

Clone Select Imager Training Guide

Plate Imaging



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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.



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Image Microplate Image a microplate	Ratch Hiccopiate Imaging Insige bitches of plates with the same configuration.	
Review Results Review the results of previous imaging runs	E-Scope Take single Nires images of your micropiate	
Maintenance Piocesses		
Plate Alignment Adjust the alignment of a plate in the system	Eject Plate Carrier Ejects the blate carrier for maintenance. [cleaning the glats atc]	
Utility Processes		
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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.

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Image a microplate.	Save process as template.
Source	Create New Process
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Barcodes	📴 Fron template .
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Setup	
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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.

Image Microplate		
Image a microplate.		
Source		
Well plates:	96 well W1500	*
Barcodes		
Read barcode:		
Setup		
Review focus:	V	
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	*





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.*

Summary Details Guide		
Image Microplate		
Image a microplate.		
Source		
Well plates:	96 well W1500	*
Barcodes		
Read barcode:	V	
Setup		
Review focus:	V	
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	*





Select Barcode Reading Option

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Well plates:	96 well W1500	*
Barcodes		
Read barcode:		
Setup		
Review focus:	V	
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	~

- 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

Image Microplate		
Image a microplate.		
Source		
Well plates:	96 well W1500	*
Barcodes		
Read barcode:	V	
Setup		
Review focus:		
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	~

- 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

image a microplate.		
Source		
Well plates:	96 well W1500	~
Barcodes		
Read barcode:		
Setup		
Review focus:		
Auto focus before run:		
Scan Type:	Full Scan 🔛	
Enhance Images:		
Processing Type:	Cell Detection Method 1	~

- 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

Image a microplate.		
Source		
Well plates:	96 well W1500	*
Barcodes		
Read barcode:		
Setup		
Review focus:	V	
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	~

- 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

Image a microplate		
Source		
Well plates:	96 well W1500	*
Barcodes		
Read barcode:		
Setup		
Review focus:		
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	~

- 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

Image a microplate.		
Source		
Well plates:	96 well W1500	*
Barcodes		
Read barcode:		
Setup		
Review focus:	V	
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	~

- 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

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image a microplate.		
Source		
Well plates:	96 well W1500	~
Barcodes		
Read barcode:		
Setup		
Review focus:		
Auto focus before run:		
Scan Type:	Full Scan 💌	
Enhance Images:		
Processing Type:	Cell Detection Method 1	~

- 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.







- 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.







- 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.







- 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.







- 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.







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



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.

- 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.





Plate Imaging

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- 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

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- 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

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- 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.

Summary Details Guide					
Image Microplate	Current Process				
Image a microplate.	Save piccase as template.				
Source	Create New Process				
Well plates: 96 well W1800	Select from categories				
Barcodes	😇 Fion template .				
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Setup					
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Support Resources

- Go to the HELP menu within CSI Software
- Support and Knowledge Base: <u>http://mdc.custhelp.com/</u>
- Request Support: <u>http://mdc.custhelp.com/app/ask</u> or via email <u>support@moldev.com</u>
- 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

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