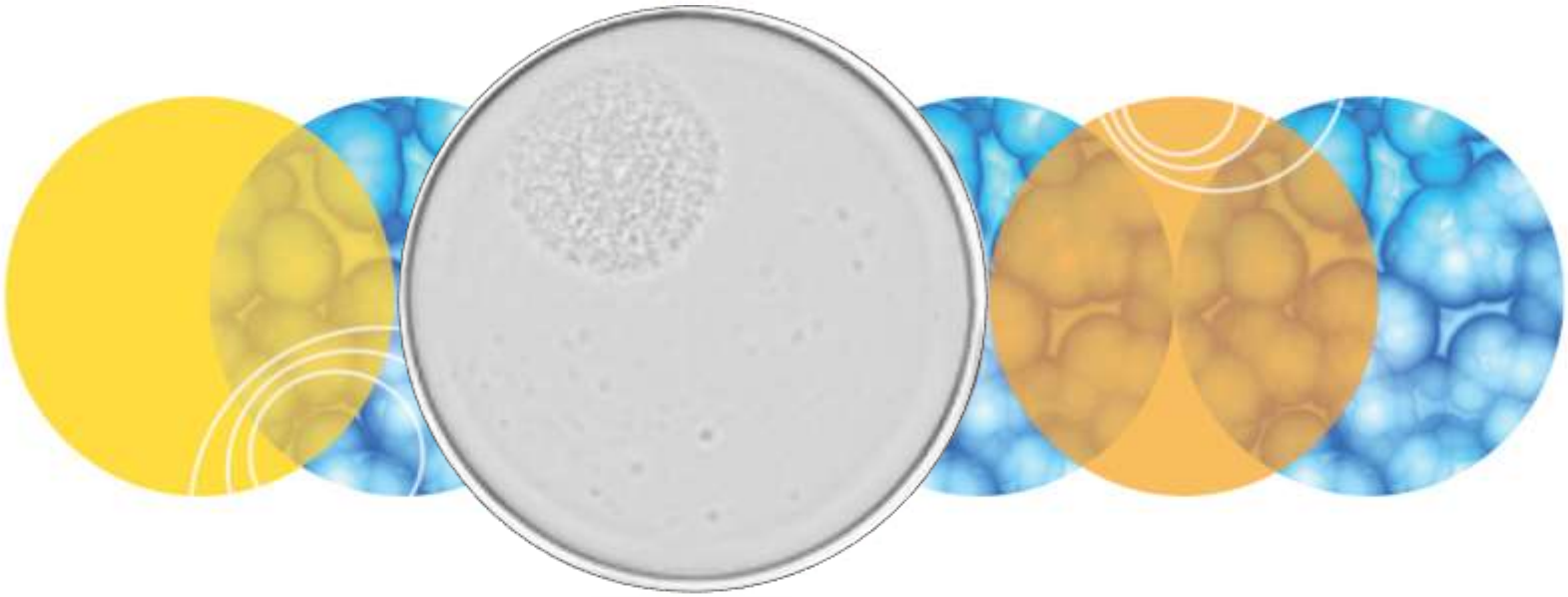


Clone Select Imager Training Guide

Plate Imaging

MOLECULAR DEVICES
UNLEASH YOUR BRILLIANCE™

Date Revised 3/22/2017 Version C

Index

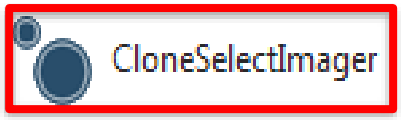
Chapter Purpose

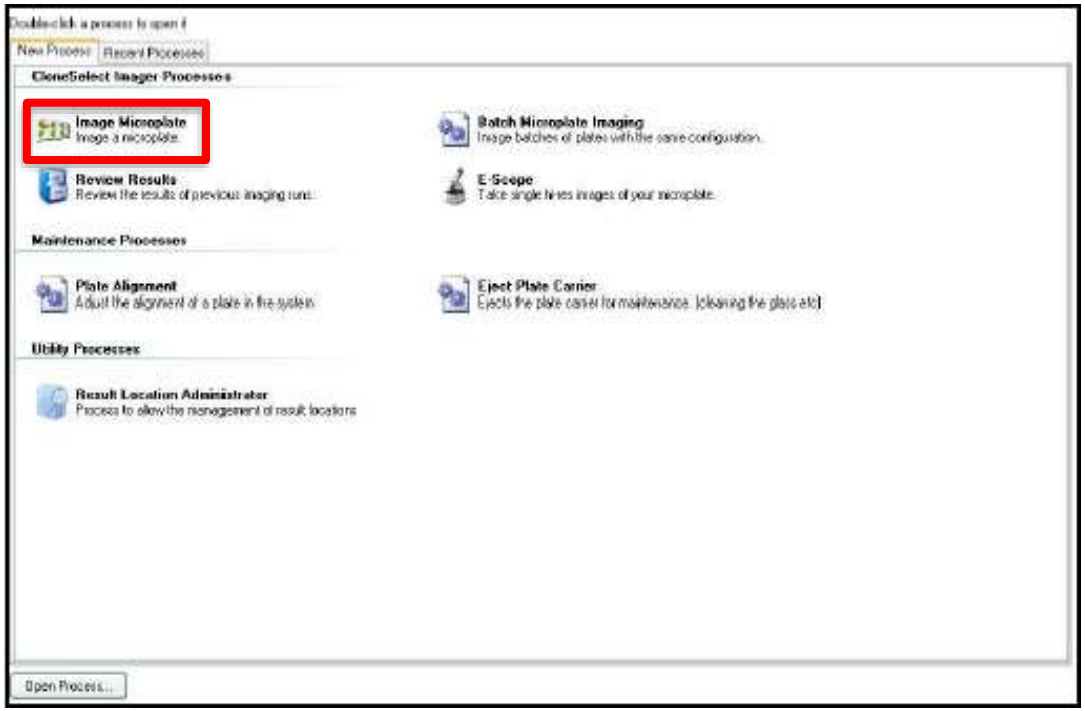
The purpose of this chapter is to illustrate the plate imaging and analysis workflow for the CloneSelect Imager (CSI).

This guide does not include detailed descriptions around the CSI hardware, reviewing data, or performing downstream analysis applications. Please refer to corresponding chapters for details on these topics.

Setup Plate Imaging

- To begin imaging your plate, first click on the **CloneSelect imager software icon** on your desktop. You will now see the **Main Navigation** screen appear. Click on the **Image Microplate icon** to launch the imaging worfkflow.



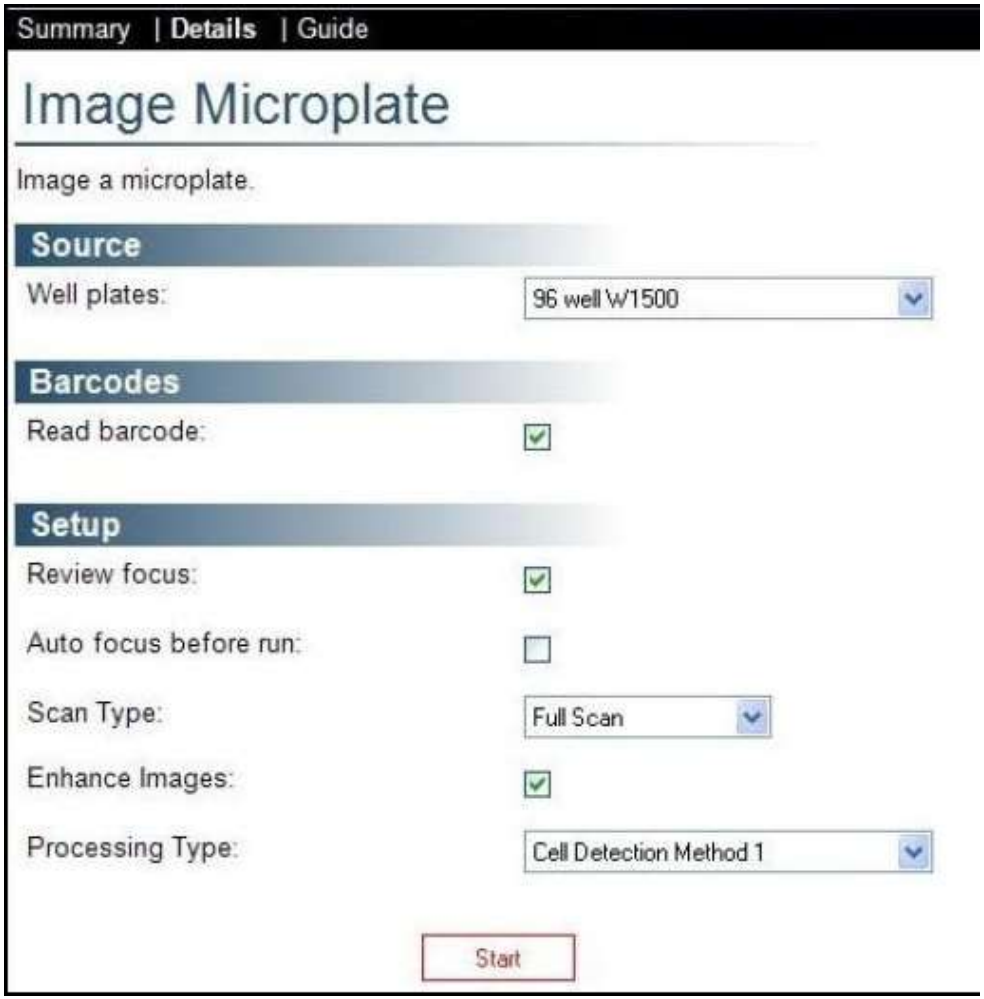


Define Microplate Imaging Settings

- The **Image Microplate** screen appears. Here you can set up the source microplate, barcode reading, and plate setup details. Click on the **Details** tab at the top of the window to define these settings.

