .bmp, .jpg or .png format. If **Show Confluence** is selected, the confluence overlay is displayed with the exported image. The zoomed position is saved within the image.

Cancel

If you click **Cancel**, you are prompted to confirm you want to end the process. Clicking **Yes** at the prompt takes you to the **Load Results** screen. See Load Results on page 34.

To finish, see Finish on page 65.

Cell Number

The **Cell Number** tab displays the estimated number of cells in each well, both graphically and as a list. The number of cells is estimated using a formula that can be created for each of your cell types from the confluence readings of a standard plate containing known numbers of cells. See Setting up Cell Number Estimation on page 89 for a detailed procedure.

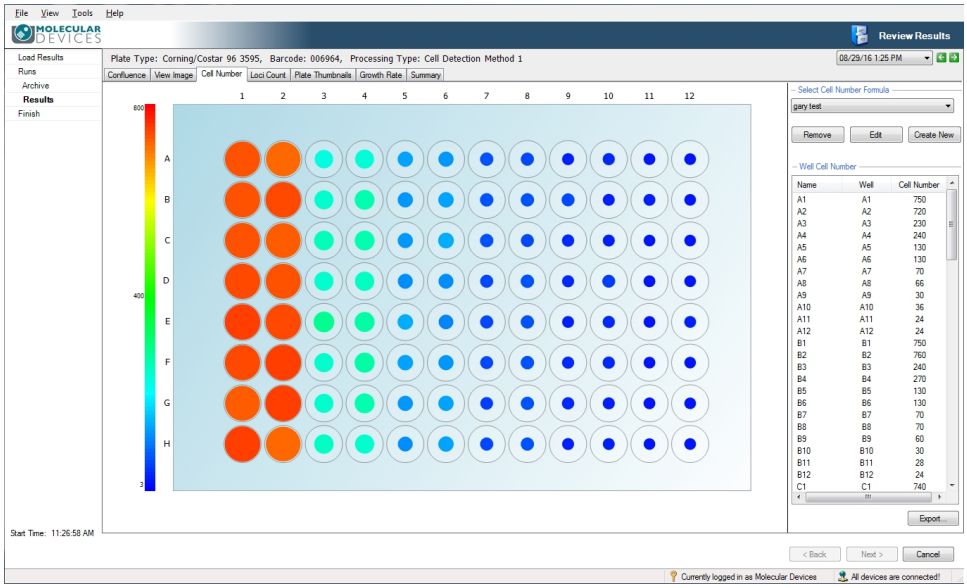


Figure 4-22: The cell number view

Each well contains a colored fill with the estimated cell number indicated by the color and size of the fill area, with a large red fill area representing a high cell number and a small blue fill area representing a low cell number. A color scale is shown to the left of the graphic. Hovering the cursor over a well displays a tool tip giving the well coordinate and the estimated cell number for that well.

Select Cell Number Formula

Use the **Select Cell Number Formula** menu to select from the created formulas for estimating the number of cells per well.

In the **Select Cell Number Formula** section, you can also do the following:

- **Remove**—Click **Remove** to delete the currently selected cell number formula from the menu list.
- **Edit**—Click **Edit** to change the currently selected cell number formula. See Edit on page 43.
- **Create New**—Click **Create New** to create a new cell number estimation formula. See Create New on page 43.

Edit

A graph displays the cell number against confluence for the formula.

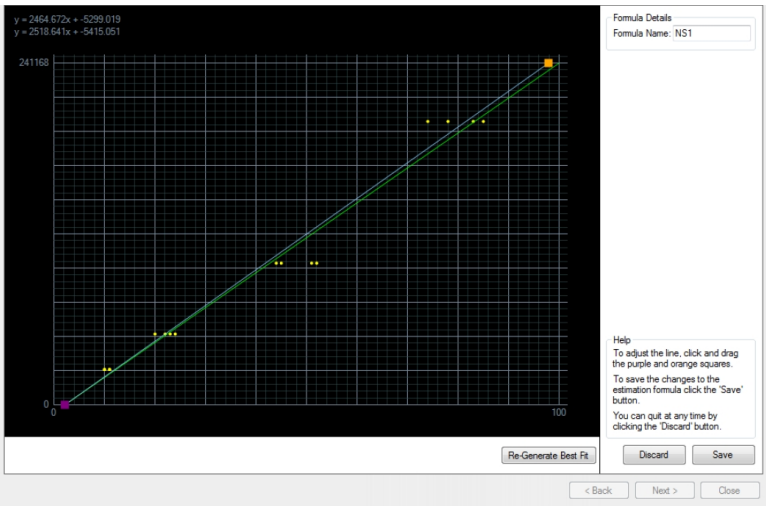


Figure 4-23: Edit Cell Number Formula screen

To edit, drag the handles at each end of the line. The formula is appears at the top left and the edited version appears immediately below it.

To automatically generate a best fitting line, click **Regenerate Best Fit**.

To change the formula name, in the **Formula Name** field, type a new name.

To cancel the edits and return to the **Cell Number** tab, click **Discard**.

To keep the edits and return to the **Cell Number** tab, click **Save**.

Create New

Ensure that the confluence results displayed in the Confluence tab are those for a standard plate containing known numbers of cells. The software automatically selects up to twelve wells with confluence values ranging between 10% and 80% and displays them at the bottom of the graph.

A screen to create a new cell number estimation formula appears. Ensure that the confluence results displayed in the Confluence tab are those for a standard plate containing known numbers of cells. The software automatically selects up to twelve wells with confluence values ranging between 10% and 80% and displays them on the left of the graph.

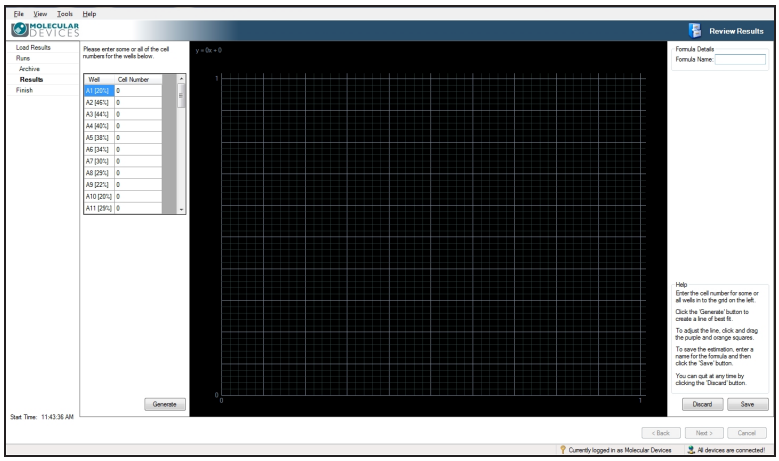


Figure 4-24: Create New Cell Number Formula screen

To name your formula, in the **Formula Name** field, type your formula name.

Enter the cell numbers for each well into the corresponding well number box to the left of the graph. After you enter all the relevant information, click **Generate**.

To cancel the edits and return to the **Cell Number** tab, click **Discard**.

To keep the edits and return to the **Cell Number** tab, click **Save**.

Well Cell Number

A list of estimated cell numbers for each well appears in the **Well Cell Number** list on the right.

To change the well name, in the **Name** column of the list, click the well name, pause, then click the well name again. When the edit field appears, type a new name.

Export

The **Export** option lets you save the well cell number list as a **Comma Separated Values (CSV)**, **Excel**, or **XML** file.

The **Data Export** wizard guides you through the process of exporting the **Cell Number** data. **Confluence** and **Loci Count** data can also be exported.

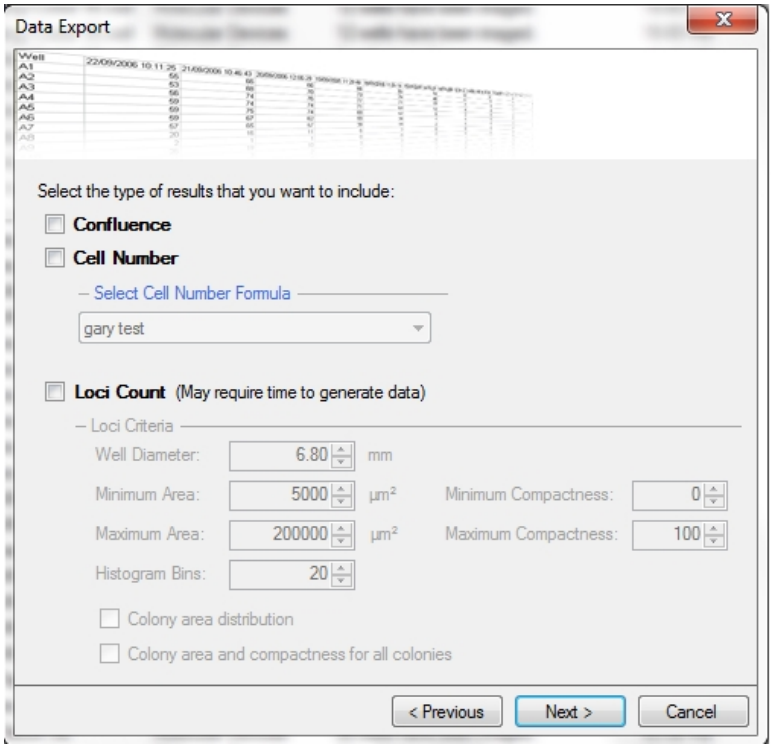


Figure 4-25: Data Export wizard dialog

Cancel

If you click **Cancel**, you are prompted to confirm you want to end the process. Clicking **Yes** at the prompt takes you to the **Load Results** screen. See Load Results on page 34.

To finish, see Finish on page 65.

Loci Count

Note: Loci Count is optional software. Contact your Molecular Devices representative to purchase.

For colony formation assays, use Loci Count to count the number and size (area) of colonies in each well.

Generated images can be processed using **Loci Count**. This detects and counts the number of loci of growth, for example, cell colonies for applications including monoclonality verification and colony forming assays. If a plate is imaged multiple times during colony growth, the history of each well with one identified colony can be viewed for visual proof that the colony is derived from a single cell progenitor.

Plate View

In the **Loci Count** tab, the plate view displays each well with a figure indicating the number of cell colonies found in the well. The default plate view displays the **Loci Count** (in the **Display Statistic** section menu), however this can be changed to **Mean Loci Area**, which displays this value within each of the wells.

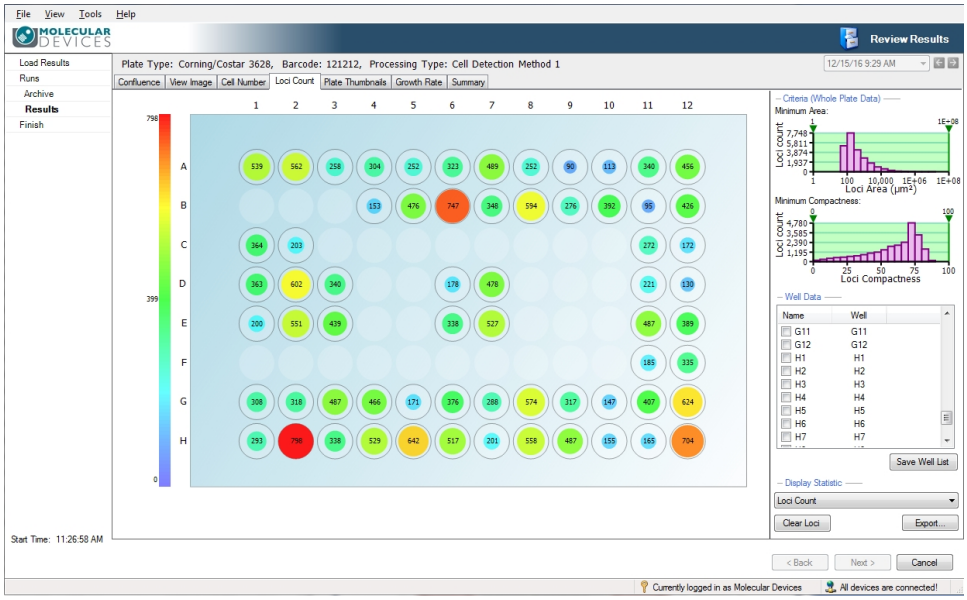


Figure 4-26: Loci Count in Plate View

Criteria

The **Criteria** section includes the following bar charts:

- **Minimum Area**—The loci count (frequency) is plotted against the loci area (μm^2) in a log scale. The bar chart has two, lower and upper, gates that can be moved accordingly to eliminate unwanted objects/debris within the well that artificially inflate the number of loci.

- **Minimum Compactness**—The loci count (frequency) is plotted against the loci compactness. The bar chart has two, lower and upper, gates that can be moved accordingly 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.

Well Data

In the Plate View **Well Data** section, you can click on a well to view the image. You can also select well check boxes to save as a well list.

To save the selected wells in the list, click **Save Well List**. This file can be saved as a .csv file. The file includes the plate barcode, run date, operator and the selected wells.

Display Statistics

The **Display Statistics** menu lets you select to view as the overview either **Loci Count** or **Mean Loci Area**.

To clear all of the current loci data processing, click **Clear Loci**.

Export

The **Export** option lets you save the wells and the corresponding Loci Count list as a **Comma Separated Values (CSV)**, **Excel**, or **XML** file.

The **Data Export** wizard guides you through the process of exporting the **Loci Count** data. **Confluence** and **Cell Number** data can also be exported.

