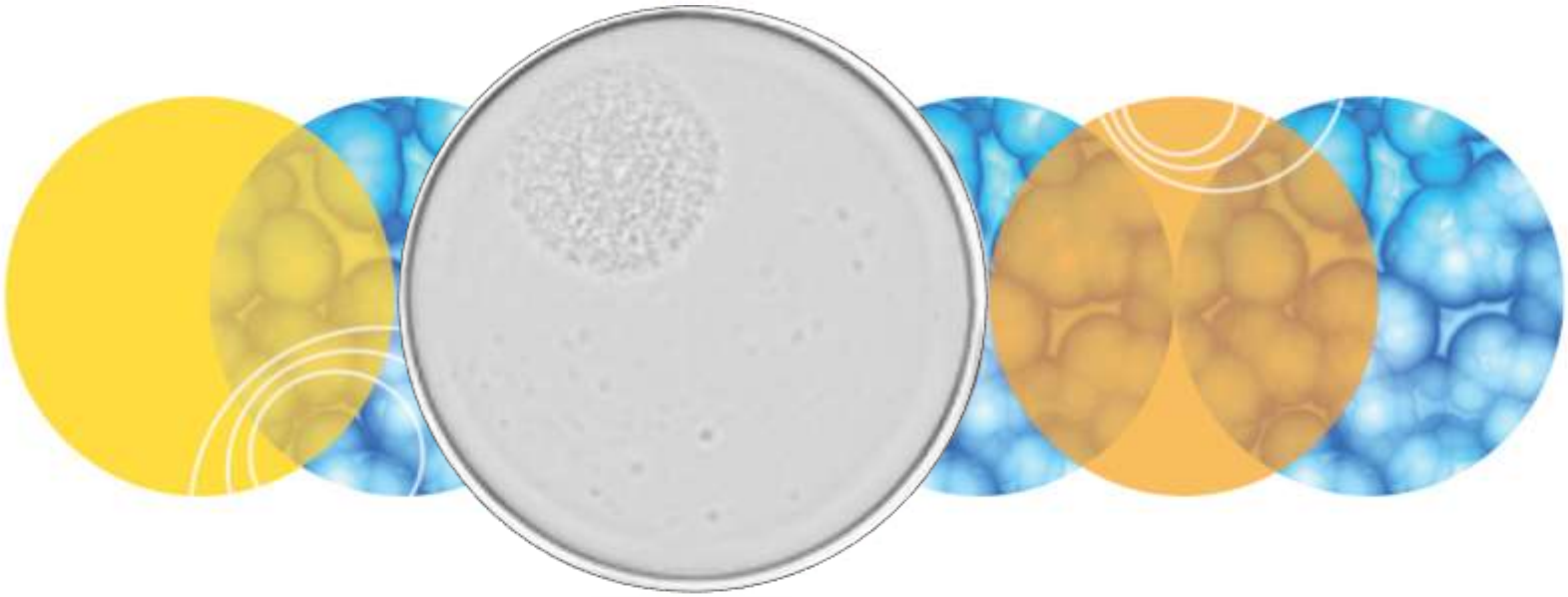


# CloneSelect Imager Training Guide

## Analyzing Data Post-Imaging

MOLECULAR DEVICES
UNLEASH YOUR BRILLIANCE™

Date Revised 3/22/2017 Version A

# Index

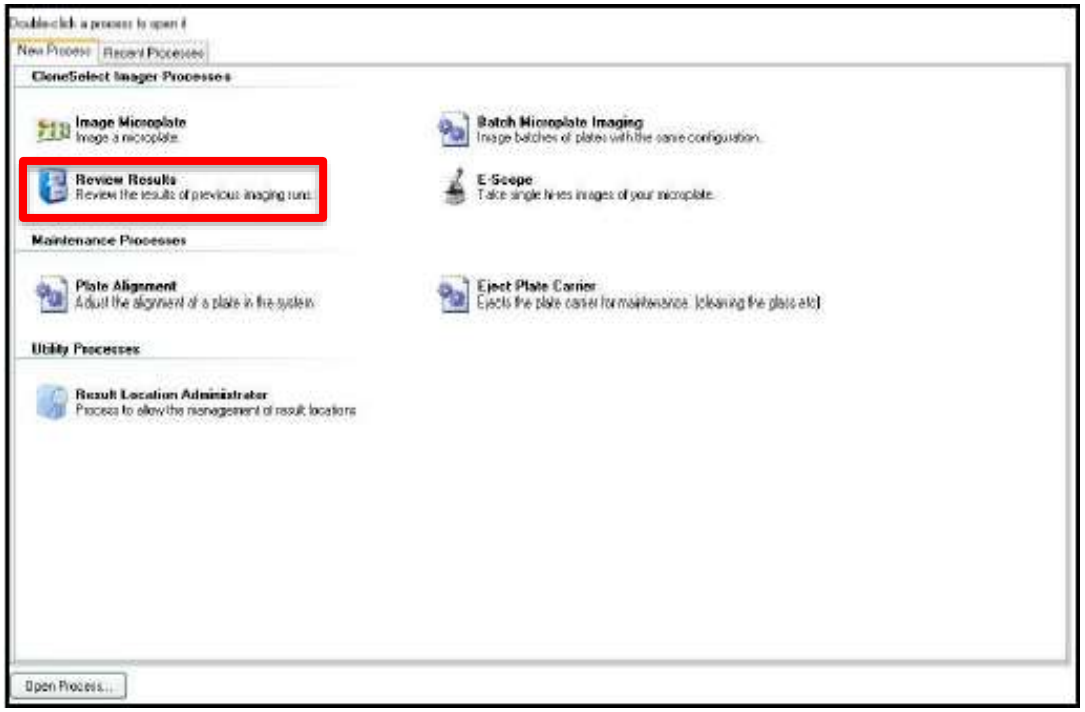
# Chapter Purpose

The purpose of this chapter is to illustrate the post-imaging analysis workflow for the CloneSelect Imager.

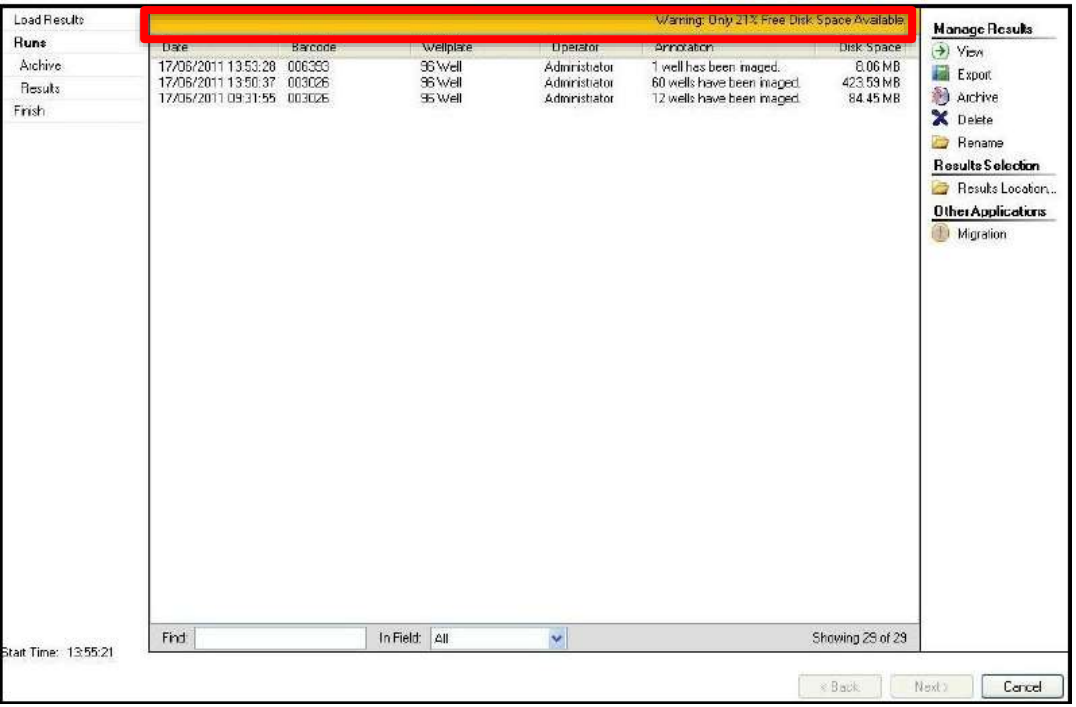
This guide does not include detailed descriptions around the CSI hardware or other specific imaging/analysis applications.  Please refer to corresponding chapters for details on these topics.

# Review Results from Previous Experiment

- To review results of your experiment, click on the **Review Results icon** to select your plate. Note that the **Review Results** dialog also automatically opens at the conclusion of an **imaging process** – this step is only necessary to review and **perform further analysis steps on data from previous experiments**.

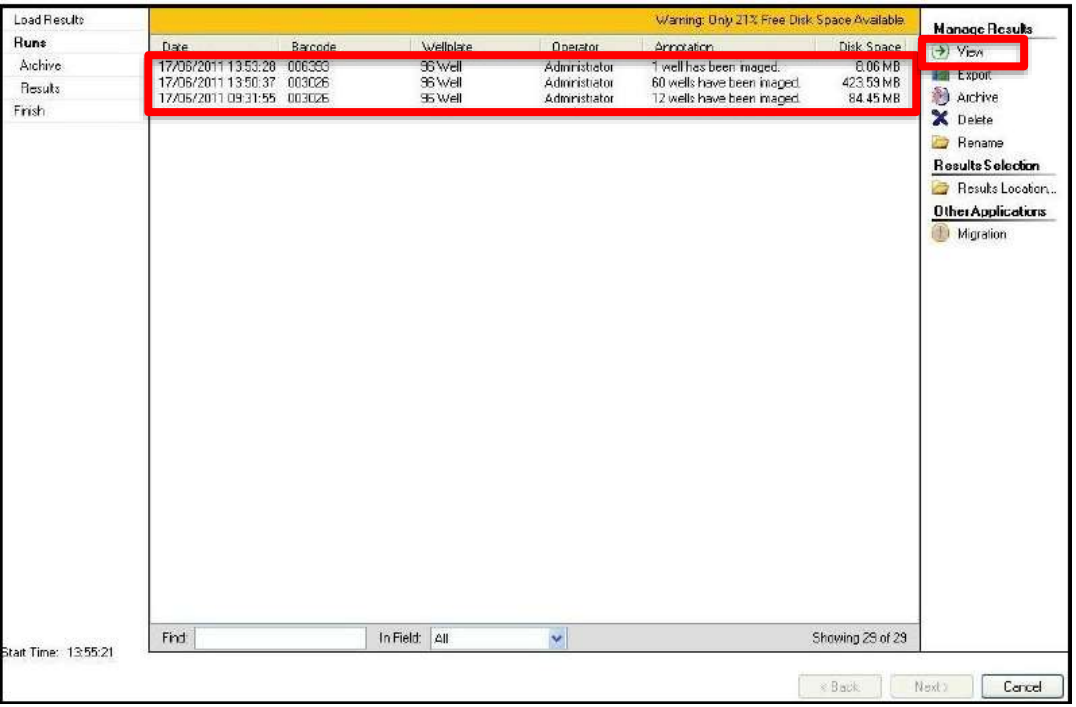


# About the Load Results Dialog




- The **Load Results** dialog appears.
- The **default location** where imaging results are stored is the **'C:\Image Archive'** folder.
- All of the **image and data files** for each individual run are stored in a **folder** that is named with the **date and time stamp** for the run, e.g. 2005-11-01T11-52-10.
- Within the **Image Archive** folder, the **run folders** are automatically organized into **separate folders for each month**, e.g. 2006-10.
- When opening the **Review Results** process on the CloneSelect Imager the user is directed straight to the results.
- Plates imaged on the system are displayed as a **list**.
- 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**.

# Select Results to View



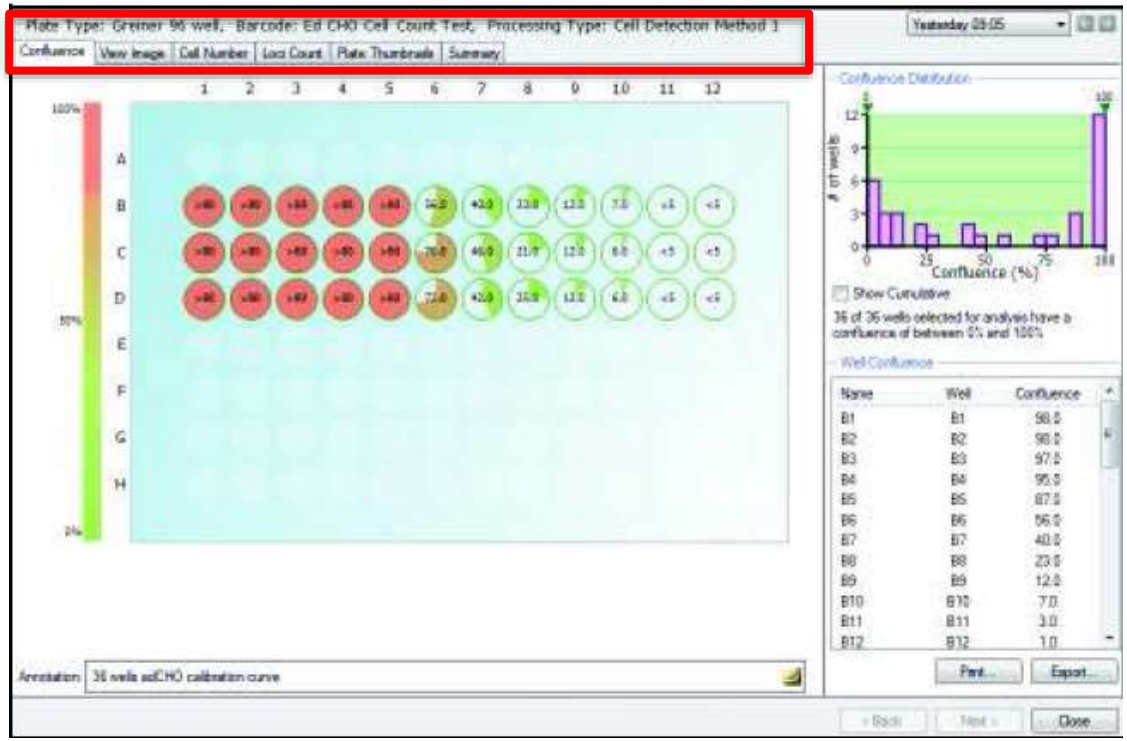

- To view **results** either **double click on the data set of interest** in the list or click to **highlight the data** and then click **View**.
- **Multiple data sets** can be selected by **holding down the Shift or Control keys** while clicking the data of interest to select contiguous or noncontiguous sets of data, respectively, then click **View**.
- ***NOTE: Selecting multiple data sets will be required to proceed with Monoclonality tracking.***

# Analyzing Results

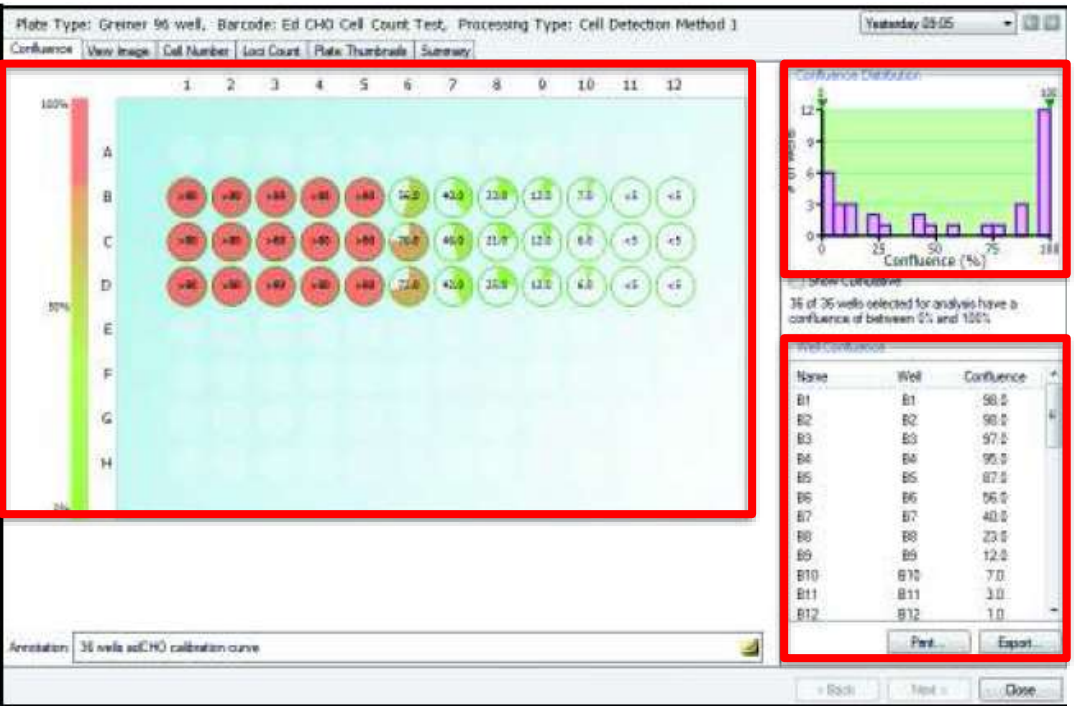
- Once an imaging process has completed the results are displayed in the **Results window**, which has several **tabs**: **Confluence, View Image, Cell Number, Loci Count, Plate Thumbnails, Growth Rate** and **Summary**.

- This window opens with the **Confluence tab displayed by default**.

- The **Plate Type, Barcode and Processing Type** are all displayed **above** all the tabs to make it clear which data set is being viewed throughout the processing.

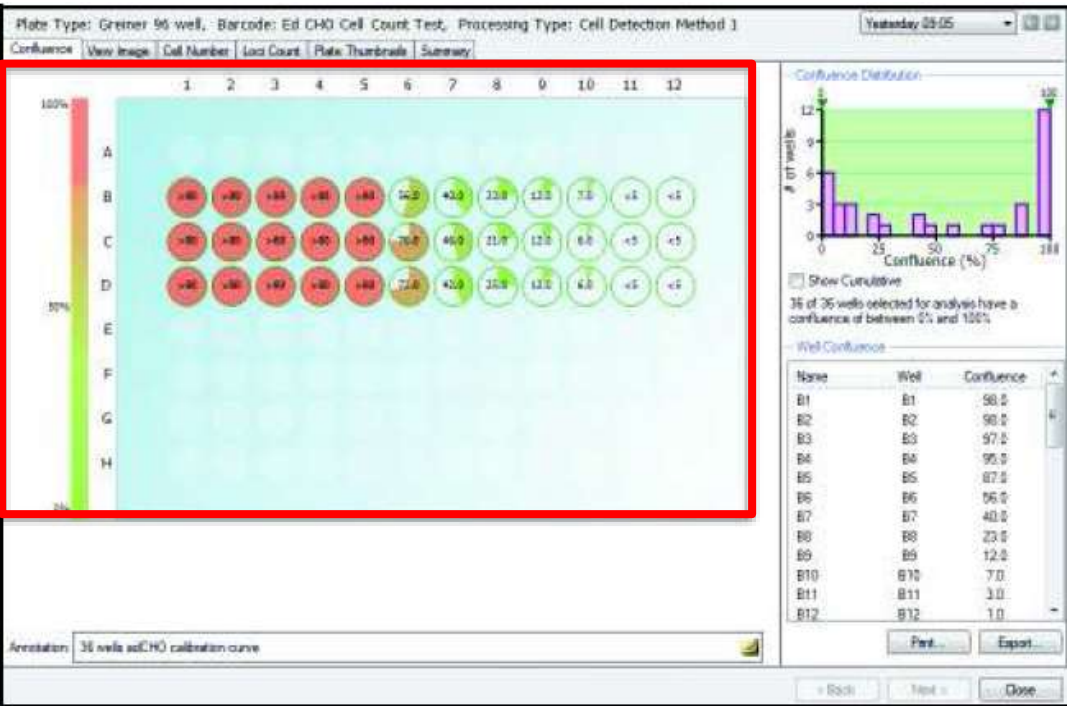


# Confluence Tab



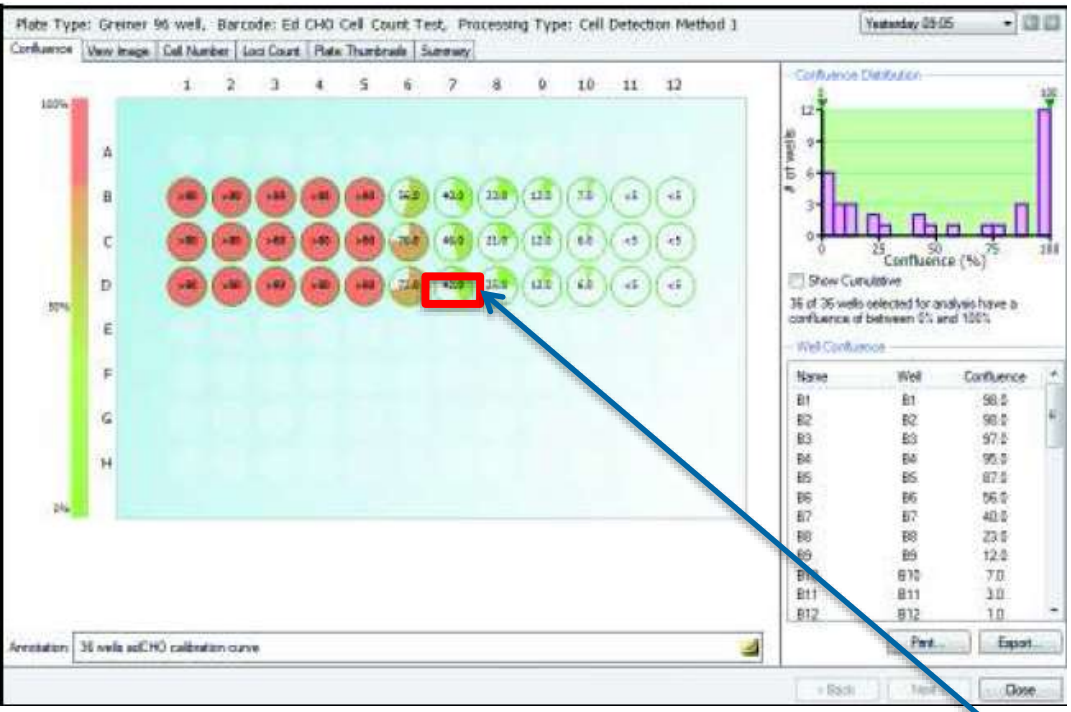

- The Confluence tab displays the **confluence level** for each **well**, both **graphically** and as a **list**.

# Confluence Tab – Pie Chart Area




- The Confluence tab displays the **confluence level** for each **well**, both **graphically** and as a **list**.
- 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.
- **Hovering the mouse** over the pie chart area causes the **percentage confluence** for all the wells to be displayed.
- **Confluence** that is detected as **lower than 5 percent is displayed at <5** and **confluence above 80 percent is**
- **displayed as >80**.

# Confluence Tab – Pie Chart Area





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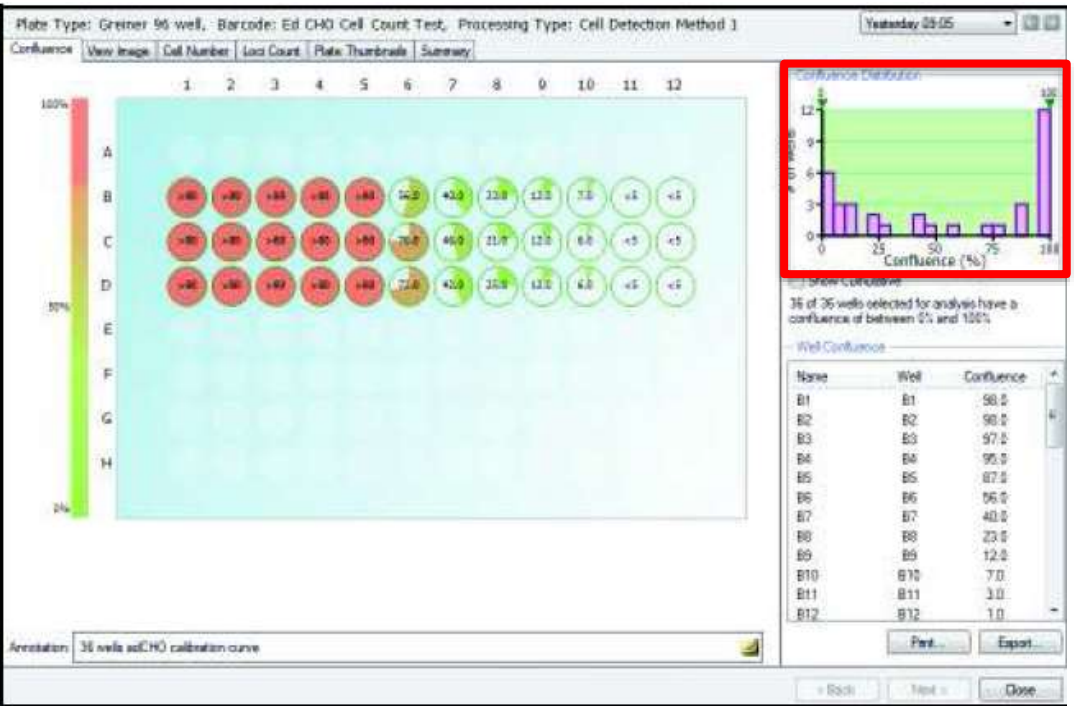
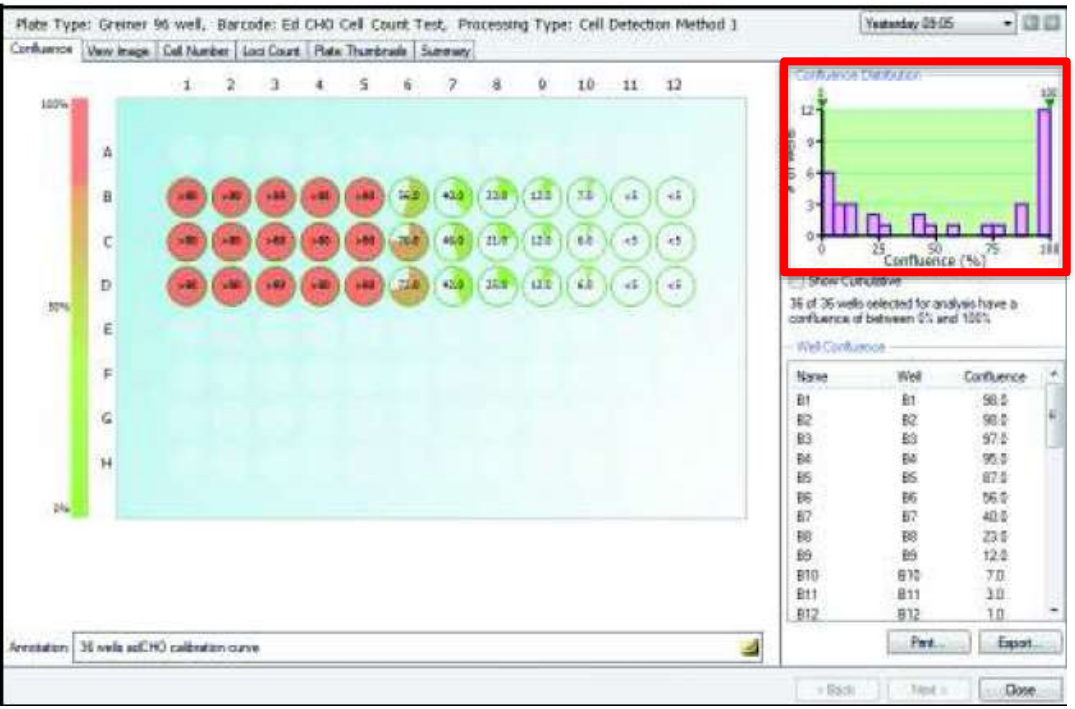
- **Hovering the mouse** over the pie chart area causes the **percentage confluence** for all the wells to be displayed.
- **Confluence** that is detected as **lower than 5 percent is displayed at <5** and **confluence above 80 percent is displayed as >80**.
- Hovering over a well causes a **tool tip** to appear displaying the **well co-ordinate** and the **confluence** for that well.
- Clicking on a well will display the **well image** in the **View Image** tab.
- **Right clicking** on the overview will allow the **overview to be copied to clipboard**.

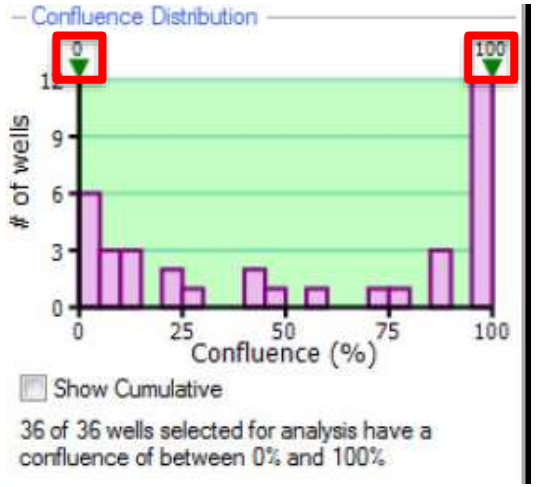
# Confluence Tab – Confluence Distribution




- The **bar chart** at the top right of the Confluence tab window plots the **number of wells with a given confluence level**.