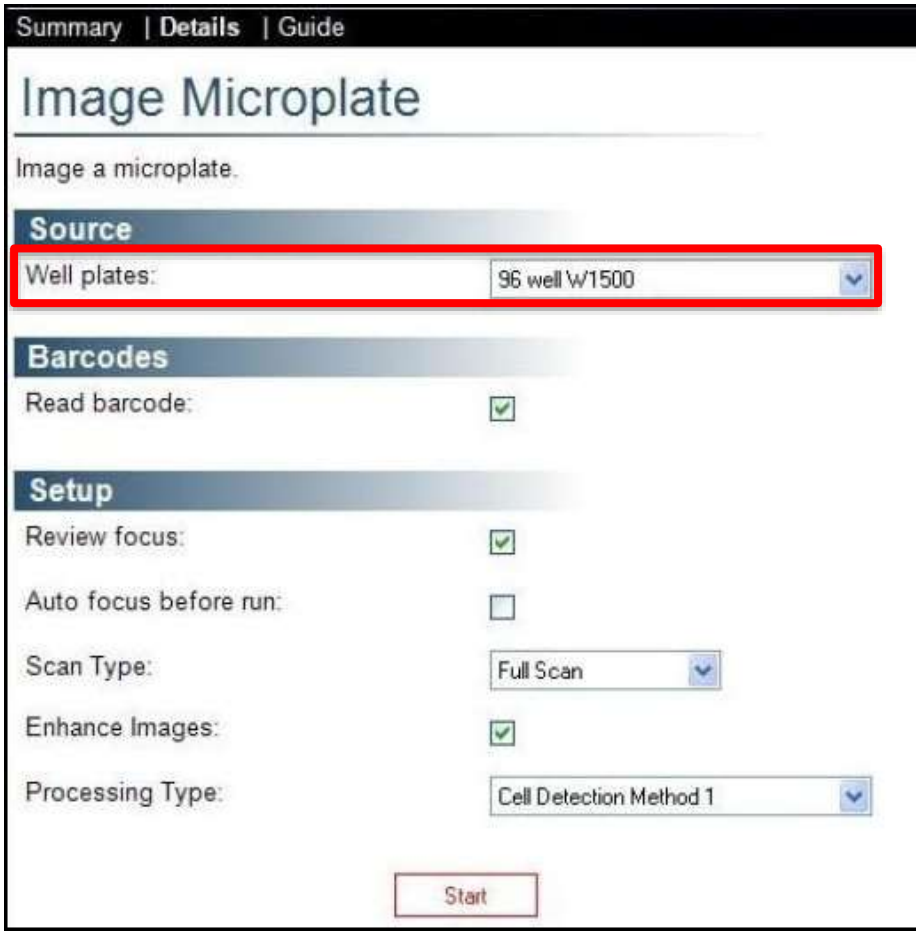
Define Microplate Imaging Settings

- The **Details** view appears. You will now see **dropdowns/tick boxes** enabled that will allow you to change the displayed settings.

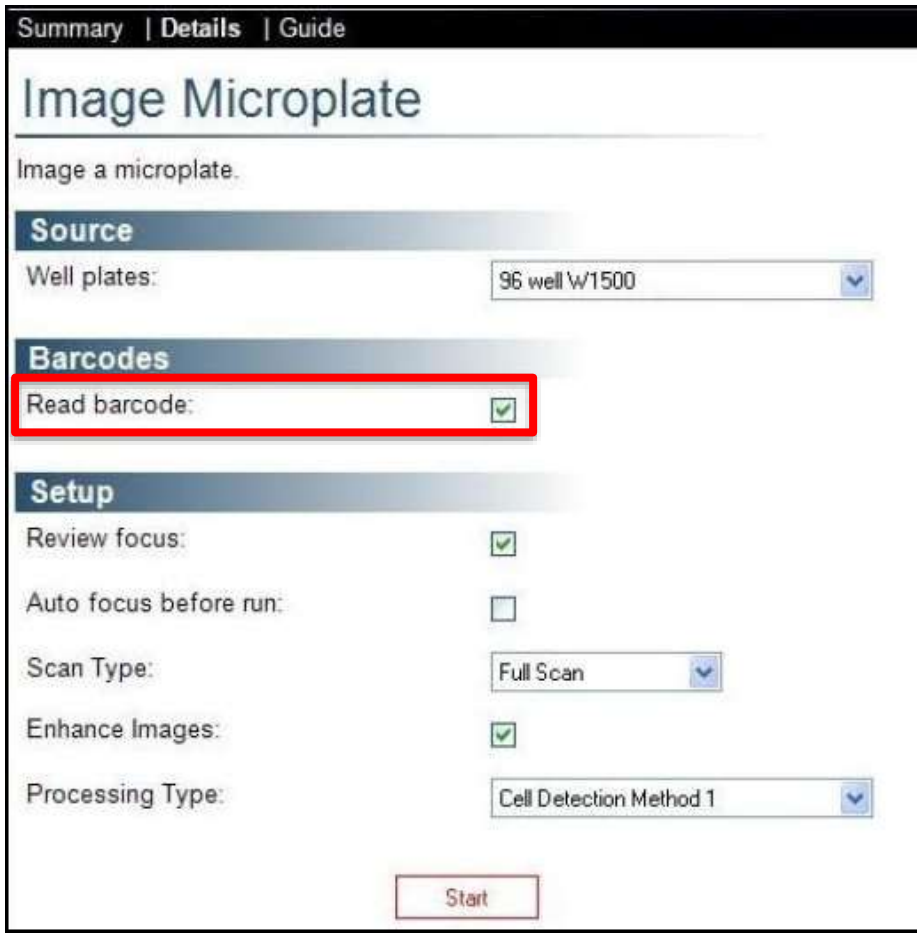


Choose Plate Type

- Click on the **Well plates dropdown** to select your desired **microplate type** for imaging. *If you do not see your chosen plate type in the list, please contact Molecular Devices Technical Support for assistance with adding to the list.*