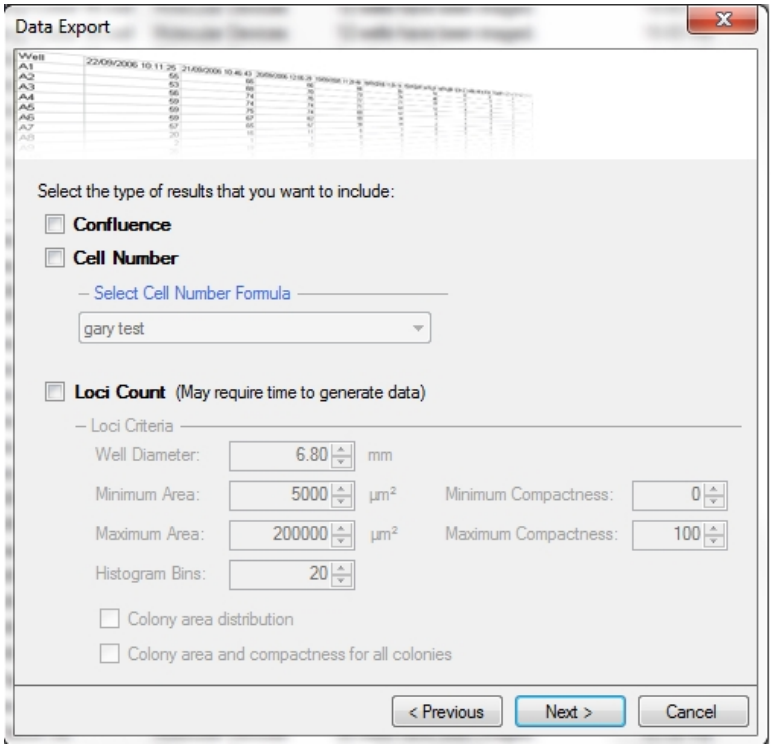


Figure 4-27: Data Export wizard dialog

The **Loci Count** criteria automatically populate from the gated **Minimum Area** and **Minimum Compactness** graphs. You can also export the following:

- **Histogram Bins**—This option is used if the Colony area distribution box is selected. This value sets the number of equi-sized bins for exporting loci frequency counts. The default value is set to 20 bins.
- **Colony area distribution**—When this box is selected, the colony area distribution data is divided into the number of bins specified in the **Histogram Bins** and displayed with any other exported data.

> **Note:** Only wells selected in the **Well Data** list export.

- **Colony area and compactness for all colonies**—When this box is selected, a list of all colonies in all wells with the area and compactness for each colony expiorts.

> **Note:** There is no filtering, so all the data is listed.

Well View

Clicking on the well of interest in the plate view of the **Loci Count** tab displays the image for the well with an overlay over areas of growth detected according to the **Minimum Area** and **Minimum Compactness** criteria.

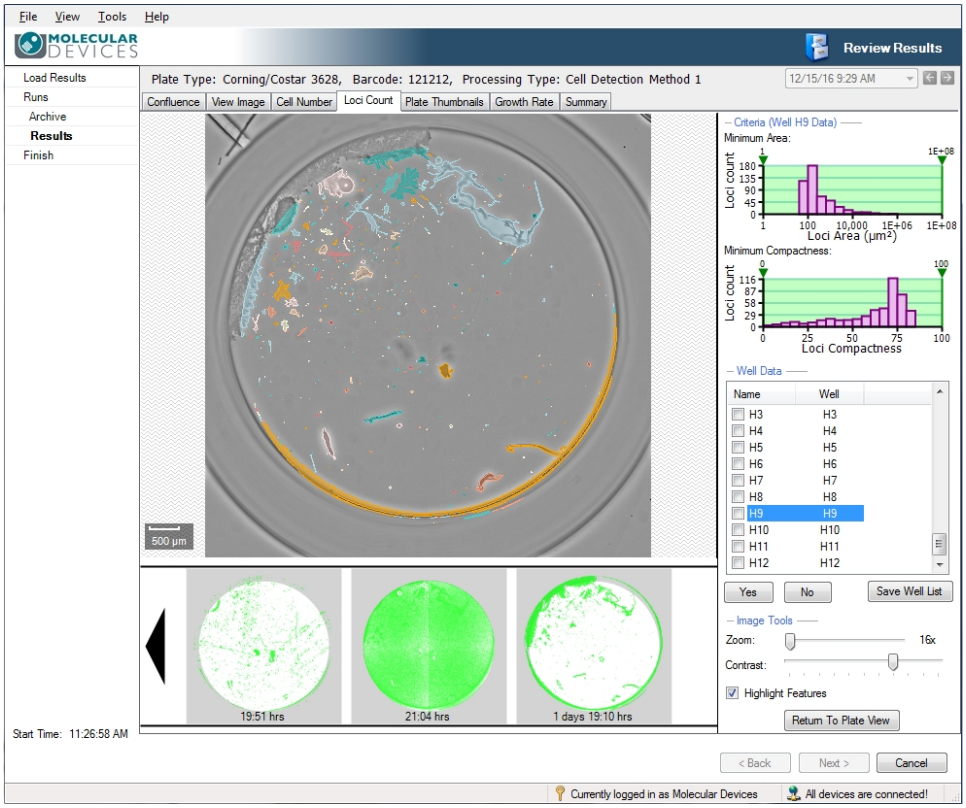


Figure 4-28: The Well View in Loci Number

If the microplate has been imaged at several time points a film strip of schematics of the well at the various time points appears across the bottom of the screen with loci colored in green. This provides a way of checking if a colony results from a single cell or from more than one cell.

To view the well image at that time point with the loci from the latest image outlined, click on any of the schematics thumbnails.

To save the sequence of images, right-click on the sequence, and select **Export image sequence**.The sequence can be saved as a .bmp, .jpg or .png file. The file includes the confluence percentage and time and day the image was acquired.

Well Data

In the Well View **Well Data** section, to see if a colony is monoclonal, click on a well in the list. Move through the list and mark wells as monoclonal in the following ways:

- Click **Yes** button to mark a well as monoclonal and scroll to the next well image. Click No to scroll to the next well without marking the well as monoclonal.
- Click on a well to view its image and click on the check box to mark it as monoclonal.
- Use the keyboard up and down arrow keys to scroll through the wells and the spacebar to mark the wells.

To save the selected wells in the list, click **Save Well List**. This file can be saved as a .csv file. The file includes the plate barcode, run date, operator and the selected wells.

Imaging Tools

The following controls are available in the **Imaging Tools** section:

- **Zoom**—Move the slide either left or right to zoom out or in respectively. The lowest magnification of the image is 18x and the highest is 144x. When the figure turns red the system is zooming digitally and can cause some pixelation of the image. When zoomed into an image, the zoomed area will be displayed on the image thumbnail to the right.
- **Contrast**—Move the slider either left or right to improve the image contrast.
- **Highlight Features**—Select to mark features with different overlay colors.
- **Return To Plate View**—Click to return to the plate view within the Loci Count tab to view other wells. See Plate View on page 46.

Cancel

If you click **Cancel**, you are prompted to confirm you want to end the process. Clicking **Yes** at the prompt takes you to the **Load Results** screen. See Load Results on page 34.

To finish, see Finish on page 65.

Plate Thumbnails

The **Plate Thumbnails** tab shows the entire plate and the wells that have been imaged as thumbnails. The thumbnails include the confluence overlay of each of the imaged wells. Use this screen to select wells to be included or excluded from analysis, which helps to be focus only on wells of interest. Any wells included or excluded in the **Plate Thumbnails** tab automatically get included or excluded in the **Confluence**, **Cell Number**, **Loci Count**, and **Growth Rate** tabs as well.

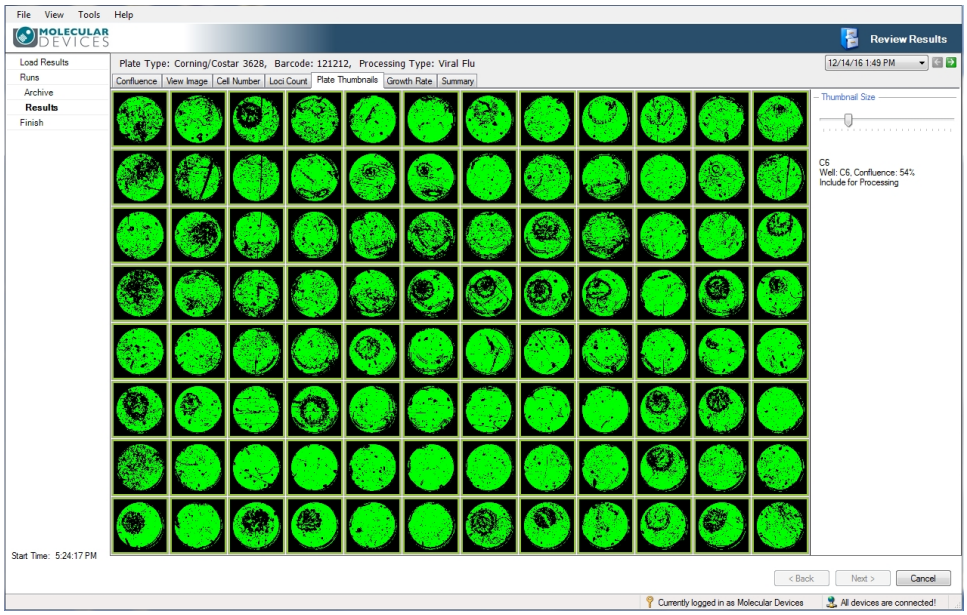


Figure 4-29: Plate Thumbnails tab screen

- A group well selection highlights in yellow.
- Excluded well selections highlight in red.
- Included well selections highlight in green.

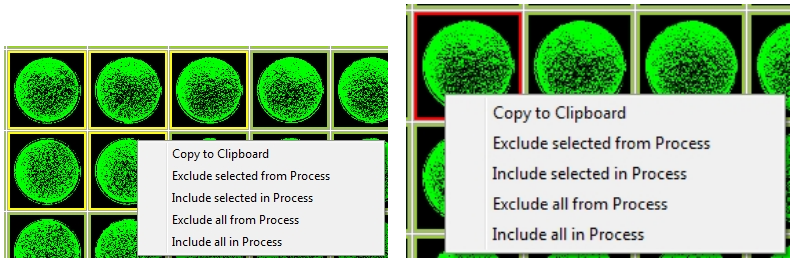


Figure 4-30: Group selected wells and excluded well with selection options menu

To specify what to do with each well for your analysis, 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 the well of interest, and then select one of the following from the options menu:
   - Copy to Clipboard
   - Exclude selected from Process
   - Include selected in Process
   - Exclude all from Process
   - Include all in Process

Cancel

If you click **Cancel**, you are prompted to confirm you want to end the process. Clicking **Yes** at the prompt takes you to the **Load Results** screen. See Load Results on page 34.

To finish, see Finish on page 65.

Growth Rate

If a microplate has been imaged previously, growth curves appear for all the wells in the plate. The system determines if the plate has been imaged by looking for the barcode in the stored results, so use barcoded microplates to enable this function. Named plates can also be used, but for the software to identify the series, the name must be typed exactly the same each time the plate is imaged. The ability to rename plates does risk the inclusion of mis-named plates in the growth curve data.

The captured images for the selected well appear in the well schematic below the graph.

To see the well coordinate for a point, hover the cursor over a data point.

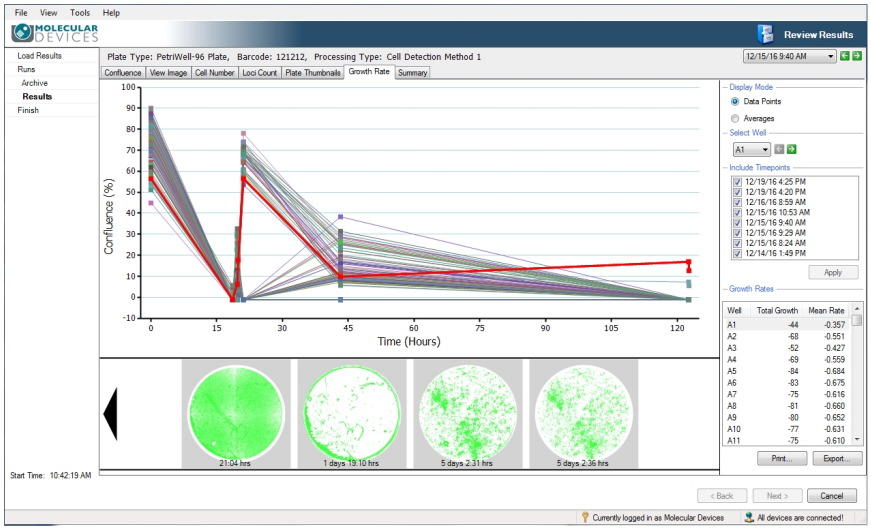


Figure 4-31: Growth Rate tab screen showing Data Points graph

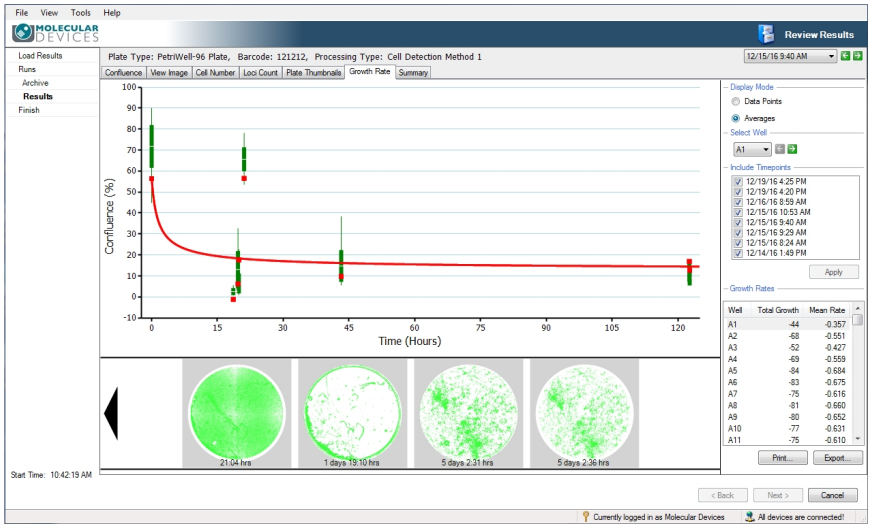


Figure 4-32: Growth Rate tab screen showing Averages graph

Display Mode

You can view the following growth rate graphs:

- **Data Points**—graphs all the well data points
- **Averages**—graphs the averages of the well data points.