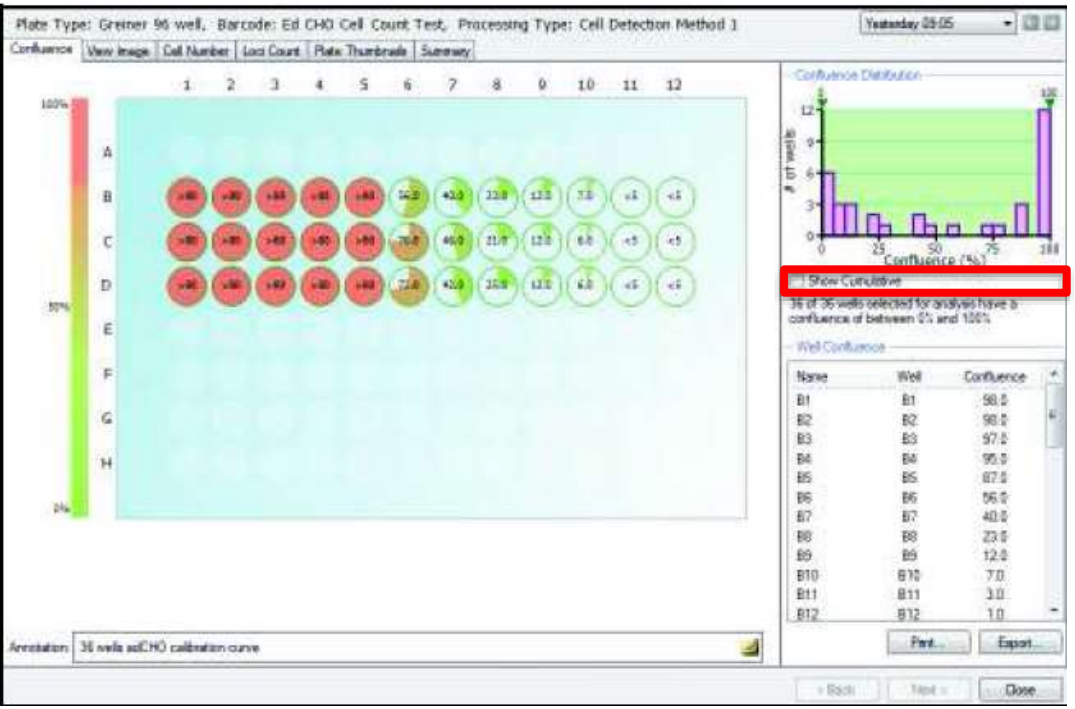
# Confluence Tab – Confluence Distribution



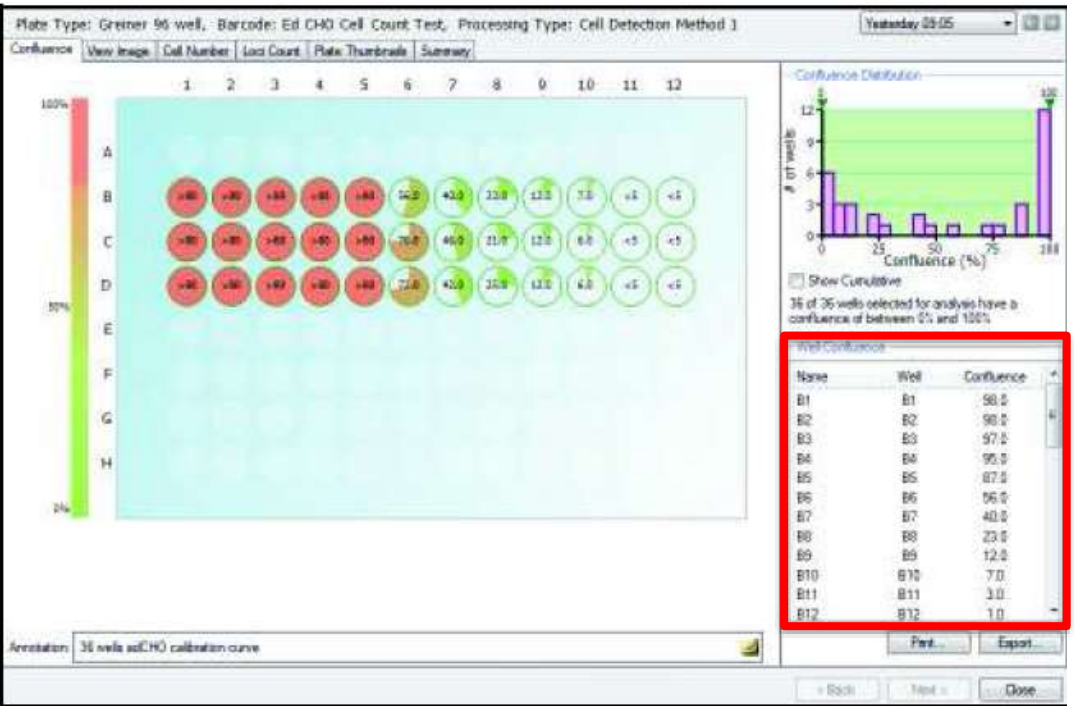

- The **bar chart** at the top right of the Confluence tab window plots the **number of wells with a given confluence level**.
- **Dual Gates:** The **green arrows** pointing down at the **top of the bar chart** can be dragged across the chart to select a **subset range** of confluence data to display.
  - Any wells that do not fall inside these lower and upper gates will be **grayed out.**
  - The **text** displayed **below** the chart will update to show the **number of wells that fall within the selected confluence range**.

# Confluence Tab – Confluence Distribution



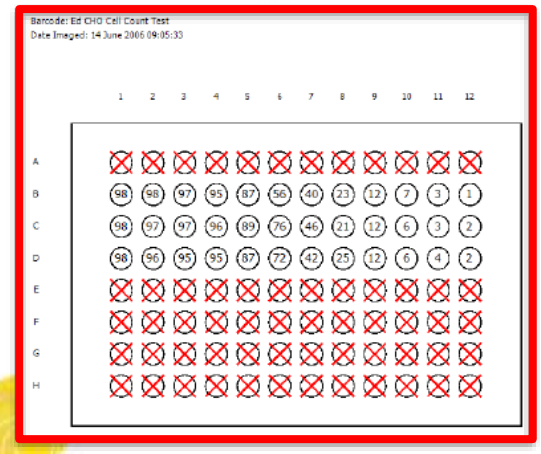

- The **bar chart** at the top right of the Confluence tab window plots the **number of wells with a given confluence level**.
- **Dual Gates:** The **green arrows** pointing down at the **top of the bar chart** can be dragged across the chart to select a **subset range** of confluence data to display.
  - Any wells that do not fall inside these lower and upper gates will be **grayed out.**
  - The **text** displayed **below** the chart will update to show the **number of wells that fall within the selected confluence range**.
- **Show Cumulative**: Select this option to **merge** the individual bars in the chart to show the confluence as **continuous data**.

# Confluence Tab – Well Confluence



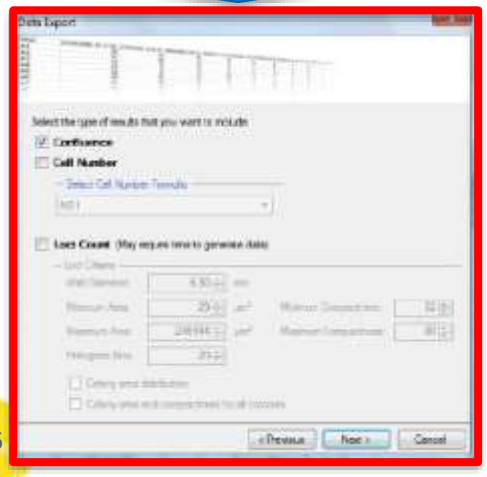

- The confluence for each well is displayed in a **list** format witih the **Well Confluence field** on the Confluence tab.
- If desired the **well name** can be **changed** in this list by **clicking twice on the well name** of choice and editing the text.

# Confluence Tab – Well Confluence


- The confluence for each well is displayed in a **list** format witih the **Well Confluence field** on the Confluence tab.
- If desired the **well name** can be **changed** in this list by **clicking twice on the well name** of choice and editing the text.
- **Print**: This option will print a **schematic presentation of the confluence overview in the plate format**. The **confluence values** are displayed **within the wells** and the **plate details** are displayed at the **top of the overview.**

# Confluence Tab – Well Confluence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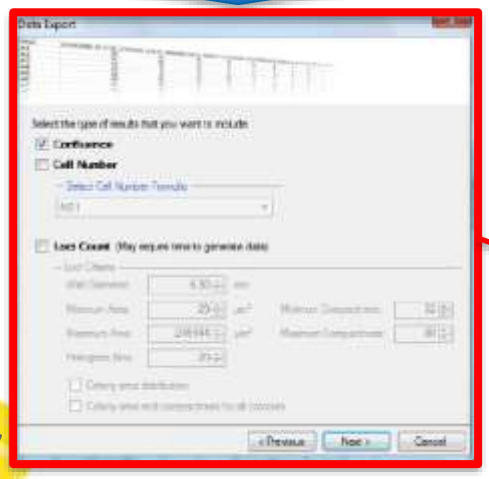
- **Export:** Launches a **Data Export wizard** that enables export of the **list of wells** and the corresponding **confluence** as a **.csv or .xml file**.
  - **Cell Number** and **Loci Count (if feature is enabled)** data can also be exported at this point.

# Confluence Tab – Well Confluence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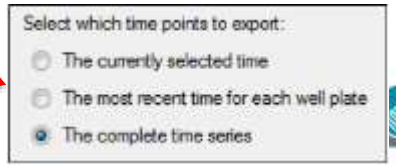
  - The wizard provides the option to export the data for the following **time points**:



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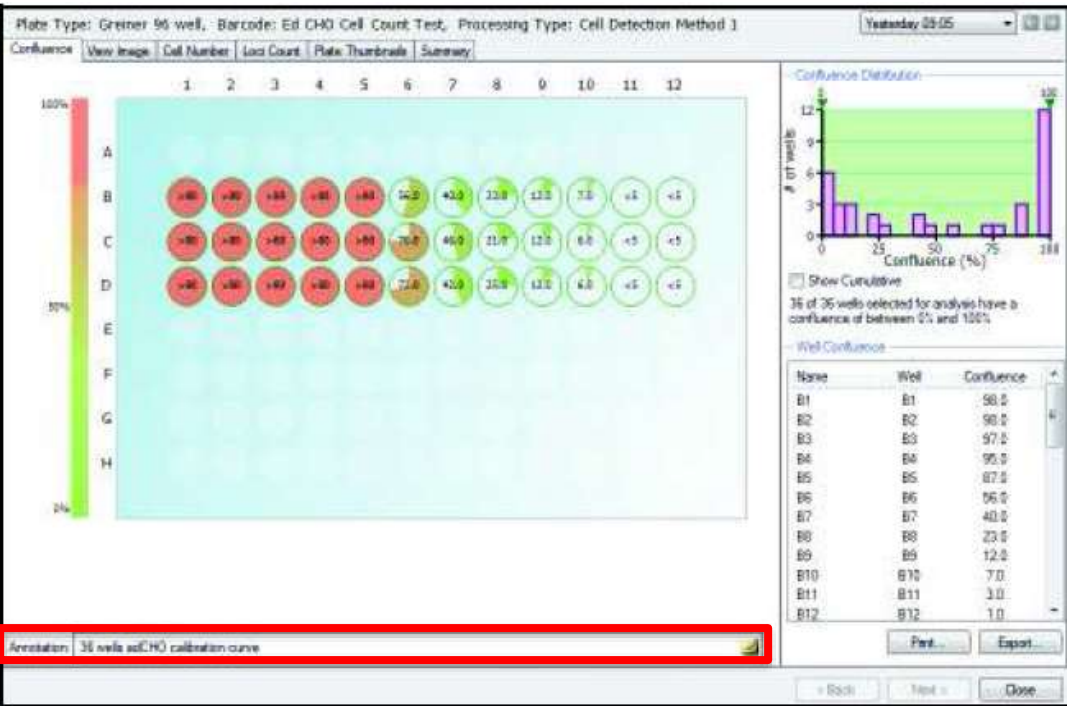


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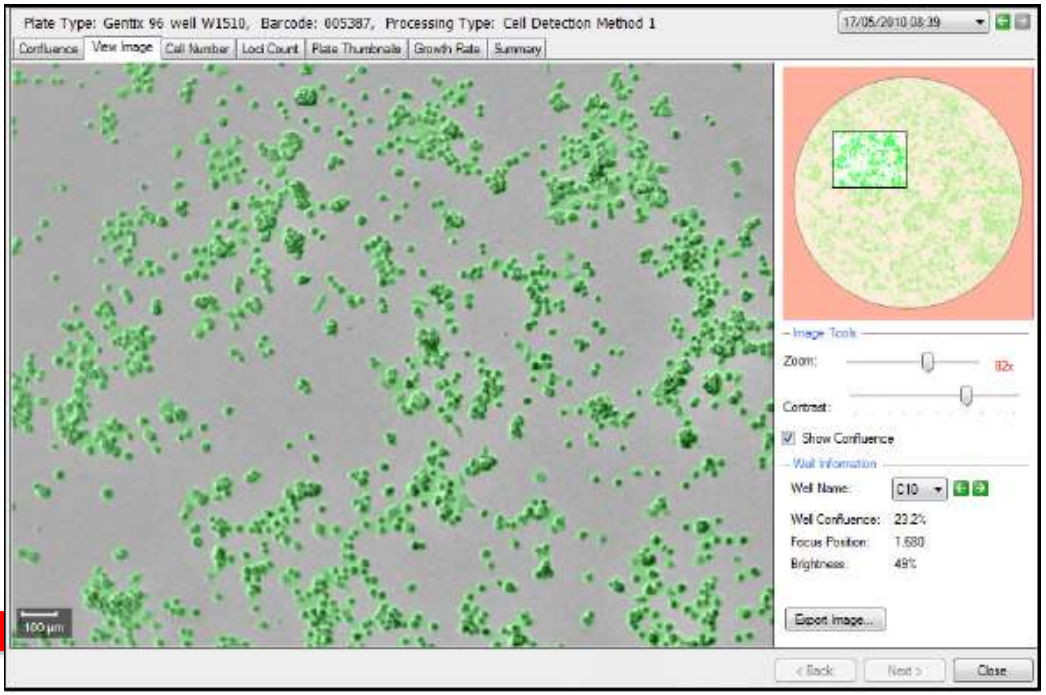
  - **Cell Number** and **Loci Count** data can also be exported at this point.
  - The wizard provides the option to export the data for the following **time points**:

**ANNOTATION**

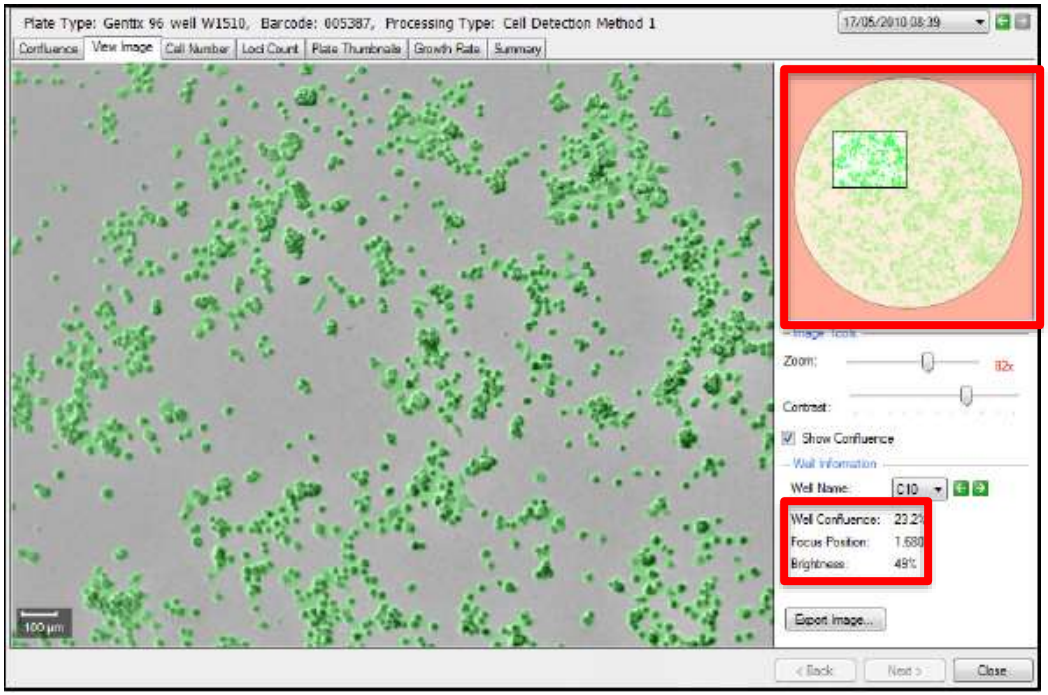
- By default the **Annotation** text box displays the **number of wells that have been imaged.**
- This field **can** be edited to more meaningful annotation.
- Note: This information **cannot** be changed when viewing the data through a **remote data viewer**.

# View Image Tab



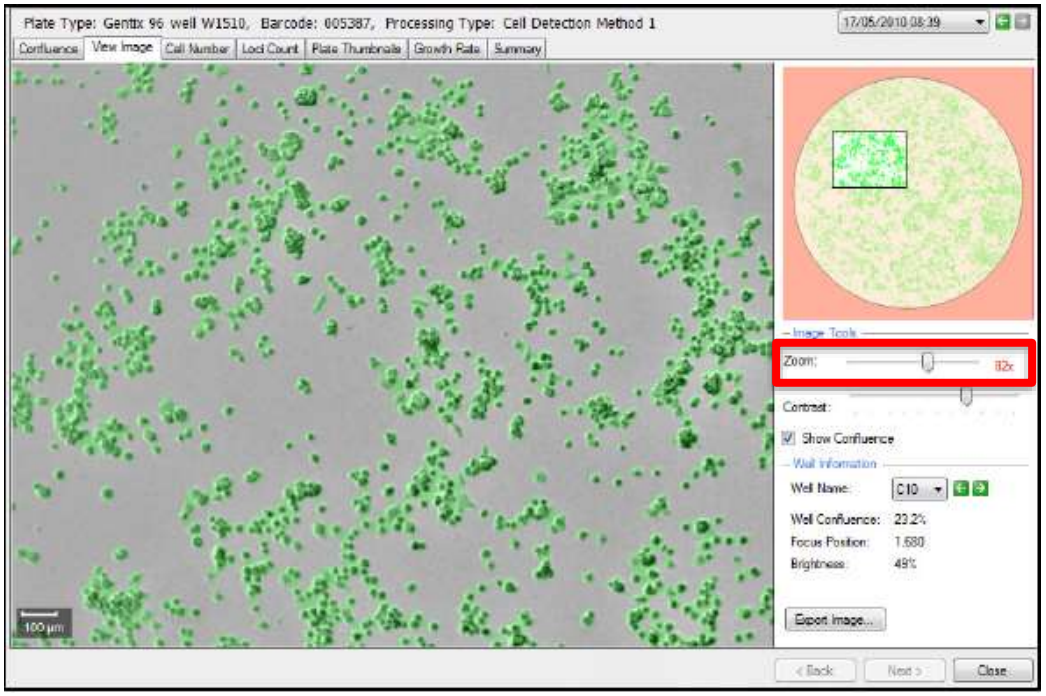

- The **View Image** tab displays the **images** for a **selected well**. The **whole well** is displayed.

# View Image Tab




- The **View Image** tab displays the **images** for a **selected well**. The **whole well** is displayed.
- **Well Schematic:** By clicking on the image of the well currently being displayed, it is possible to navigate to different areas of the well and this is reflected on the **image schematic** to the right. The **confluence levels** of the currently selected whole well are displayed **below the schematic**.

# View Image Tab

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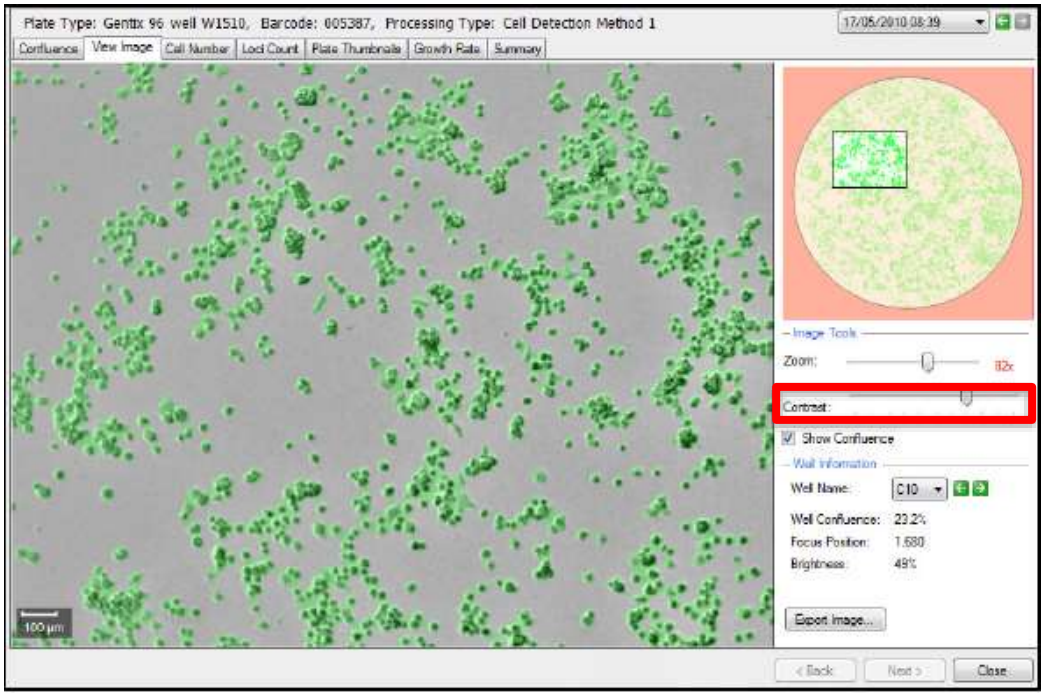
- **Image Tools:**
  - **Zoom** – This **slider** can be moved either **left or right** to zoom **out or in** respectively. When the figure turns **red** the system is zooming digitally and which may cause some **pixilation** of the image. When zoomed into an image, the **zoomed area** will be **displayed on the image thumbnail** to the right.

# View Image Tab

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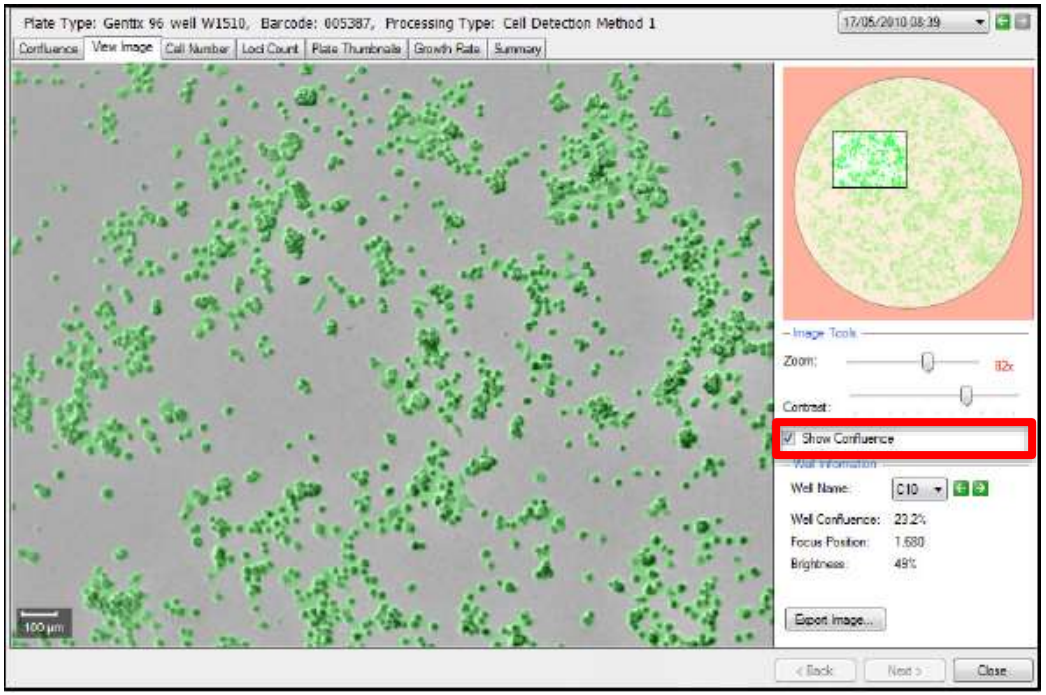
  - **Contrast**: The **contrast** of the image displayed can be altered by changing this **slider**.

# View Image Tab

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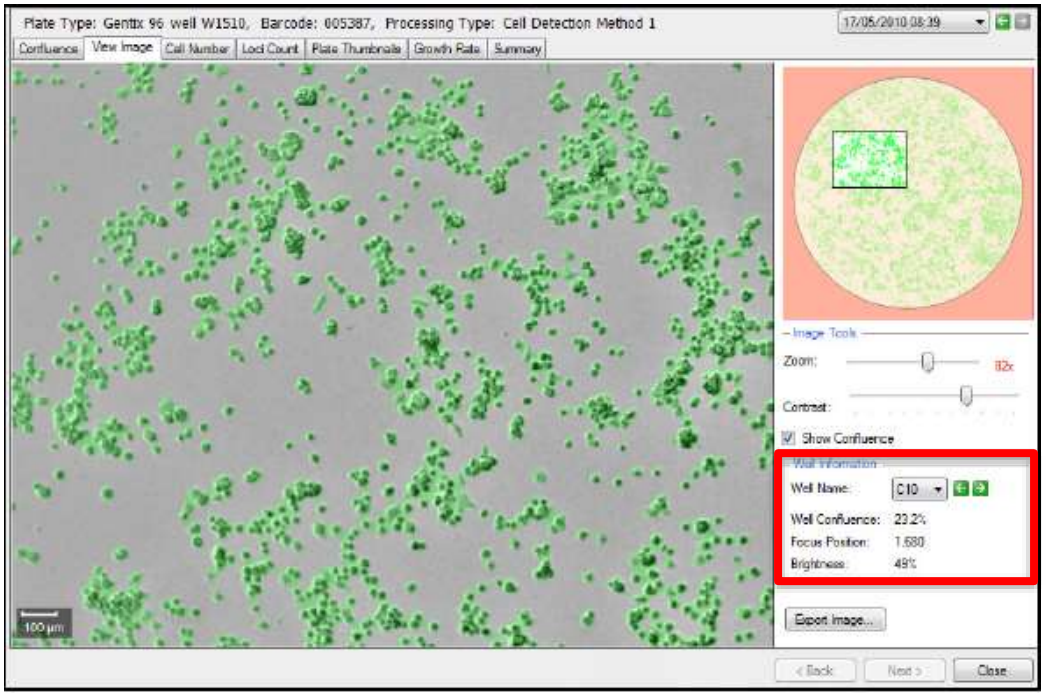
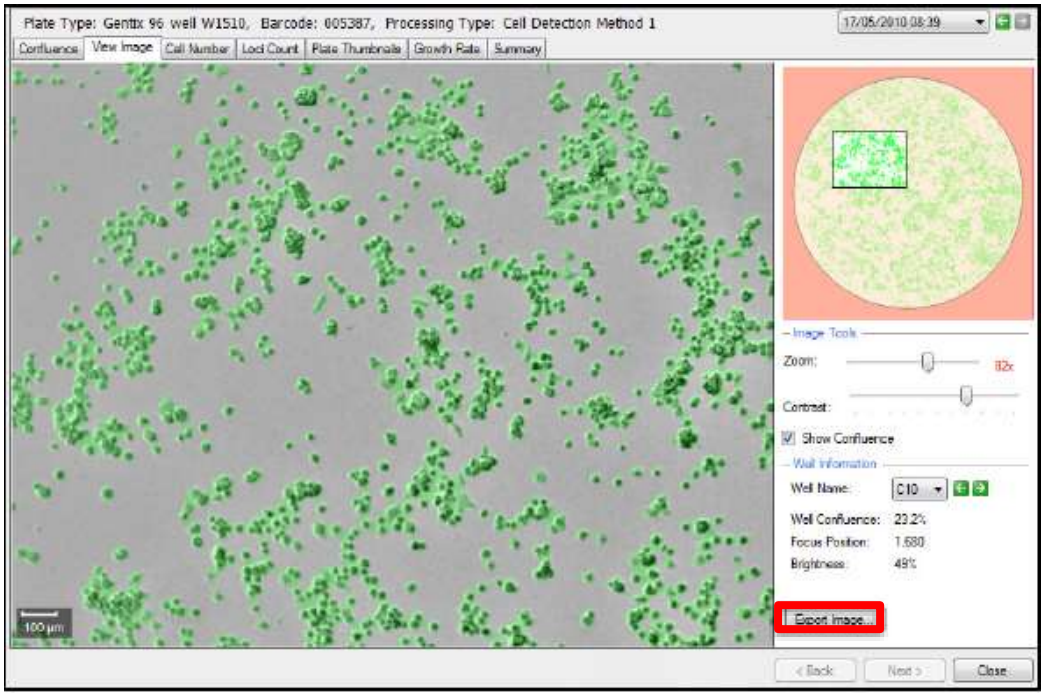
  - **Contrast**: The **contrast** of the image displayed can be altered by changing this **slider**.
  - **Show Confluence**: Checking this box will display the **confluence detected** within the well. **Detected objects** are displayed in **green**.

# View Image Tab




- The **Well Information field** contains the following information about the displayed image:
  - **Well Name:** Using the **drop down** menu or the **arrows** it is possible to **toggle** to the well of choice.
  - **Well Confluence**: The **well confluence percentage** is displayed here for the selected well.
  - **Focus Position**: The **focus point** of the image when captured is displayed here.
  - **Brightness:** The **brightness level used to capture the image** is displayed as a percentage here.

# View Image Tab

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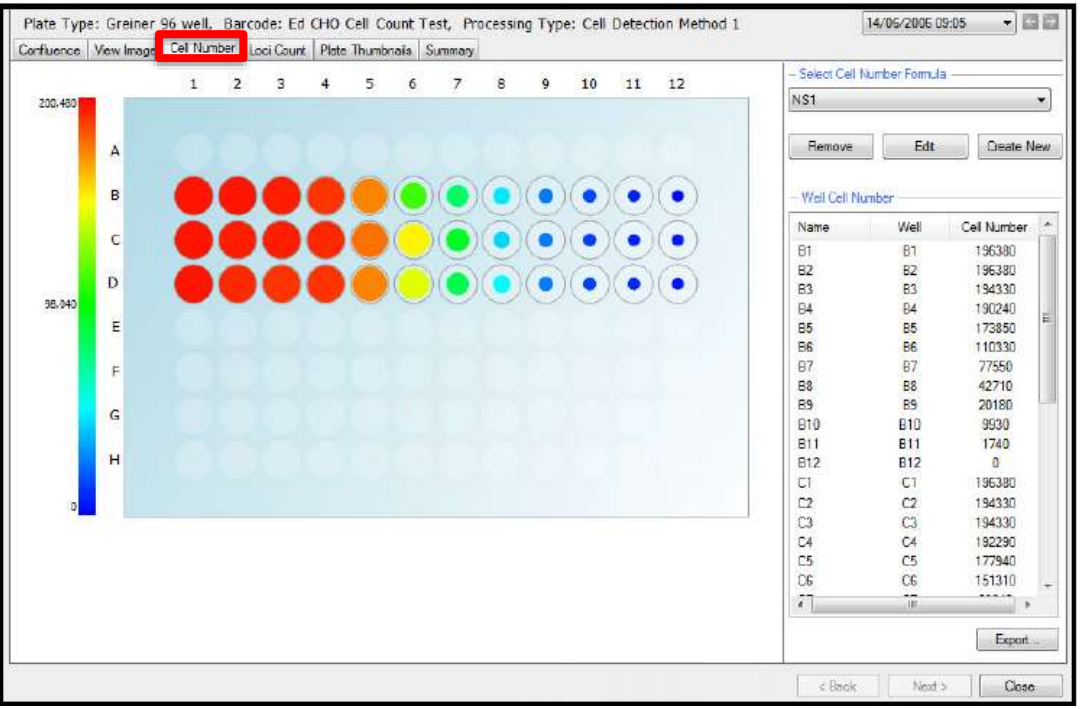
  - **Focus Position**: The **focus point** of the image when captured is displayed here.
  - **Brightness:** The **brightness level used to capture the image** is displayed as a percentage here.