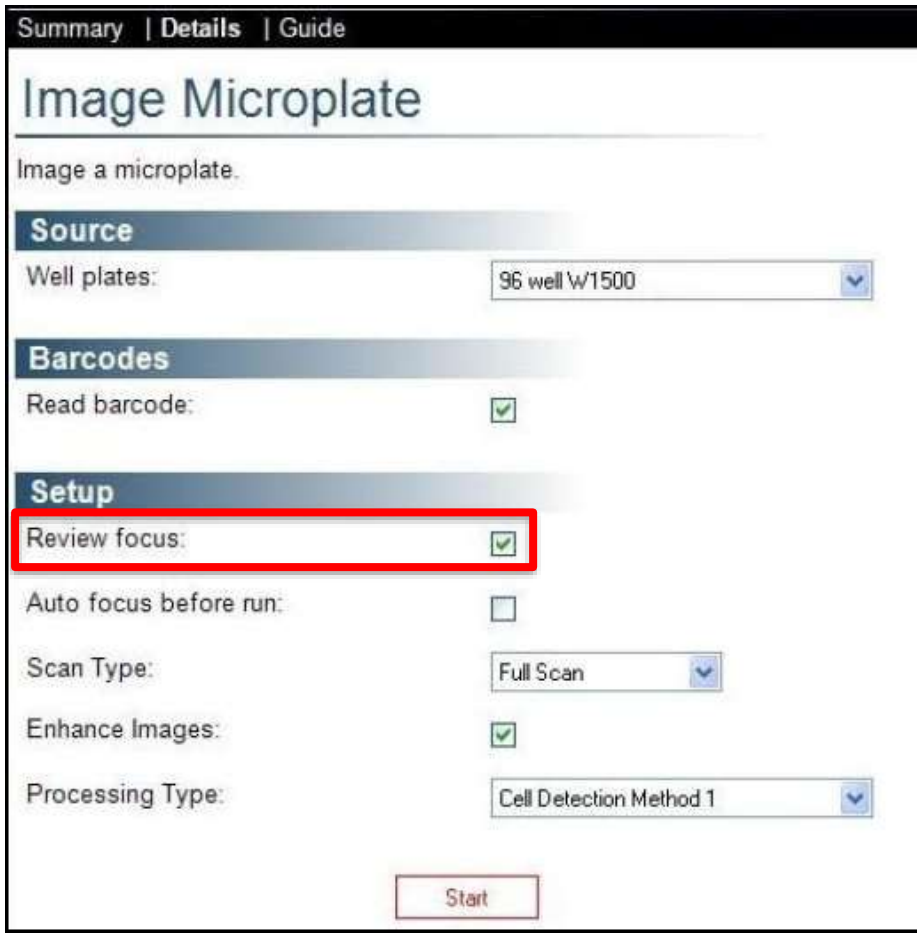


Select Barcode Reading Option



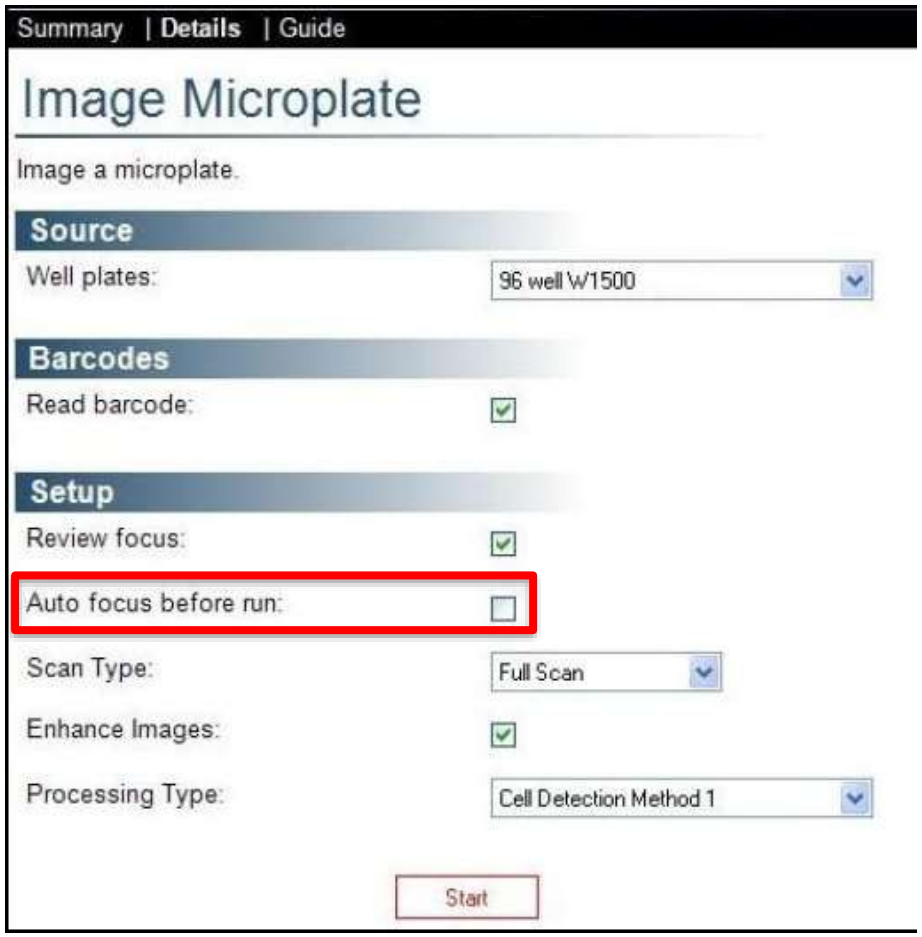
- Enable or disable **barcode reading** by **ticking this box**.
- If barcode reading is **enabled** and the instrument fails to read the barcode, or barcode reading is not enabled, a screen is displayed prompting for a **barcode/plate name** to be entered **manually**.
- The barcode/name can also be edited within **Review Results** at a later stage if required.
- ***NOTE: For the Monoclonality application, if you choose to manually enter a barcode you MUST enter the exact same barcode name each time you read the plate in order to track growth.***

Plate Setup Options: Review Focus



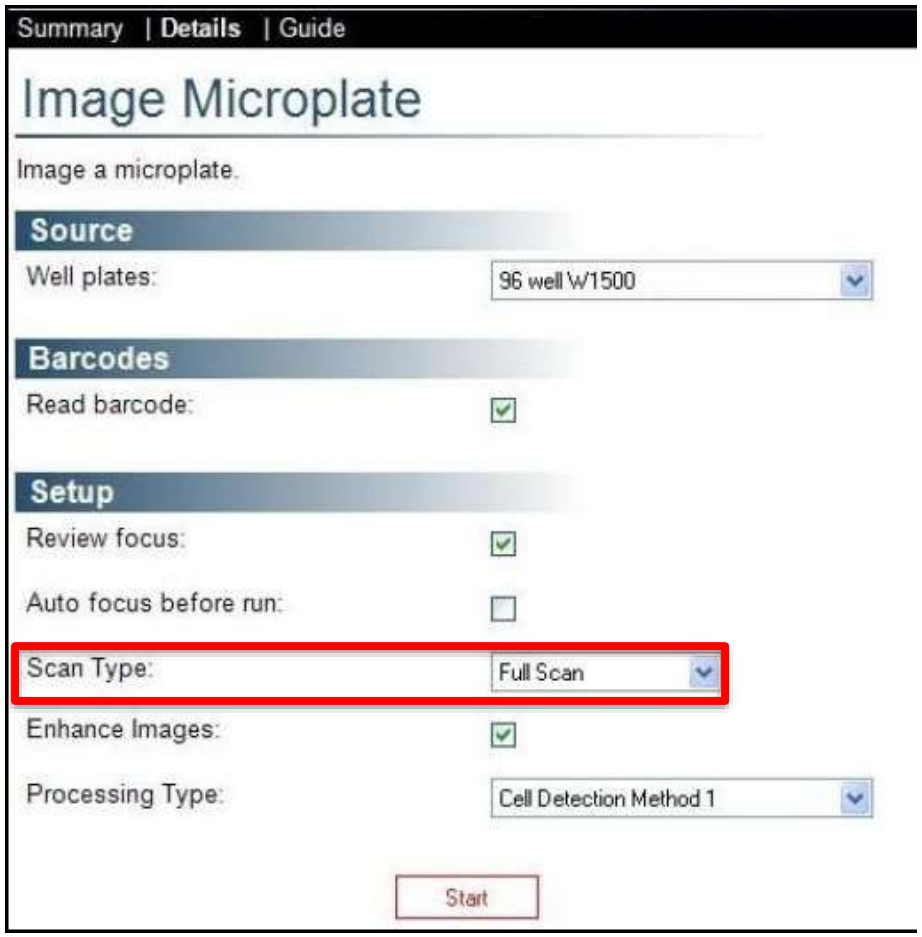
- Enable or disable the **Review Focus** option by **ticking this box**.
- If enabled the **Focus window** is displayed **before** the microplate is imaged to allow the **focus and brightness settings** to be adjusted before imaging a microplate (recommended).
- Note that individual images can also be **saved** from the **Focus** window.

Plate Setup Options: Auto Focus Before Run



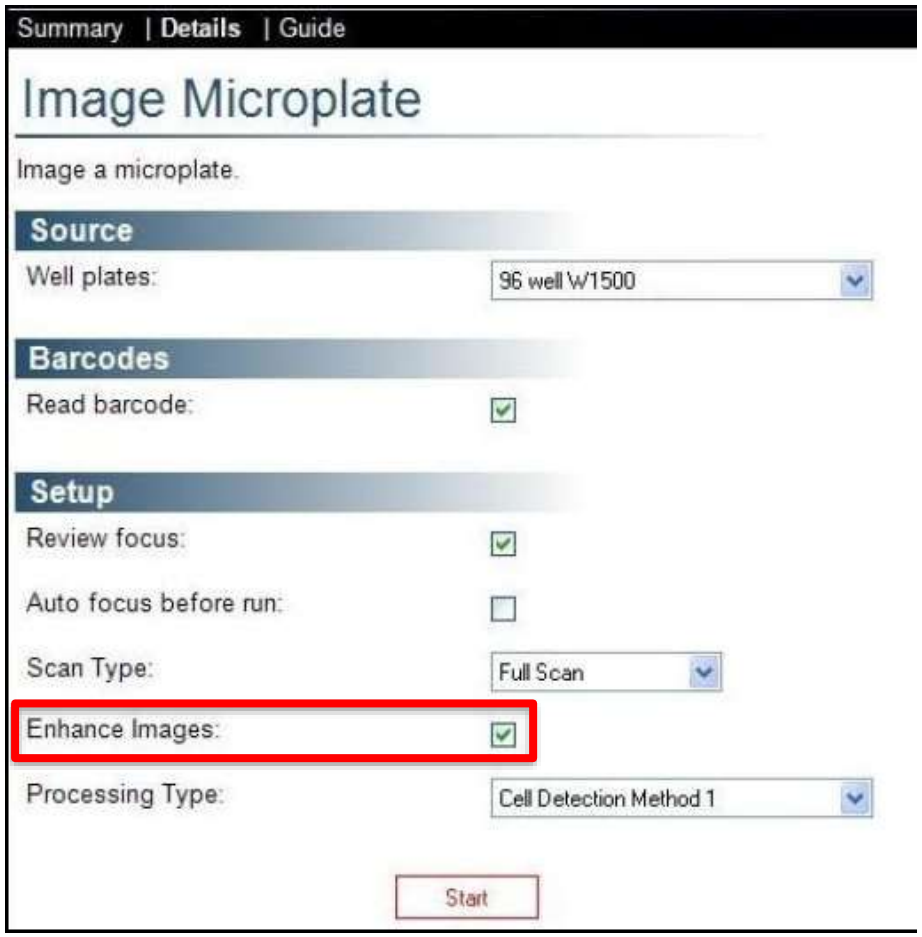
- Enable or disable the **Auto focus before run** option by **ticking this box**.
- If enabled the instrument carries out an **auto focus** procedure **before** imaging the microplate.
  - ***NOTE:*** *If you are working with a sample that **does not have wells with high contrast cells** included, we recommend leaving this feature **disabled**.*