The thick red line within both graphs shows the growth rate for the selected well.

Select Well

Use the menu or the arrows to select a different well.

Include TimePoints

Use the **Include Timepoints** list to select which time points get included or excluded from the growth rate output. All plate scans with the same barcode are automatically selected for inclusion in the growth rate data unless you deselect them here.

To change time point selections:

- Adjust the time point selections, and then click **Apply**.

Growth Rates

The **Growth Rates** list displays the **Well**, **Total Growth** and **Mean Rate** for each well in the data set.

Print

The **Print** option prints the growth curve graph.

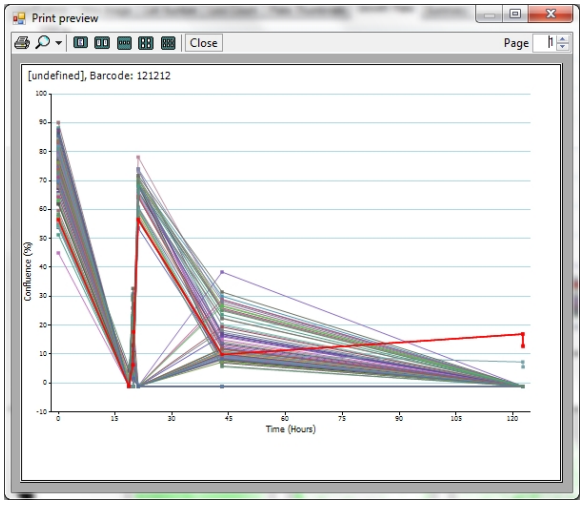


Figure 4-33: Growth Curve Graph Print Preview

Export

The **Export** option lets you save all the data used to construct the growth curve as a **Comma Separated Values (CSV)**, **Excel**, or **XML** file.

The **Data Export** wizard guides you through the process of exporting the **Confluence** data. **Cell Number** and **Loci Count** data can also be exported.

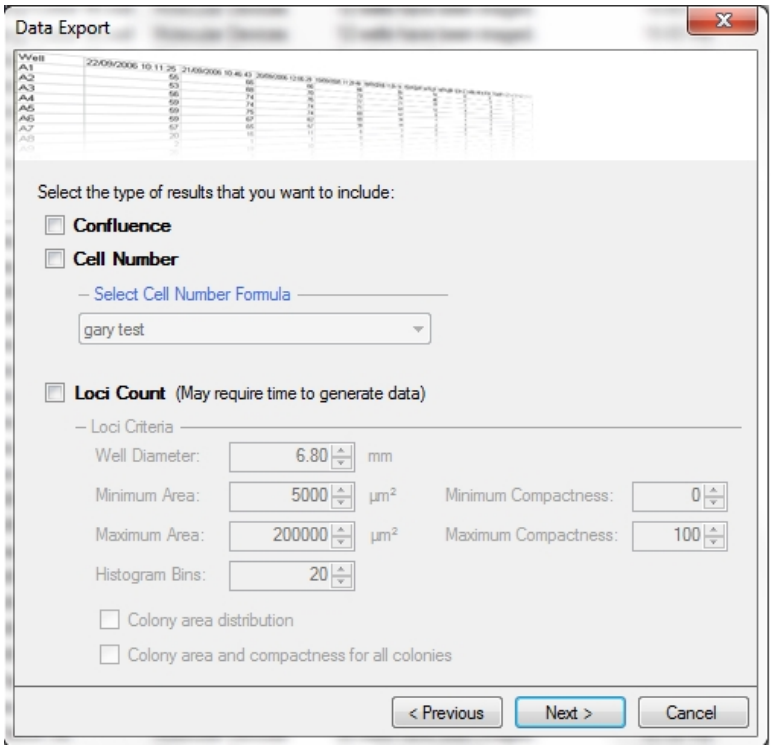


Figure 4-34: Data Export wizard dialog

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.

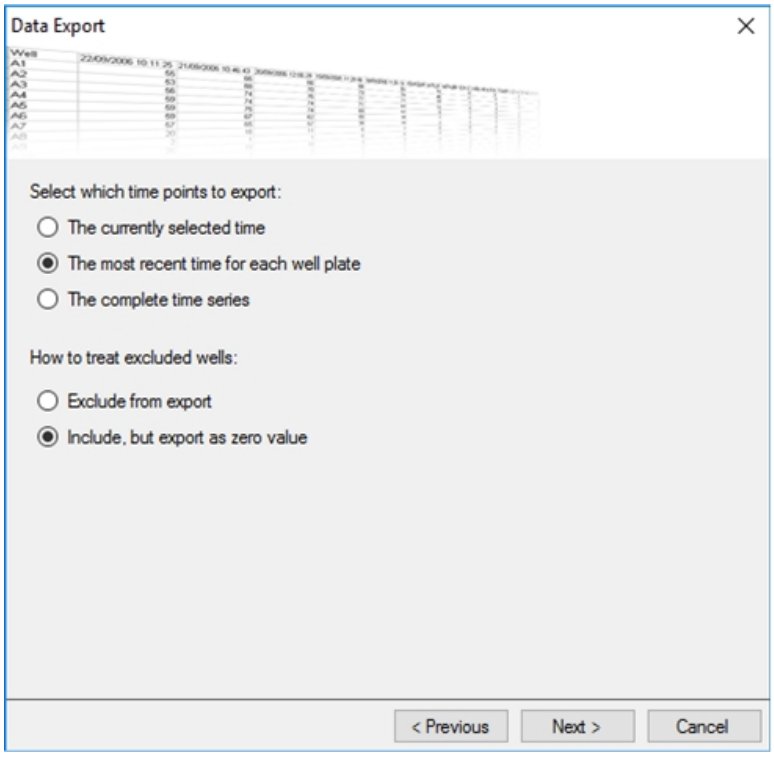


Figure 4-35: Data Export wizard with excluded wells options

When the option **Include, but export as zero** value is selected, 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

Figure 4-36: Example export data with Well A1 and Well A3 excluded

Cancel

If you click **Cancel**, you are prompted to confirm you want to end the process. Clicking **Yes** at the prompt takes you to the **Load Results** screen. See Load Results on page 34.

To finish, see Finish on page 65.

Summary

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

The table lists the **Date Imaged**, **Elapsed Time**, **Operator**, **Imaged on**, **Min Confluence**, **Mean Confluence** and **Max Confluence** for each time the plate was imaged.

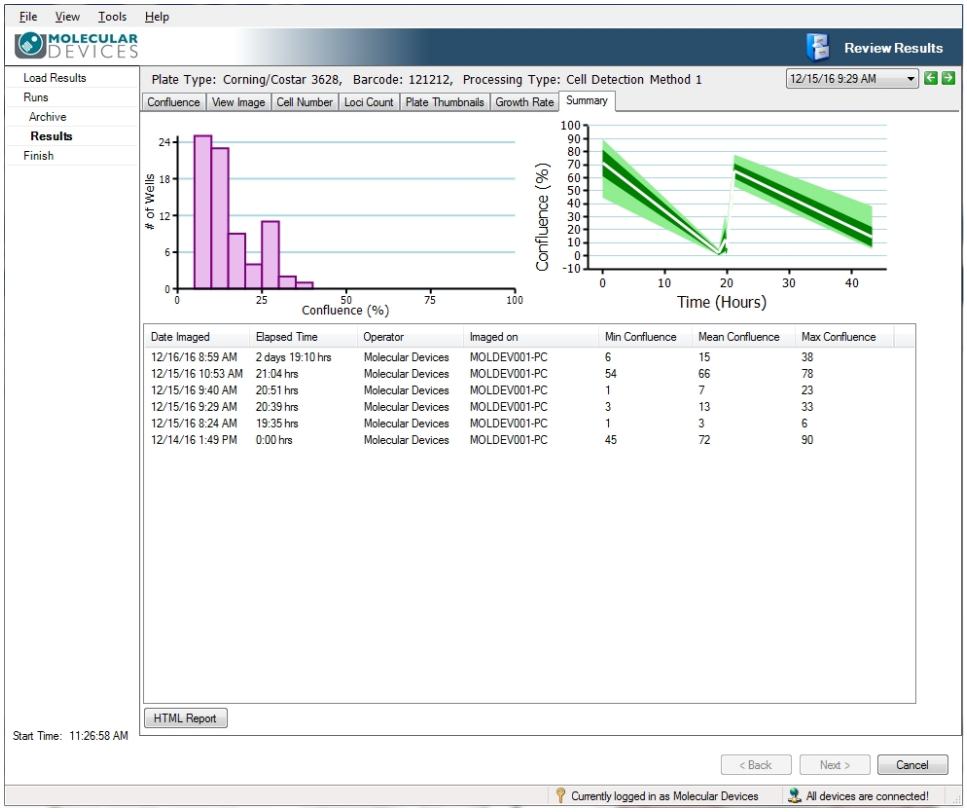


Figure 4-37: Summary tab screen

HTML Report

The **HTML Report** button lets you generate an HTML formatted report of the various information relating to the data that you select, such as confluence, growth rate, and cell number.

To create an HTML report:

1. In the **Review Results > Results > Summary** tab, click **HTML Report**.
2. In the **HTML Report Wizard** dialog, select the information you want included in the report.

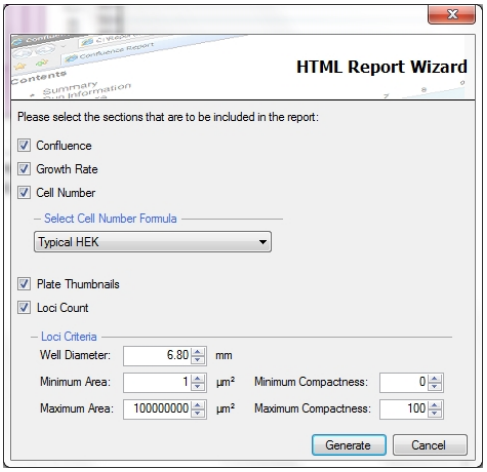


Figure 4-38: HTML Report Wizard dialog

Note: The loci data are automatically populated using the Minimum and Maximum Area and Minimum and Maximum Compactness data set in the **Loci Count** tab. See Loci Count on page 46.

3. To save the report as an .html file, click **Generate**.

Cancel

If you click **Cancel**, you are prompted to confirm you want to end the process. Clicking **Yes** at the prompt takes you to the **Load Results** screen. See Load Results on page 34.

To finish, see Finish on page 65.

Migration Analysis

Note: (Optional) You must purchase a license for this software module.

Do Migration analysis on data that has been imaged over a number of time points.

To view the data for a migration assay:

- In the **Results Archive** list, double-click on the run date you want to use, and then click **Migration**.

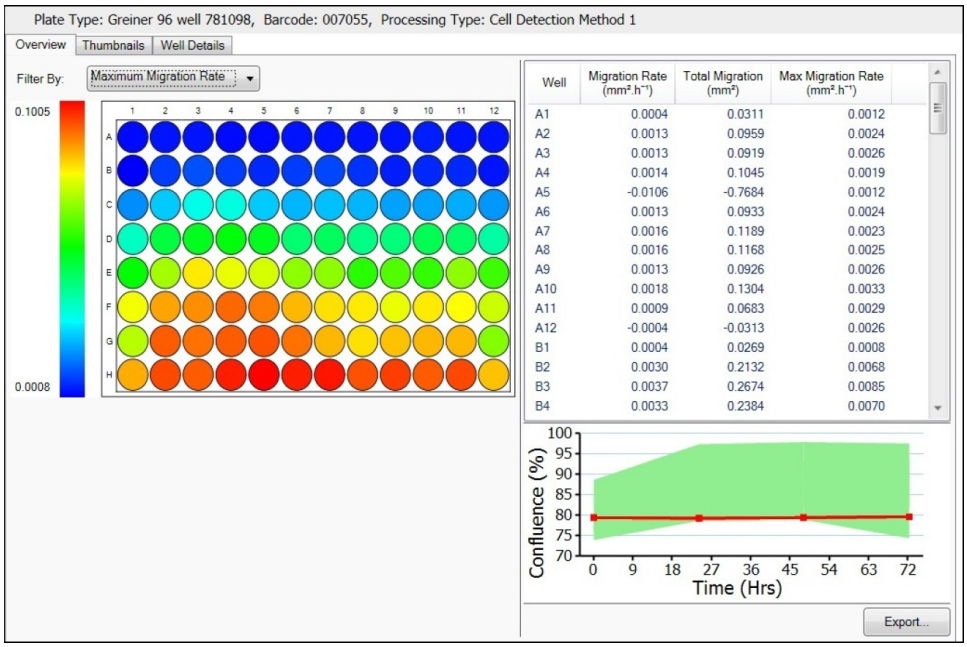


Figure 4-39: Migration analysis Overview screen

The Migration data analysis screen includes three tabs:

- Overview on page 59
- Thumbnails on page 60
- Well Details on page 61

For each data set analyzed, the **Plate Type**, the **Barcode**, and the **Processing Type** information appears at the top of the screen for reference.

Overview

The **Overview** tab provides an overview of the migration assay data. A heat map is generated and can be displayed using one of three **Filter By** menu options:

- **Migration Rate**—measures the rate of increase in cell movement (mm^2 per hour) over the entire time period.
- **Total Migration**—measures the increase in area of cell movement (mm^2) over the entire time period.
- **Maximum Migration Rate**—measures the largest rate of increase in cell movement (mm^2 per hour) between any two adjacent time points.