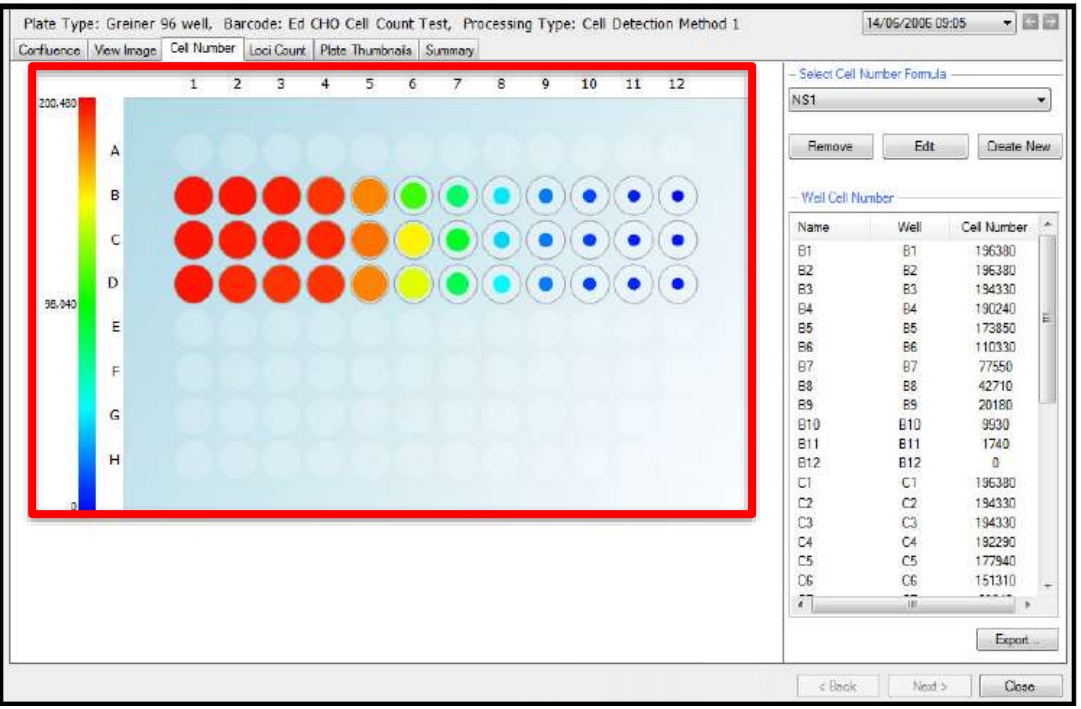
- **Export Image:** Click this button to export the current image as it is displayed.
  - It is possible to the export the currently displayed image in **.bmp, .jpg or .png** format.
  - If **Show Confluence** has been selected, the **confluence overlay** is displayed with the exported image.
  - The **zoomed position** is also saved within the image.

# Cell Number Tab



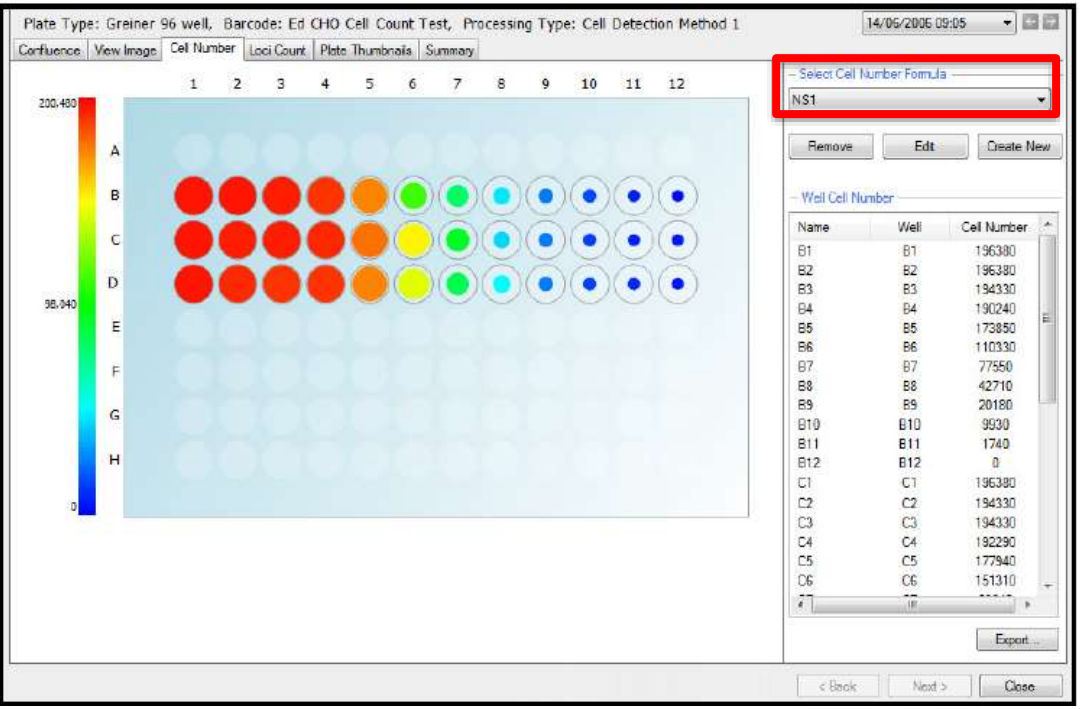

- The **Cell Number** tab displays the **estimated number of cells in each well**, both graphically and as a list.
- The number of cells is estimated using a **formula** that can be created for each **cell type** from the **confluence readings of a standard plate containing known numbers of cells** (see **Appendix C of the User Manual** for detailed protocol).

# Cell Number Tab



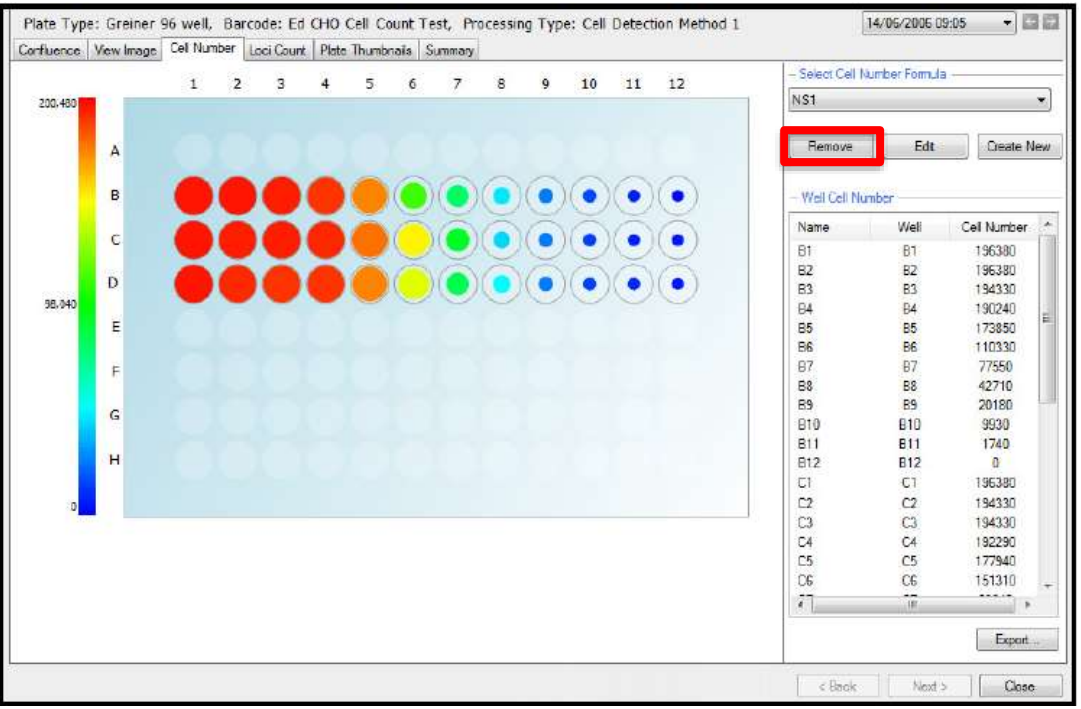

- The **Cell Number** tab displays the **estimated number of cells in each well**, both graphically and as a list.
- The number of cells is estimated using a **formula** that can be created for each **cell type** from the **confluence readings of a standard plate containing known numbers of cells** (see **Appendix C of the User Manual** for detailed protocol).
- Each well in the display 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 mouse over a well displays a **tool tip** giving the **well co-ordinate and the estimated cell number** for that well.

# Cell Number Tab: Select Cell Number Formula



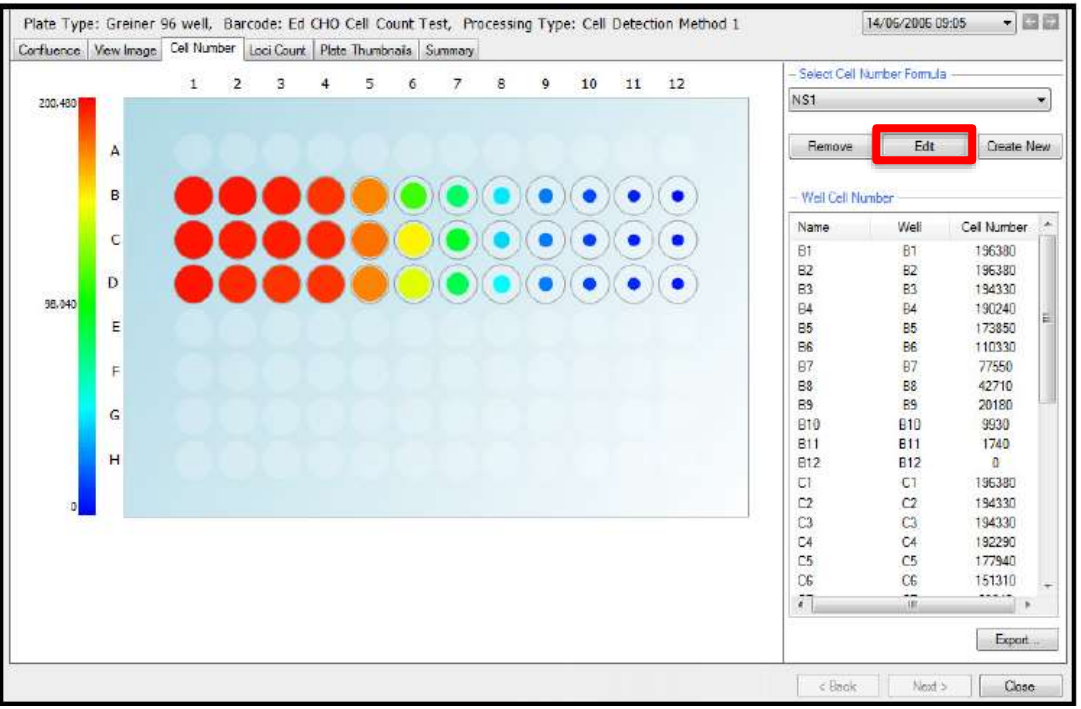

- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.

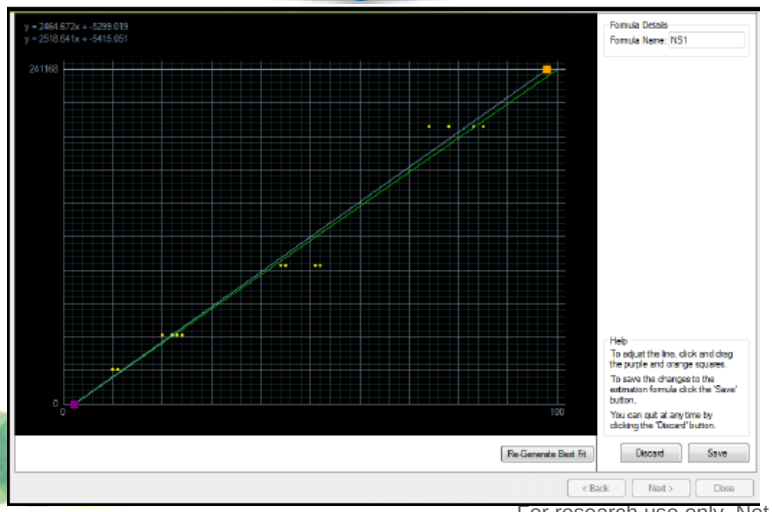
# Cell Number Tab: Remove Cell Number Formula




- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Remove:** Clicking this button will **remove** the **currently selected cell number formula** from the **drop down** menu.

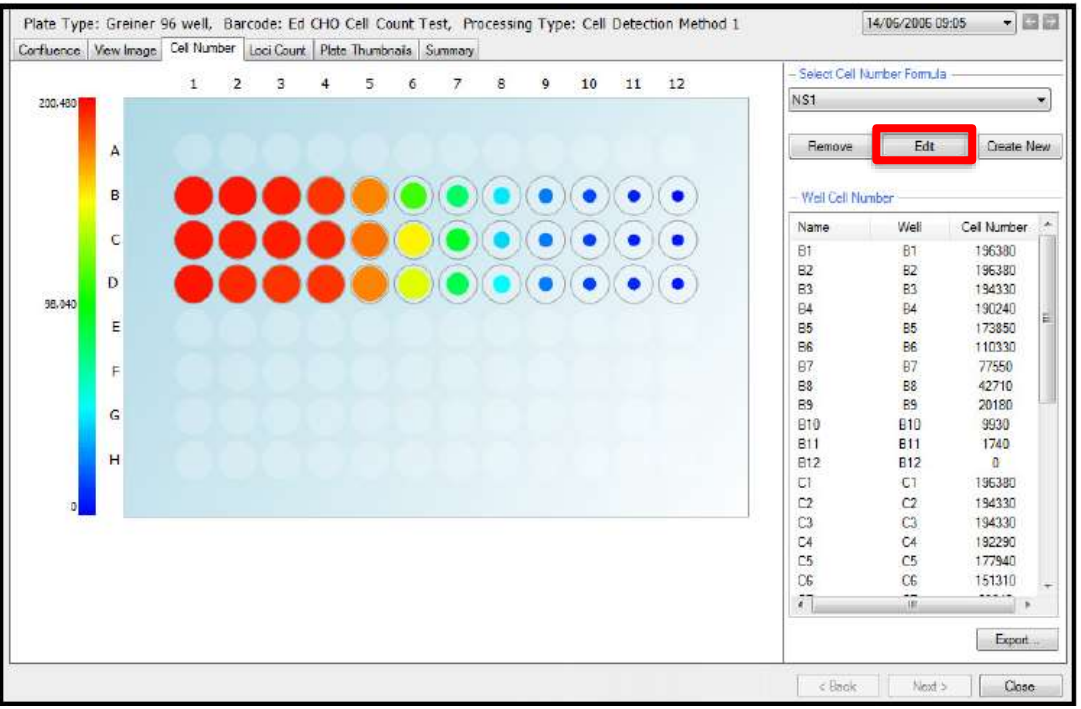
# Cell Number Tab: Edit Cell Number Formula

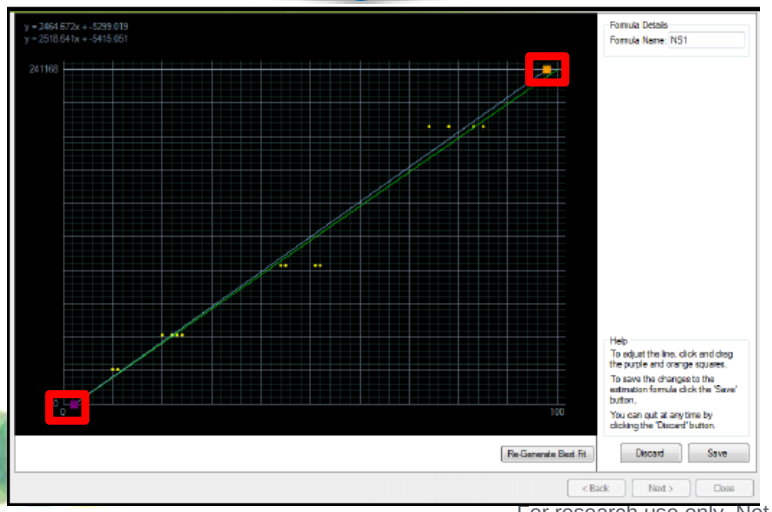



- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Remove:** Clicking this button will **remove** the **currently selected cell number formula** from the **drop down** menu.
- **Edit:** Clicking this will allow editing to be carried out on the currently **selected cell number formula** from the drop down menu.
  - A **graph** of **cell number against confluence** for the formula is displayed.

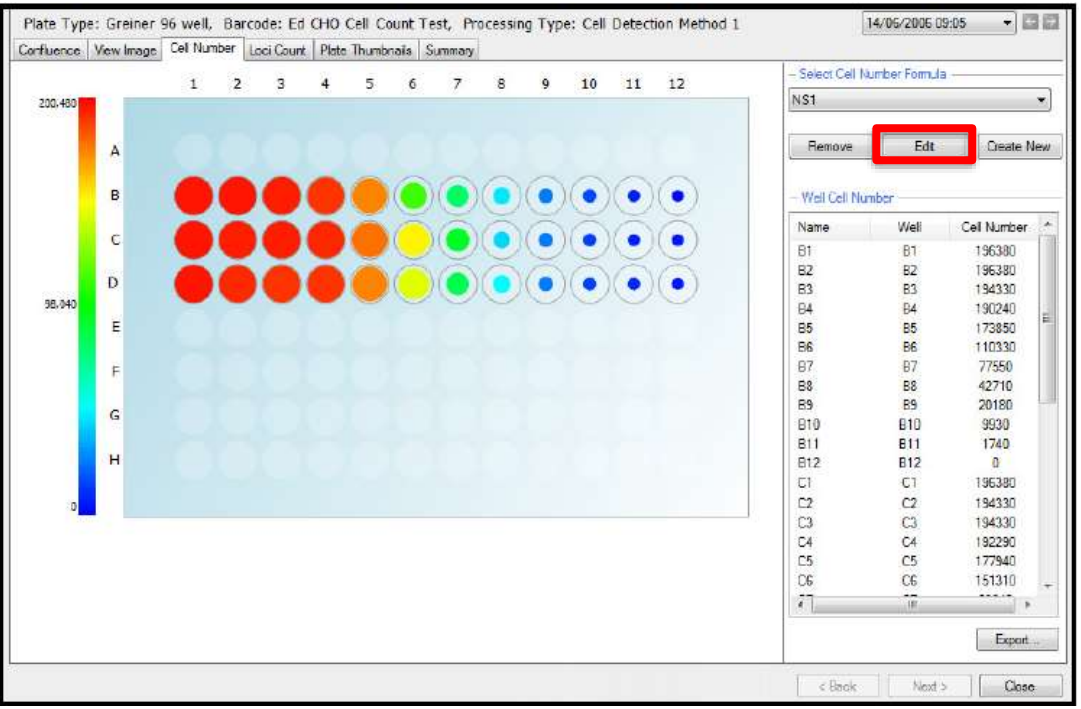
# Cell Number Tab: Edit Cell Number Formula

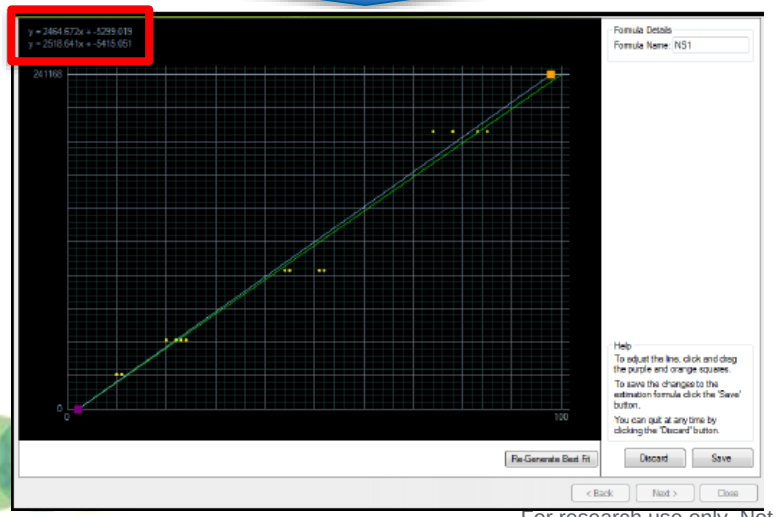



- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Remove:** Clicking this button will **remove** the **currently selected cell number formula** from the **drop down** menu.
- **Edit:** Clicking this will allow editing to be carried out on the currently **selected cell number formula** from the drop down menu.
  - A **graph** of **cell number against confluence** for the formula is displayed.
  - The **handles** at each end of the line can be **dragged to edit the formula**.

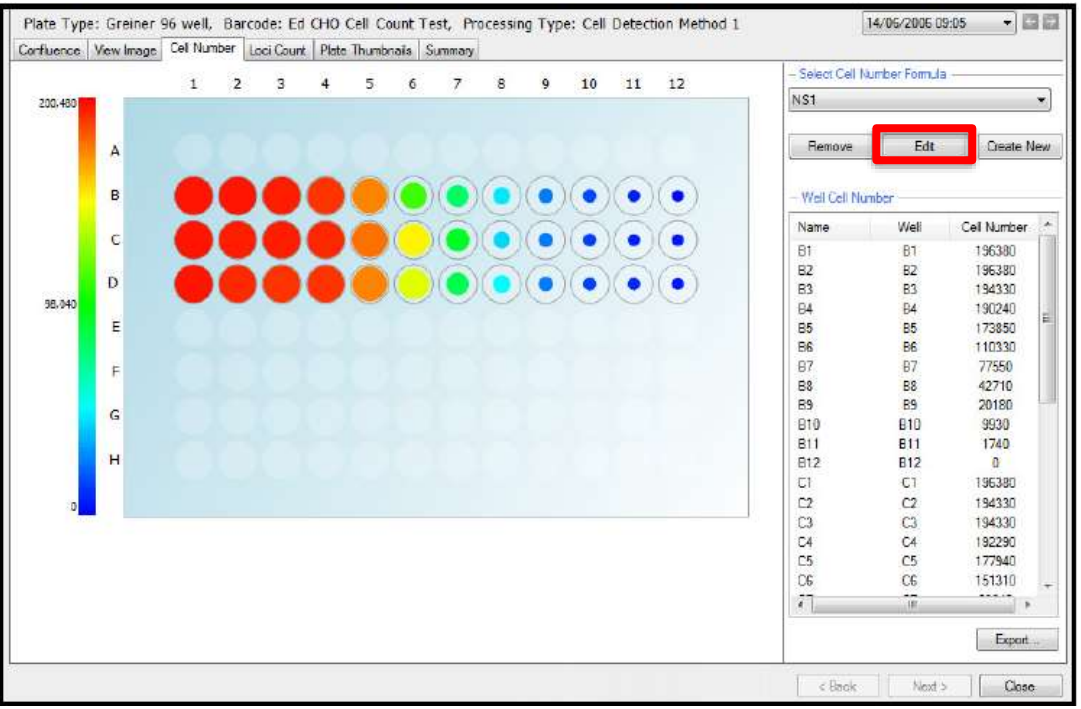
# Cell Number Tab: Edit Cell Number Formula

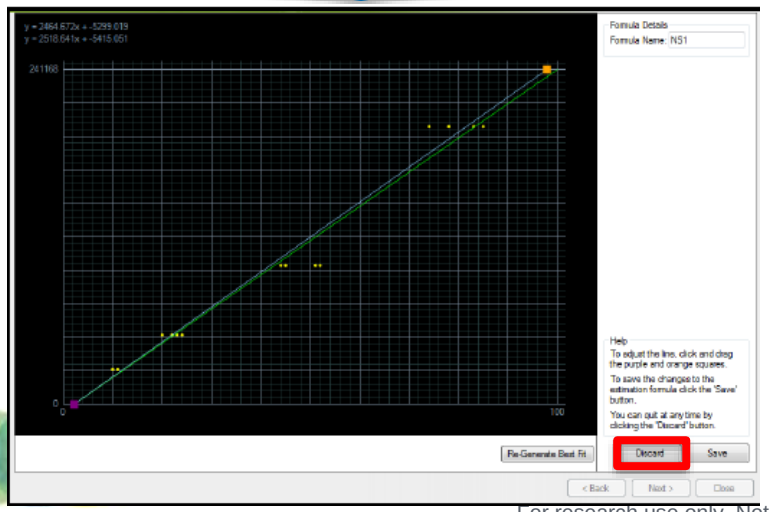



- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Remove:** Clicking this button will **remove** the **currently selected cell number formula** from the **drop down** menu.
- **Edit:** Clicking this will allow editing to be carried out on the currently **selected cell number formula** from the drop down menu.
  - A **graph** of **cell number against confluence** for the formula is displayed.
  - The **handles** at each end of the line can be **dragged to edit the formula**.
  - The **formula** is displayed at the **top left** and the **edited version** is displayed **immediately below** it.

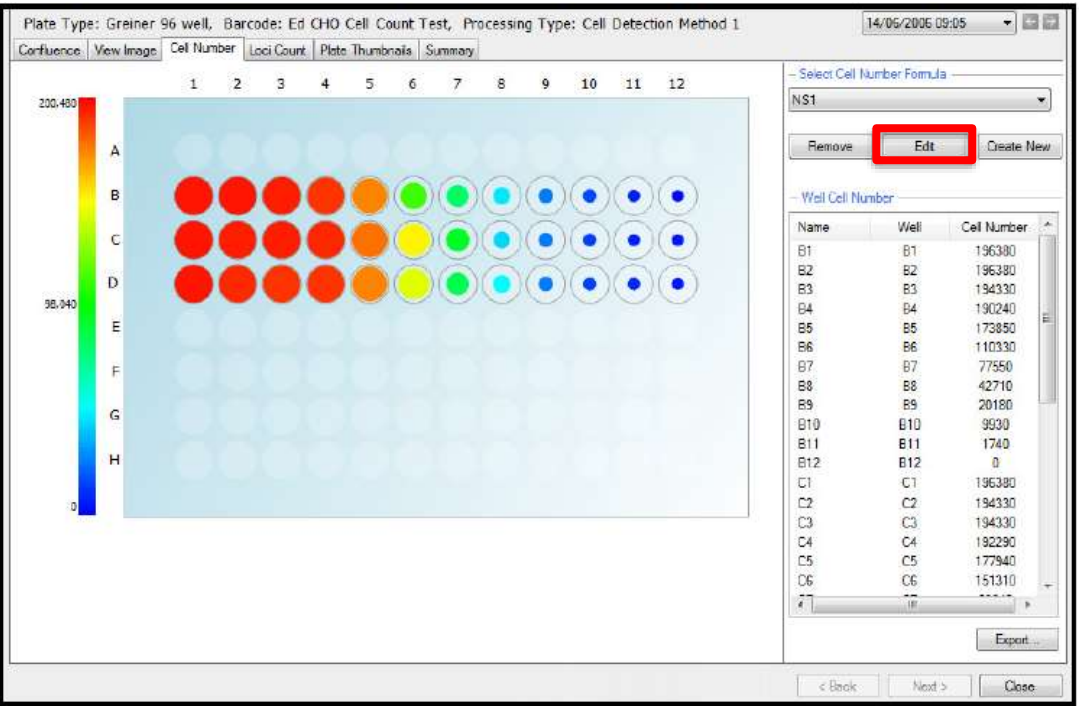
# Cell Number Tab: Edit Cell Number Formula

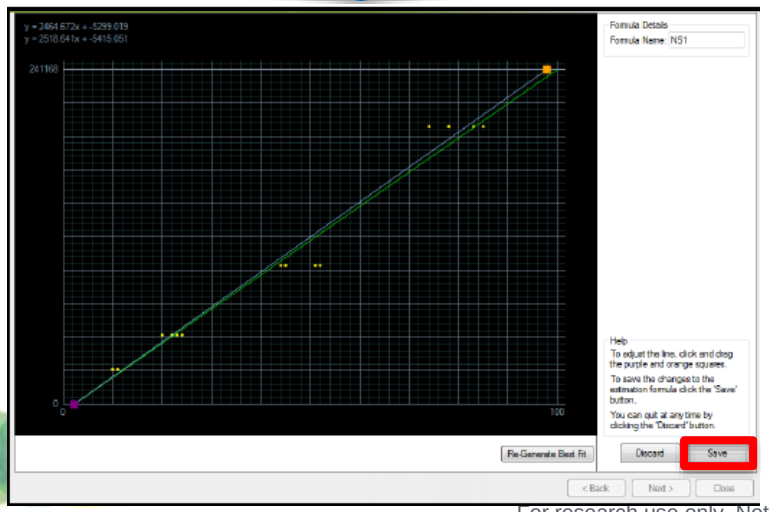



- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Remove:** Clicking this button will **remove** the **currently selected cell number formula** from the **drop down** menu.
- **Edit:** Clicking this will allow editing to be carried out on the currently **selected cell number formula** from the drop down menu.
  - A **graph** of **cell number against confluence** for the formula is displayed.
  - The **handles** at each end of the line can be **dragged to edit the formula**.
  - The **formula** is displayed at the **top left** and the **edited version** is displayed **immediately below** it.
  - **Discard:** Click this button to **abandon the edits** and return to the **Cell Number** tab.

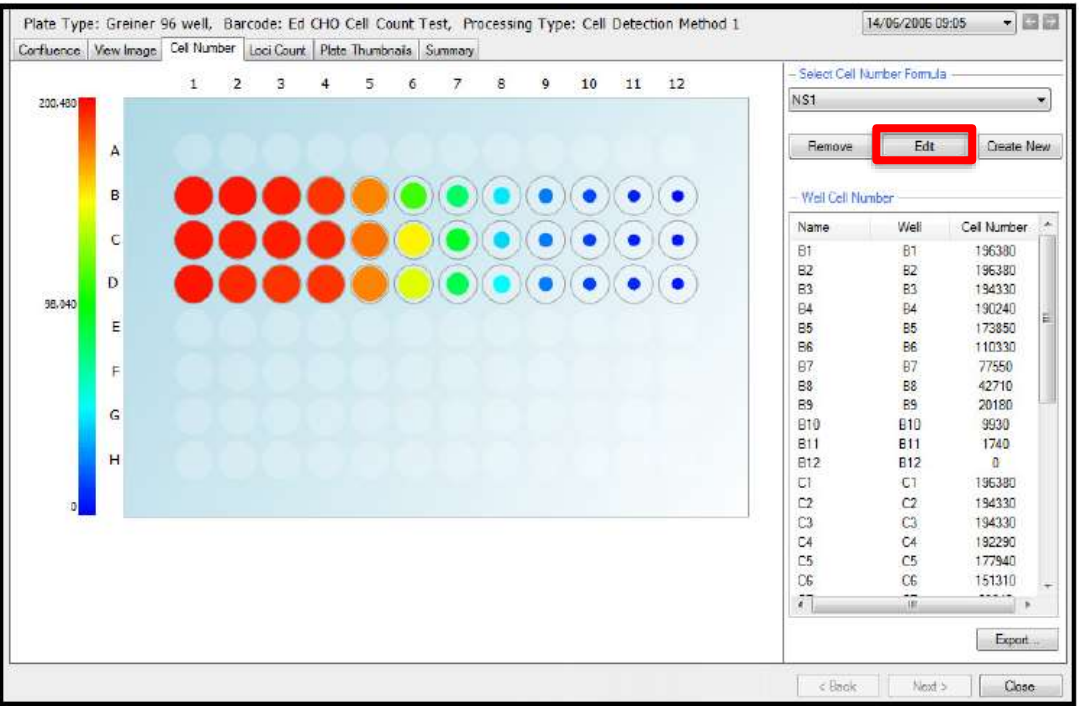
# Cell Number Tab: Edit Cell Number Formula

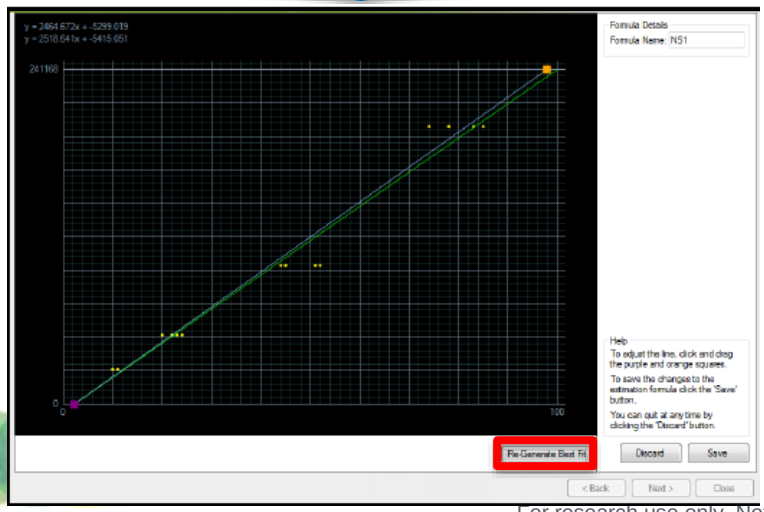



- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Remove:** Clicking this button will **remove** the **currently selected cell number formula** from the **drop down** menu.
- **Edit:** Clicking this will allow editing to be carried out on the currently **selected cell number formula** from the drop down menu.
  - A **graph** of **cell number against confluence** for the formula is displayed.
  - The **handles** at each end of the line can be **dragged to edit the formula**.
  - The **formula** is displayed at the **top left** and the **edited version** is displayed **immediately below** it.
  - **Discard:** Click this button to **abandon the edits** and return to the **Cell Number** tab.
  - **Save:** Click here to save the **changes** made to the **cell number estimation formula**.