Plate Setup Options: Scan Type



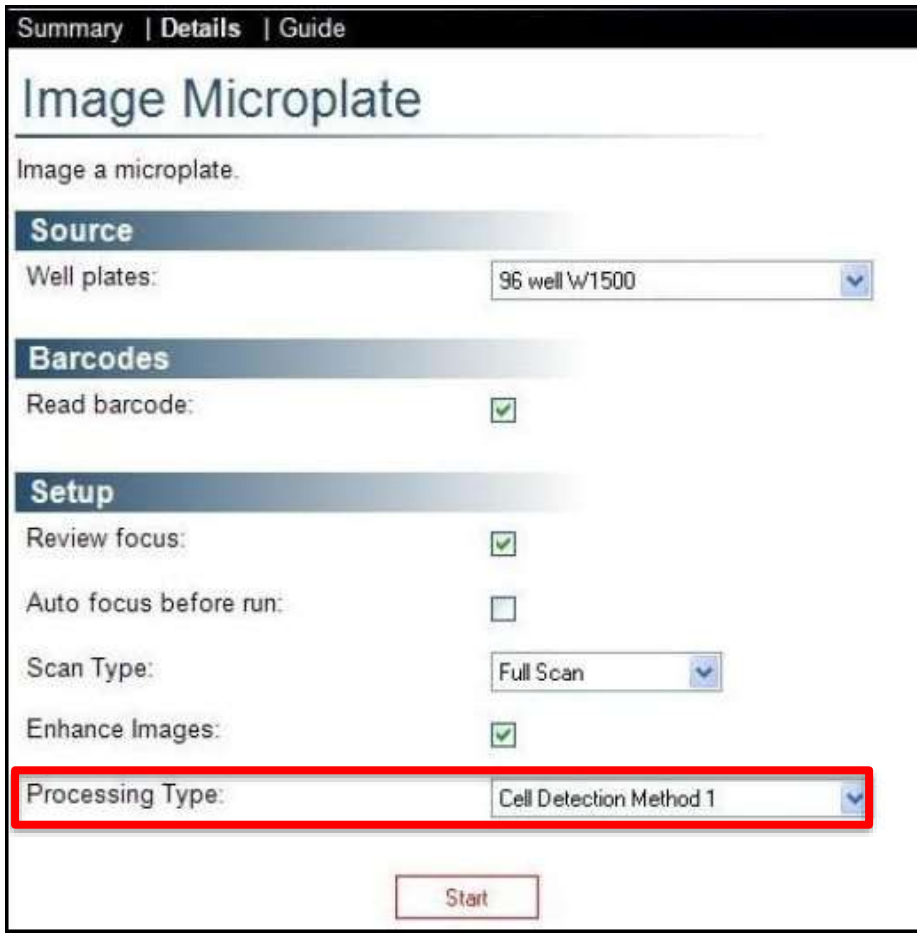
- Click on the the **Scan Type dropdown** to select well scanning options (**Full Scan** or **Partial Scan**).
- Select the **Full Scan** option to image the **entire area of each well** in the microplate. *NOTE: This setting is recommended for the Monoclonality application.*
- If the **Partial Scan** option is selected, an area from the center of each well is imaged. This speeds up imaging and is suitable when the data from the center of each well is representative of the well as a whole.

Plate Setup Options: Enhance Images



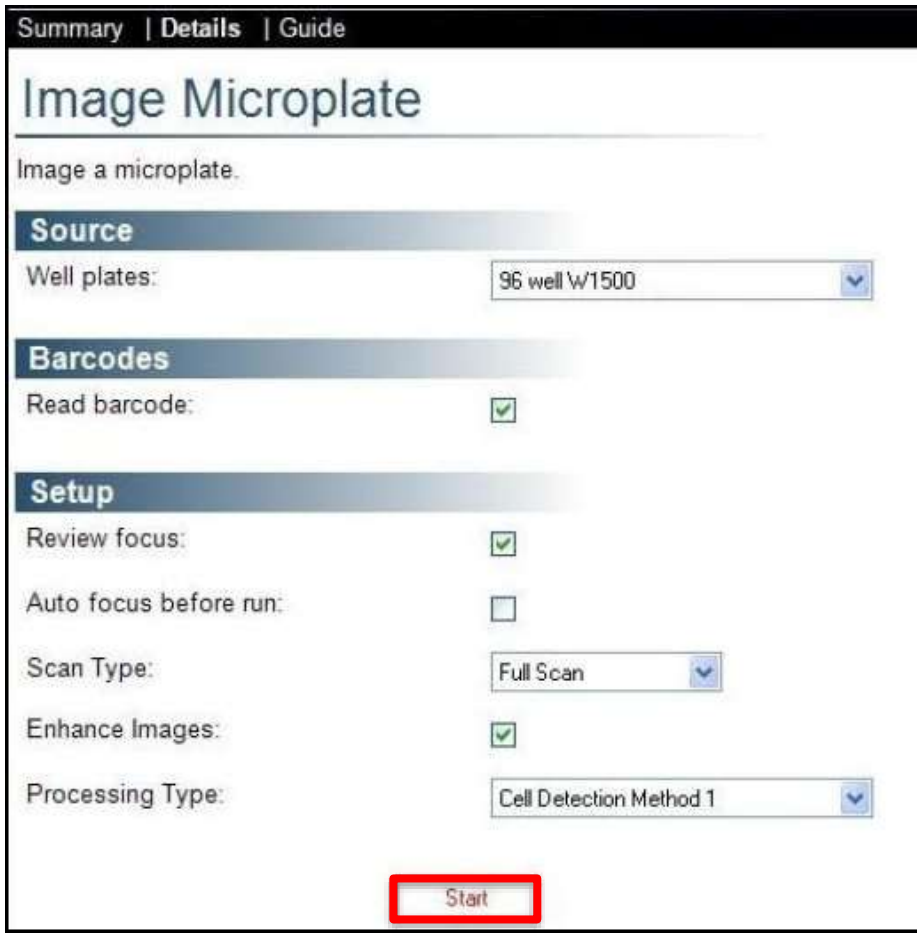
- Enable or disable the **Enhance Images** option by **ticking this box**.
- Selecting this option will **normalize / flatten the background** of the images to enhance them for display.
- *NOTE: Enabling this option is recommended for the **Monoclonality** application.*

Plate Setup Options: Processing Type



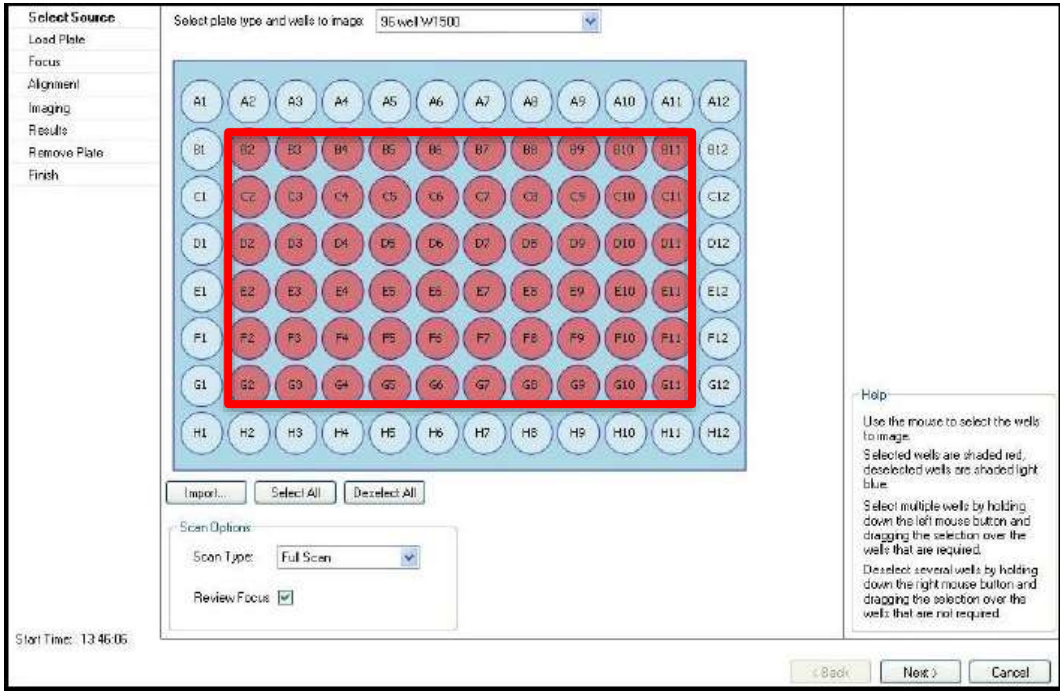
- You can select an image **processing (analysis) type** by selecting an option from this **dropdown**.
- **Cell Detection Method 1**: Otherwise known as 'Typical Adherent Cell Confluence', this algorithm is ideal for settled **suspension cells and adherent cells with good contrast.**
- **Cell Detection Method 2:** This algorithm helps to detect cell samples when **contrast is low**, or when **cells are clumped or at the edge of the well**.
- **Cell Detection Method 3**: This algorithm is designed to **detect colonies** and **distinguish them from any artifacts** around the **edges** of the colonies.
- ***NOTE: You can change your Processing Type choice once the process is started to optimize detection of your cells.***