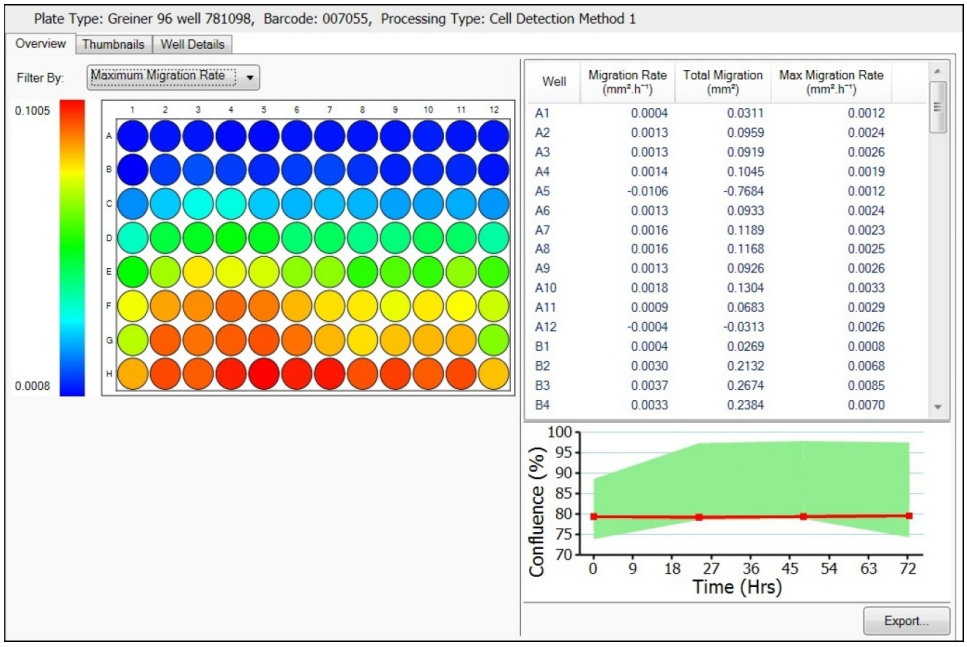


Figure 4-40: Migration analysis Overview screen

The table to the right of the heat map also displays these statistics for each well.

To reorder the data, click on any of the table headers.

To copy the heat map to the clipboard, right-click anywhere on the map and select **Copy to clipboard**.

To highlight the well on the information table and on the Confluence (%) graph, click on any well of interest.

To view a well in the **Well Details** tab, double-click on the well of interest.

Export

The **Export** option lets you save data for either **Selected Time Points** or for **All Time points**. You can also export the confluence data if required.

The **Data Export** wizard guides you through the process of exporting. The data can be exported as a **Comma Separated Values (CSV)**, **Excel**, or **XML** file.

Thumbnails

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

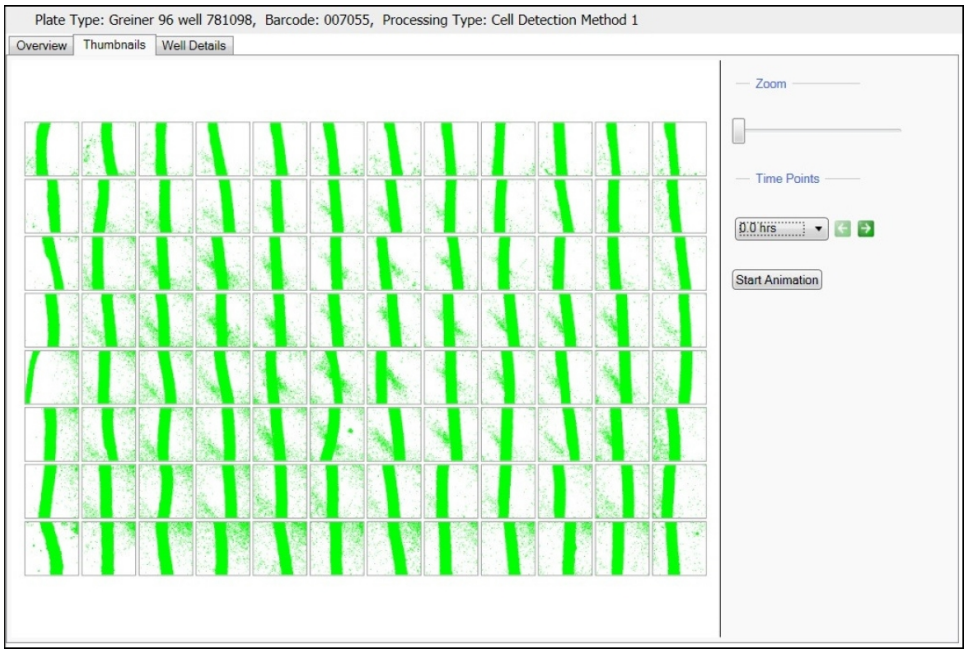


Figure 4-41: Migration analysis Thumbnails screen

To copy the thumbnails to the clipboard, right-click anywhere on the thumbnail map and select **Copy to clipboard**.

The following controls are available:

- **Zoom**—Move the slide either left or right to zoom out or for the whole thumbnail map.
- **Time Points**—Use the drop down menu or the arrows to select a different time point.

  Note: If the time points available are restricted by the **End Time Point** option in the **Display Graph** section of the **Well Details** tab, only the selected time points appear here.

- **Start Animation**—To observe the migration taking place in the wells, click this button to animate the thumbnails from the starting time point to the final time point.

  Note: If the time points available are restricted by the **End Time Point** option in the **Display Graph** section of the **Well Details** tab, only the available time points animate here.

Well Details

The **Well Details** tab provides all the details generated from individual wells. A whole well image appears center screen with time point thumbnails in the film strip below.

To display the enlarged image for a time point in the film strip below, click on any of the thumbnails.

By default **Well Details** opens in the **Display Image** view. You can also view as a graph by clicking **Display Graph** in the right side screen controls.

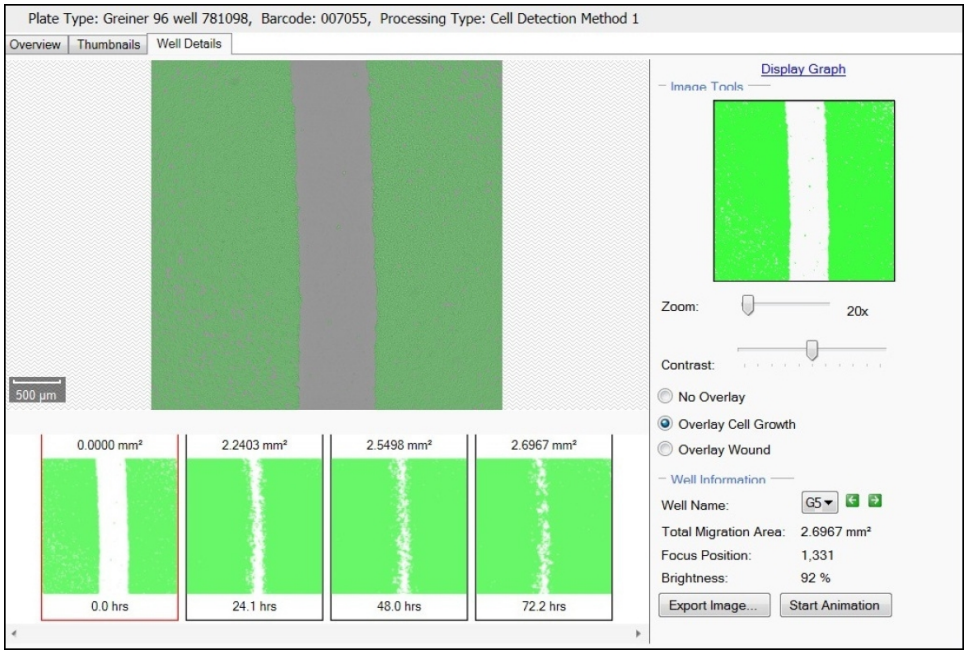


Figure 4-42: Migration analysis Well Details screen

Display Image

The **Display Image** controls include the following:

- **Image Tools**

  Image Tools include the following:

  - **Zoom**—Move the slide either left or right 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 is zooming digitally and can cause some pixelation of the image. When zoomed into an image, the zoomed area will be displayed on the image thumbnail to the right.
  - **Contrast**—Move the slider either left or right to improve the image contrast.
  - **No Overlay**—Select to hide all overlay highlights.
  - **Overlay Cell Growth**—Select to highlight in green all areas with cells.
  - **Overlay Wound**—Select to highlight in green all areas without cells.

- **Well Information**

  Well Information include the following:

  - **Well Name**—Use the drop down menu or the arrows to select a different well.
  - **Total Migration Area**—Shows as a value the measurement of the total cell growth in mm^2 over the entire time period for the selected well.
  - **Focus Position**—Shows the focus position used for the microplate.
  - **Brightness**—Shows the brightness value used for imaging.

- **Export Image**—Lets you save the currently displayed image in .bmp, .jpg, or .png format.
- **Start Animation**—To observe the migration taking place in the wells, click this button to animate the thumbnails from the starting time point to the final time point.

Note: If the time points available are restricted by the **End Time Point** option in the **Display Graph** section of the **Well Details** tab, only the available time points animate here.

Display Graph

The Display Graph view shows the information gathered from the individual wells and displays it in graph format. The graph appears center screen with Migration Area (mm^2) plotted against Time (hours). A time point thumbnail of the selected well, denoted by a red line on the graph, appears below the graph. Each thumbnail image in the film strip displays the total migration at that time point and time passed since the first time point in the series. When hovering over a data point on the graph for the selected well (denoted by a red line on the graph), the corresponding thumbnail will be highlighted in red.

To return to the image view, click **Display Image** in the right side screen controls.

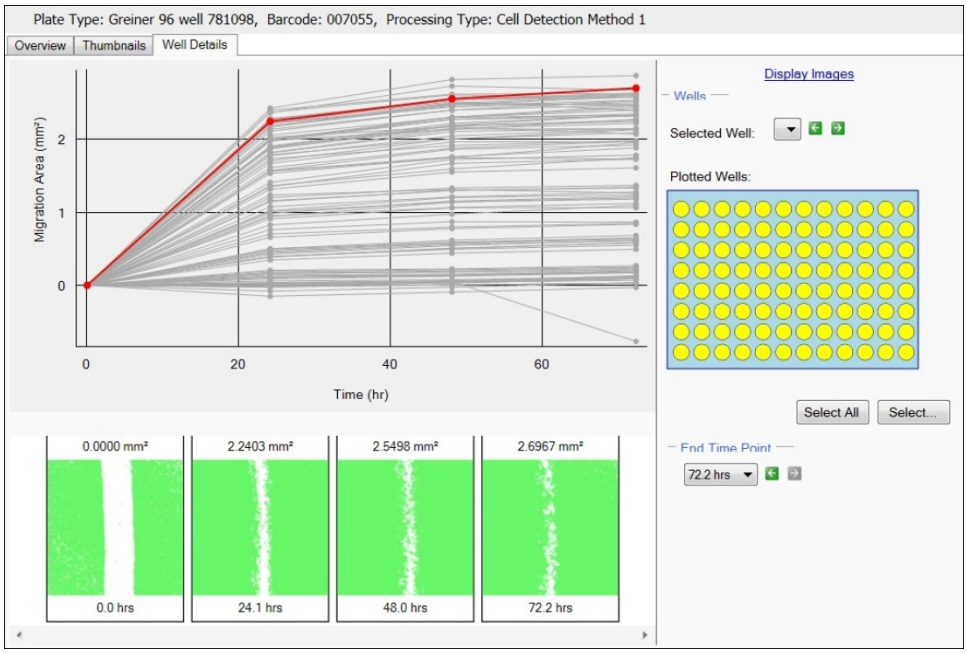
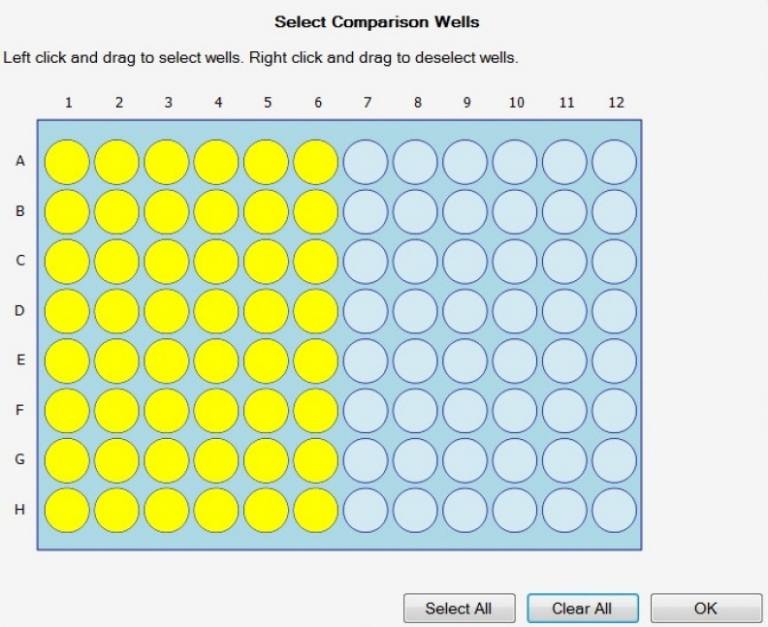


Figure 4-43: Migration analysis Well Details Display Graph screen

The **Display Graph** controls include the following:

- **Wells**
  Wells include the following:

- **Selected Wells**—Use the drop down menu or the arrows to select the well currently displayed on the graph as a bold red line.
- **Plotted Wells**—Use to select the wells for plotting on the graph. The selection and deselection of these wells only apply to the graph and not the overview. Shows on a mini plate map all of the wells that were imaged and are plotted on the graph. When wells are selected they are yellow in the plate map. When wells are deselected they are blue in the plate map.



- **End Time Point**—Use the drop down menu or the arrows to select an end time point. When the end time point is selected the graph shows only the time points before and including the end point selected.

Note: When changing the **End Time Point**, only the selected time points appear on the **Thumbnails** tab. Data on the **Overview** tab is also recalculated using the selected time points.

Finish

When you finish the **Review Results** process, you return to the **Review Results** process start screen. See Review Results on page 33.

To finish the **Review Results** process from a results tab:

1. In any review results tab, click **Cancel**. See Analyzing Results on page 37.
2. In the **Load Results** screen, click **Cancel**. See Load Results on page 34.
3. In the **Finish** screen, click **Finish**.