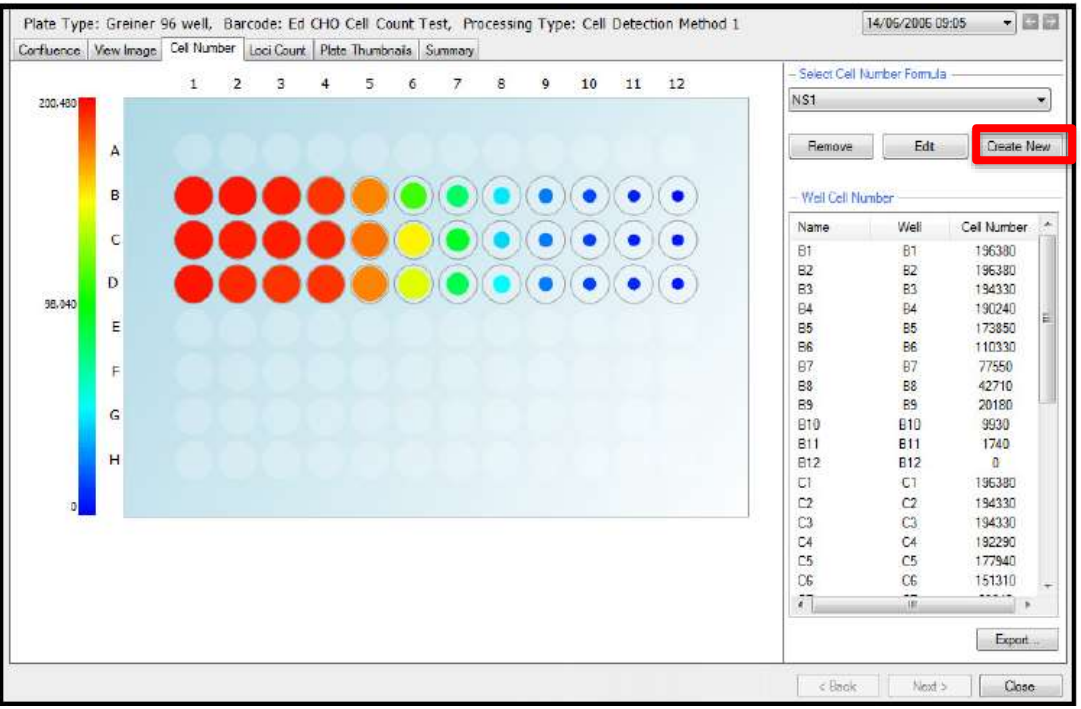
# Cell Number Tab: Edit Cell Number Formula

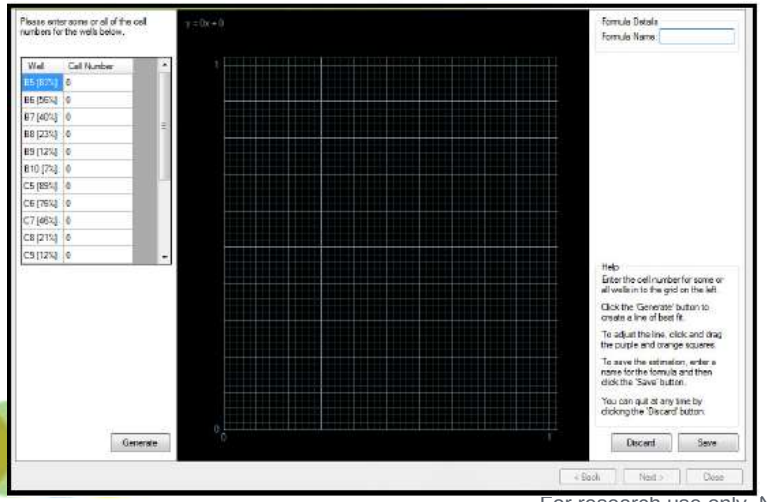



- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Remove:** Clicking this button will **remove** the **currently selected cell number formula** from the **drop down** menu.
- **Edit:** Clicking this will allow editing to be carried out on the currently **selected cell number formula** from the drop down menu.
  - A **graph** of **cell number against confluence** for the formula is displayed.
  - The **handles** at each end of the line can be **dragged to edit the formula**.
  - The **formula** is displayed at the **top left** and the **edited version** is displayed **immediately below** it.
  - **Discard:** Click this button to **abandon the edits** and return to the **Cell Number** tab.
  - **Save:** Click here to save the **changes** made to the **cell number estimation formula**.
- **Re-Generate Best Fit:** Clicking on this will **automatically** generate the line of best fit if required.

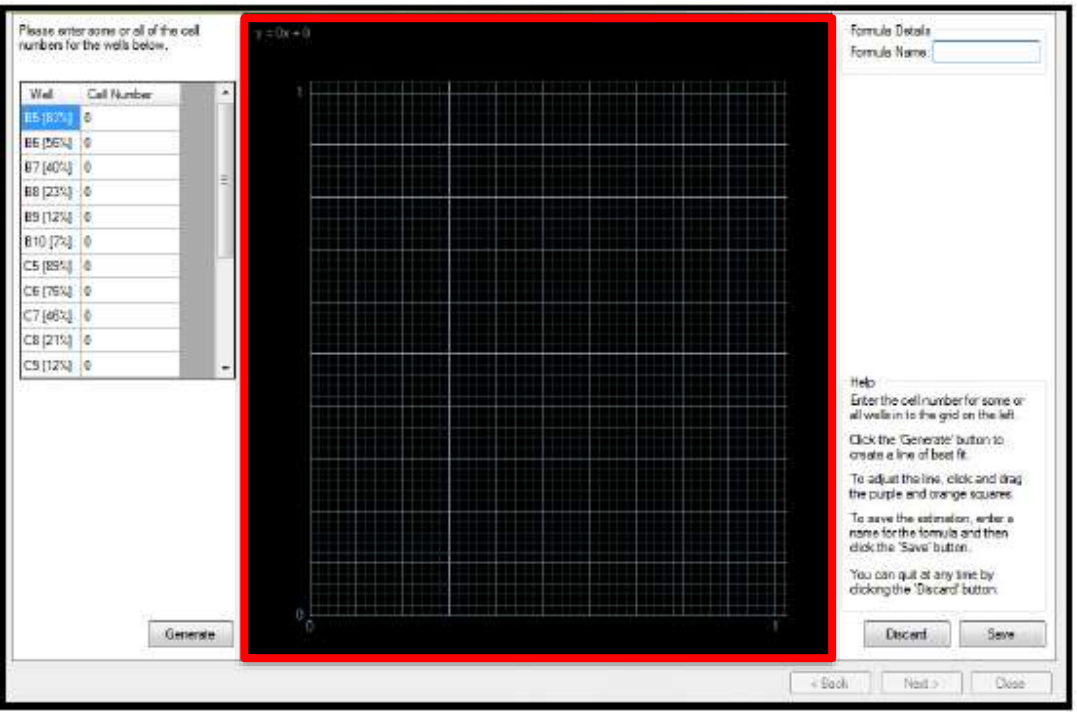
# Cell Number Tab: Create New Cell Number Formula




- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Create New:** Clicking this will enable a **new cell number estimation formula** to be created in a separate dialog.
  - Before proceeding with this step, ensure that the **confluence results displayed in the Confluence Tab are those for a standard plate containing known numbers of cells**.

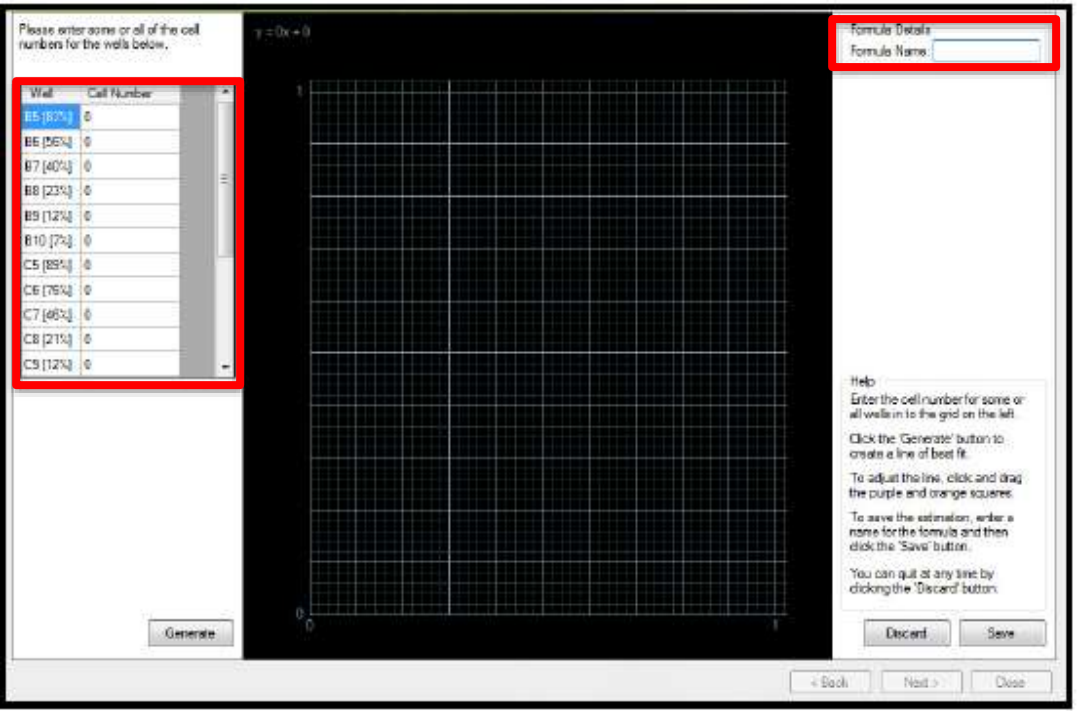
# Cell Number Tab: Create New Cell Number Formula




- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.

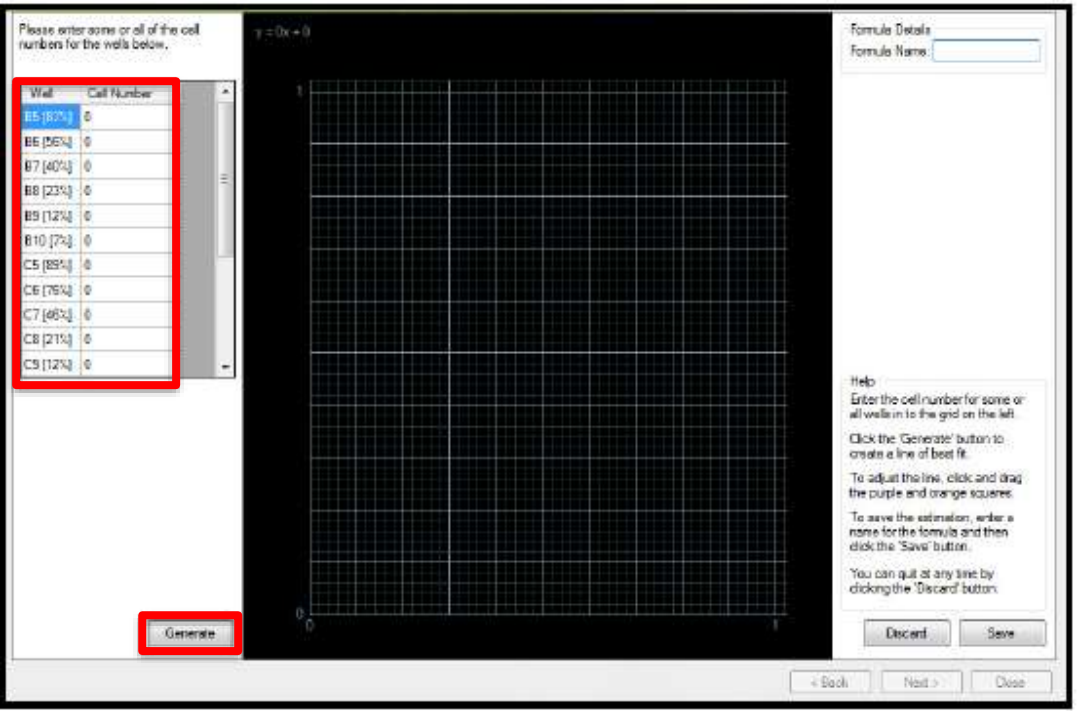
- **Create New:** Clicking this will enable a **new cell number estimation formula** to be created in a separate dialog.
  - Before proceeding with this step, ensure that the **confluence results displayed in the Confluence Tab are those for a standard plate containing known numbers of cells**.
  - The software will **automatically select up to twelve wells with confluence values ranging between 10 % and 80 %** and display them at the bottom of the graph

# Cell Number Tab: Create New Cell Number Formula




- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.
- **Create New:** Clicking this will enable a **new cell number estimation formula** to be created in a separate dialog.
  - Before proceeding with this step, ensure that the **confluence results displayed in the Confluence Tab are those for a standard plate containing known numbers of cells**.
  - The software will **automatically select up to twelve wells with confluence values ranging between 10 % and 80 %** and display them at the bottom of the graph
  - The **name** for the new formula should be entered in the **top right hand corner** and the **cell numbers for each well** should be entered into the corresponding **well number box** to the left of the graph.

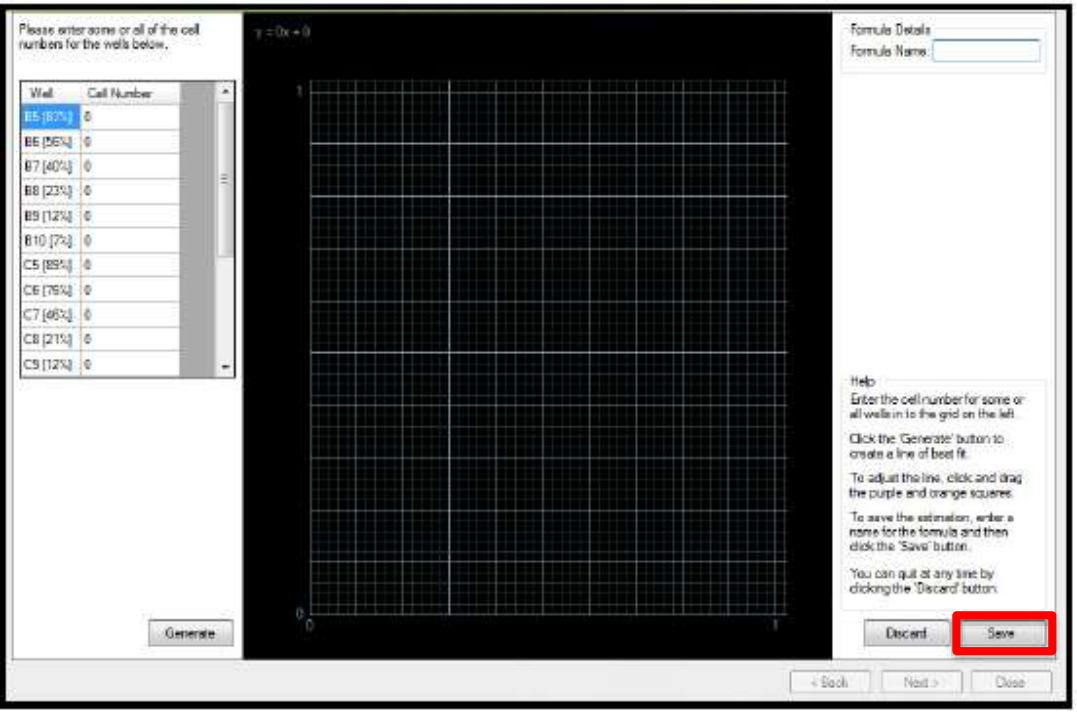
# Cell Number Tab: Create New Cell Number Formula




- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.

- **Create New:** Clicking this will enable a **new cell number estimation formula** to be created in a separate dialog.
  - Before proceeding with this step, ensure that the **confluence results displayed in the Confluence Tab are those for a standard plate containing known numbers of cells**.
  - The software will **automatically select up to twelve wells with confluence values ranging between 10 % and 80 %** and display them at the bottom of the graph
  - The **name** for the new formula should be entered in the **top right hand corner** and the **cell numbers for each well** should be entered into the corresponding **well number box** to the left of the graph.
  - Enter the **seeded cell numbers** for the **selected wells** in the **table**, then click **Generate** after all relevant information is entered will **create the new formula**.

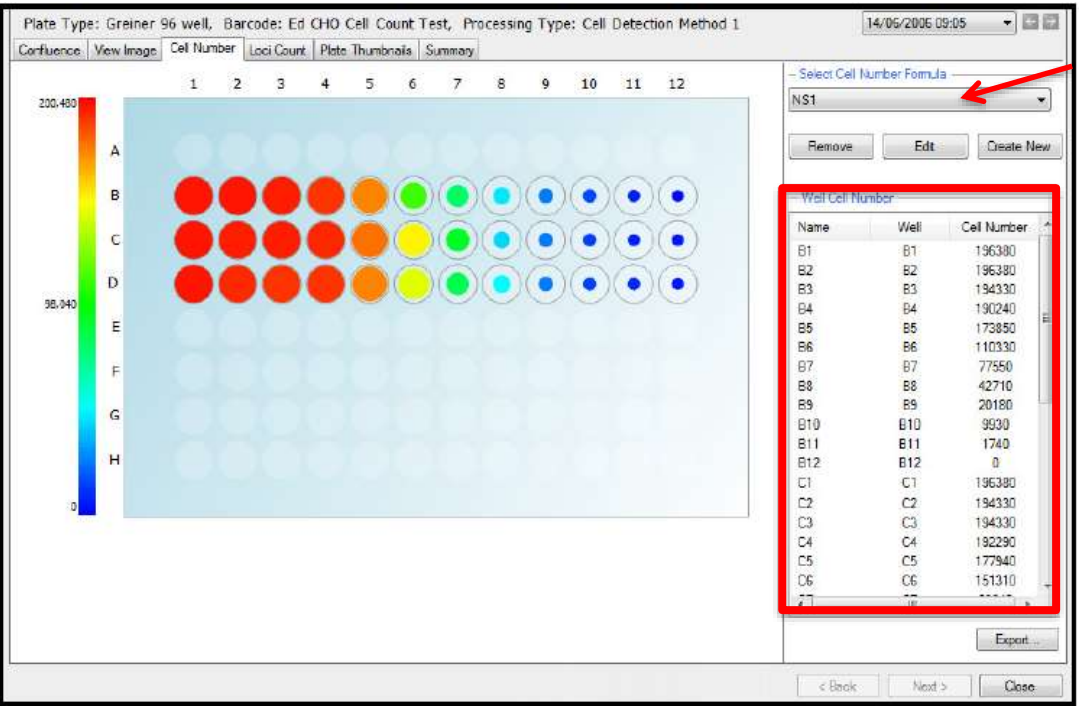
# Cell Number Tab: Create New Cell Number Formula




- **Select Cell Number Formula:** This drop down menu will list all the formulas created for estimating the number of cells per well.

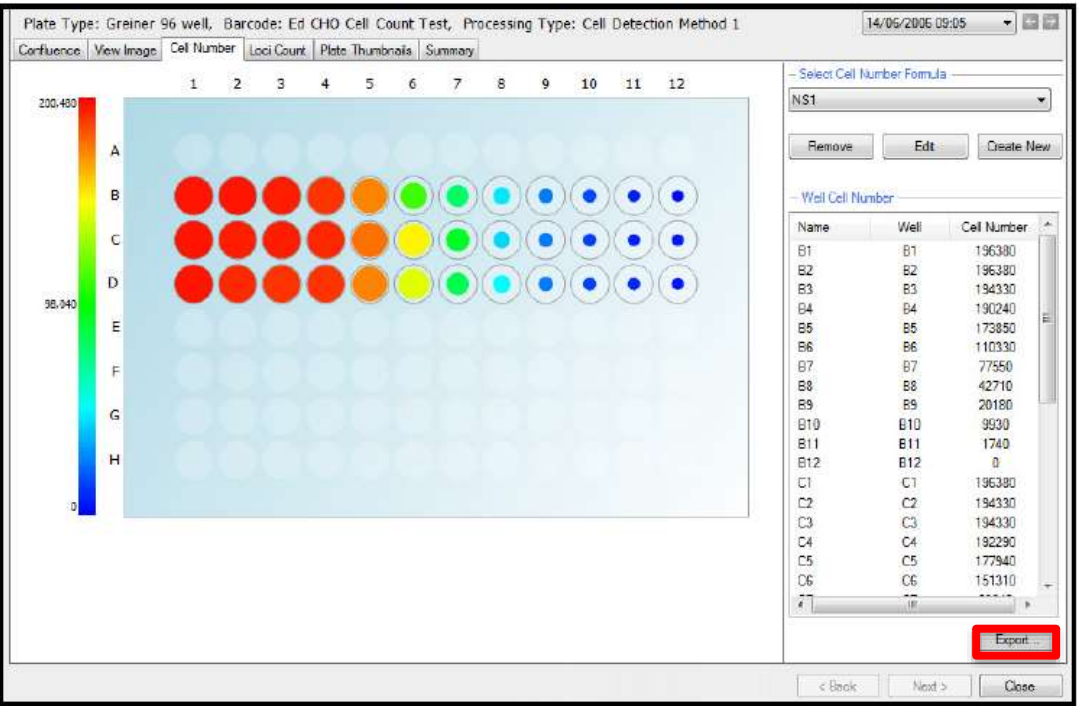
- **Create New:** Clicking this will enable a **new cell number estimation formula** to be created in a separate dialog.
  - Before proceeding with this step, ensure that the **confluence results displayed in the Confluence Tab are those for a standard plate containing known numbers of cells**.
  - The software will **automatically select up to twelve wells with confluence values ranging between 10 % and 80 %** and display them at the bottom of the graph
  - The **name** for the new formula should be entered in the **top right hand corner** and the **cell numbers for each well** should be entered into the corresponding **well number box** to the left of the graph.
  - Enter the **seeded cell numbers** for the **selected wells** in the **table**, then click **Generate** after all relevant information is entered will **create the new formula**.
  - Click **Save** to return to the **Results view**.

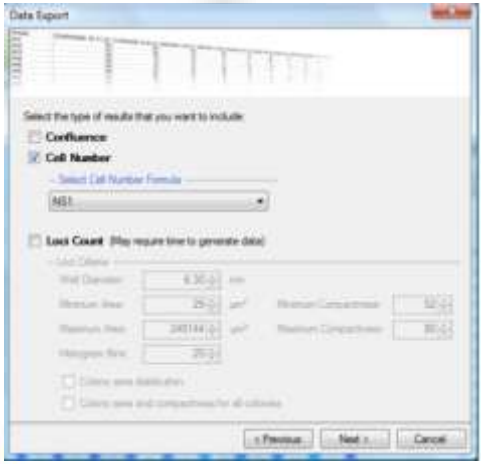
# Cell Number Tab: Well Cell Number Data Field




- **Well Cell Number Field:** A list of **estimated cell numbers** is displayed to the right of the plate overview.
  - ***NOTE:*** *These values are calculated based on the **Cell Number Formula** selected in the dropdown above.*

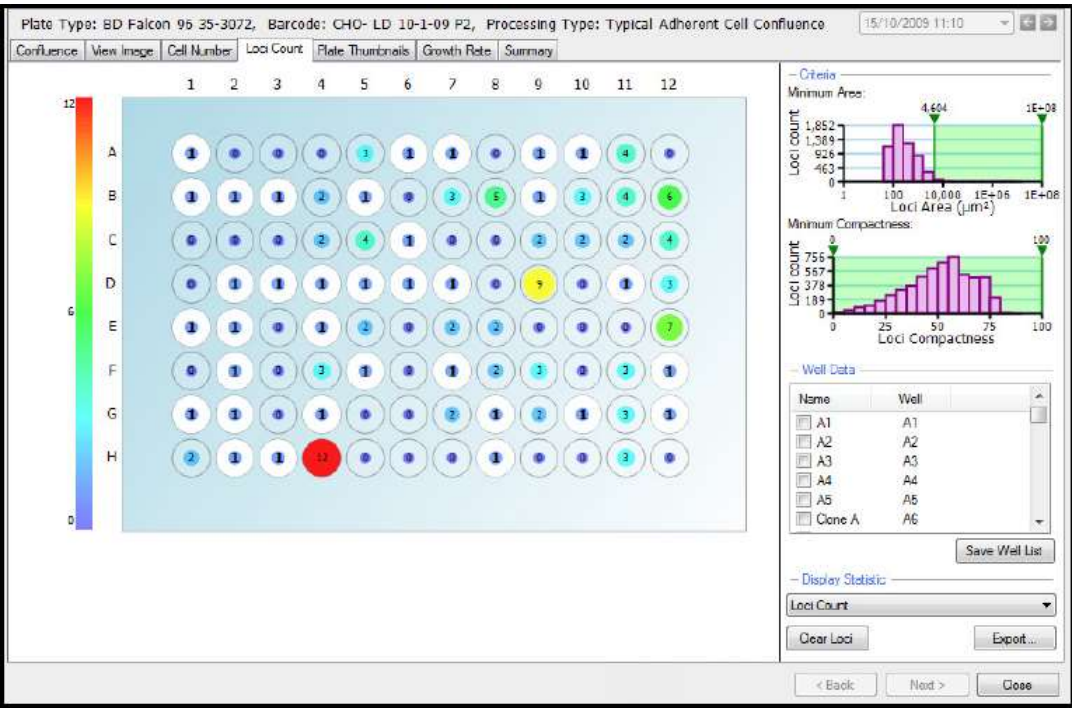
# Cell Number Tab: Well Cell Number Data Field



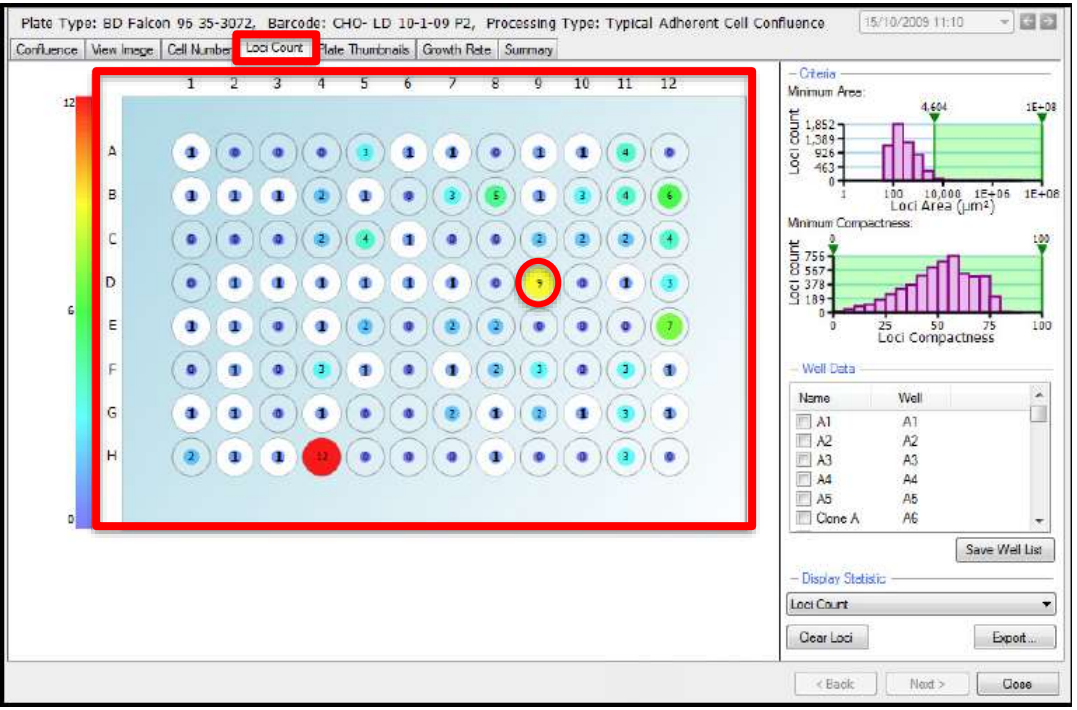

- **Well Cell Number Field:** A list of **estimated cell numbers** is displayed to the right of the plate overview.
  - ***NOTE:*** *These values are calculated based on the **Cell Number Formula** selected in the dropdown above.*
- Click **Export** to launch the Data Export Wizard enable the lists of wells and the corresponding Cell Number to be exported as a **.csv or .xml** file.
  - The **Data Export wizard** will guide the process of exporting the **Cell Number** data.
  - **Confluence** and **Loci Count** data can also be exported at this point

# Loci Count Tab: General Info



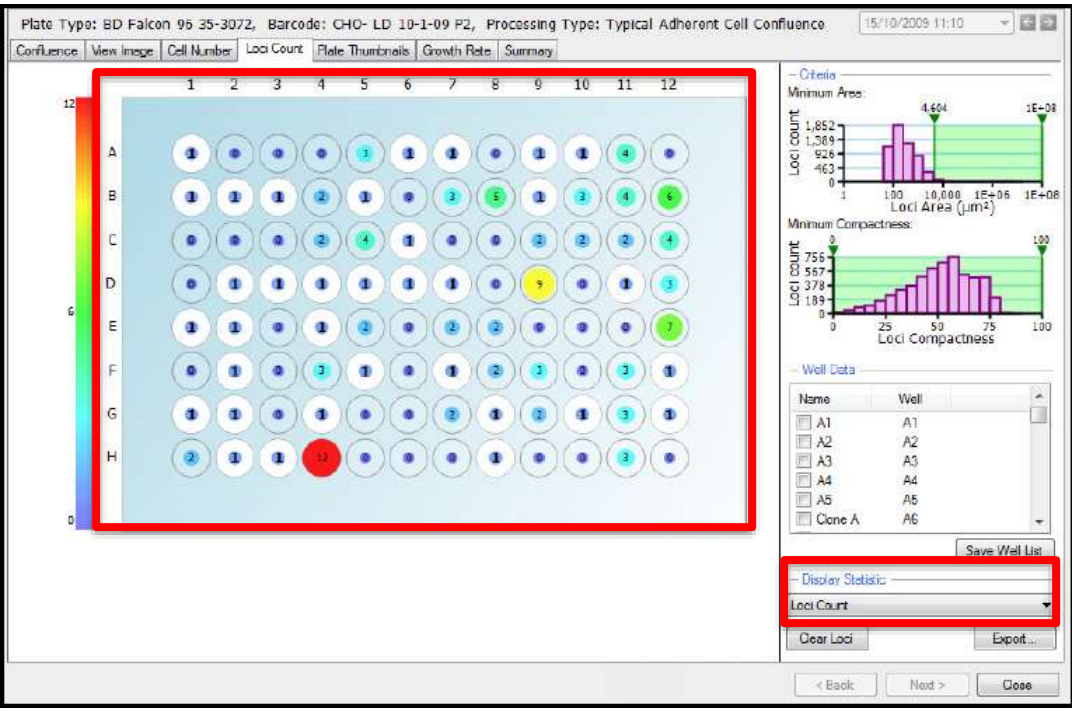

- Generated images can be processed using the **loci count** feature.
  - ***NOTE:*** *This is a **licensable feature** of the software – please contact **Molecular Devices Technical Support** if you require this functionality.*
- This **detects** and **counts** the number of **loci of growth**, **i.e. 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**.
- For **colony formation** assays, the Loci Count feature can be used to **count the number and size (area) of colonies** in each well.