Starting the Imaging Process



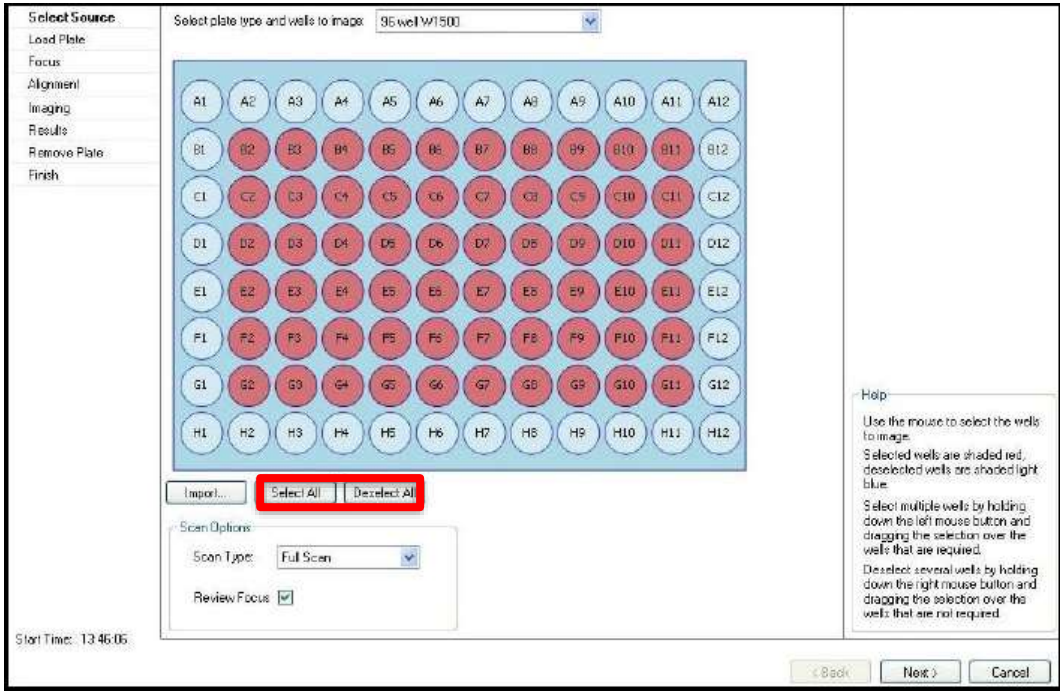
- Click on the **Start** button to launch the **microplate imaging process**.
- A **wizard** will now be launched that will allow you to further modify the settings that were selected in the **Image Microplate Details** dialog.

Select Source & Define Wells To Image



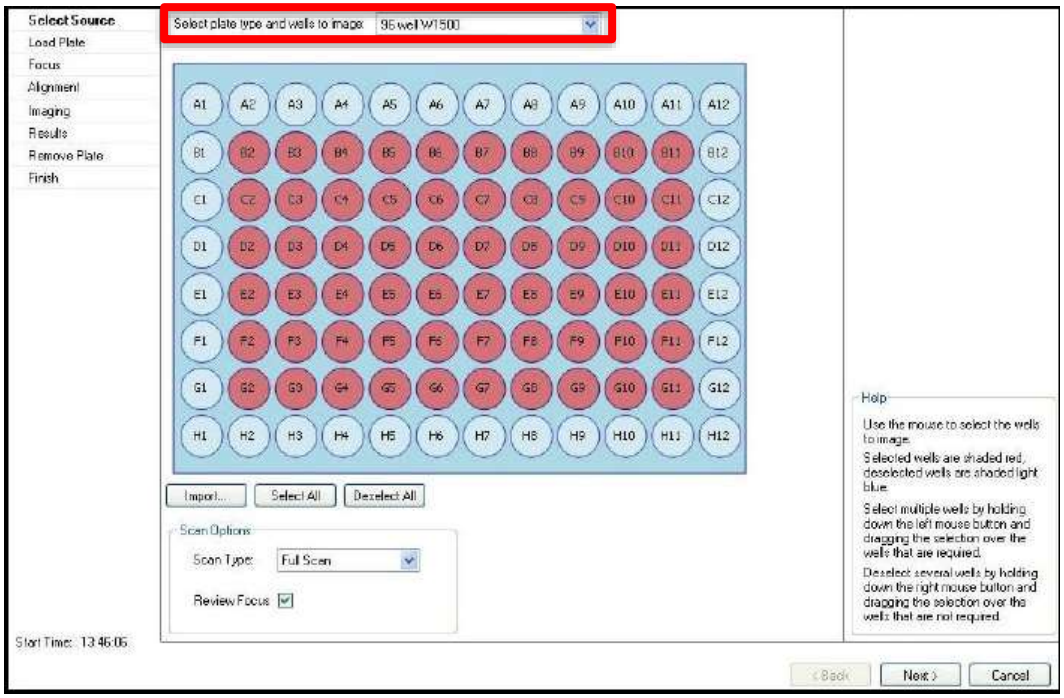
- In this dialog you can select the **wells to be imaged**.
- Wells **selected to be imaged** are shown in **red**, those **not selected for imaging** are shown in **light blue**.
- **Left click** on a well to **select** it and **right click** to **deselect**.
- **Groups** of wells can be **selected or deselected** by clicking and dragging over the wells.

Select Source & Define Wells To Image



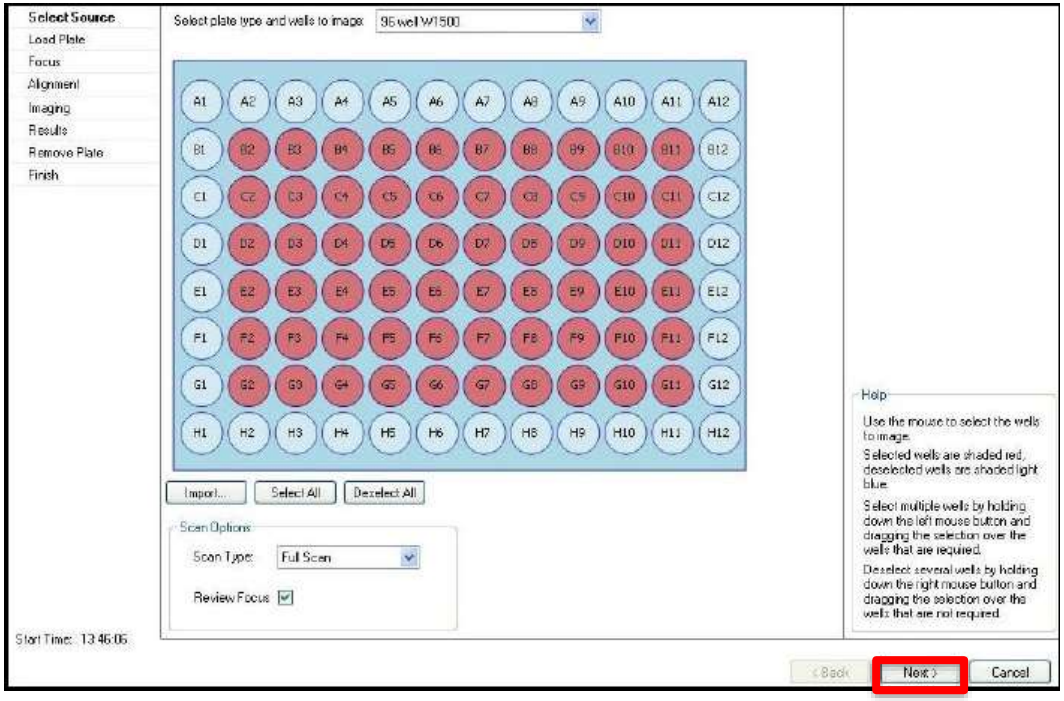
- In this dialog you can select the **wells to be imaged**.
- Wells **selected to be imaged** are shown in **red**, those **not selected for imaging** are shown in **light blue**.
- **Left click** on a well to **select** it and **right click** to **deselect**.
- **Groups** of wells can be **selected or deselected** by clicking and dragging over the wells.
- You can also choose to **select or deselect all wells** by clicking the appropriate button below the plate map.