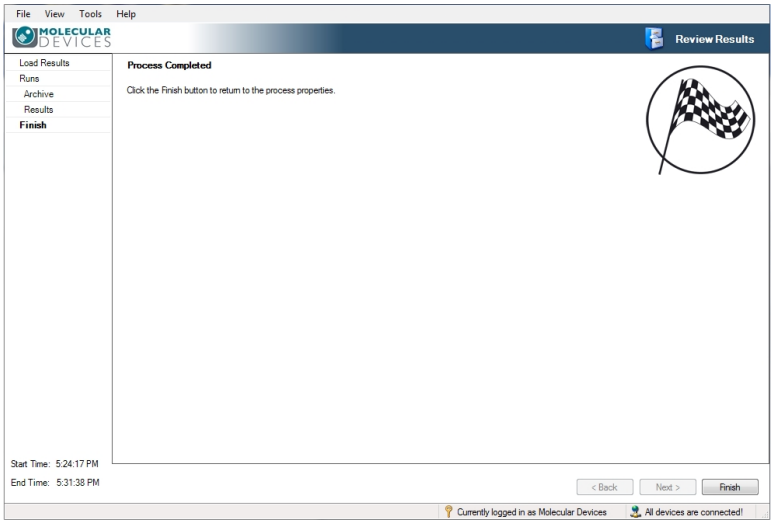


Figure 4-44: Review Results process Finish screen

Batch Microplate Imaging

Batch Microplate Imaging enables you to image multiple plates with the same configuration without reviewing the focus and alignment between plates.

To enter a Batch Microplate Imaging process, in the Navigation screen, click **Batch Microplate Imaging**. See Navigation Screen on page 13.

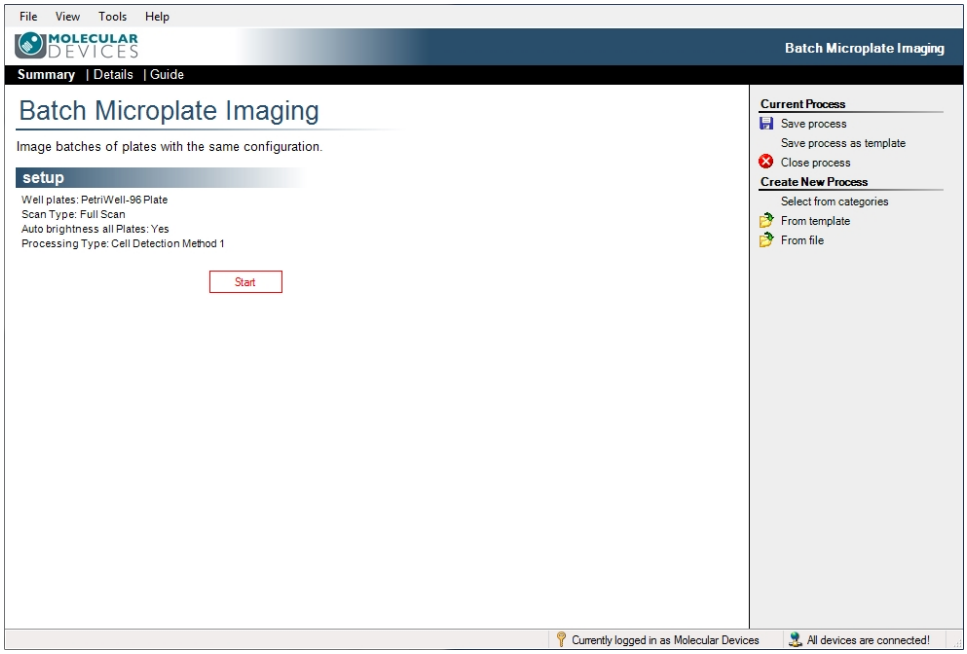


Figure 4-45: Batch Microplate Imaging Summary screen

To set the properties for the Batch Microplate Imaging process, the process screen opens in the Process Properties screen in the **Summary** view. Change the screen view, as needed, and change properties as needed. See Process Properties Screen on page 16. Alternatively, you can click the **Setup** header, and then select from the available properties.

The **Auto brightness all Plates** option automatically selects the appropriate brightness for the image.

This process automatically looks for barcodes on plates, but if not detected, you are prompted to manually enter a plate barcode or plate name.

To begin the process, click **Start**. The first step of the Batch Microplate Imaging process is called **Select Source**.This and the remaining process steps are the same as the Image Microplate process. See Image Microplate on page 17.

E-Scope

The **E-Scope** process lets you view cells at a high resolution (1.85 µm) without performing any image analysis. Images are not saved.

Frames to be imaged can be freely selected by clicking with the mouse on the screen.

To enter an **E-Scope** process, in the Navigation screen, click **E-Scope**. See Navigation Screen on page 13.

How to Image Using E-Scope

Note: Images are not saved.

To image using E-Scope:

1. To set up the properties screen, click the **Source** header, and then select from the available plates.

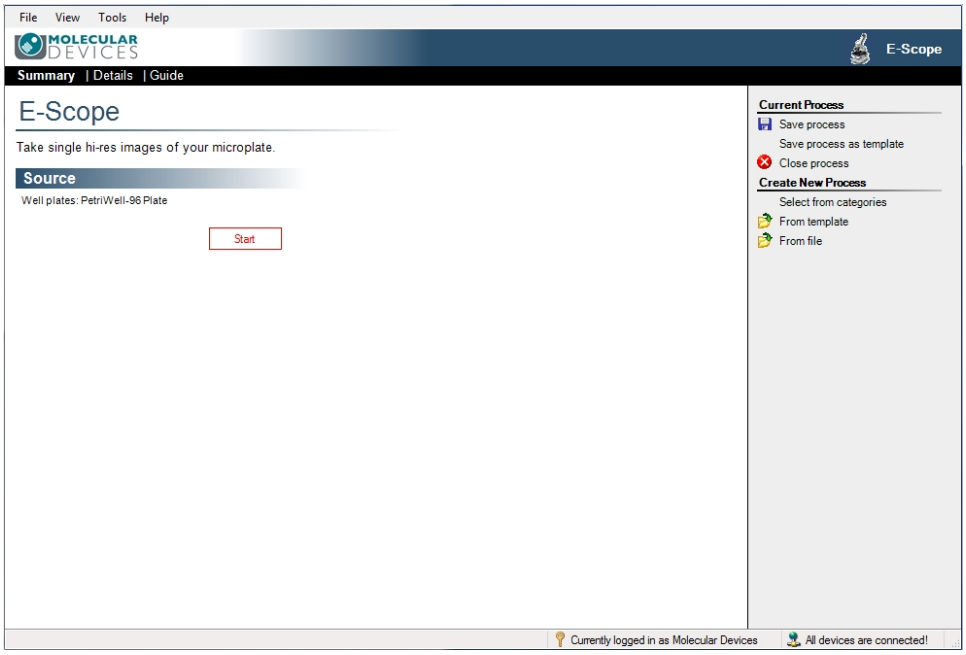


Figure 4-46: E-Scope Summary screen

2. To begin the process, click **Start**.
3. When the plate carrier ejects, place your microplate on the carrier with the A1 well in the back right corner, and then click **Next** to **Load Plate**.

4. Use the **Alignment** screen to check that the camera aligns properly with the wells of the microplate. Four images are taken 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. Adjust as needed.
   - If the well edges do not fall in the middle of the box formed by the four red lines on screen, click and drag on the lines to correct the alignment.
   - If needed, you can also adjust the image brightness by moving the **Brightness** slider to the percentage value you want to use.

     Note: Brightness changes only for the alignment image.

   - When the button is pink, click **Re-image** to recheck the alignment.
   - To finish Alignment and continue, click **Next**.
5. In the **Select Location** screen, in the displayed schematic of the microplate, to view a real-time image, click on one of the available image frames.

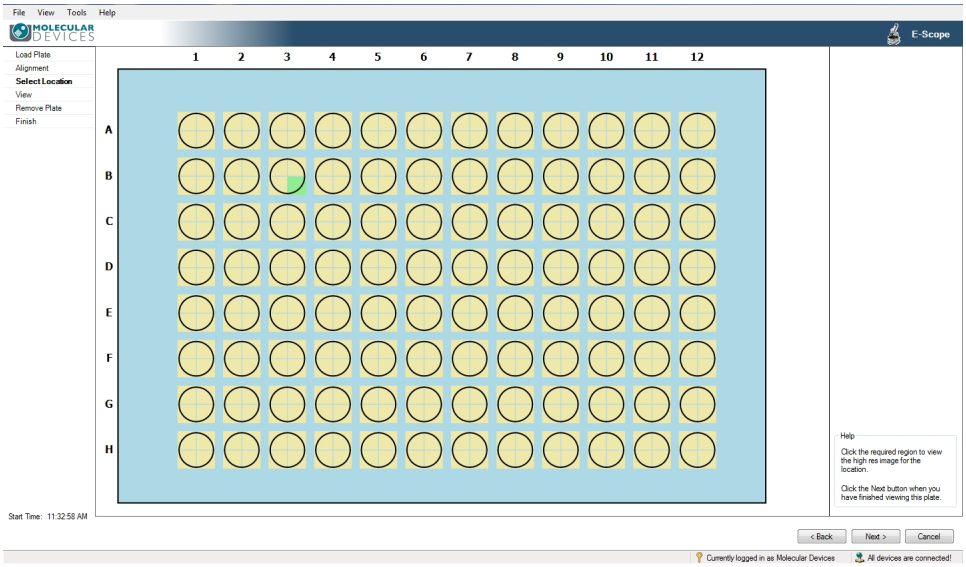


Figure 4-47: E-Scope Select Location screen

6. The **View** screen opens automatically with the selected image frame.

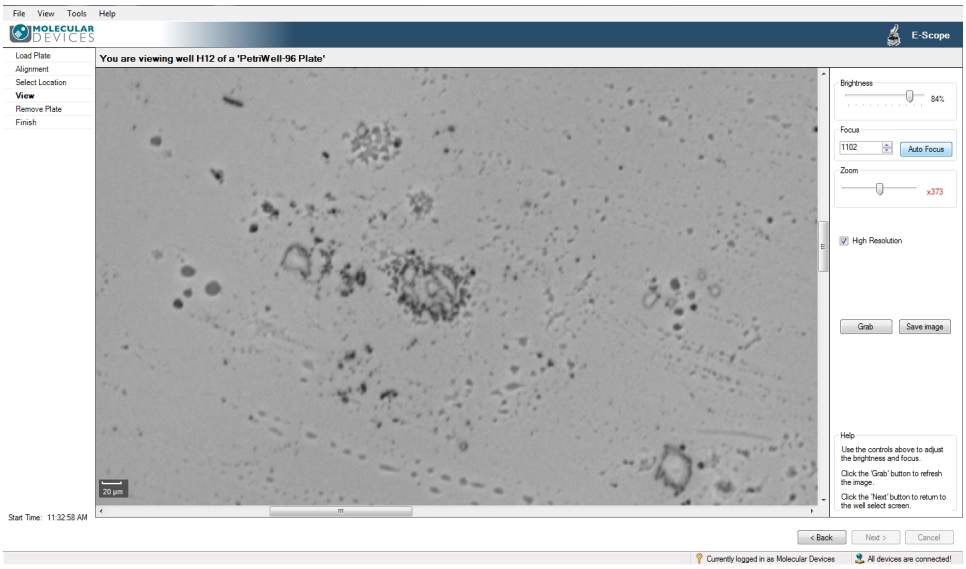


Figure 4-48: E-Scope View screen

You can do any of the following in 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 type a new value in the field.
- **AutoFocus**—Click to automatically adjust the image focus.
- **Zoom**—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 of the image window automatically adjusts.

  Tip: When the zoom setting figure on the right of the slider is black, the system is zooming optically. When the figure turns red, the system is zooming digitally and you might see some pixelation of the image.

- **High Resolution**—Select to use high resolution imaging (1.85 Om), deselect to use standard resolution imaging (3.7Om).
- **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.

7. To select another image or well, click **Back** to go back to the **Select Location** screen. See step 5.
8. To continue, click **Next**.
9. When the plate carrier ejects, remove your plate, and then click **Next**.
10. In the **Finish** screen, click **Finish** to return to the E-Scope process start screen. See step 1.

Plate Alignment

The **Plate Alignment** process is one of the **Maintenance Processes** on the Navigation screen. See Navigation Screen on page 13. It enables the alignment of the plate within the system like the **Alignment** step within the **Image Microplate** process, except as a stand alone process without imaging. See Alignment on page 24.

Eject Plate Carrier

The **Eject Plate Carrier** process is one of the **Maintenance Processes** on the Navigation screen. See Navigation Screen on page 13. It enables a way to eject the plate carrier for cleaning or if a microplate has been left in the instrument.

To eject the plate carrier:

1. To enter the **Eject Plate Carrier** process, in the Navigation screen, click **Eject Plate Carrier**.
2. In the **Eject Plate Carrier** start screen, click **Start**.
3. Clean the plate carrier or remove a microplate.
4. To close the plate carrier and continue, click **Next**.
5. In the **Finish** screen, click **Finish** to return to the Eject Plate Carrier process start screen.