# Loci Count Tab: Plate View



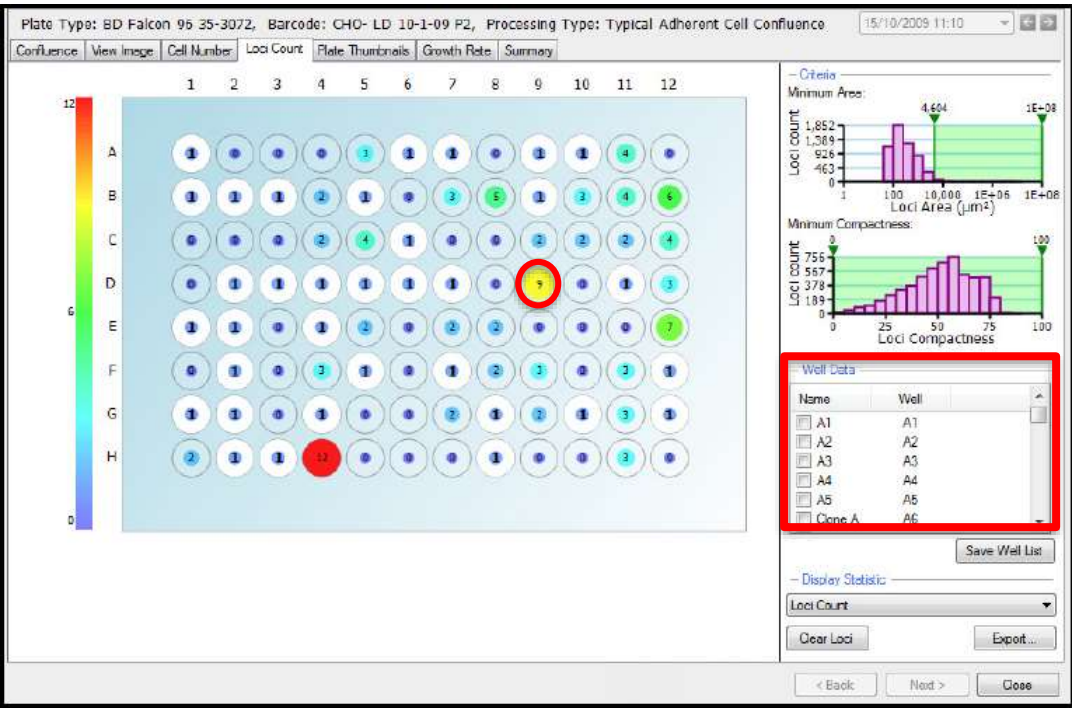

- In the **Loci Count** tab, the **plate view** displays each well with a figure indicating the **number of cell colonies** found in the well.

# Loci Count Tab: Plate View



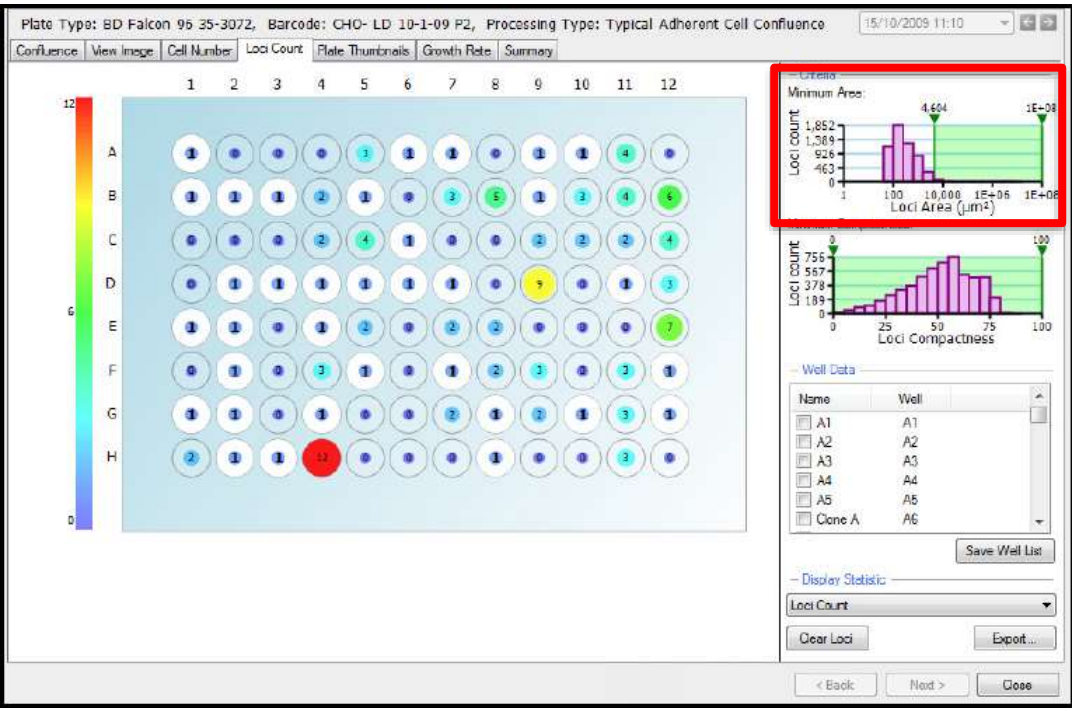

- In the **Loci Count** tab, the **plate view** displays each well with a figure indicating the **number of cell colonies** found in the well.
- The **default plate view** displays the **Loci Count (Under Display Statistic)** however this can be changed to **Mean Loci Area** which will display this value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

# Loci Count Tab: Well View



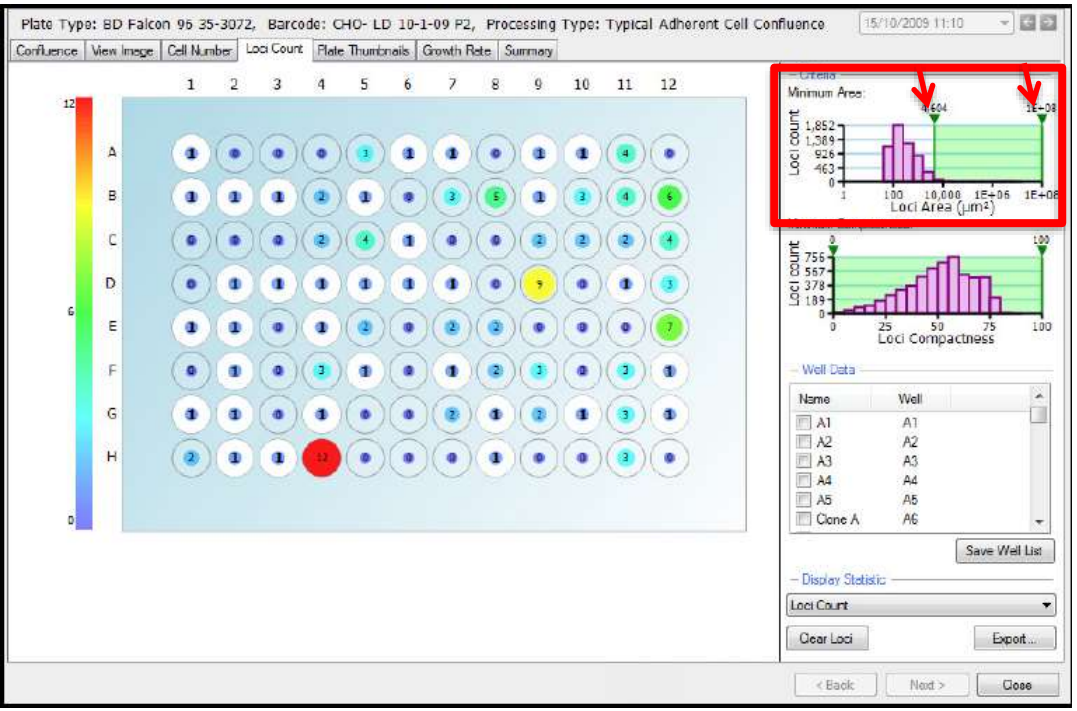

- In the **Loci Count** tab, the **plate view** displays each well with a figure indicating the **number of cell colonies** found in the well.
- The **default plate view** displays the **Loci Count (Under Display Statistic)** however this can be changed to **Mean Loci Area** which will display this value within each of the wells.
- To display the **Well View** for a well, **click on the desired well in Plate View** or in the **Well Data list.**

# Loci Count Tab: Criteria



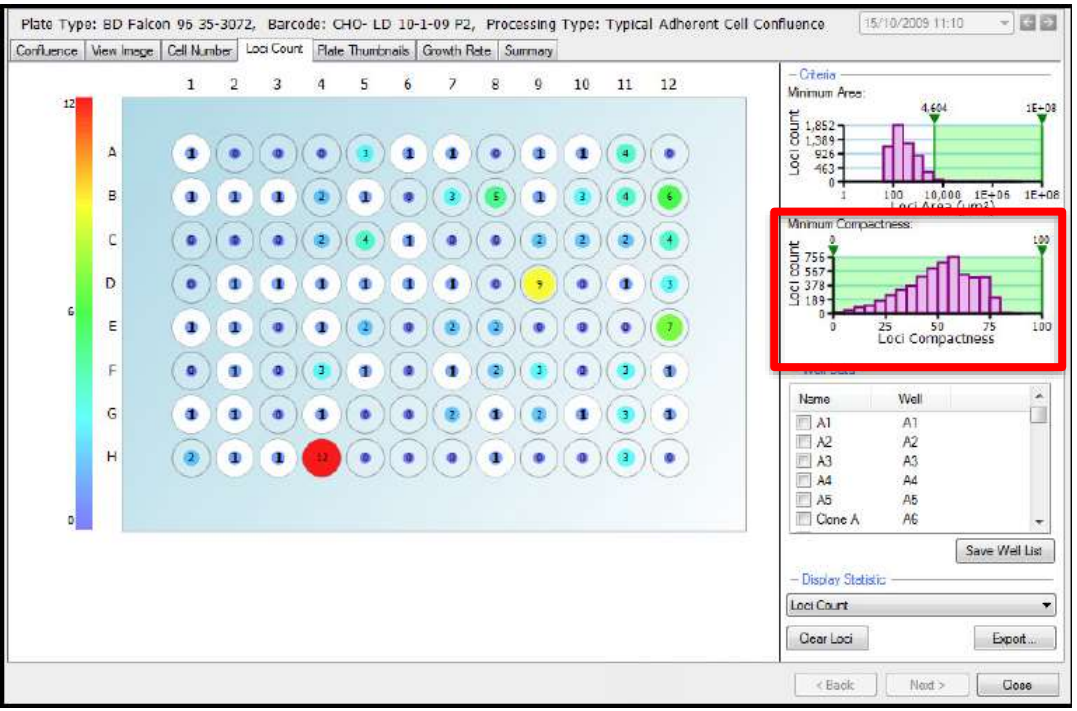

- **CRITERIA: Minimum Area**
  - The **loci count (frequency)** is plotted against the **loci area (µm2)** in a **log scale**.

# Loci Count Tab: Criteria



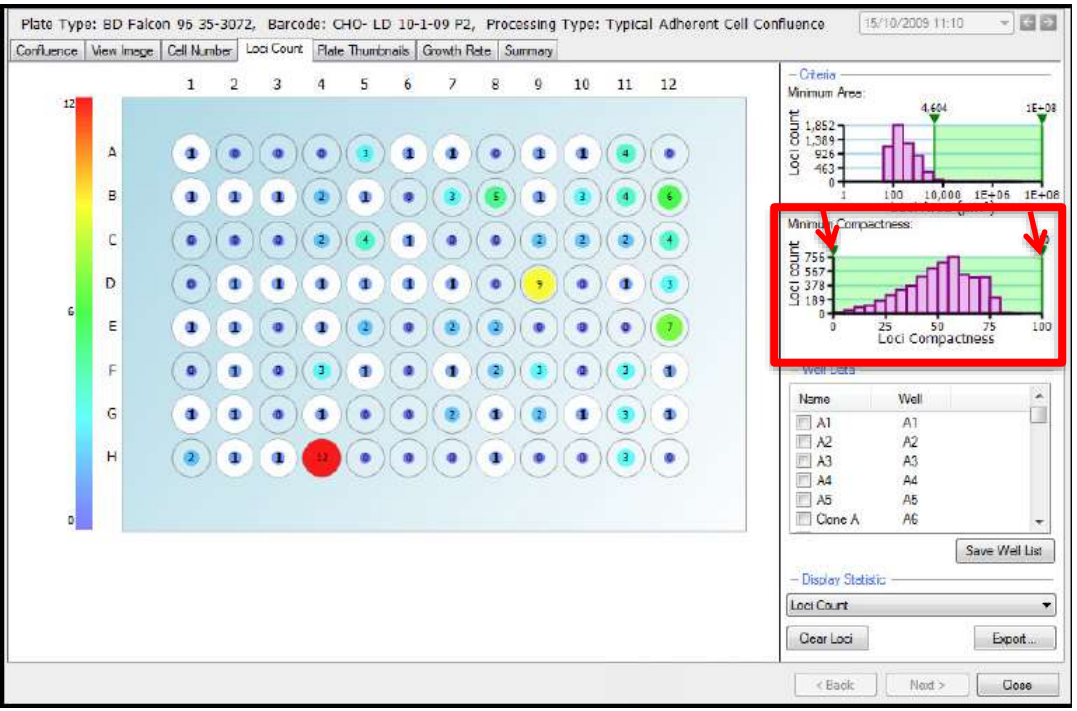

- **CRITERIA: Minimum Area**
  - The **loci count (frequency)** is plotted against the **loci area (µm2)** in a **log scale**.
  - The **bar chart** has two, **lower and upper, gates** that can be moved accordingly to **eliminate unwanted objects/debris** within the well that artificially inflate the number of loci.

# Loci Count Tab: Criteria



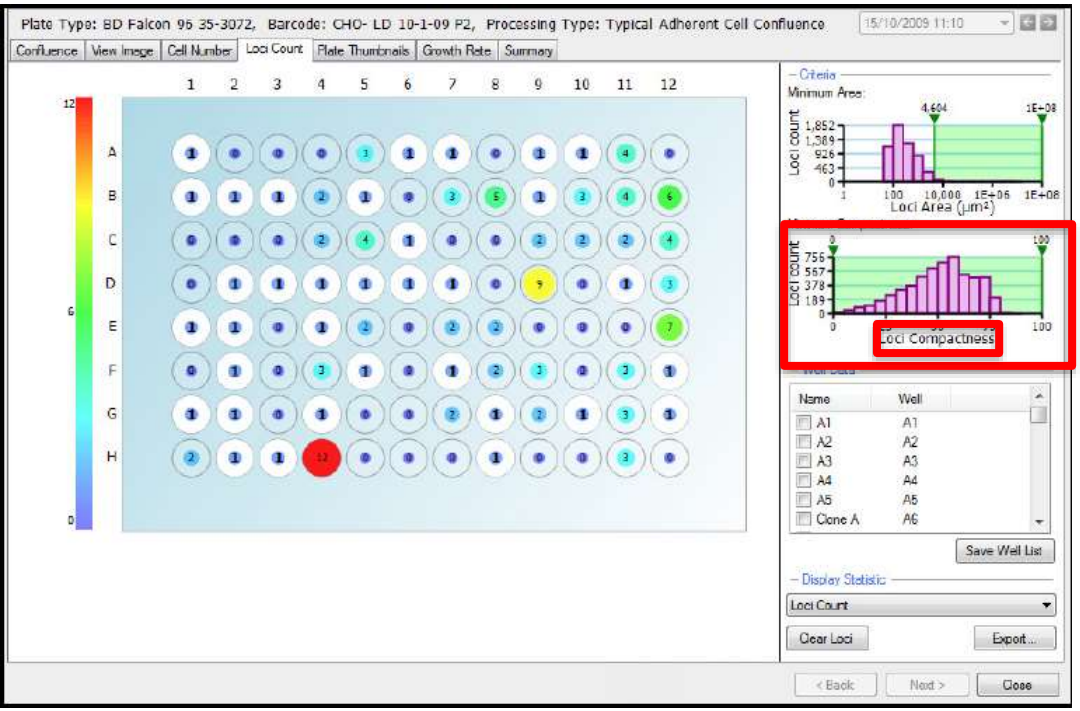

- **CRITERIA: Minimum Area**
  - The **loci count (frequency)** is plotted against the **loci area (µm2)** in a **log scale**.
  - The **bar chart** has two, **lower and upper, gates** that can be moved accordingly to **eliminate unwanted objects/debris** within the well that artificially inflate the number of loci.
- **CRITERIA: Minimum Compactness**
  - The **loci count** (frequency) is plotted against the **loci compactness**.

# Loci Count Tab: Criteria




- **CRITERIA: Minimum Area**
  - The **loci count (frequency)** is plotted against the **loci area (µm2)** 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.
- **CRITERIA: 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.

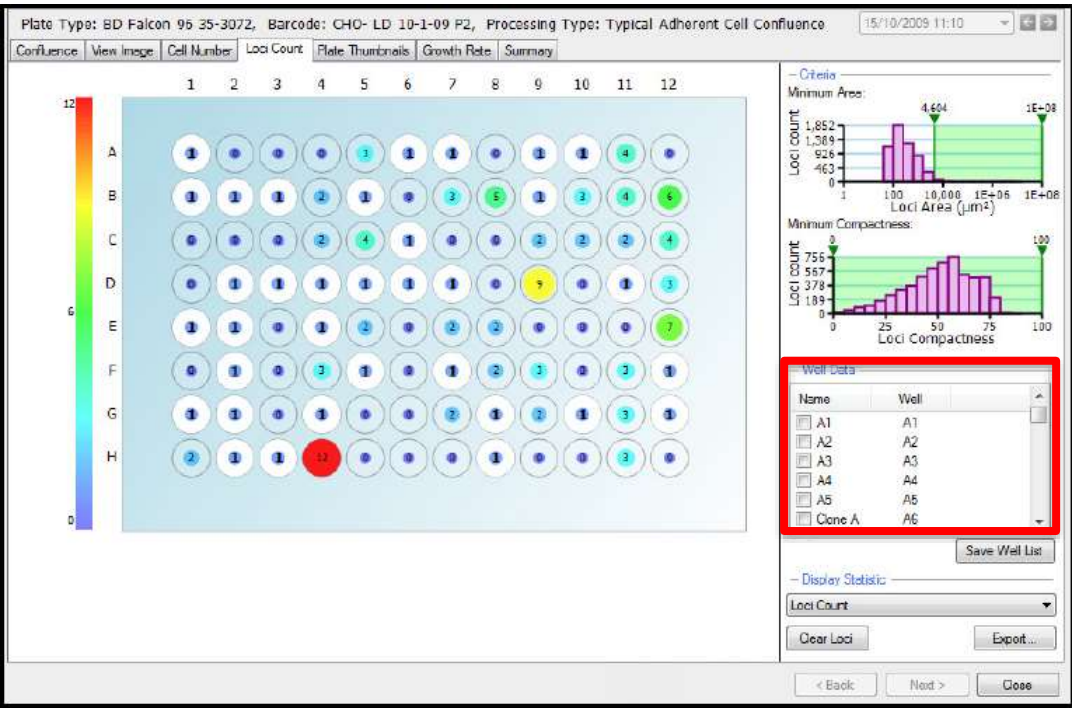
# Loci Count Tab: Criteria




- **CRITERIA: Minimum Area**
  - The **loci count (frequency)** is plotted against the **loci area (µm2)** 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.

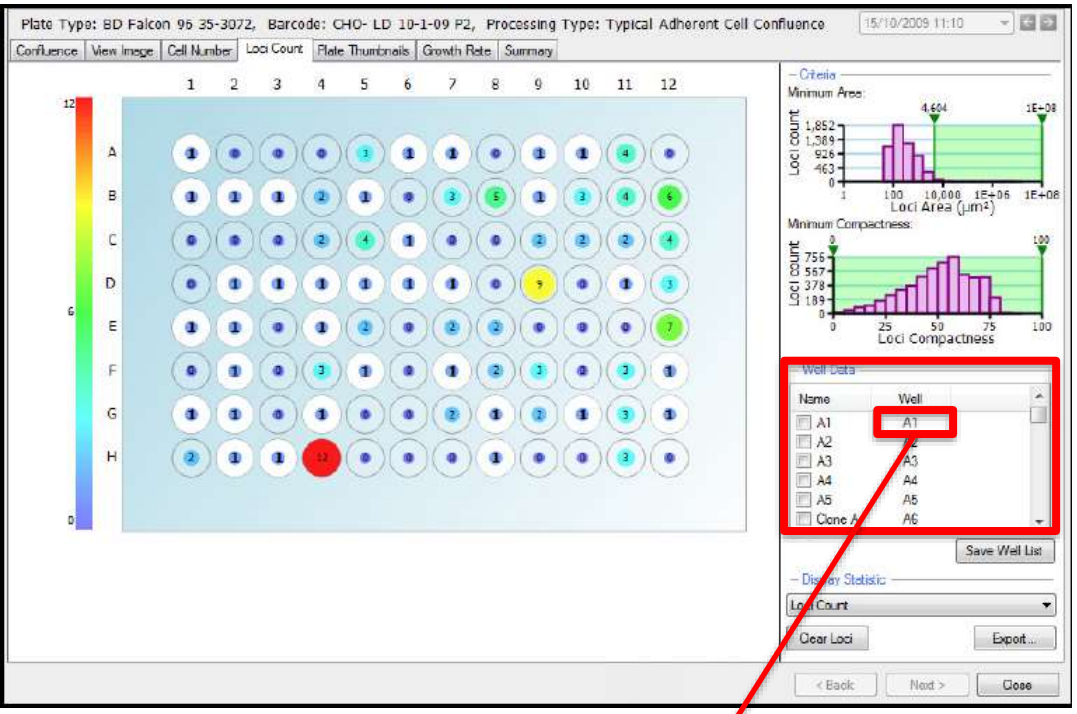
- **CRITERIA: Minimum Compactness**
  - The **loci count** (frequency) is plotted against the **loci compactness**.
  - The **bar chart** has two, **lower and upper, gates** that can be moved accordingly to **eliminate irregularly shaped objects** from the loci count.
  - **Loci Compactness** is the **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**.

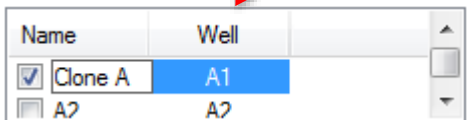
# Loci Count Tab: Well Data




- **WELL DATA: This section lists the wells.**

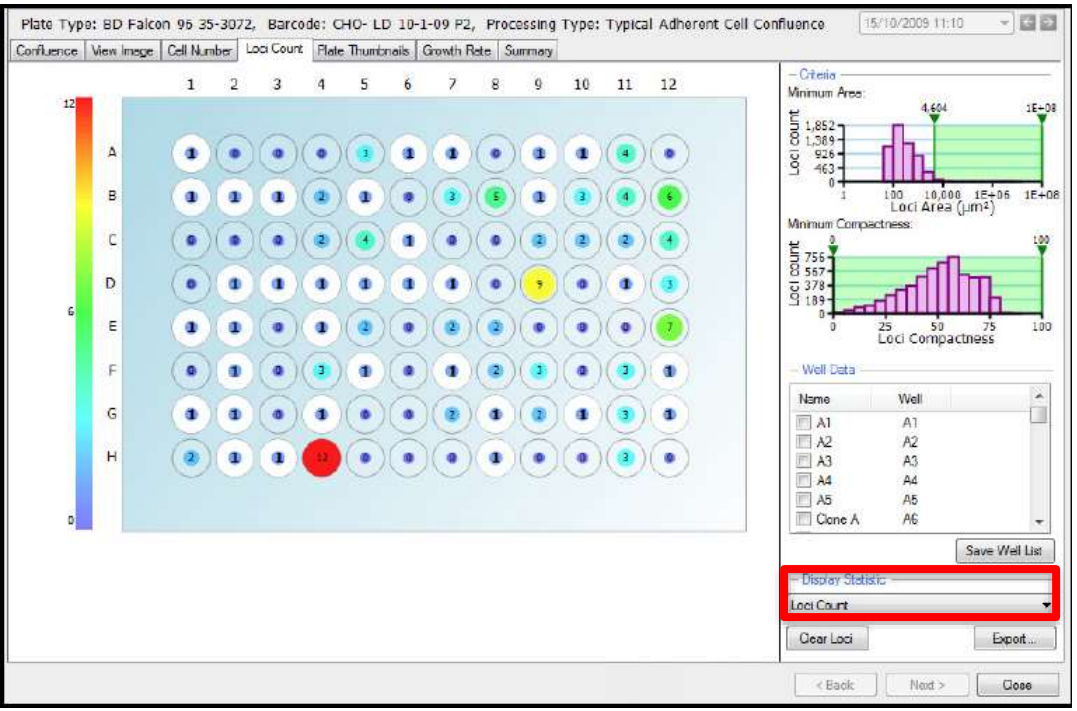
# Loci Count Tab: Well Data



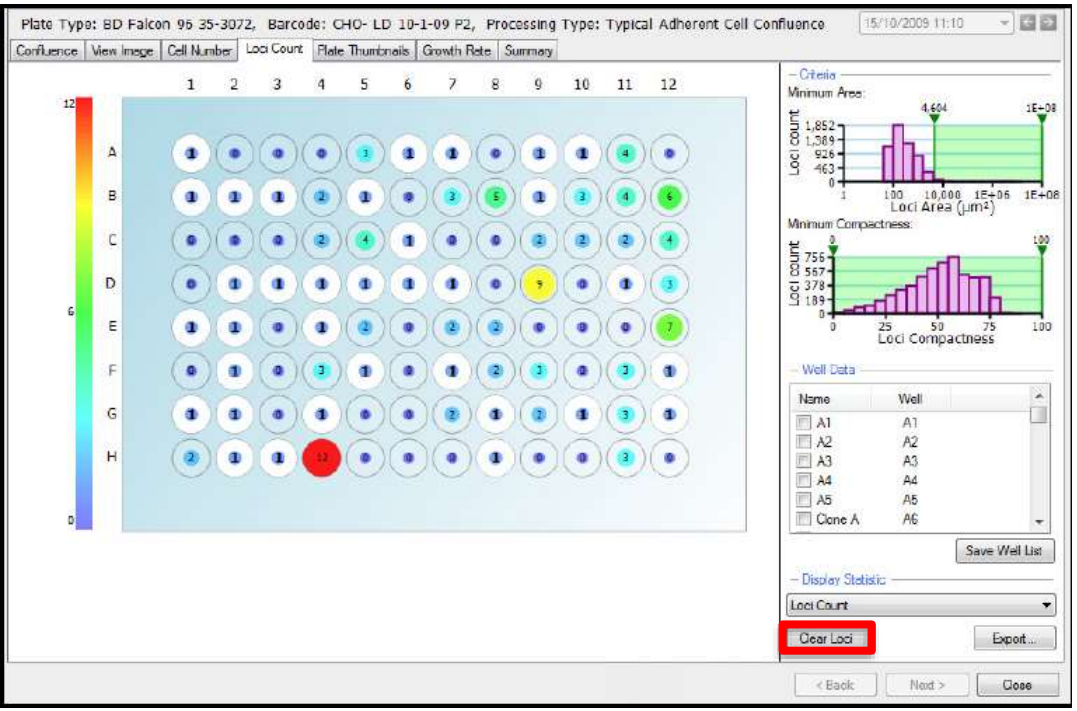

- **WELL DATA: This section lists the wells.**
- The **well name** can be changed if required by **clicking twice** on the well name and **typing in the desired name**.
  - *NOTE: This feature is utilized for tagging of monoclonal colonies in the **Monoclonality Assay** workflow.*

# Loci Count Tab: Display Statistic



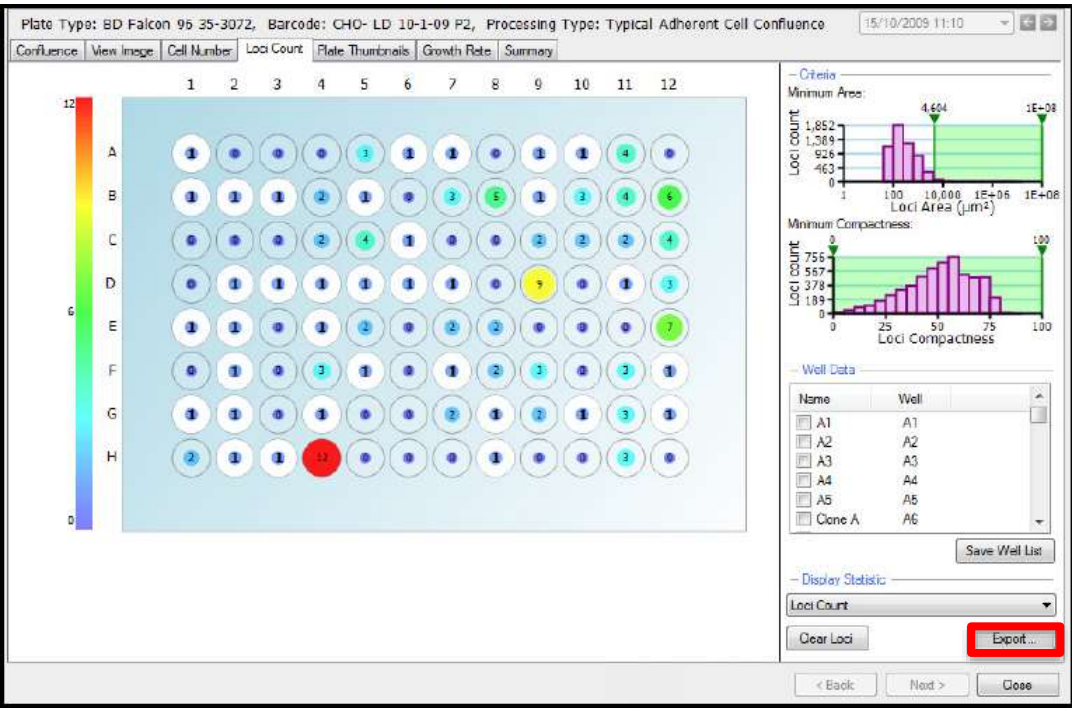

- **DISPLAY STATISTIC**: The drop down menu will display either the **'Loci Count'** or the **'Mean Loci Area'** on the overview.