Select Source & Define Wells To Image



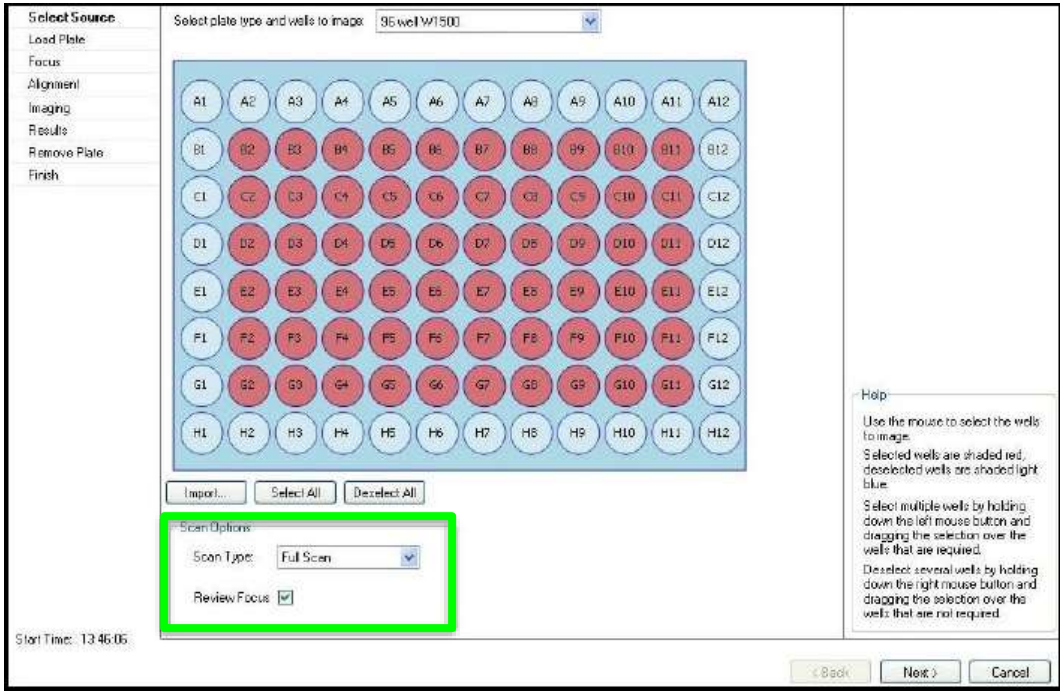
- In this dialog you can select the **wells to be imaged**.
- Wells **selected to be imaged** are shown in **red**, those **not selected for imaging** are shown in **light blue**.
- **Left click** on a well to **select** it and **right click** to **deselect**.
- **Groups** of wells can be **selected or deselected** by clicking and dragging over the wells.
- You can also choose to **select or deselect all wells** by clicking the appropriate button below the plate map.
- The **microplate type** to be imaged can be altered via the **Select plate type and wells to image drop down** list.

Select Source & Define Wells To Image



- In this dialog you can select the **wells to be imaged**.
- Wells **selected to be imaged** are shown in **red**, those **not selected for imaging** are shown in **light blue**.
- **Left click** on a well to **select** it and **right click** to **deselect**.
- **Groups** of wells can be **selected or deselected** by clicking and dragging over the wells.
- You can also choose to **select or deselect all wells** by clicking the appropriate button below the plate map.
- The **microplate type** to be imaged can be altered via the **Select plate type and wells to image drop down** list.
- Once your wells to image are defined, click the **Next** button to proceed.

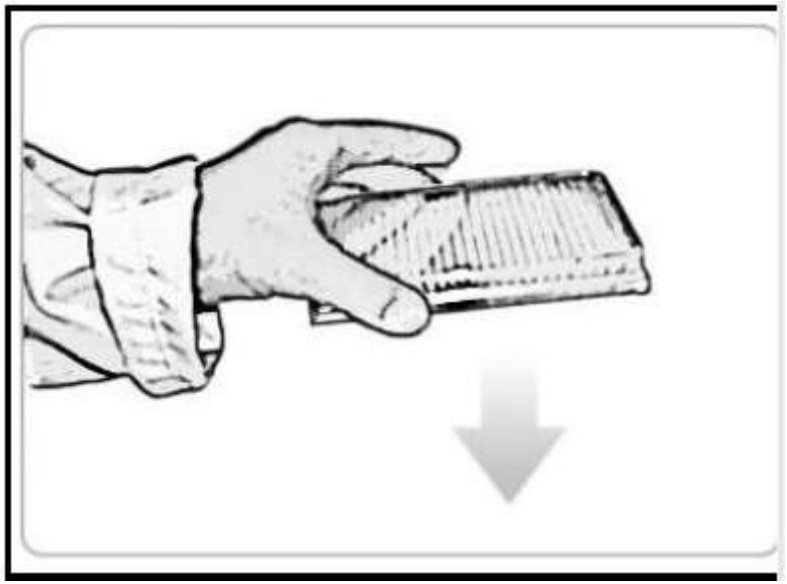
Select Source & Define Wells To Image



NOTE: You can change your Scan Type & Preview Focus options from this dialog as well if desired. For Monoclonality, be sure to choose Full Scan and select Preview Focus.

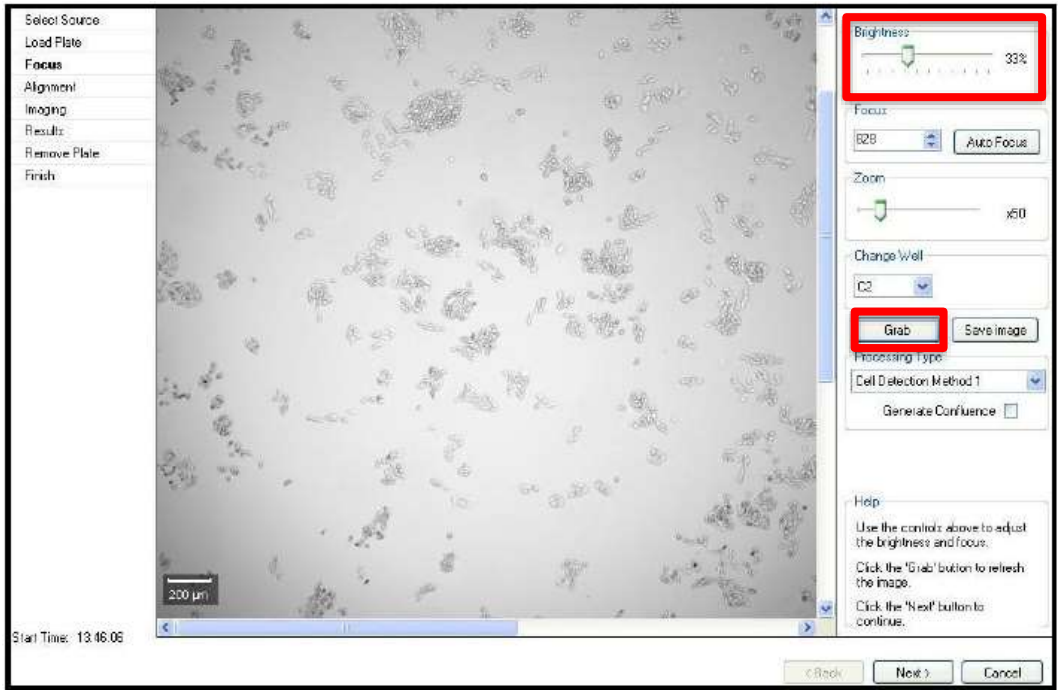
- In this dialog you can select the **wells to be imaged**.
- Wells **selected to be imaged** are shown in **red**, those **not selected for imaging** are shown in **light blue**.
- **Left click** on a well to **select** it and **right click** to **deselect**.
- **Groups** of wells can be **selected or deselected** by clicking and dragging over the wells.
- You can also choose to **select or deselect all wells** by clicking the appropriate button below the plate map.
- The **microplate type** to be imaged can be altered via the **Select plate type and wells to image drop down** list.
- Once your wells to image are defined, click the **Next** button to proceed.

Load Plate



- The **Load Plate** dialog appears.
- The **stage door** will **open** and the **plate carrier** will be **ejected**.
- Insert your plate, ensuring that **well A1** is aligned to the **back right corner** of the **plate carrier**.
- Click the **Next** button to **load the plate** and proceed.