Chapter 5: Data Analysis Examples

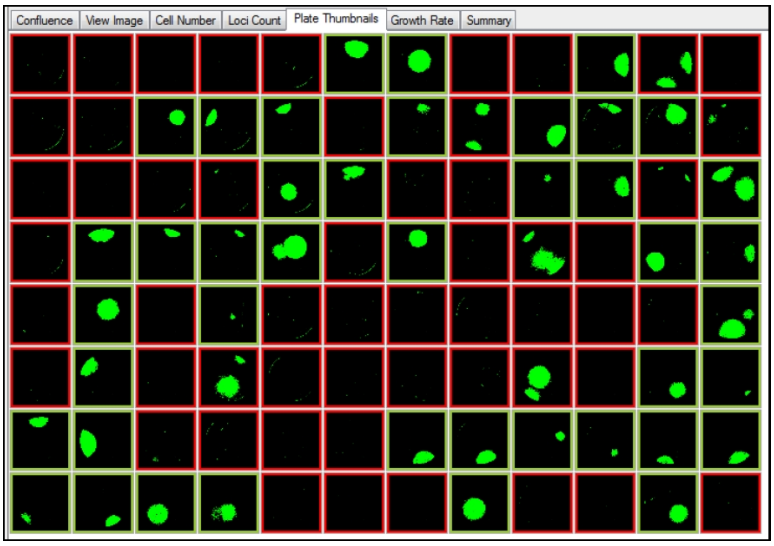
5

The following are some examples of how to use the features of the CloneSelect Imager Software to analyze various data sets.

Monoclonality Workflow

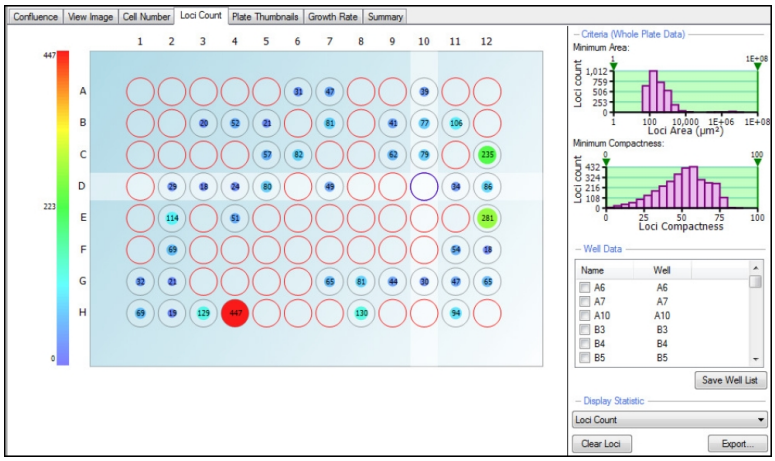
Note: Loci Count is optional software. Contact your Molecular Devices representative to purchase.

1. After imaging the plate of interest at several time points, open the latest data point in **Review Results**. The software automatically opens the data in the **Confluence** tab.
2. Click **Thumbnails** to display all the imaged wells, and then to save processing time, exclude wells without at least 1 colony within the well. See Plate Thumbnails on page 50.

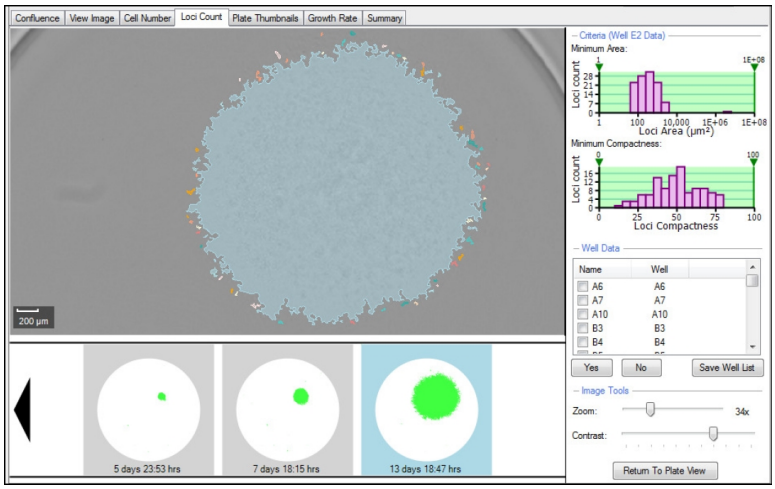


3. Click **Loci Count** and adjust the well diameter as needed. See Loci Count on page 46.

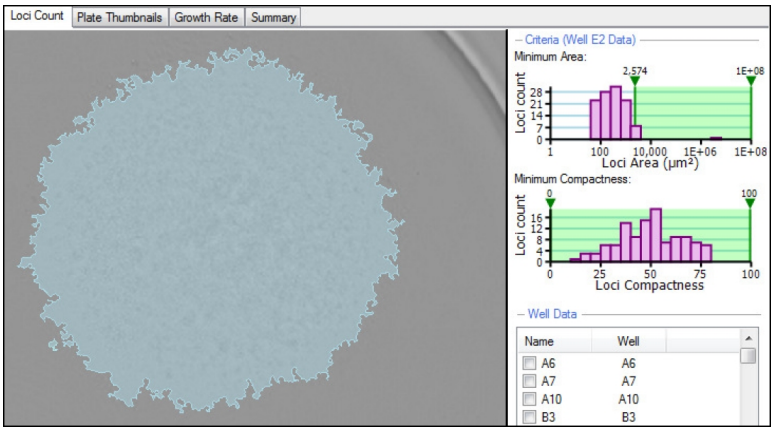
4. Click **Process** to produce the loci counts for the selected wells. The **Loci Count** tab screen changes to display an overview of all the selected wells and their corresponding loci count.



5. Click on one of the wells to display the well image. The image shows the loci processing.

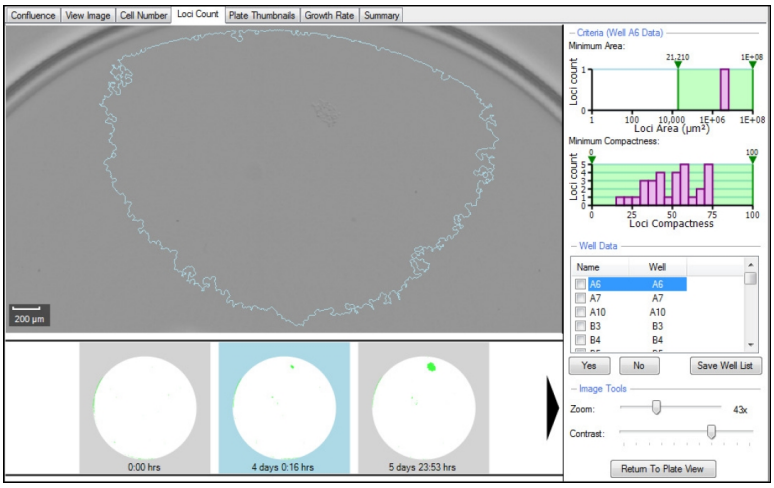


6. Move the Minimum Area and Minimum Compactness lower and uppers sliders on the graphs to gate out any unwanted debris and misshaped colonies within the wells.

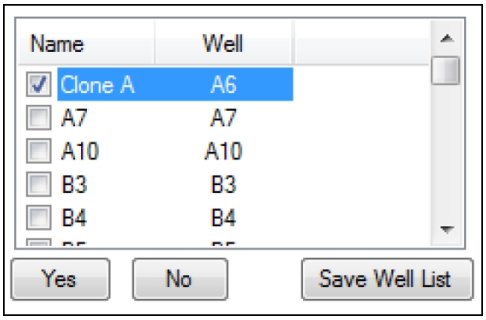


7. Click on **Return To Plate View** and check through some of the other wells to make sure all debris and misshapen colonies are excluded. The plate overview reflects the processing and displays the new loci count number in each of the wells.

8. Click through each well in the **Well Data** list. The well image appears and monoclonality can be determined by looking back through the series of well images and identifying if the colony originated from a single cell.



9. If the colony did originate from a single cell, select the well by either selecting the check box or clicking **Yes**. The well name can also be changed to reflect the clone if needed.

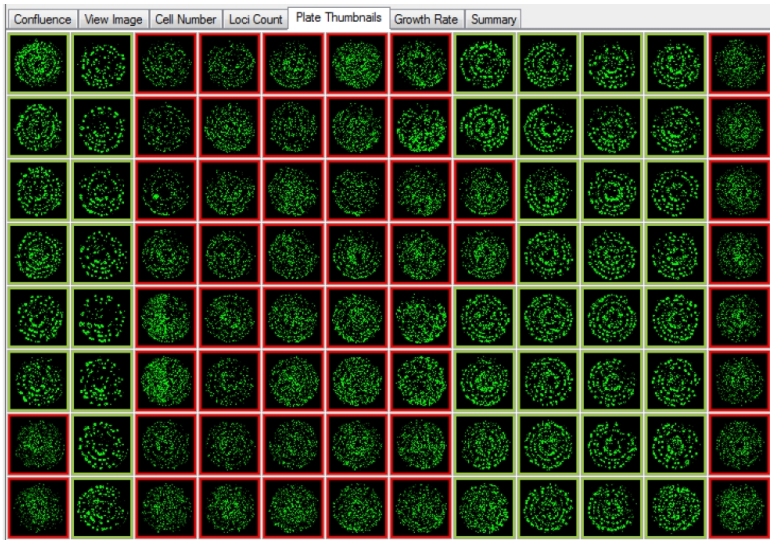


After all the wells have been selected, the list of selected wells containing a monoclonal colony can be saved and printed for further use.

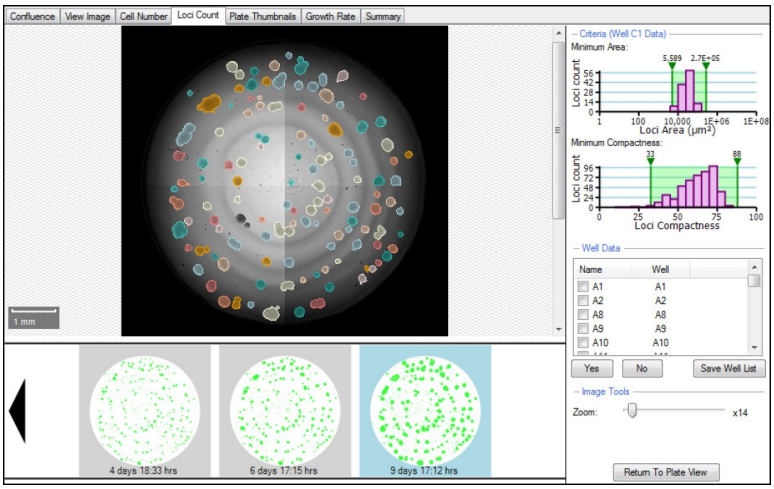
Colony Forming Assay Data Workflow

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

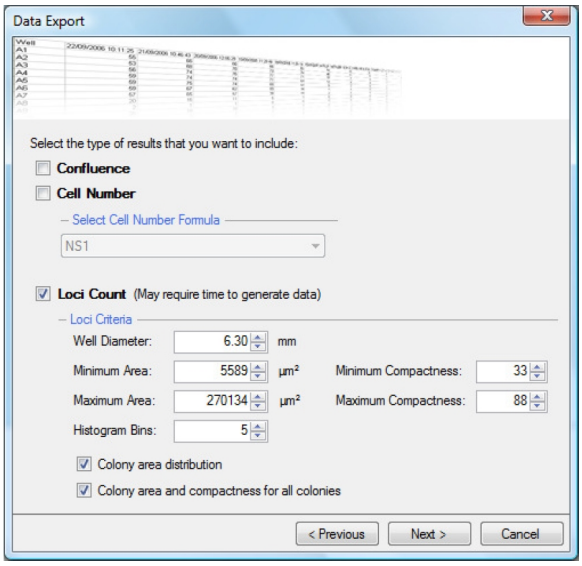
1. After imaging the plate of interest at several time points, open the latest data point in **Review Results**. The software automatically opens the data in the **Confluence** tab.
2. Click **Thumbnails** to display all the imaged wells, and then to save processing time, exclude wells without at least 1 colony within the well. See Plate Thumbnails on page 50.



3. Click **Loci Count** and adjust the well diameter as needed. See Loci Count on page 46.
4. Click **Process** to produce the loci counts for the selected wells. The **Loci Count** tab screen changes to display an overview of all the selected wells and their corresponding loci count.
5. Move the Minimum Area and Minimum Compactness lower and uppers sliders on the graphs to gate out any unwanted debris and misshaped colonies within the wells.

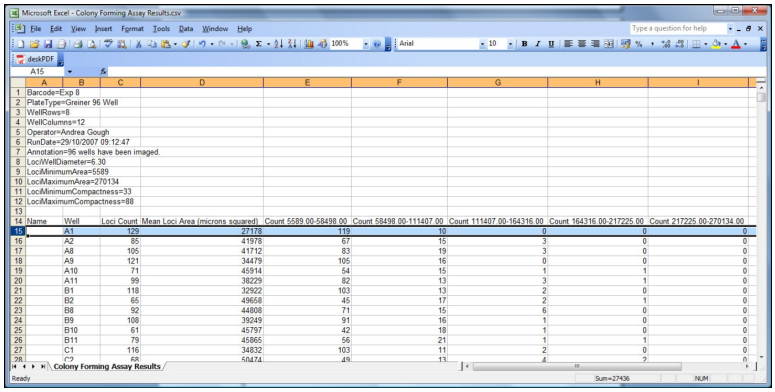


6. When satisfied with the gating of the data and the loci count produced, the data can be exported. The **Well Data** list is used for accepting wells based on monoclonality so it is not necessary to select any of these wells. The data exports for all wells when exporting the data via the wizard.



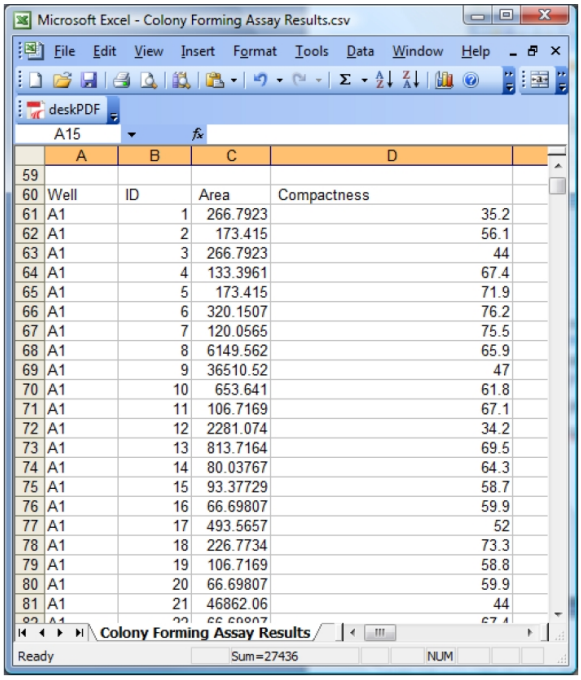
7. In the **Data Export** wizard, select **Loci Count**. The criteria automatically populate. The default number of bins is 20, but in the example it has been changed to 5 bins. Select **Colony area distribution** and **Colony area and compactness for all colonies** and then click **Next** to finish exporting the data.