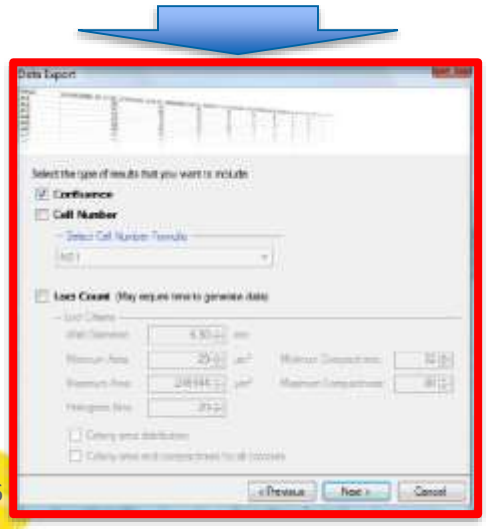
# Loci Count Tab: Clear Loci




- The **'Clear Loci'** button allows the **loci processing** to be **cleared.**
- When selected, a **prompt** will appear warning that all the **current loci data** will be **lost** if continuing**.**

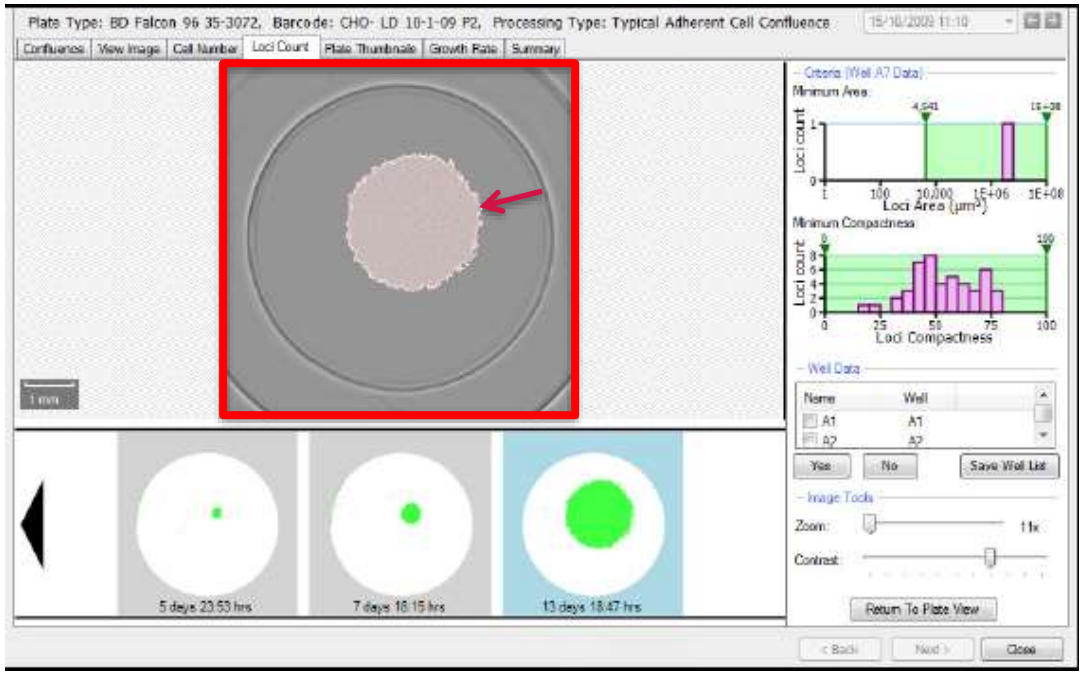
# Loci Count Tab: Export




- **Export:** Launches a **Data Export wizard** that enables export of the **list of wells** and the corresponding **confluence** as a **.csv or .xml file**.
  - **Cell Number** and **Loci Count (if feature is enabled)** data can also be exported at this point.

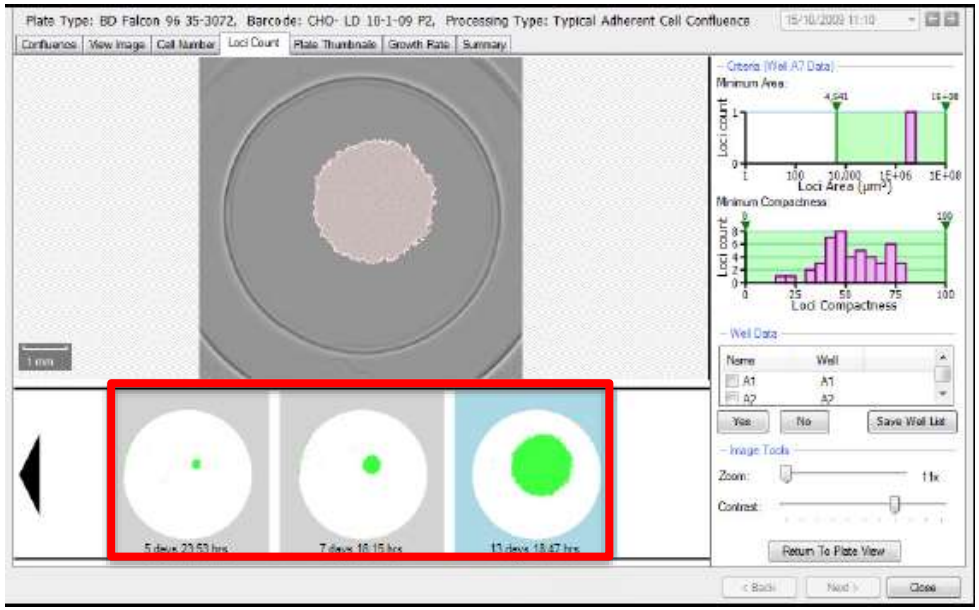
# Loci Count Tab: Well View




- To display the **Well View** for a well, **click on the desired well in Plate View** or in the **Well Data list.**

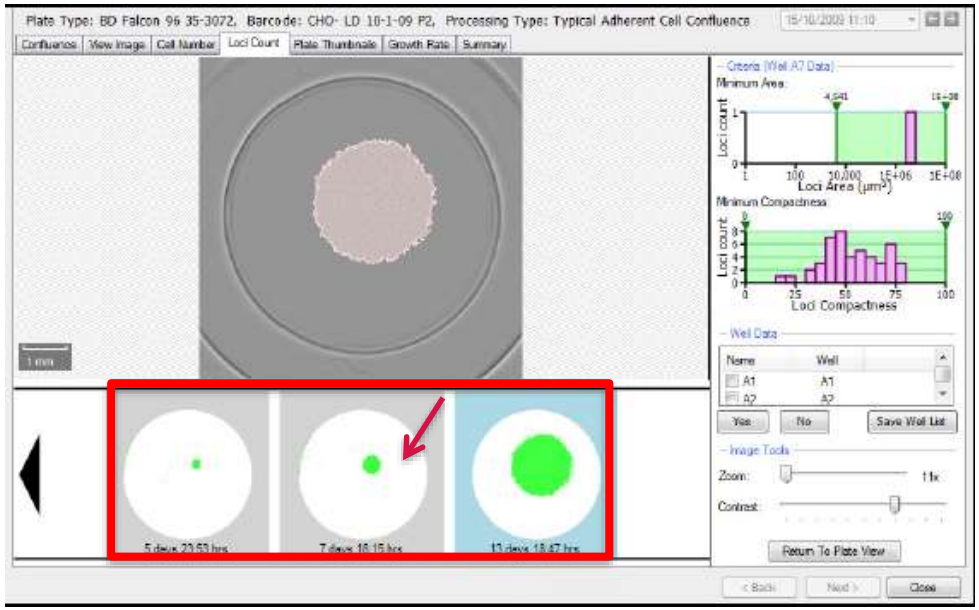
- You will now see the **image** for the selected well with an **overlay** over areas of growth **detected** according to the **Minimum Area** and **Minimum Compactness** criteria that were set on the graphs.

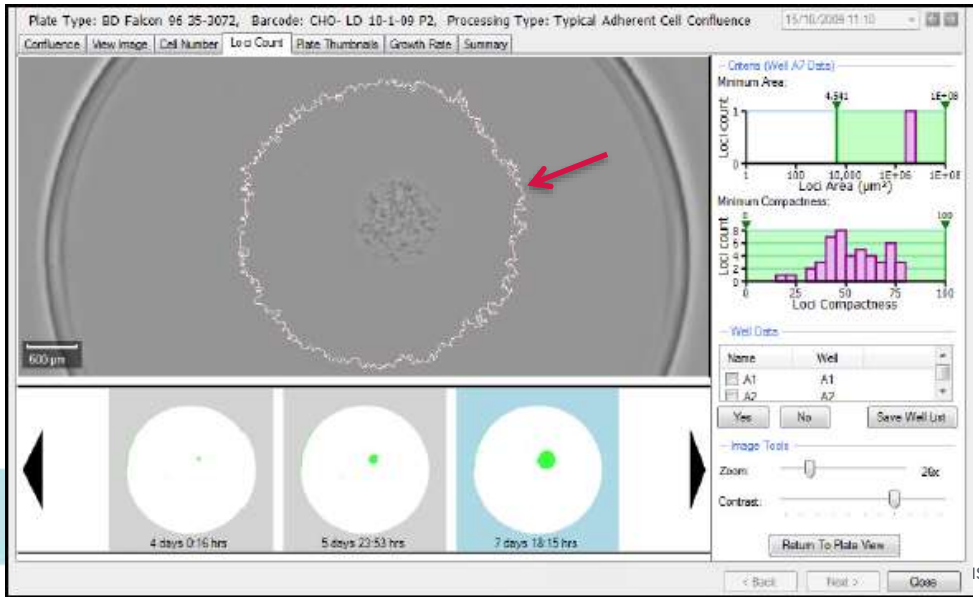
# Loci Count Tab: Well View – Filmstrips




- To display the **Well View** for a well, **click on the desired well in Plate View** or in the **Well Data list.**
- You will now see the **image** for the selected well with an **overlay** over areas of growth **detected** according to the **Minimum Area** and **Minimum Compactness** criteria that were set on the graphs.
- **Filmstrip View:** If the **microplate** has been **imaged** at **several time points** a **film strip** of **schematics** of the **well** at these time points is displayed across the bottom of the screen with detected **loci** colored in **green**.

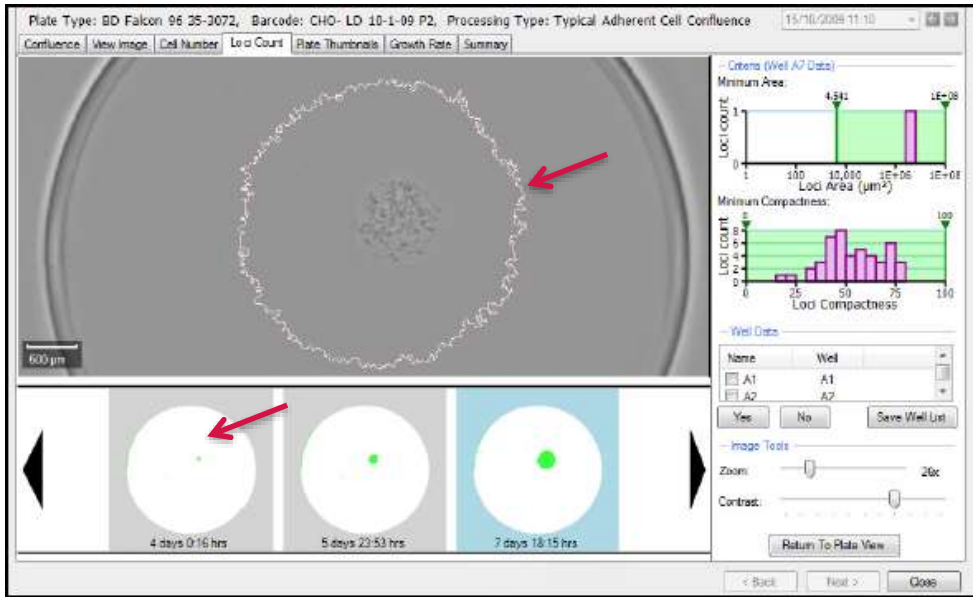
# Loci Count Tab: Well View – Filmstrips




- To display the **Well View** for a well, **click on the desired well in Plate View** or in the **Well Data list.**
- You will now see the **image** for the selected well with an **overlay** over areas of growth **detected** according to the **Minimum Area** and **Minimum Compactness** criteria that were set on the graphs.
- **Filmstrip View:** If the **microplate** has been **imaged** at **several time points** a **film strip** of **schematics** of the **well** at these time points is displayed across the bottom of the screen with detected **loci** colored in **green**.
- Click on any of the **schematics** in the **Filmstrip View** to **display** the **well image** at that **time point** with the **loci** from the **most recently captured image outlined**.

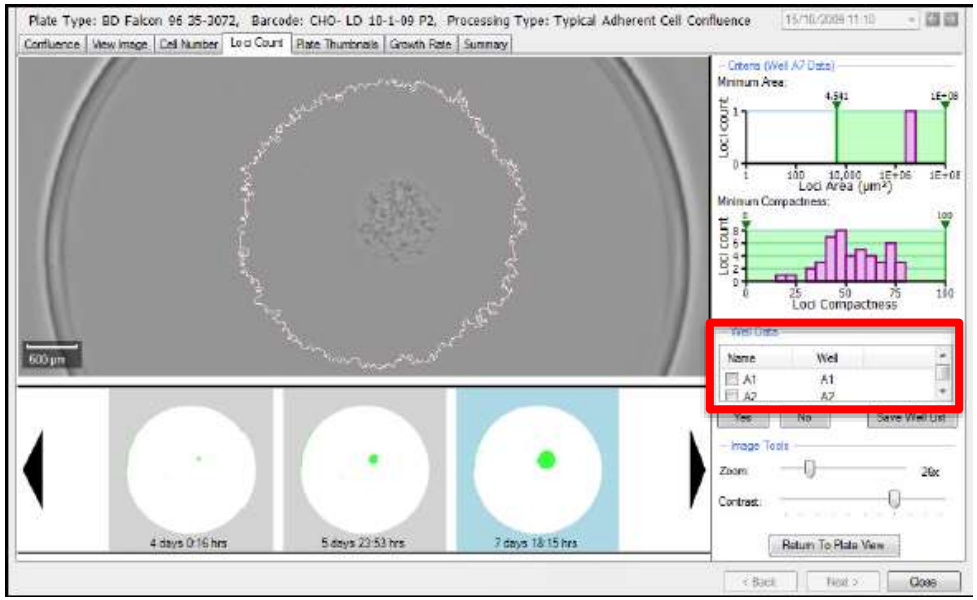
# Loci Count Tab: Well View - Filmstrips




***TIP:*** *To **export** the **Image Sequence, right-click** on the sequence - this can then be saved as **a .bmp, .jpg or .png file**. The **file** will include the **confluence percentage** and **time/day** that the images were acquired.*

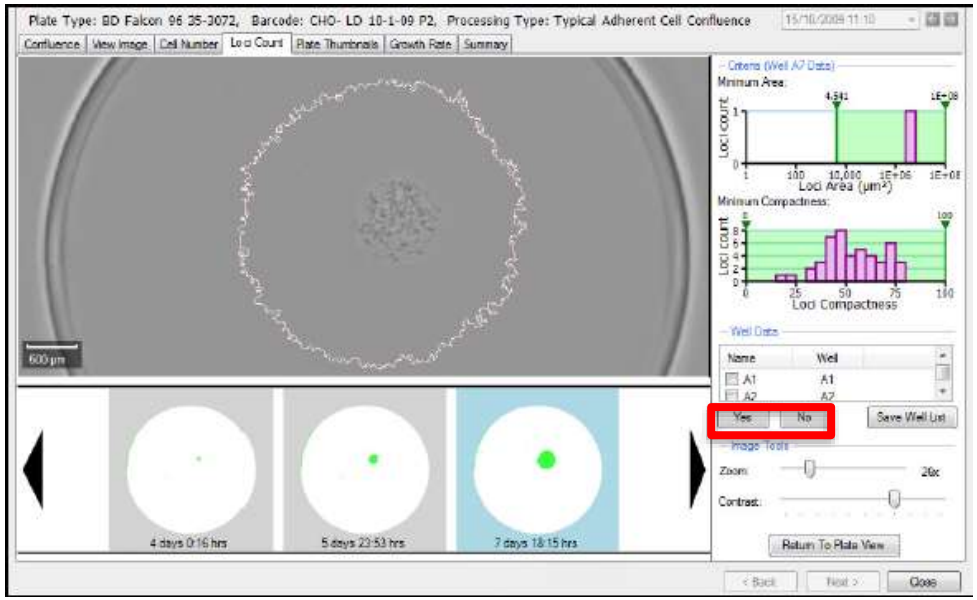
- To display the **Well View** for a well, **click on the desired well in Plate View** or in the **Well Data list.**
- You will now see the **image** for the selected well with an **overlay** over areas of growth **detected** according to the **Minimum Area** and **Minimum Compactness** criteria that were set on the graphs.
- **Filmstrip View:** If the **microplate** has been **imaged** at **several time points** a **film strip** of **schematics** of the **well** at these time points is displayed across the bottom of the screen with detected **loci** colored in **green**.
- Click on any of the **schematics** in the **Filmstrip View** to **display** the **well image** at that **time point** with the **loci** from the **most recently captured image outlined**.
- This provides a means of **checking** if a **colony** has **resulted from a single cell** or from more than one cell. For an example of analyzing **monoclonality data** see the **dedicated training module**.

# Loci Count Tab: Well View – Marking Monclonals




- **Marking Monoclonals:** It is possible to click on a well in the **Well Data** list to go to its corresponding **Well View** and check if the **colony** is **monoclonal**.

# Loci Count Tab: Well View – Marking Monclonals

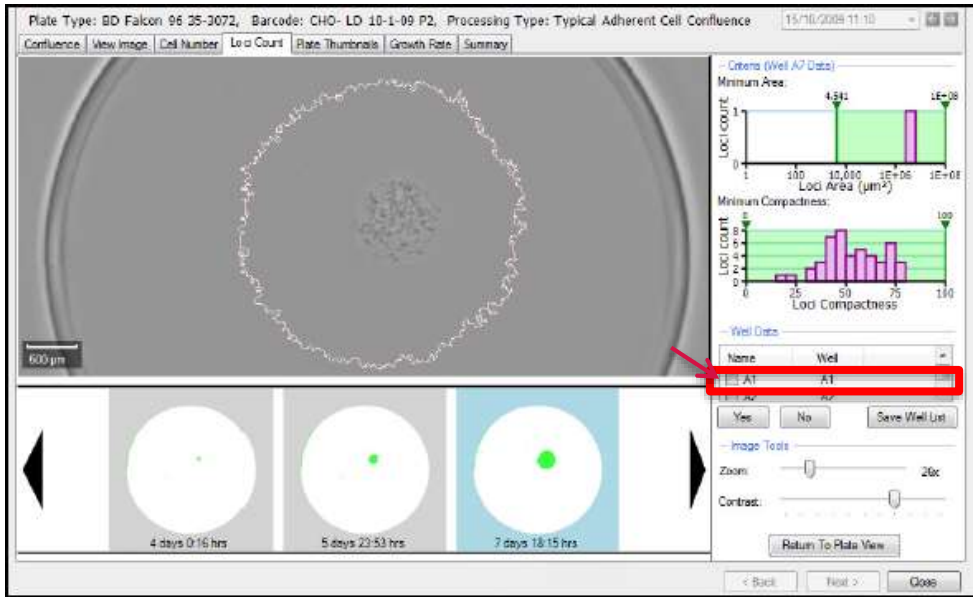



- **Marking Monoclonals:** It is possible to click on a well in the **Well Data** list to go to its corresponding **Well View** and check if the **colony** is **monoclonal**.

- There are **three** ways to move through the **well list** and **mark wells** as **monoclonal:**

  1. **Click** the **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**.

# Loci Count Tab: Well View – Marking Monclonals



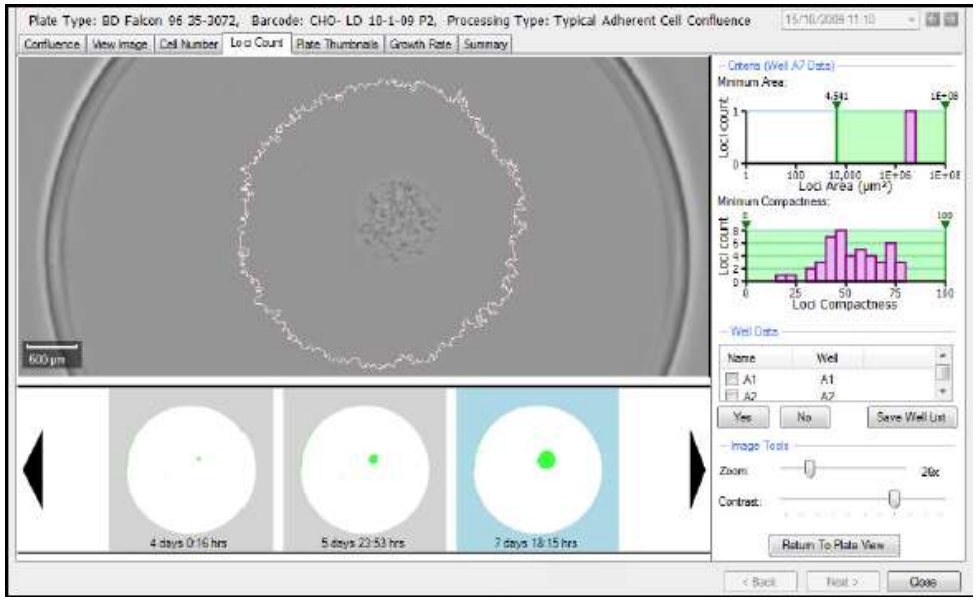

- **Marking Monoclonals:** It is possible to click on a well in the **Well Data** list to go to its corresponding **Well View** and check if the **colony** is **monoclonal**.

- There are **three** ways to move through the **well list** and **mark wells** as **monoclonal:**

1. **Click** the **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**.

2. **Click** on a **well** to **view** its **image** and **click** on the **check box** to **mark** it as **monoclonal**.

# Loci Count Tab: Well View – Marking Monclonals





**Up/Down Arrow Keys = Scroll through wells**

**Space Bar = Mark selected well as monoclonal**

- **Marking Monoclonals:** It is possible to click on a well in the **Well Data** list to go to its corresponding **Well View** and check if the **colony** is **monoclonal**.

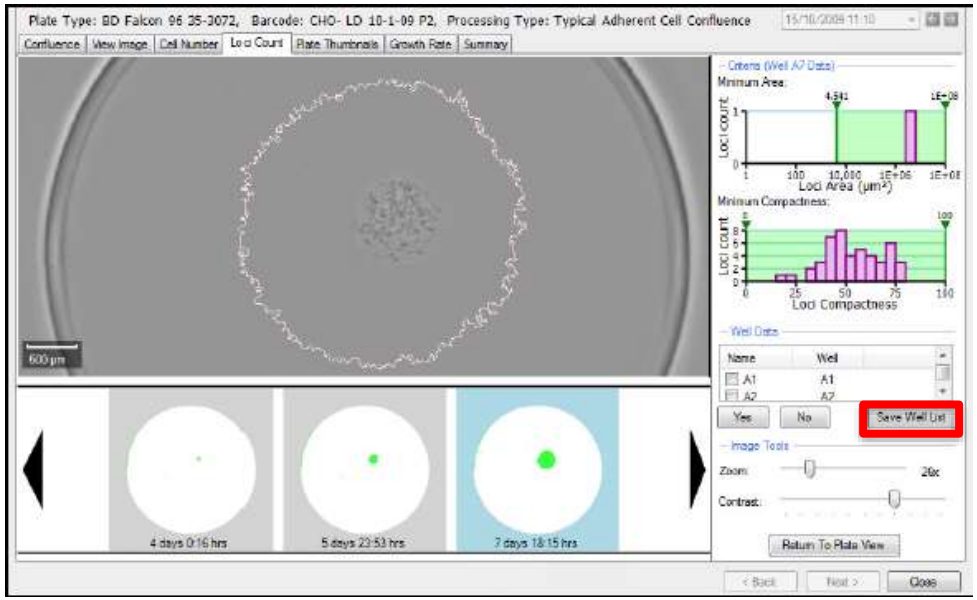
- There are **three** ways to move through the **well list** and **mark wells** as **monoclonal:**

1. **Click** the **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**.

2. **Click** on a **well** to **view** its **image** and **click** on the **check box** to **mark** it as **monoclonal**.

3. Use the **keyboard up/down arrow keys** to **scroll through the wells** and the **spacebar** to **mark** the **wells** as **monoclonal**.

# Loci Count Tab: Well View – Marking Monclonals




- **Marking Monoclonals:** It is possible to click on a well in the **Well Data** list to go to its corresponding **Well View** and check if the **colony** is **monoclonal**.

- There are **three** ways to move through the **well list** and **mark wells** as **monoclonal:**

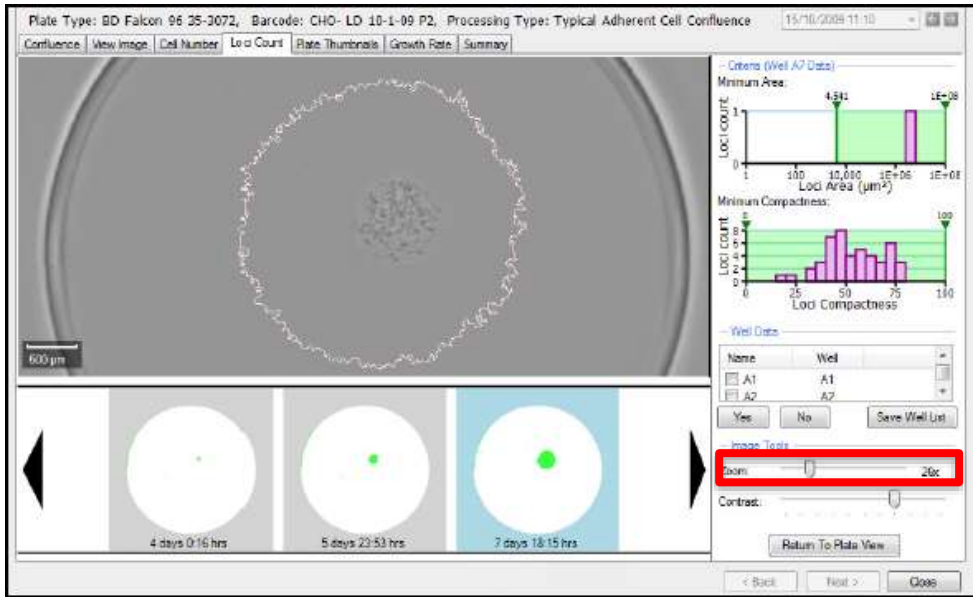
  1. **Click** the **Yes button** to **mark** a **well** as **monoclonal** and **scroll** to the **next well image**. Click **No** to **scroll** to the **next well without marking** the **well** as **monoclonal**.

  2. **Click** on a **well** to **view** its **image** and **click** on the **check box** to **mark** it as **monoclonal**.

  3. Use the **keyboard up/down arrow keys** to **scroll through the wells** and the **spacebar** to **mark** the **wells** as **monoclonal**.

- **Save Well List:** The **selected wells** in the **list** can be **saved** by clicking on the **Save Well List** button. This **file** can be saved as a **.csv** file. The **file** will display the **plate barcode, run date, operator** and the **selected wells.**

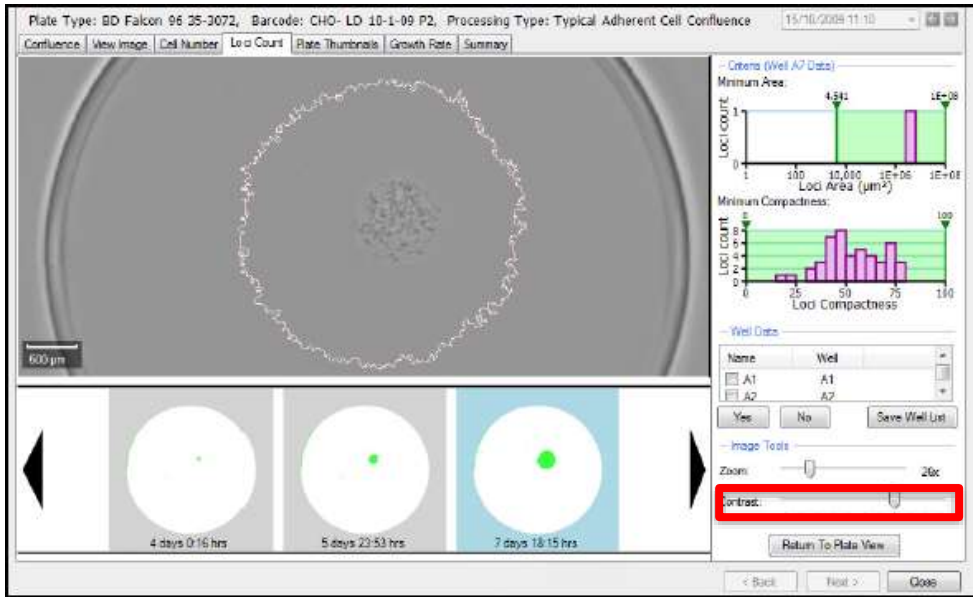
# Loci Count Tab: Well View – Image Tools




- **Zoom:** This **slider** can be moved 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 which may cause some **pixilation** of the image.
  - When **zoomed into an image**, the **zoomed area** will be displayed on the **image thumbnail** to the **right**.