Focus Dialog: Adjust Brightness Setting

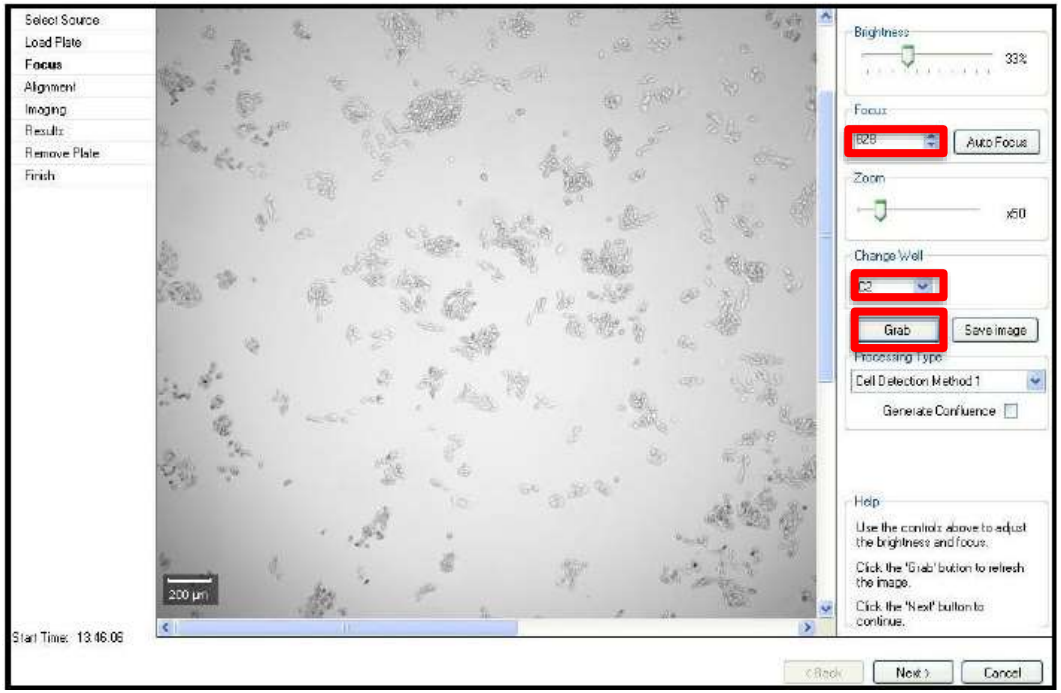


- The **Focus** dialog appears.
- **Brightness:** The software **automatically** determines the optimum brightness setting for imaging.
- This setting can be adjusted via the **Brightness slider**.
- After adjusting the brightness, you must click on the **Grab** button to retake the image using the new settings.
- **Saturated pixels** will be displayed in **red**, so it is important not to make the image too bright.

Focus Dialog: Adjust Focus Setting

- **Focus:** The system stores the **last focus setting** used for each **plate type**. After a plate type has been imaged for the first time the focus setting should not need to be altered significantly.
- After altering the focus setting, click the **Grab** button to **retake the image** using the **new setting**. The altered focus setting now becomes the default focus setting for the plate type being used in this run.
- Clicking the **Auto Focus** button will start the autofocus procedure. This may take a minute or two.

Focus Dialog: Adjust Focus Setting

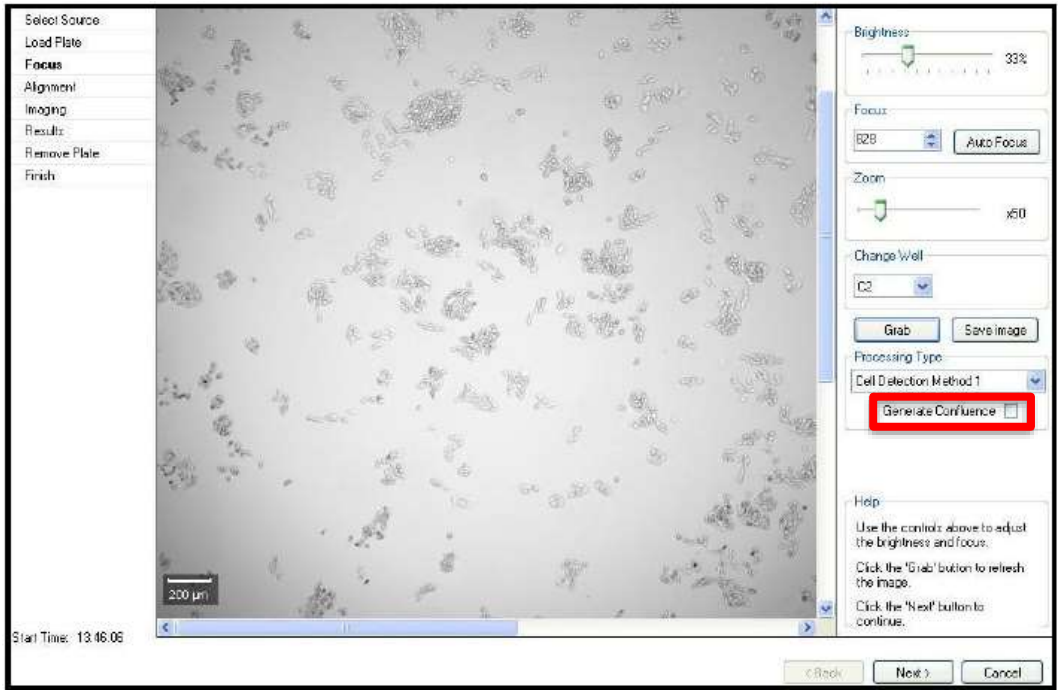


- **Focus:** The system stores the **last focus setting** used for each **plate type**. After a plate type has been imaged for the first time the focus setting should not need to be altered significantly.
- After altering the focus setting, click the **Grab** button to **retake the image** using the **new setting**. The altered focus setting now becomes the default focus setting for the plate type being used in this run.
- Clicking the **Auto Focus** button will start the autofocus procedure. This may take a minute or two.
- NOTE: If there is **debris** on the bottom of the plate or on the **glass plate carrier** the autofocus may focus on these instead of the cells. If this is the case, the focus will have to be adjusted **manually**. To avoid debris, you can also choose to change the **well to image** from the **Change Well** dropdown and clicking **Grab**.

Focus Dialog: Choose Processing Type & Enable Confluence Measurement

- **Processing Type:** This drop down menu displays the four **Cell Detection Methods** to choose from:
- **Cell Detection Method 1**: Otherwise known 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 helps to detect cell samples when **contrast is low**, or when cells are **clumped or at the edge of the well**.
- **Cell Detection Method 3**: This algorithm is designed to detect **colonies** and distinguish them from any artifacts around the edges of the colonies.
- **Very Low Contrast:** This algorithm is designed to detect cells with **very low inherent contrast**.