8. Within the exported results produced, a summary of the experiment criteria appears in the first rows the spreadsheet (top left).

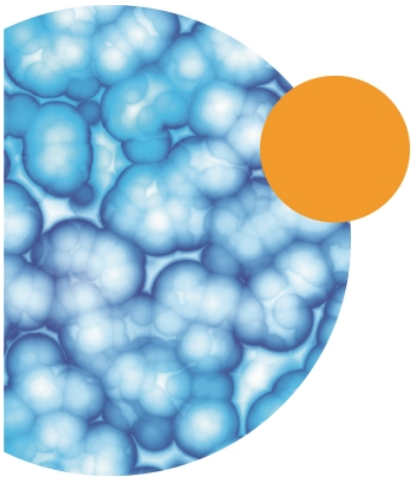


9. When the **Colony area distribution** is selected, the following information is produced for each well:
   - Name (if changed within the software)
   - Well number (eg A1)
   - Loci Count (this is the total)
   - Mean Loci Area
   - Bins equally splitting the loci area range into equal groups so that 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 selecting the **Colony area and compactness for all colonies**, the area and compactness of each identified loci is displayed:



	A	B	C	D
59				
60	Well	ID	Area	Compactness
61	A1	1	266.7923	35.2
62	A1	2	173.415	56.1
63	A1	3	266.7923	44
64	A1	4	133.3961	67.4
65	A1	5	173.415	71.9
66	A1	6	320.1507	76.2
67	A1	7	120.0565	75.5
68	A1	8	6149.562	65.9
69	A1	9	36510.52	47
70	A1	10	653.641	61.8
71	A1	11	106.7169	67.1
72	A1	12	2281.074	34.2
73	A1	13	813.7164	69.5
74	A1	14	80.03767	64.3
75	A1	15	93.37729	58.7
76	A1	16	66.69807	59.9
77	A1	17	493.5657	52
78	A1	18	226.7734	73.3
79	A1	19	106.7169	58.8
80	A1	20	66.69807	59.9
81	A1	21	46862.06	44



Chapter 6: Health and Safety

6

Transport and Storage

The CloneSelect Imager must be stored and transported in temperatures within the range –25°C to +55°C.

Lifting Points

The CloneSelect Imager is intended for bench top operation. Care should be taken when moving the CloneSelect Imager. It is recommended that 2 people lift it.

External Covers

WARNING! If any of the external covers on the CloneSelect Imager are removed the power supply is not automatically interrupted. If it is necessary to remove any of the external covers you must ensure that the power is switched off first and do not attempt to use the robot until the covers are replaced.

Electrical Safety

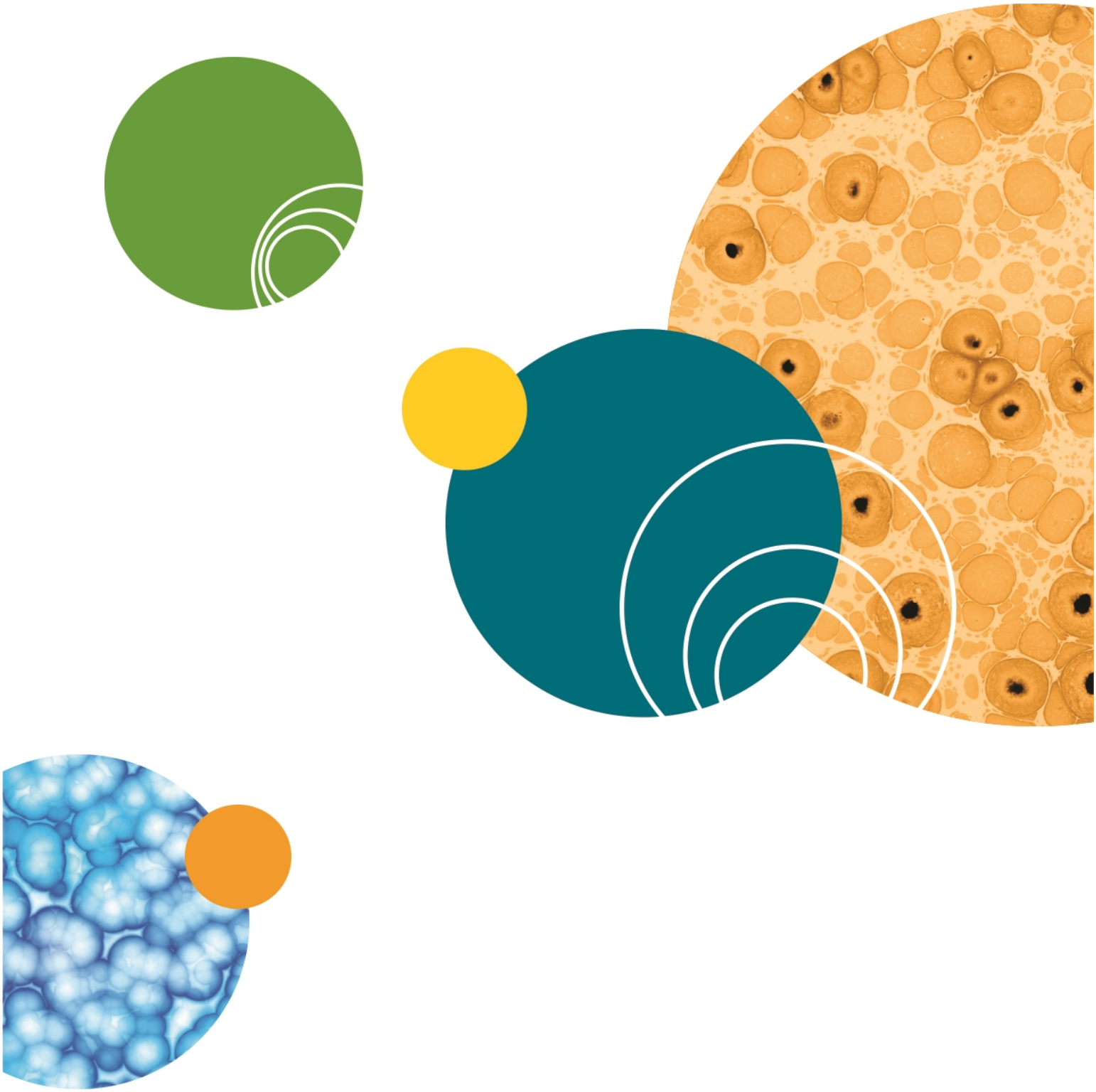
WARNING! HIGH VOLTAGE. The CloneSelect Imager must be connected to a properly earthed power outlet to protect users from the risk of electric shock. The main chassis of the machine is earthed together with all associated electrical components. Do not remove any of the fixed covers, as there are no user serviceable parts inside. All internal work should be referred 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 7: General Maintenance

7

Cleaning The CloneSelect Imager

- The machine should be cleaned each day.
- Clean the machine using 80% ethanol or dilute detergent and a lint-free cloth. Organic solvents and abrasive cleaners should not be used, as they will damage the cover.
- Do not pour cleaning solution directly on to the machine or other objects. Apply using a suitable lint-free cloth.

Regular Maintenance

Daily

- Ensure that the CloneSelect Imager is free from dirt and dust.

Weekly

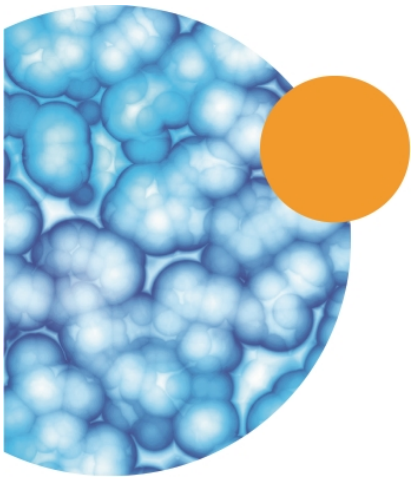
- Check operation of equipment.
- Check calibration.
- Check for damage.

Annually

- Maintenance by manufacturer.
- Molecular Devices strongly recommends that maintenance be carried out regularly and by a Molecular Devices-approved service engineer.
- Maintenance contracts can be obtained from Molecular Devices. See Obtaining Support on page 6.

General Precautions

- All waste must be disposed of according to local regulation.
- Do not use in explosive environments.



Chapter 8: Technical Specifications

8

Manufactured by Molecular Devices.

Table 8-1: Dimensions (Total assembled)

Item	Description
Size	575 mm (width) x 720 mm (depth) x 438 mm (height) Excluding ancillary equipment
Weight	45 kg

Table 8-2: 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

Table 8-3: Electrical Supply

Item	Description
Voltage	100 to 200 VAC, 50 yo 60 Hz, single phase
Power	100 VA
Connections	IEC input
Fuses	F1 - F3A

Table 8-4: Symbols on Equipment

Item	Description
	Beware moving parts. Refer to user manual for operating instructions.
	The year of manufacture is given on the serial number label which is located on the rear of the unit.

Table 8-4: Symbols on Equipment (continued)

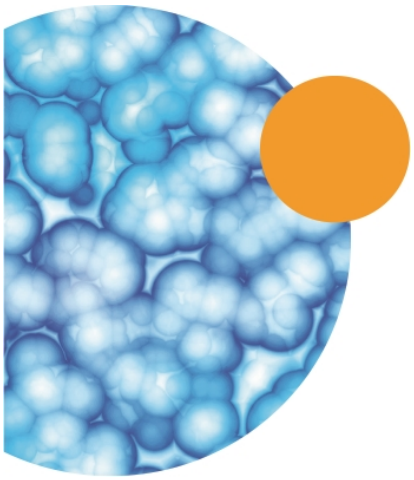
Item	Description
CE	**European Economic Area (EEA)** This mark on the product indicates compliance with the following EEC Directives: - 98/37/EEC, relating to The Supply of Machinery (Safety) Regulations 1992 - 89/336/EEC with amendments 92/31/EEC, relating to Electromagnetic Compatibility - 73/23/EEC, the Low Voltage Directive

Appendix A: Compatible Plate Types

The standard CloneSelect Imager is configured to use the following plate types:

Manufacturer	Catalogue number	Well format
Corning / Costar	3300	96
Greiner	781182	384

Note: See the Knowledge Base for more plate compatibility information.
www.moleculardevices.com/support



Appendix B: Well List File Format

B

Note: You can only have one Well List File per Plate Type.

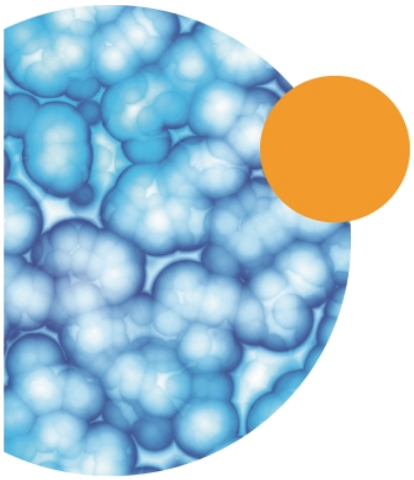
Use the following format for your Well List File:

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

and so on.

To create a Well List File:

1. As 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 you want 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 20.

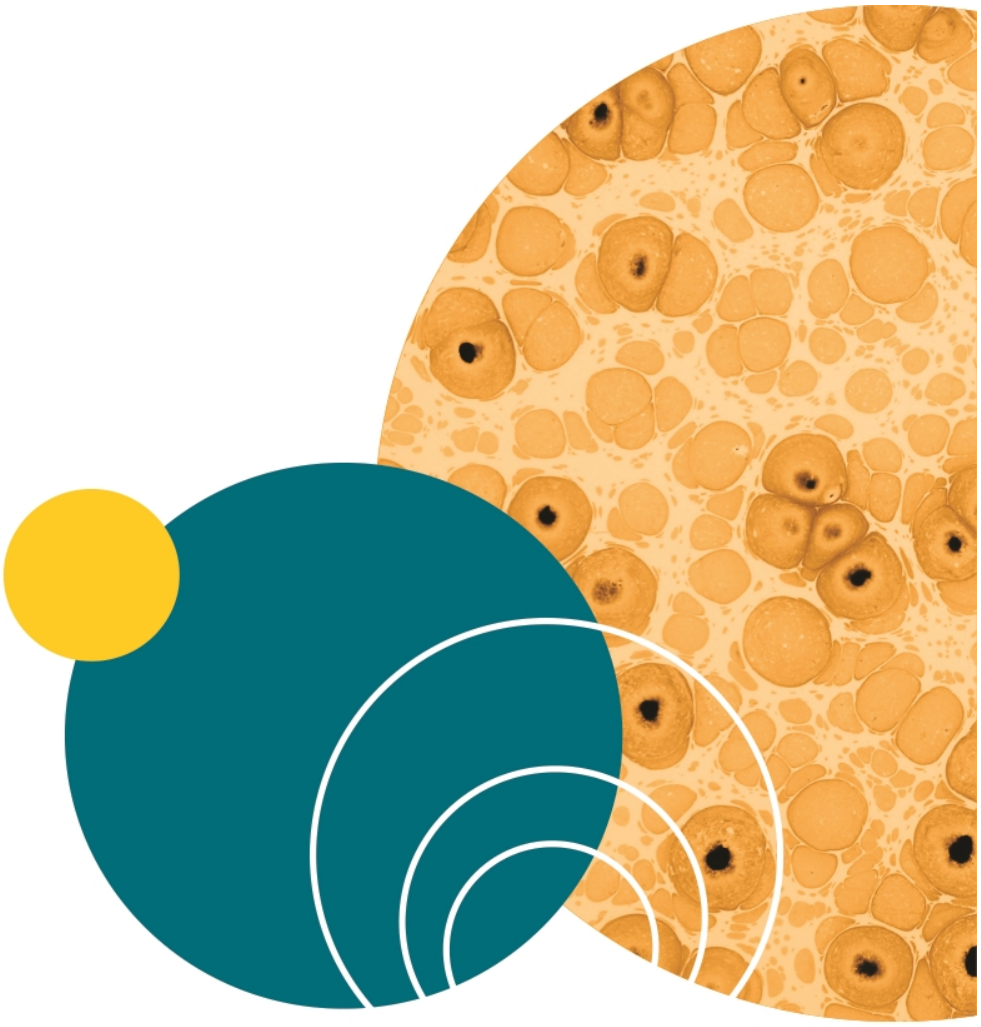


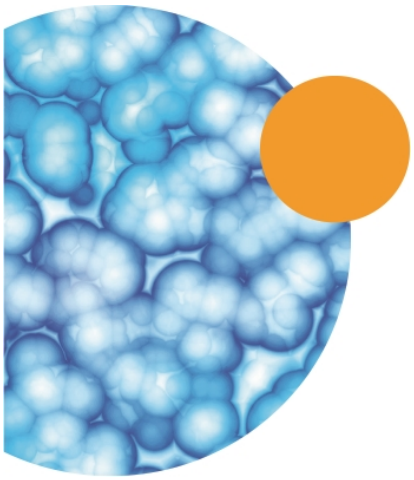
Appendix C: Setting up Cell Number Estimation

C

When creating 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.