***TIP:*** *You can also use your **mouse scroll wheel** to **zoom** on the **image**. Simply **navigate** to the **image** with your **cursor** and move the mouse scroll wheel **forward** to **zoom in**, in **reverse** to **zoom out**.*

# Loci Count Tab: Well View – Image Tools

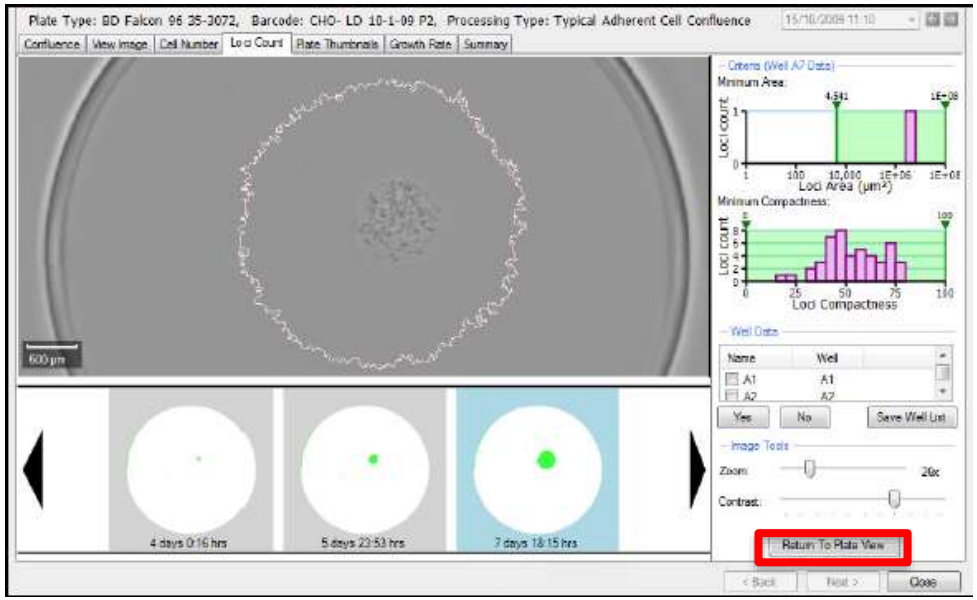


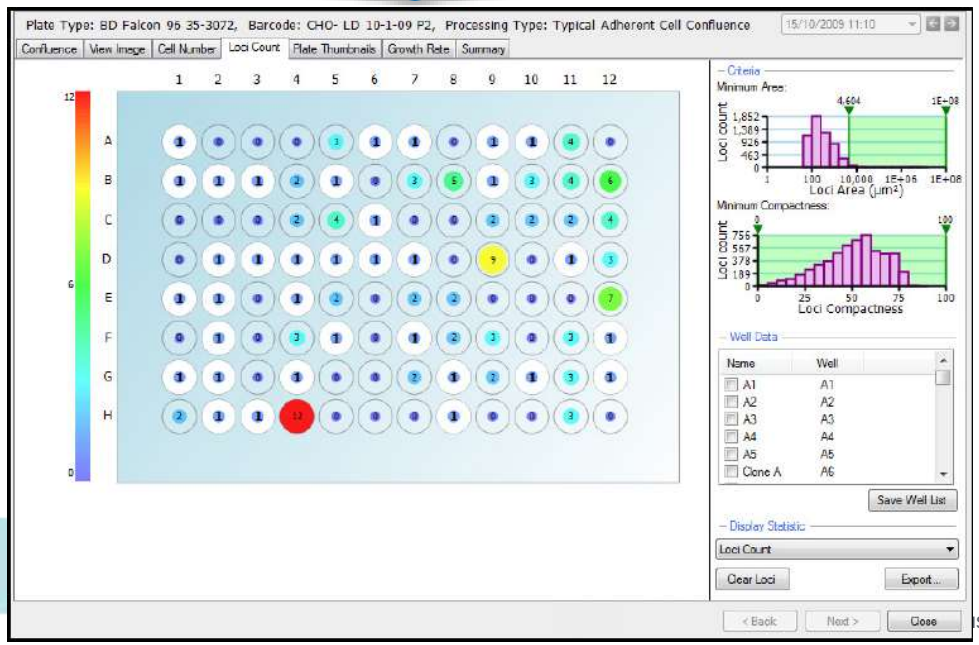

- **Zoom:** This **slider** can be moved either **left or right** to **zoom out or in respectively**.
  - The **lowest magnification** of the image is **18x** and the **highest** is **144x**.
  - When the **figure** turns **red** the system is **zooming digitally** and which may cause some **pixilation** of the image.
  - When **zoomed into an image**, the **zoomed area** will be displayed on the **image thumbnail** to the **right**.

- **Contrast:** The **displayed contrast** of the **image** can be altered by moving this **slider** to the **left or right**.

  ***Note:*** *Changing the **display contrast does not alter** the **image** itself.*

# Loci Count Tab: Well View – Image Tools



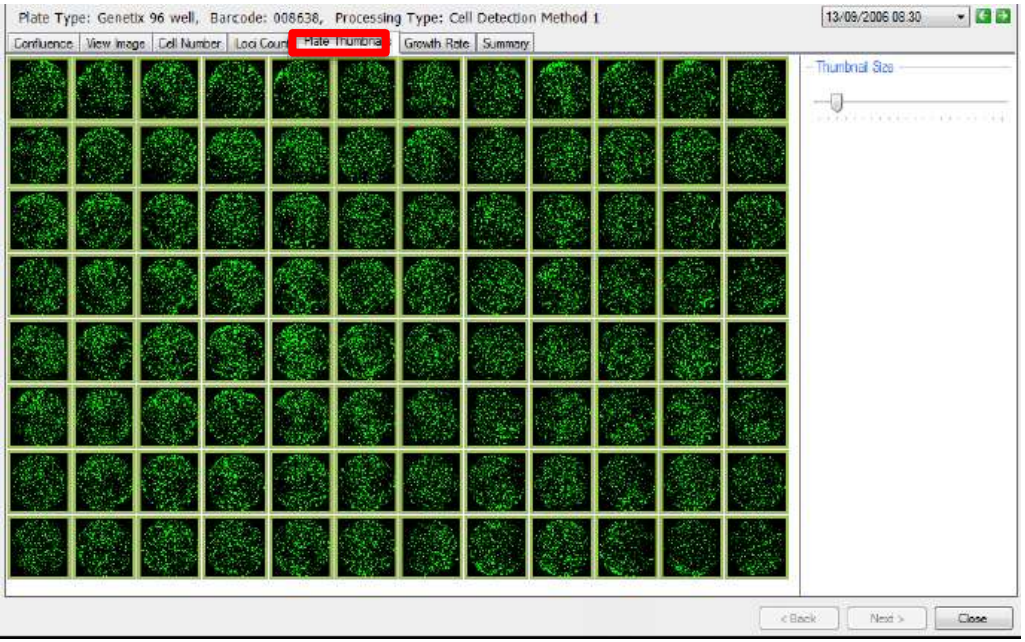

- **Zoom:** This **slider** can be moved either **left or right** to **zoom out or in respectively**.
  - The **lowest magnification** of the image is **18x** and the **highest** is **144x**.
  - When the **figure** turns **red** the system is **zooming digitally** and which may cause some **pixilation** of the image.
  - When **zoomed into an image**, the **zoomed area** will be displayed on the **image thumbnail** to the **right**.

- **Contrast:** The **displayed contrast** of the **image** can be altered by moving this **slider** to the **left or right**.

  ***Note:*** *Changing the **display contrast** **does not alter** the **image** itself.*

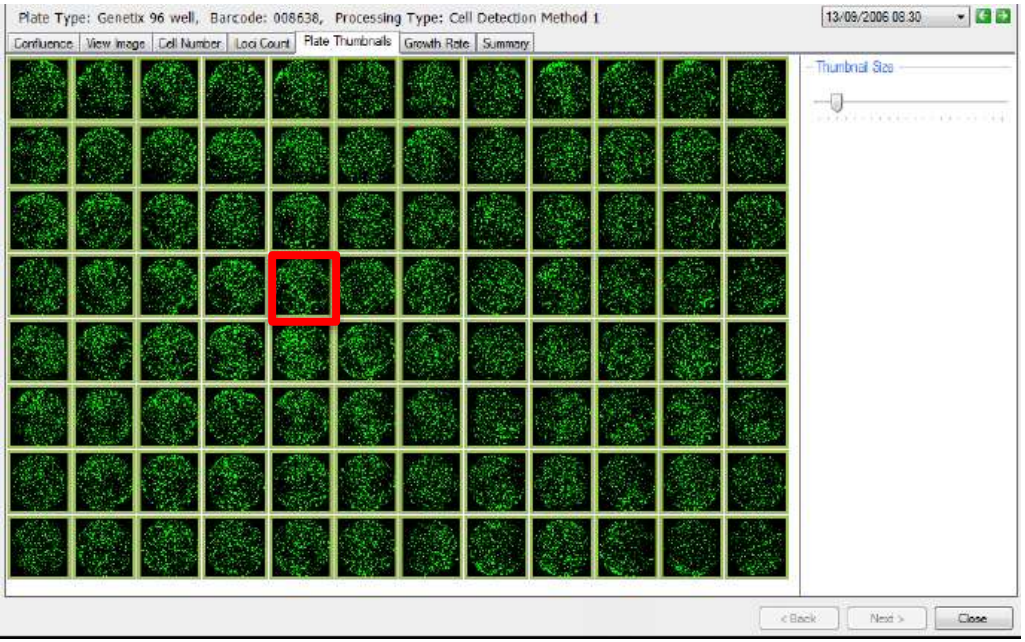
- **Return to Plate View:** Clicking this button will **return** the **screen** to the **Plate View** within the **Loci Count Tab**.

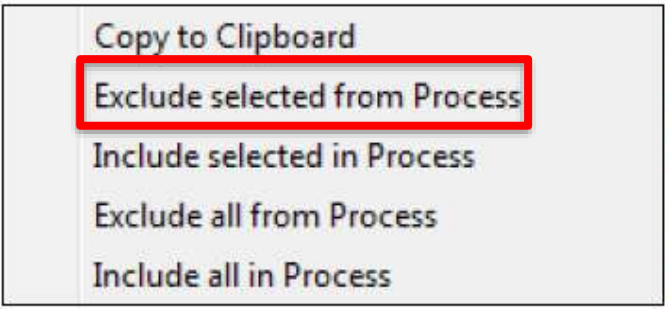
# Plate Thumbnails Tab - Overview

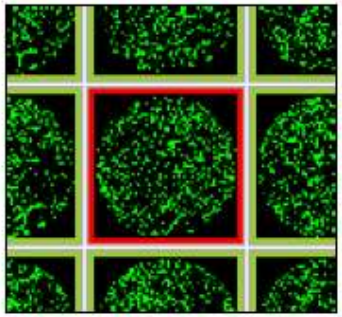



- **Plate Thumbnails Tab:** This tab displays **thumbnail images** of the **wells** for the **entire plate**. These **thumbnail images** display the **green confluence overlay** of each of the imaged **wells**.

# Plate Thumbnails Tab – Including & Excluding Wells

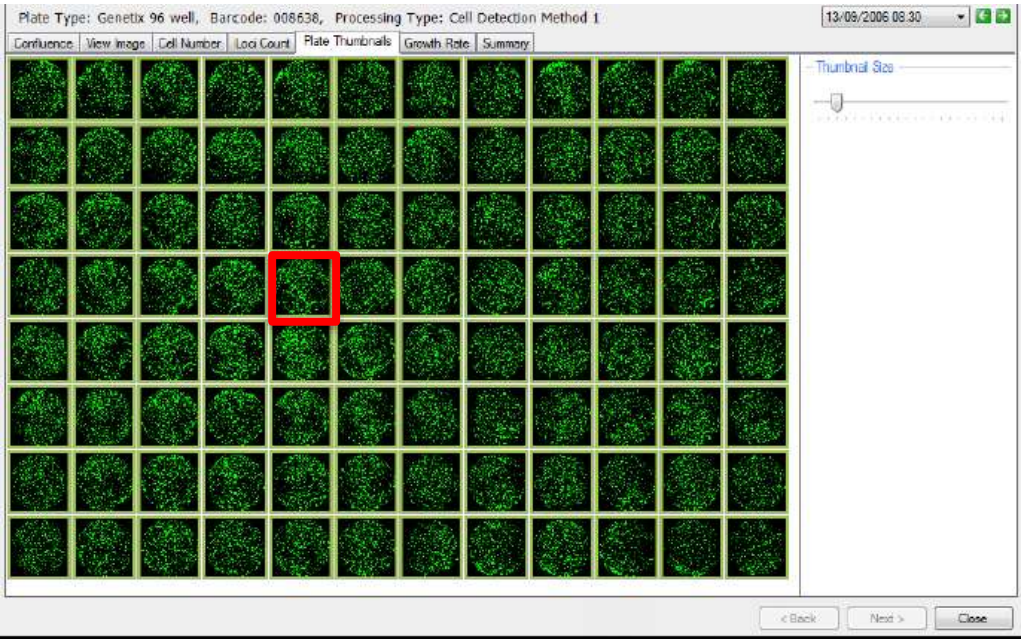


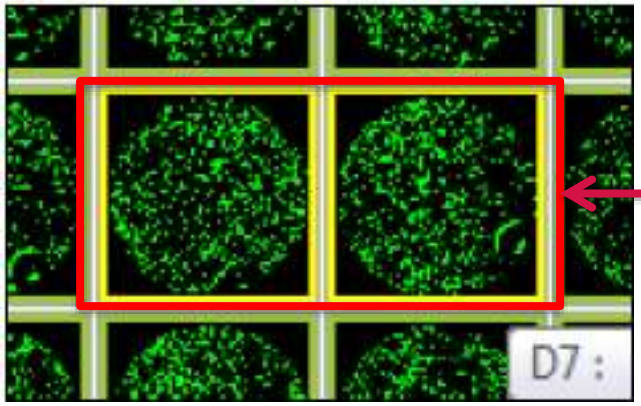



- **Selected wells** can be **included or excluded from analysis** from this view. Any **wells included or excluded** will be **updated** in the **Confluence, Cell Number, Loci Count** and **Growth Rate tabs** as well. You can **select wells** as follows:
  - **Right mouse clicking** on the **well of interest** will display an **options menu** where a **single or all wells** can be **excluded or included**. **Excluded wells** are displayed with a **red** outline. **Included wells** are displayed with a **green** outline.

# Plate Thumbnails Tab – Including & Excluding Wells

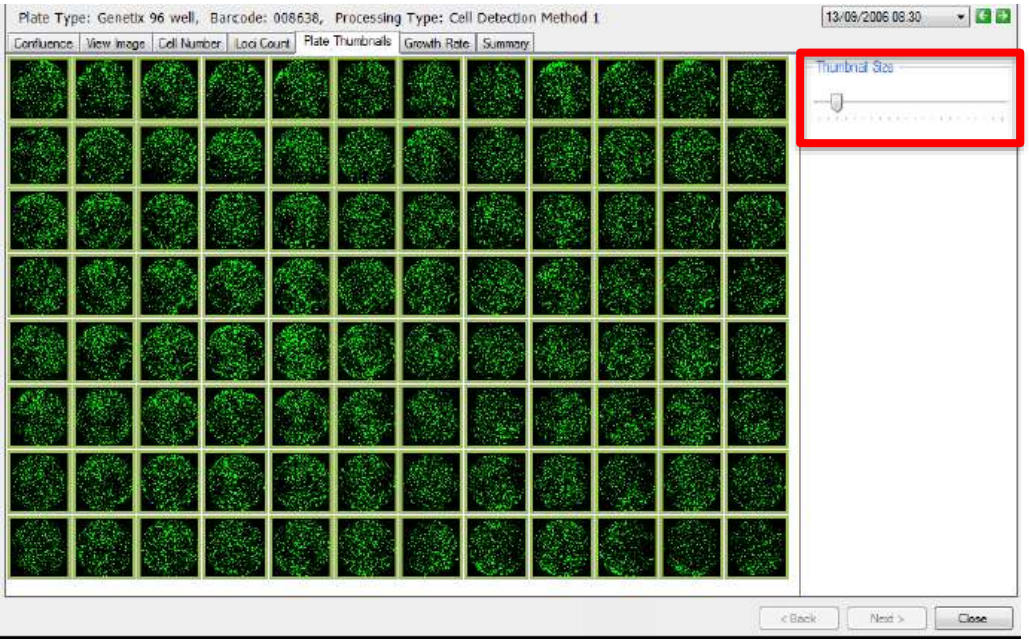



*Hold down CTRL + Left Mouse Click to Select Multiple Wells to Include or Exclude*

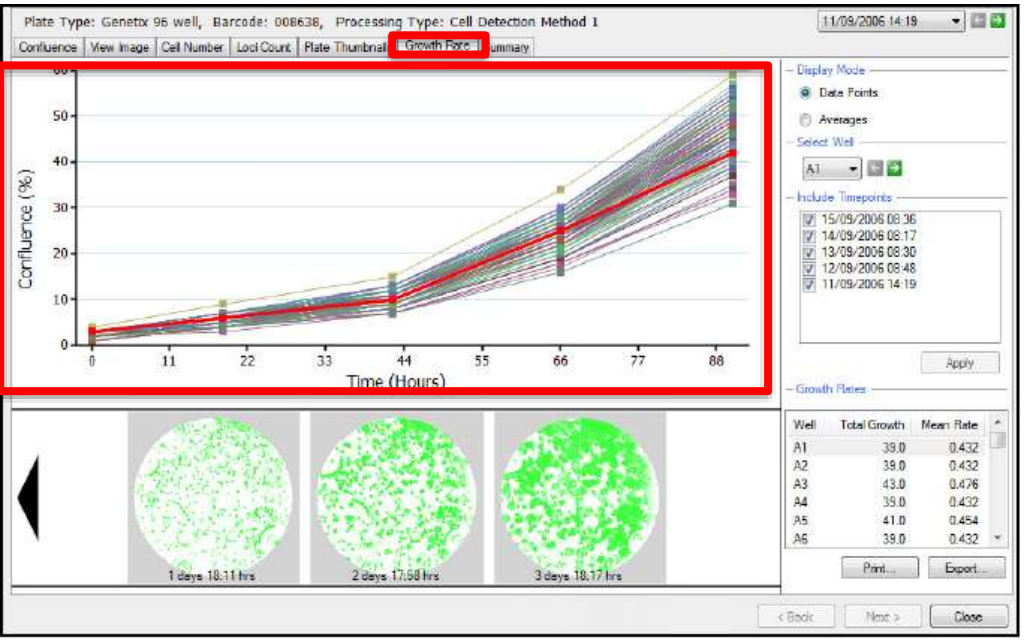
- **Selected wells** can be **included or excluded from analysis** from this view. Any **wells included or excluded** will be **updated** in the **Confluence, Cell Number, Loci Count** and **Growth Rate tabs** as well. You can **select wells** as follows:
  - **Right mouse clicking** on the **well of interest** will display an **options menu** where a **single or all wells** can be **excluded or included**. **Excluded wells** are displayed with a **red** outline. **Included wells** are displayed with a **green** outline.
  - **Holding down** the **Control key** on the **keyboard** and the **left mouse button** allows **several wells** to be **selected**. These will be highlighted in **yellow** and the **selecting** from the pop-up **options menu** will enable these wells to be **included or excluded** from the **data set**.

# Plate Thumbnails Tab – Changing Thumbnail Zoom



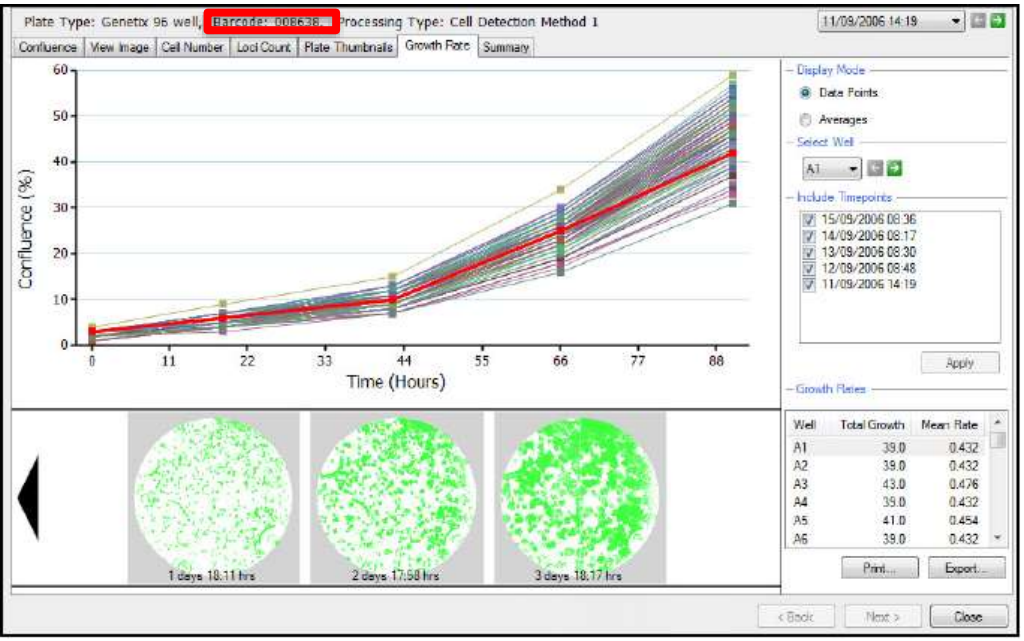

- Click and drag the **Thumbnail Size slider** to **zoom** the thumbnail view **in or out**.

# Growth Rate Tab - Overview




- **Growth Rate Tab:** If a **selected microplate** has been **imaged** at **multiple time points**, **growth curves** are displayed for **all wells** in the plate on this tab. The default graph view plots **% Confluence** of the wells vs. **Time (Hours).**

# Growth Rate Tab - Overview



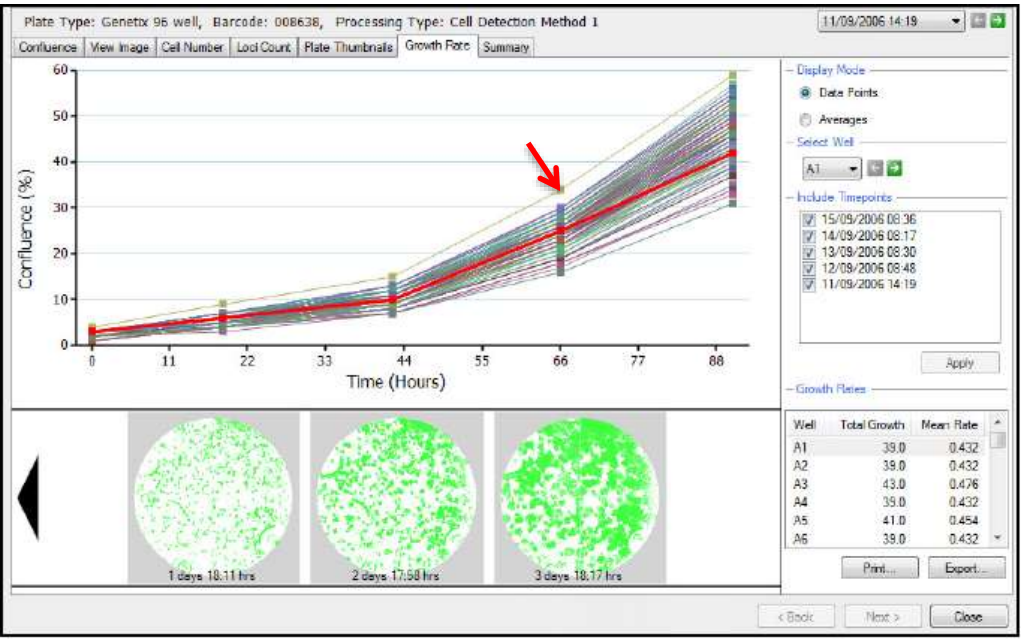

- **Growth Rate Tab:** If a **selected microplate** has been **imaged** at **multiple time points**, **growth curves** are displayed for **all wells** in the plate on this tab. The default graph view plots **% Confluence** of the wells vs. **Time (Hours).**

- The **software** determines if the plate has been imaged by **collating data** from **the same barcoded plate**, so ideally **barcoded microplates** should be used to ensure all data is gathered properly for a given plate over time.

**HINT:** **Named plates** can also be used but the ***name must be typed in exactly the same each time that the plate is imaged***, so that the software can collate the results over time.

The ability to **rename plates** also enables **mis-named plates** to be **included** in the **growth curve data** if required.

# Growth Rate Tab - Overview

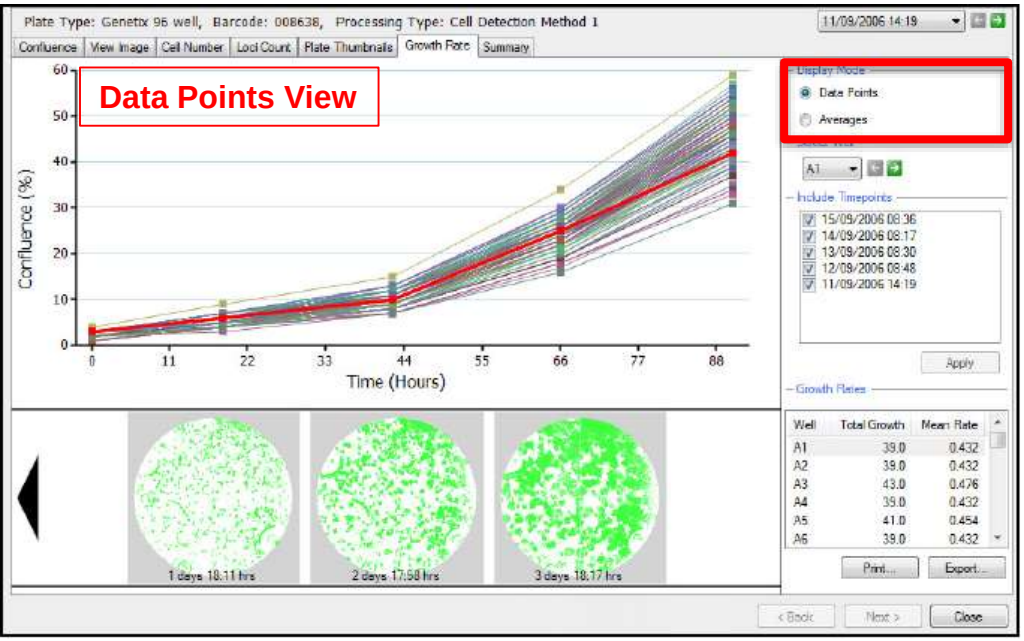


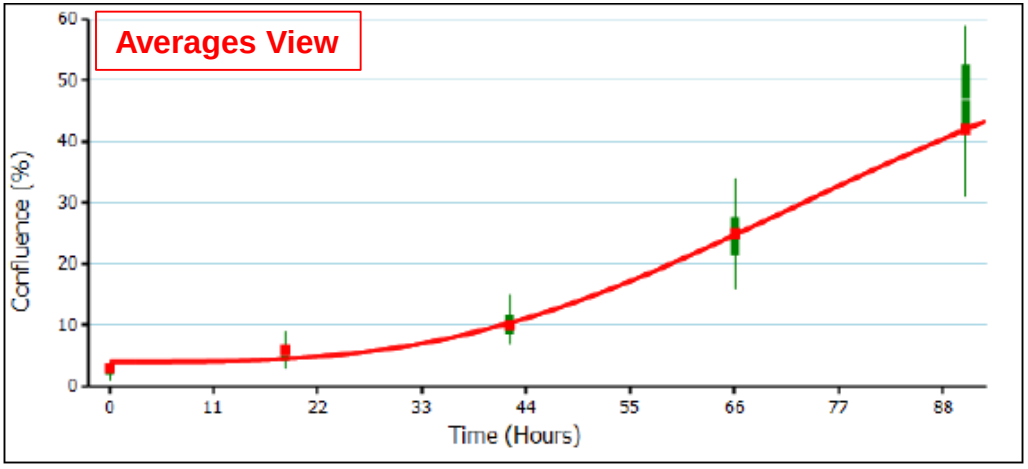

- **Growth Rate Tab:** If a **selected microplate** has been **imaged** at **multiple time points**, **growth curves** are displayed for **all wells** in the plate on this tab. The default graph view plots **% Confluence** of the wells vs. **Time (Hours).**

- The **software** determines if the plate has been imaged by **collating data** from **the same barcoded plate**, so ideally **barcoded microplates** should be used to ensure all data is gathered properly for a given plate over time.

- Hovering your **cursor** over **each data point** in the **graph** displays the **well coordinate** for that **point**.

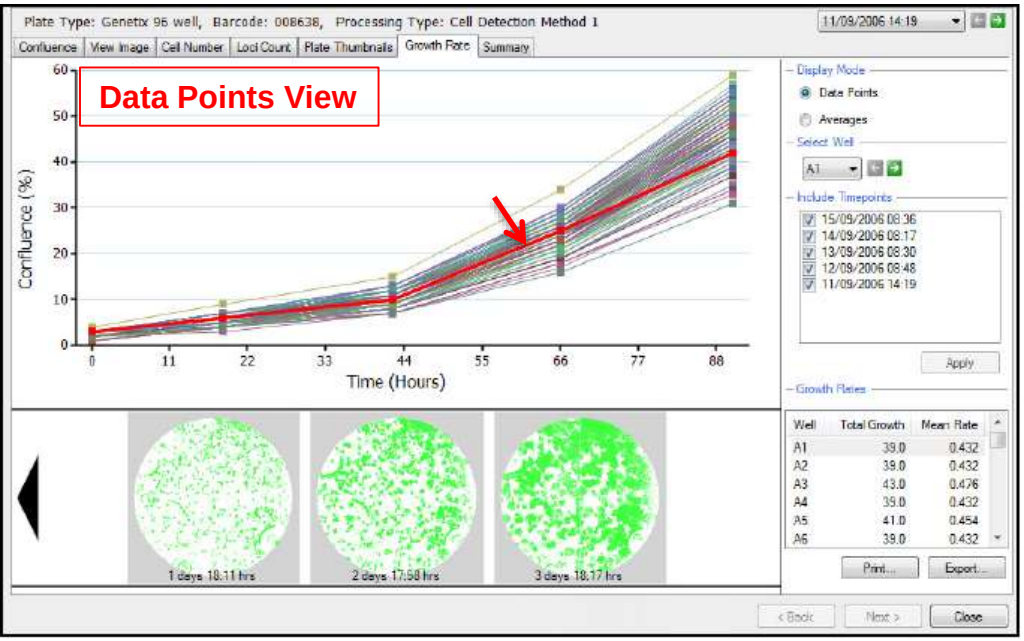
# Growth Rate Tab – Display Mode

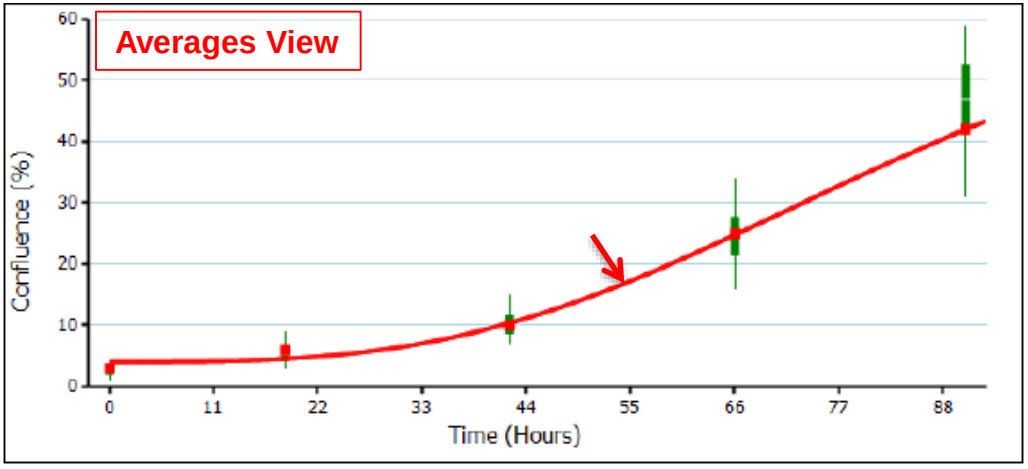



- **Display Mode:** The **growth rate graph** can be displayed with **all well data points** by selecting the **Data Points** option or as **averages** by selecting the **Averages** option.