Focus Dialog: Choose Processing Type & Enable Confluence Measurement

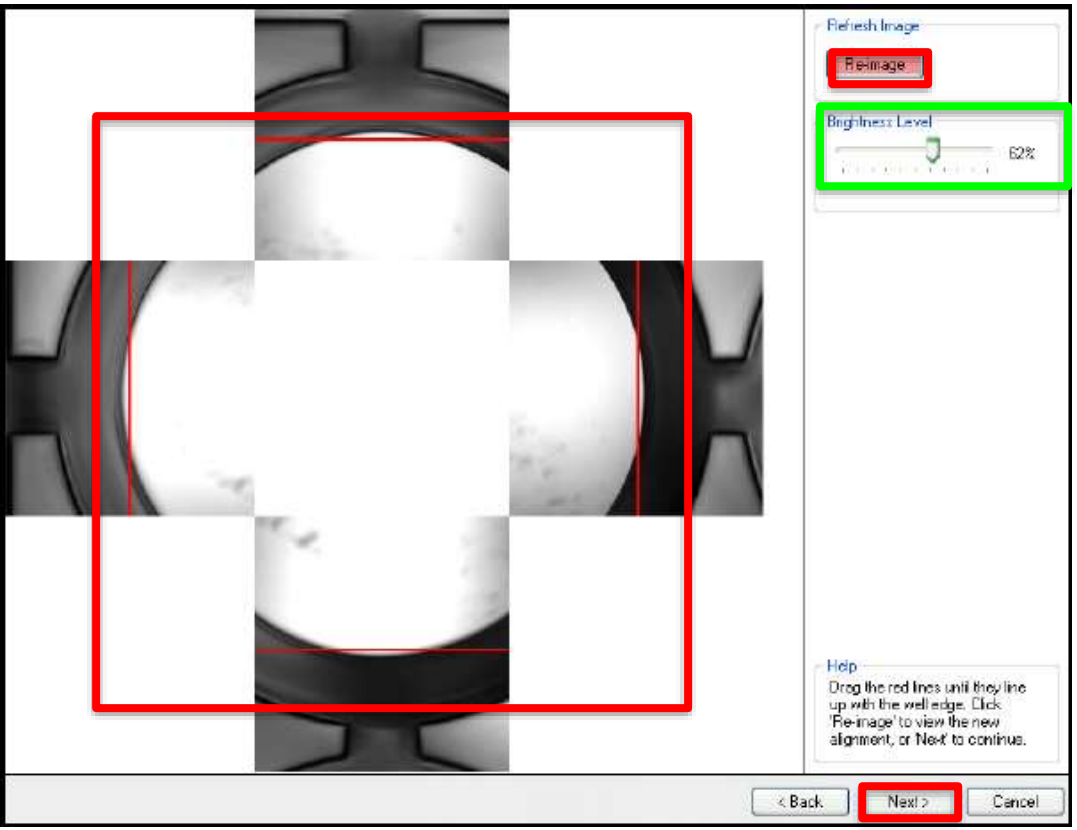


- **Processing Type:** This drop down menu displays the three **Cell Detection Methods** to choose from:
- **Cell Detection Method 1**: Otherwise known as 'Typical Adherent Cell Confluence', this algorithm is ideal for **settled suspension cells** and **adherent cells with good contrast**.
- **Cell Detection Method 2**: This algorithm helps to detect cell samples when **contrast is low**, or when cells are **clumped or at the edge of the well**.
- **Cell Detection Method 3**: This algorithm is designed to detect **colonies** and distinguish them from any artifacts around the edges of the colonies.
- **Generate Confluence:** Selecting this box will **identify and highlight in green (on the image)** all objects within the well (recommended).

Focus Dialog: Choose Processing Type & Enable Confluence Measurement

- **Processing Type:** This drop down menu displays the three **Cell Detection Methods** to choose from:
- **Cell Detection Method 1**: Otherwise known 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 helps to detect cell samples when **contrast is low**, or when cells are **clumped or at the edge of the well**.
- **Cell Detection Method 3**: This algorithm is designed to detect **colonies** and distinguish them from any artifacts around the edges of the colonies.
- **Generate Confluence:** Selecting this box will **identify and highlight in green** all objects within the well (recommended).
- Once your settings are optimized, click **Next** to proceed.

Optmizing Well Alignment for Imaging



- The **Alignment** dialog now appears.
- The **Alignment** window ensures that the **camera** is properly aligned 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 line up with the **four red lines**.
- If they **do not** line up, **clicking and dragging the lines** will correct the alignment.
- After making adjustments, click on the **Re-image button** to check the alignment.
- Click **Next** to proceed.

NOTE: You can change the brightness of the alignment image by clicking and dragging the Brightness Level slider. Note that this will not change the brightness level used during imaging of your plate.

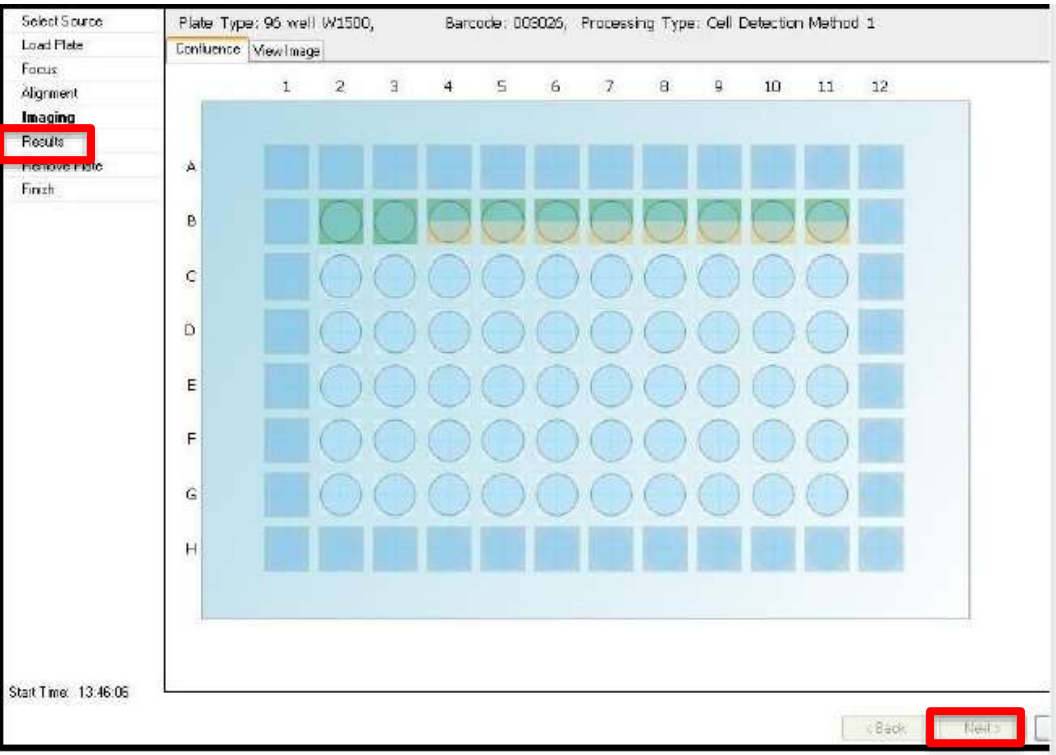
Plate Imaging

- The **Imaging** dialog now appears.
- During the imaging run, a **schematic** of the **plate** is displayed with the **image frames** superimposed in **light blue**.
- As images are captured and processed the **frame** changes color to **light brown** then **green**.
- Once a frame has changed to **green**, it is possible to click on it to display the **image** in the **View Image** tab.
- Clicking on the **Confluence** tab will return the screen to the **plate view**.
- Once imaging is complete, click **Next** to proceed to the **Results** view.

Plate Imaging

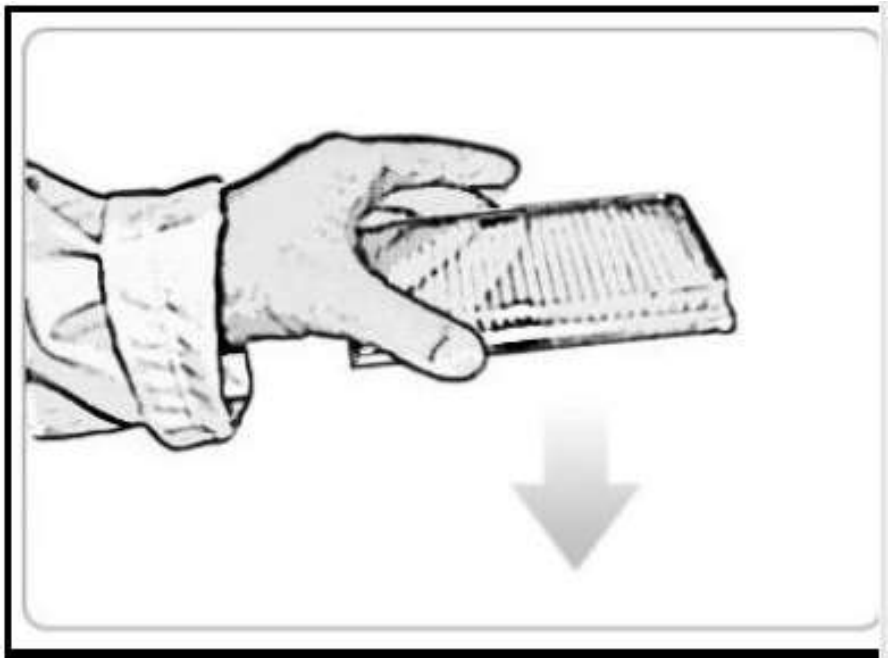
- The **Imaging** dialog now appears.
- During the imaging run, a **schematic** of the **plate** is displayed with the **image frames** superimposed in **light blue**.
- As images are captured and processed the **frame** changes color to **light brown** then **green**.
- Once a frame has changed to **green**, it is possible to click on it to display the **image** in the **View Image** tab.
- Clicking on the **Confluence** tab will return the screen to the **plate view**.
- Once imaging is complete, click **Next** to proceed to the **Results** view.

Results



- The **Results** view dialog appears.
- The **results** of the **Image Microplate process** are displayed.
- The images can be reviewed here or they can be viewed later via **Review Results** once the process has finished completely.
- Click **Next** to proceed to the **Remove Plate** step.

Remove Plate



- The **Remove Plate** dialog appears.
- When imaging is complete the **microplate** will be **ejected** from the system.
- Within this screen there is the option to **repeat** the **imaging with another plate** or move onto the **next step** of **ending the process**.
- **Image Another Plate**: Another plate can be imaged using the same configuration. To do this, click on the **Image Another Plate button** instead of Next.
  - The **plate carrier** remains in the **ejected** position and the **Select Source** screen is displayed ready to continue.
- Once imaging is complete, click **Finish** to **end the process.**

Saving Imaging Process & Closing Process

- Once the imaging process is **finished**, you will be returned to the **Image Microplate** screen.
- Here you can save your current process by clicking on the **Save process** option.
- Click on the **Close Process** option to close the current process and return to the **Main Navigation** screen.

Support Resources

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

MOLECULAR DEVICES

ADVANCING PROTEIN AND CELL BIOLOGY