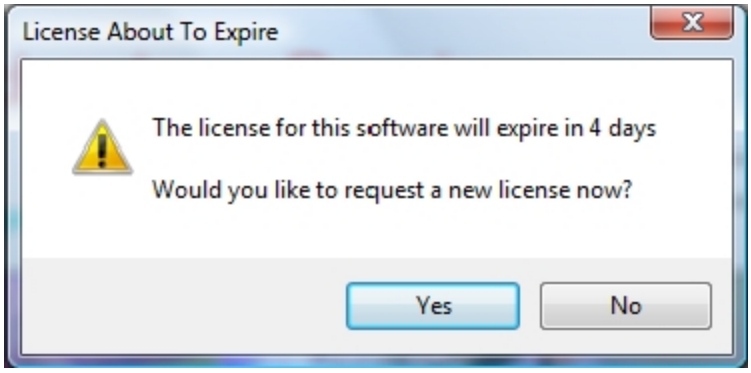




Appendix D: Obtaining a Software License

After starting the CloneSelect Imager Software, if the following message appears, you need to do the following:

1. Request a Software License on page 91
2. Install a Software License on page 93

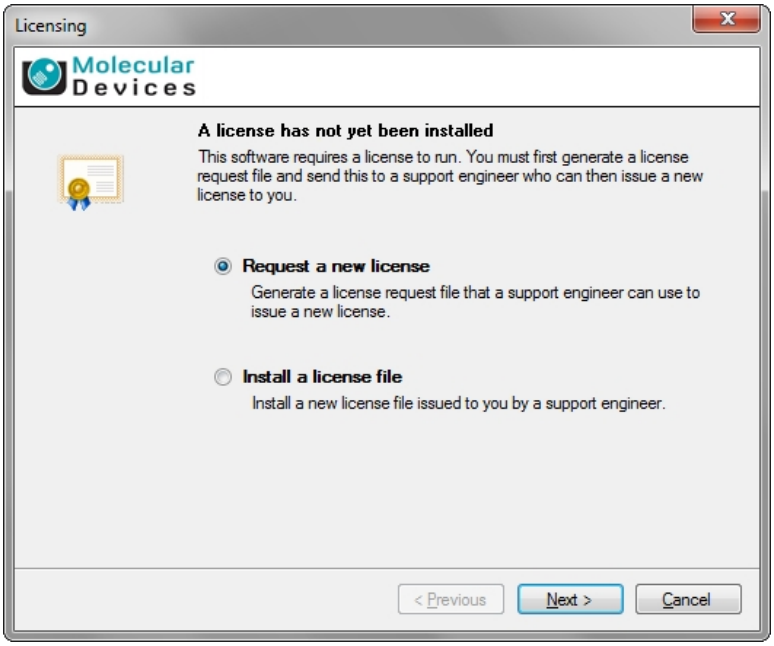


Request a Software License

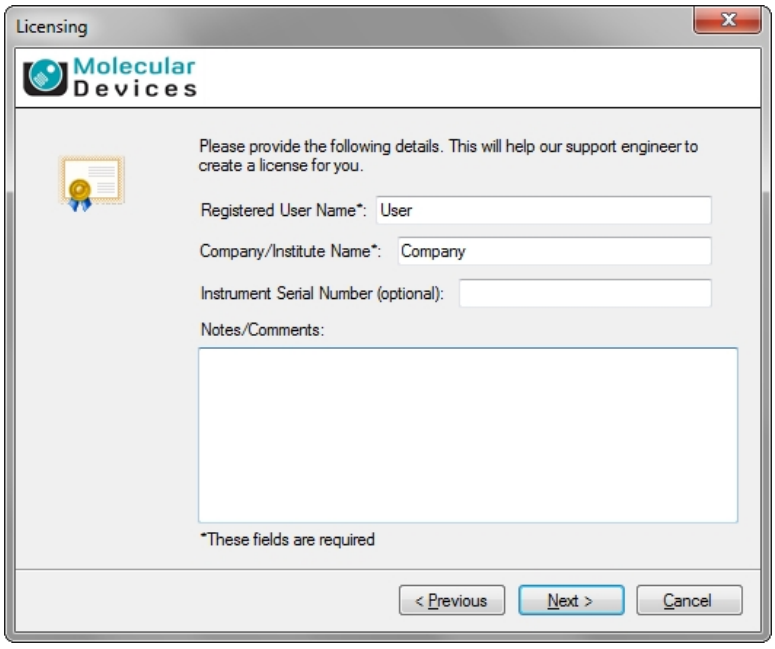
If you need to request a new software license, do the following:

To request a new software license:

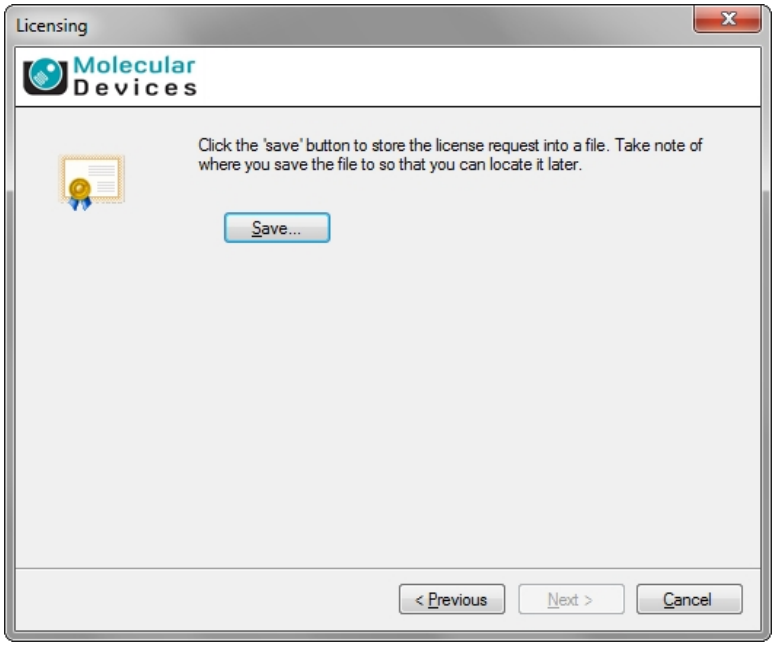
1. In the license message dialog, click **Yes**.
2. In the license request wizard, select **Request a new license**, and then click **Next**.



3. Enter the requested information, and then click **Next**.



4. To save the request file to a location on your computer for attaching it to an email, click **Save**.



5. In the **Save the request to a file** dialog, save the file in a location where you can easily find it.
6. To close the wizard, click **Finish**.
7. Change the **LicenseRequest.req** file name to a **LicenseRequest.txt** file, and then email it to Molecular Devices Technical Support. See www.moleculardevices.com/support.

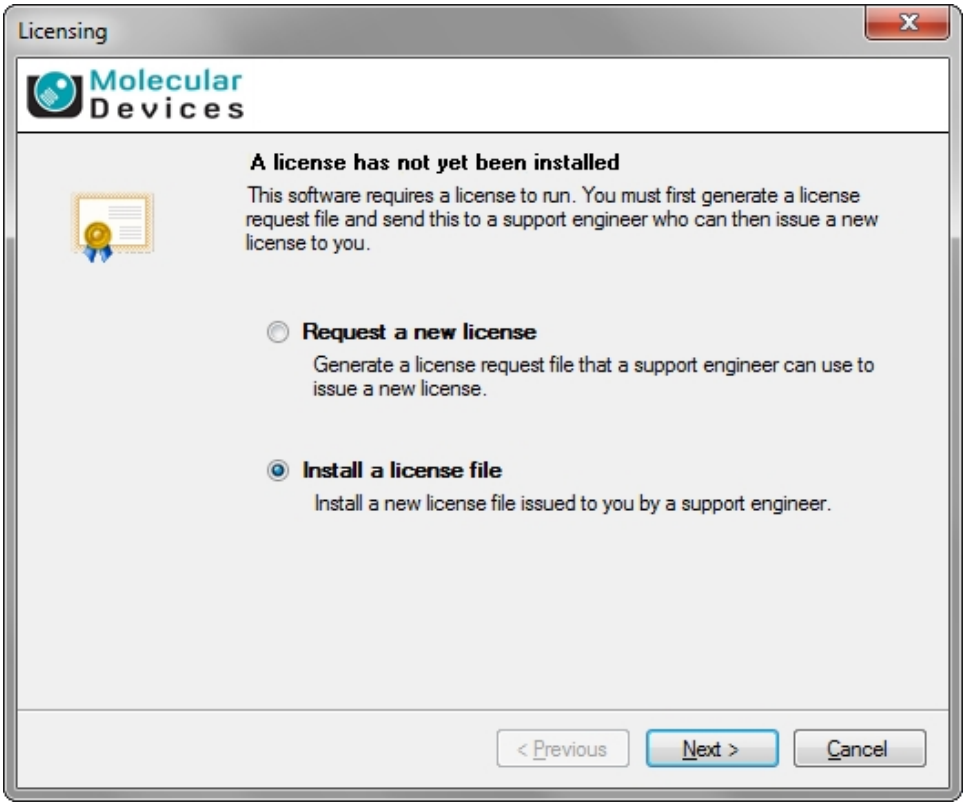
8. After you receive the license file, save it on the system computer where it is easily accessible, and then install the license.

Install a Software License

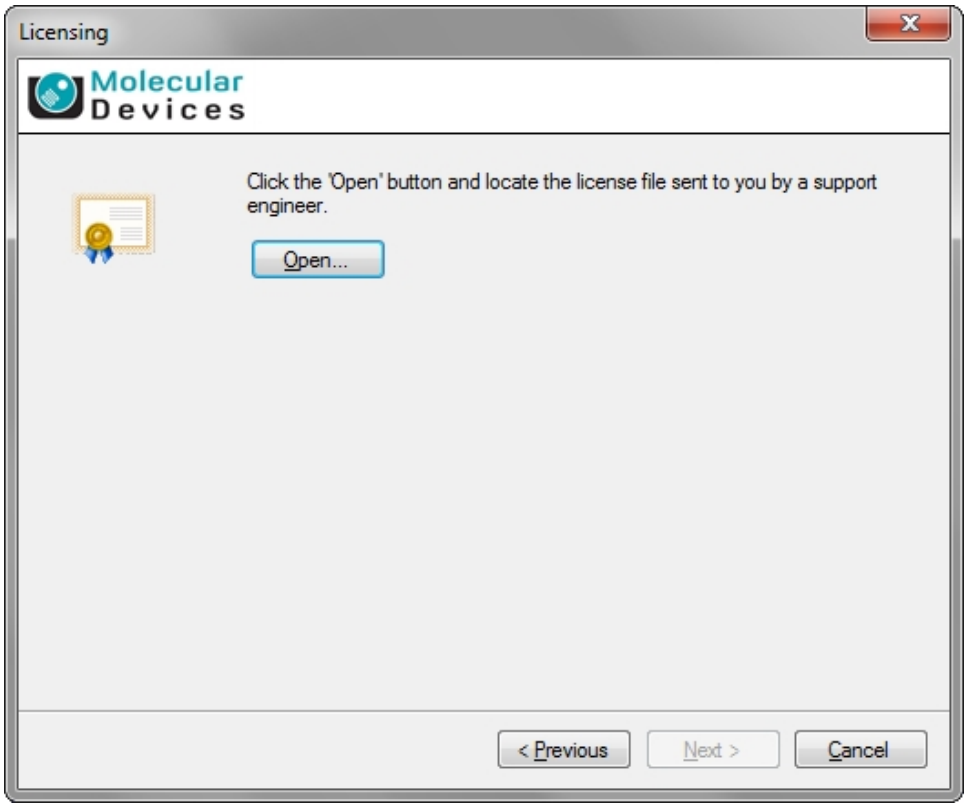
After you receive your license from Molecular Devices Technical Support, do the following:

To install a software license:

1. From the computer desktop, double-click the CloneSelect Imager icon to start the software.
2. In the **Licensing** dialog, select **Install a license file** and then click **Next**.



3. Click **Open**.



4. In the **Select the License File to install** dialog, locate and select the license file you previously saved, and then click **Open**.
5. After the license file automatically installs, click **Finish**.

Contact Us

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

Visit our website for a current listing of worldwide distributors.

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