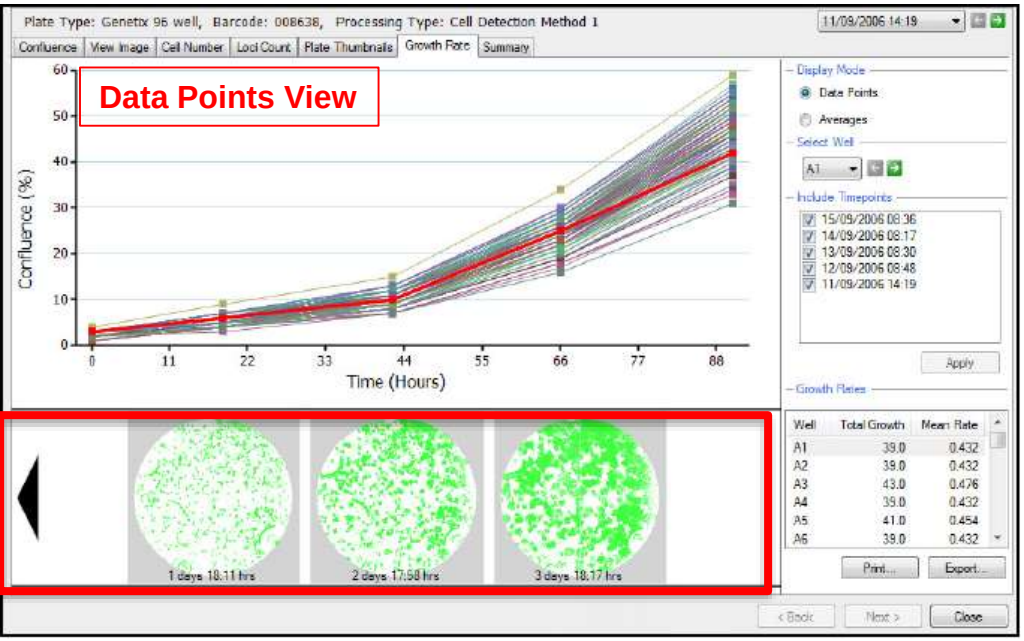
# Growth Rate Tab – Display Mode

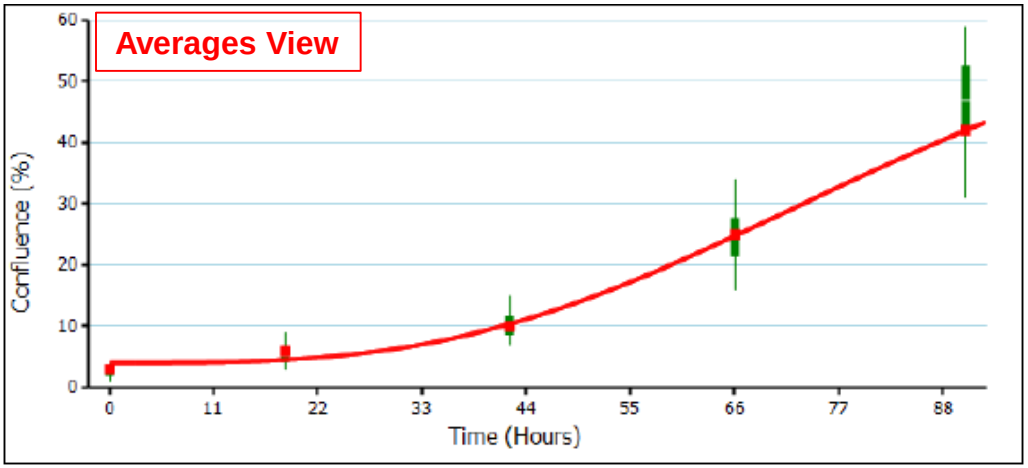



- **Display Mode:** The **growth rate graph** can be displayed with **all well data points** by selecting the **Data Points** option or as **averages** by selecting the **Averages** option.
- The **thick red line** within **both graph views** is displaying the **growth rate** for the **selected well**, e.g. A1.

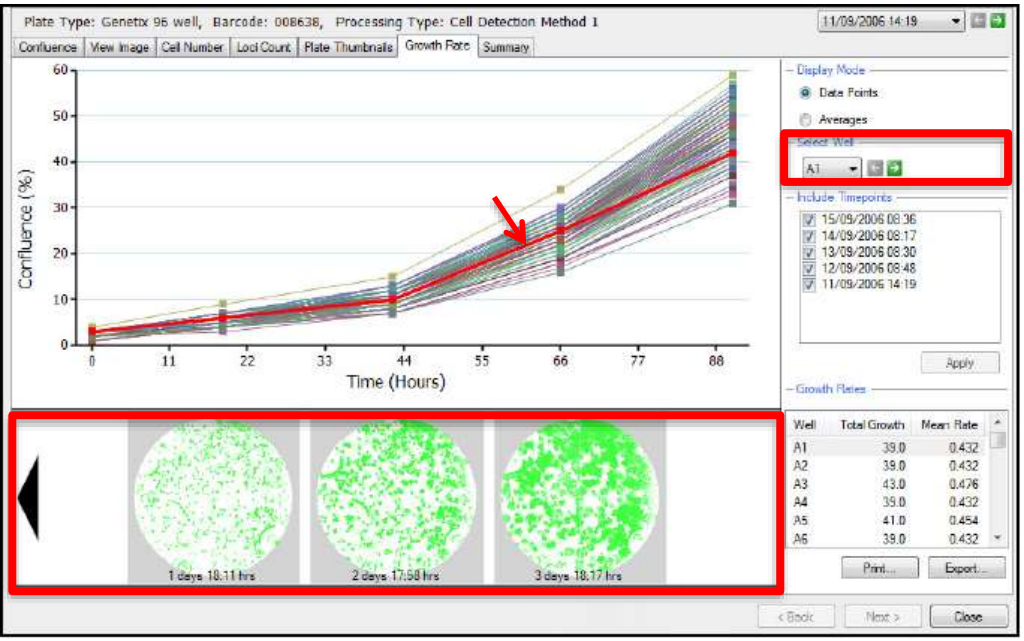
# Growth Rate Tab – Display Mode



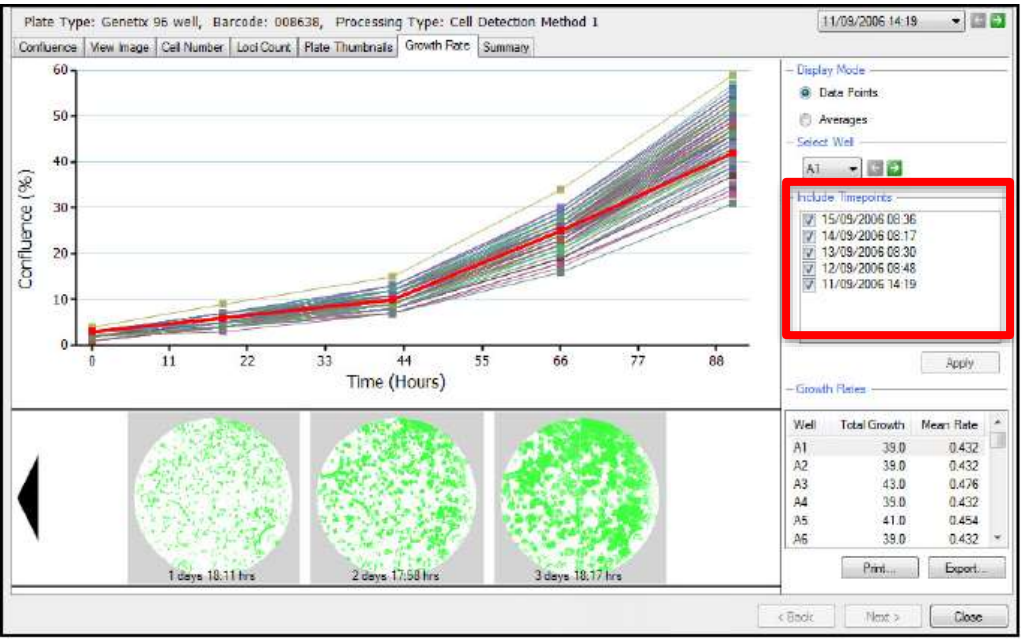

- **Display Mode:** The **growth rate graph** can be displayed with **all well data points** by selecting the **Data Points** option or as **averages** by selecting the **Averages** option.
- The **thick red line** within **both graph views** is displaying the **growth rate** for the **selected well**, e.g. A1.
- The **well schematic** below the **graph** is also displaying the **captured images** for the **selected well** over **time**.

# Growth Rate Tab – Select Well



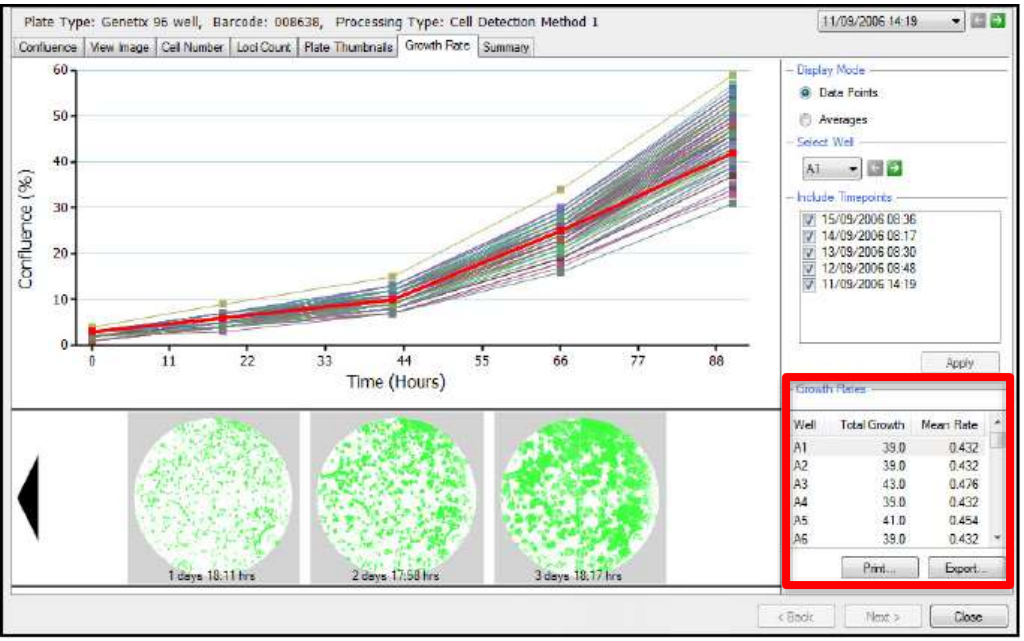

- **Select Well:** This **drop down menu** allows the displayed well to be changed.
- The **selected well** will be highlighted by a **thick red line** in the **graph view**, and the **filmstrip view** will update as well.
- You can also **advance through the wells** using the **arrow keys** next to this dropdown.

# Growth Rate Tab – Including & Excluding Timepoints



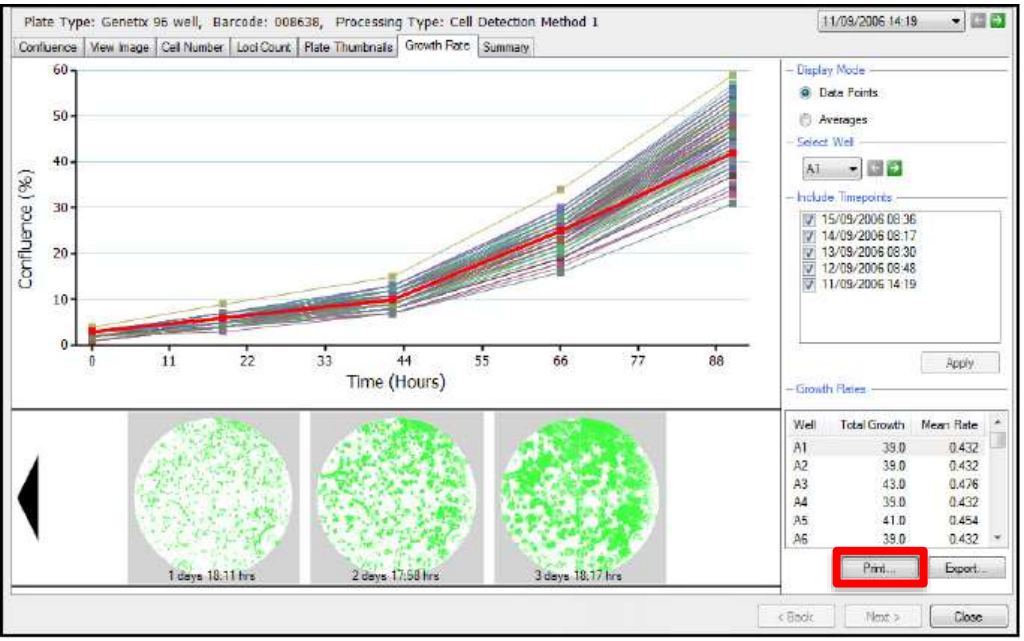

- **Include Timepoints:** This field allows for **inclusion or exclusion** of **selected timepoints** from the **displayed data**.
- **All timepoints** for a given **plate** are **selected** (checked) in the list **by default**.
- **Uncheck** the **box** next to a **selected timepoint** to **exclude** it.

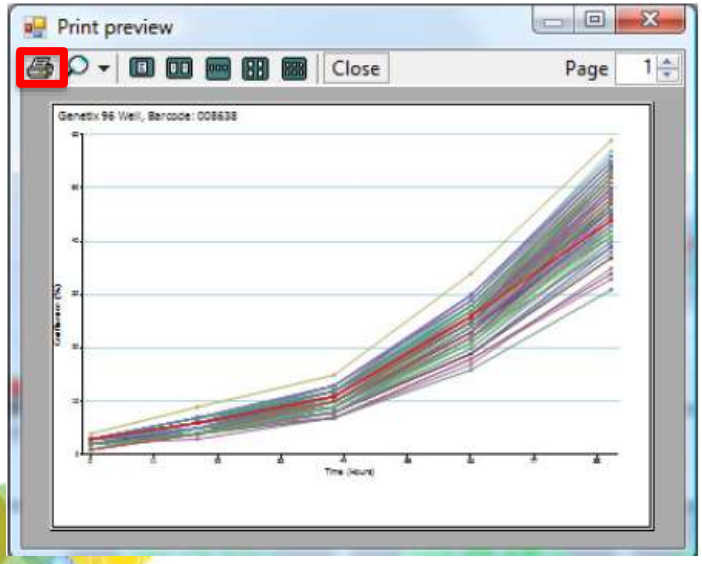
# Growth Rate Tab – Growth Rates Table




- **Growth Rates:** This field displays the **Well Name, Total Growth and Mean Rate** for each well in the data set.

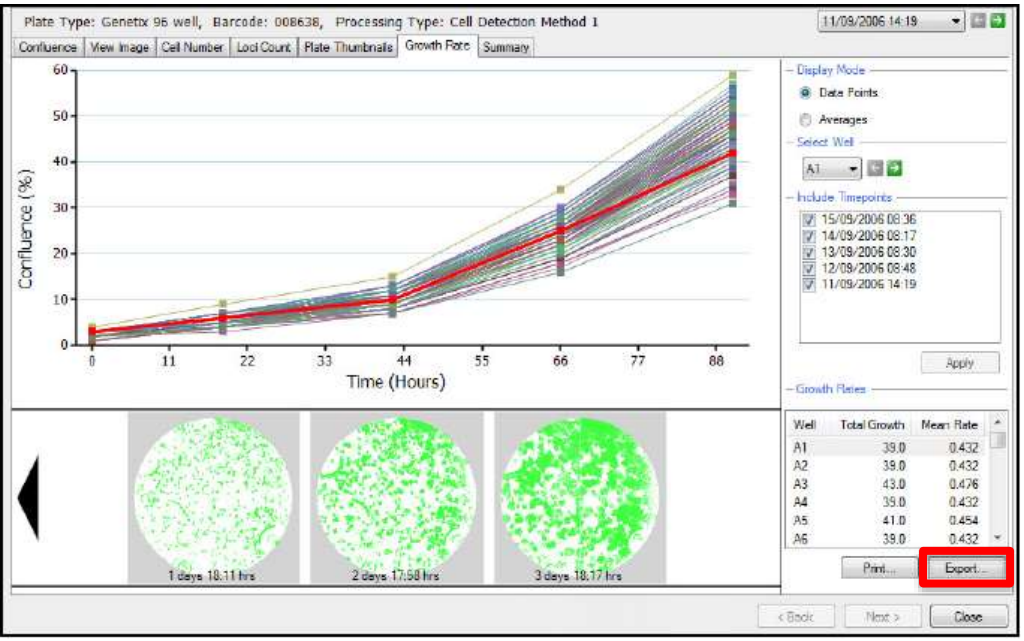
# Growth Rate Tab – Print Graph

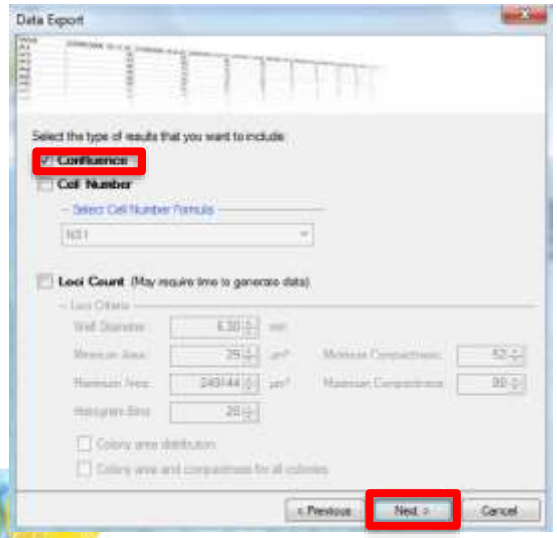


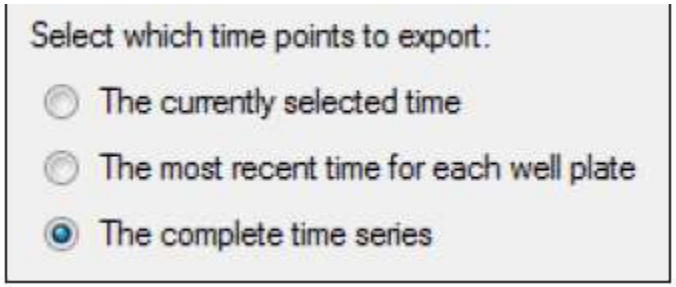

- **Print:** Click this button to open a **print preview copy** of the **growth curves**. If your **computer** is **networked** to a **printer,** you can print the **graph** from this dialog.

# Growth Rate Tab – Export Data

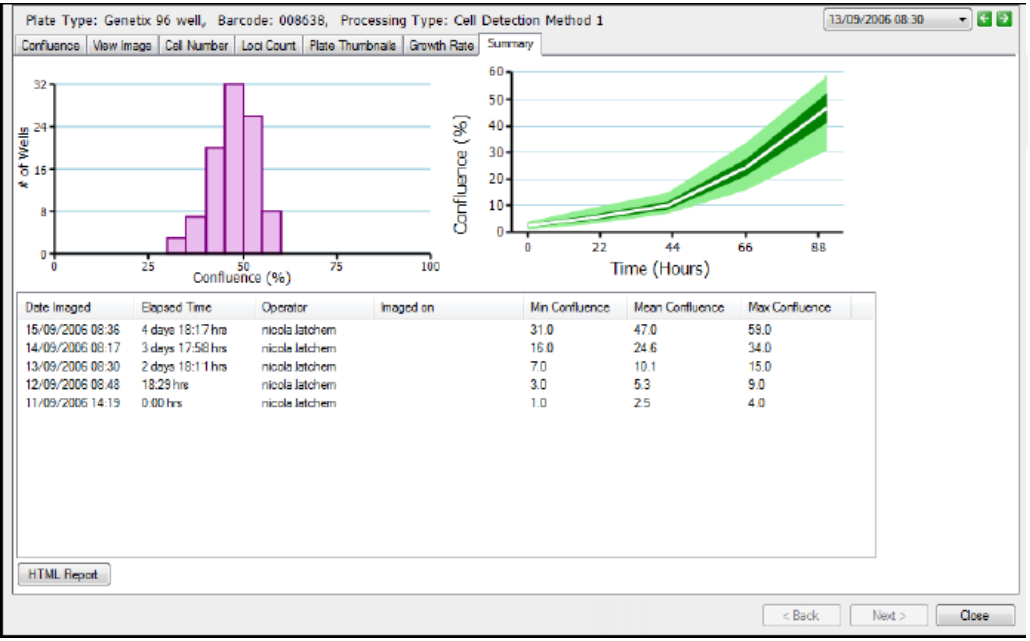



- **Export:** Click this button to launch a **Data Export Wizard** that will guide you through the process of exporting the **confluence data** as a **.csv or .xml** file.
- Be sure to select the **Confluence** option, then click **Next**.
- The wizard now provides the option to export the data for the **following time points**:



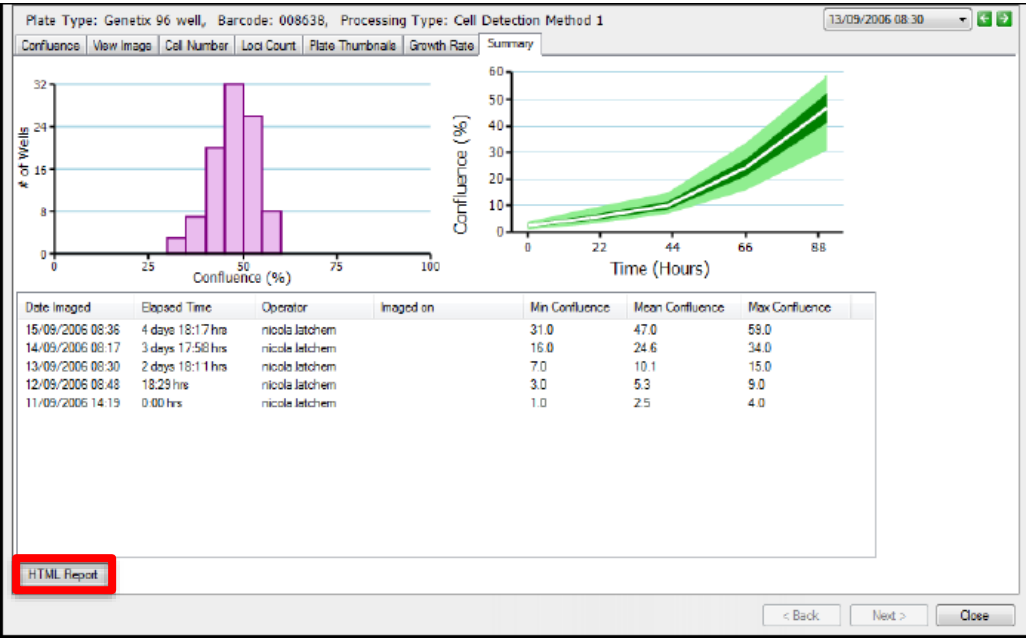
# Summary Tab – Overview

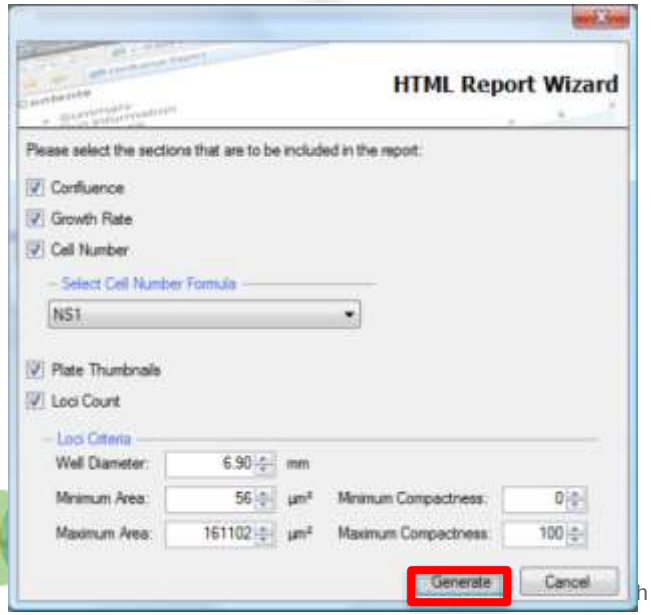



- **Summary Tab:** This tab displays a **summary** of the **confluence data**, **growth curve data** and **imaging credentials**.

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

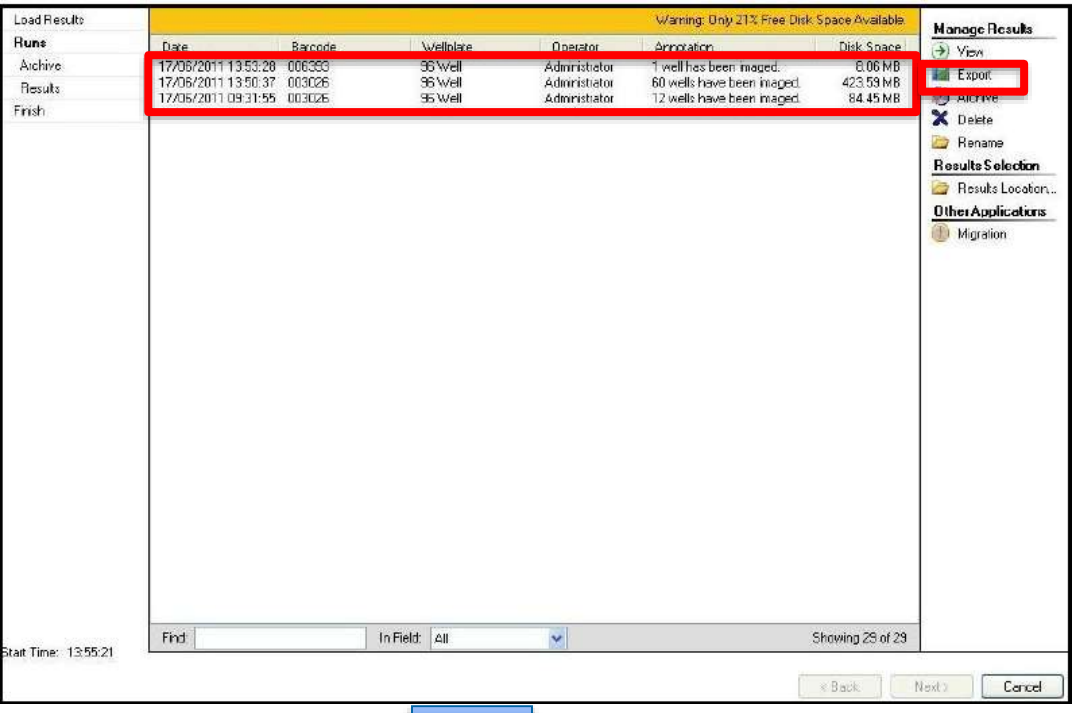
# Summary Tab – Generating an HTML Report

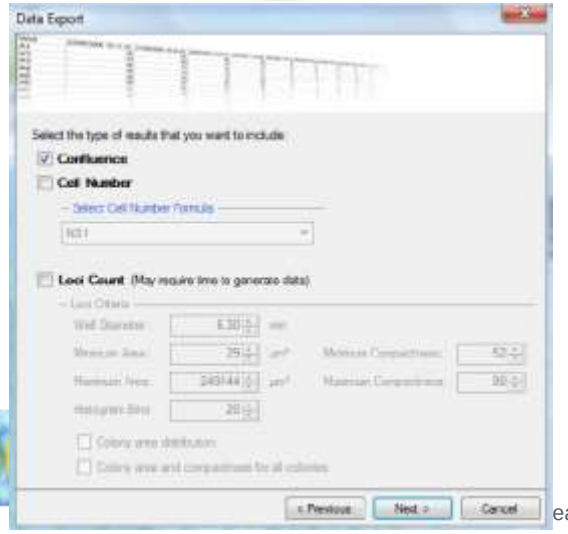



- **HTML Report:** Click this button to launch the **HTML Report Wizard** that will guide you through generating a **report** containing the desired information in **HTML format**.
- **Data points and visualizations** including **confluence, growth rate, cell number** etc., can be selected for **inclusion** in the **report** by **enabling** the **respective check boxes** in the **wizard**.
- Clicking **Generate** will **create** the report which can be saved as a **.html** file.

# Exporting Selected Results: Bulk Data Export




- To export selected **results**, **shift-click** or **control-click** to **highlight the data** set(s) of interest in the list and then click **Export**.
- A **Data Export wizard** will now be displayed that allows data from **the selected results** to be **exported** in **.csv** or **.xml** format.



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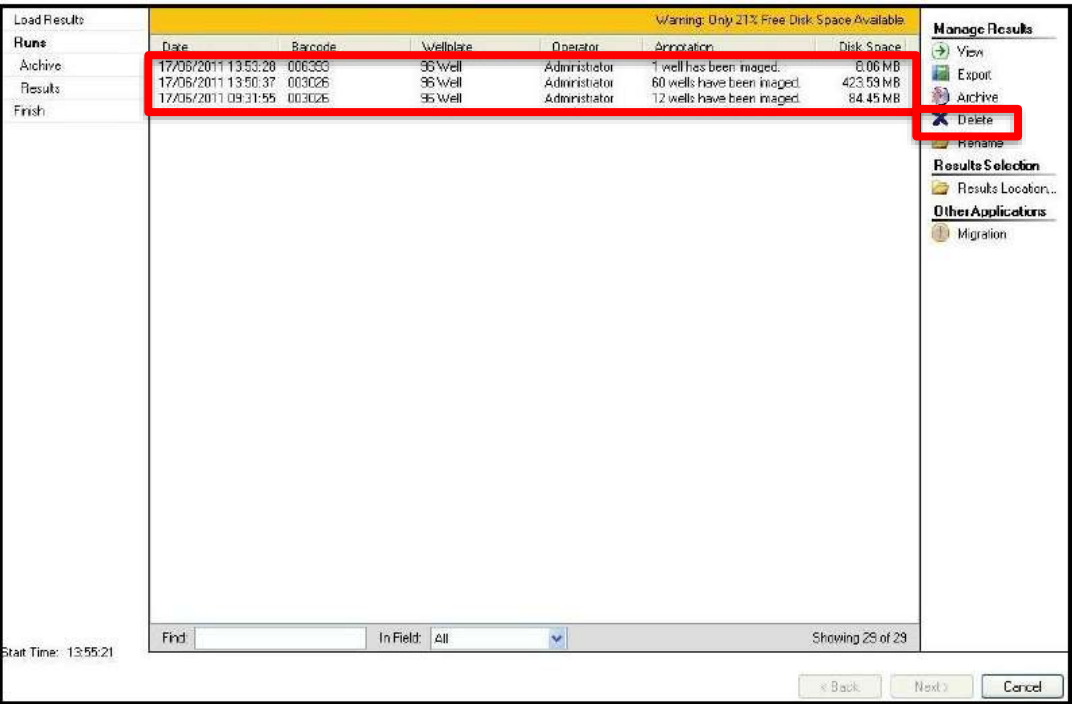
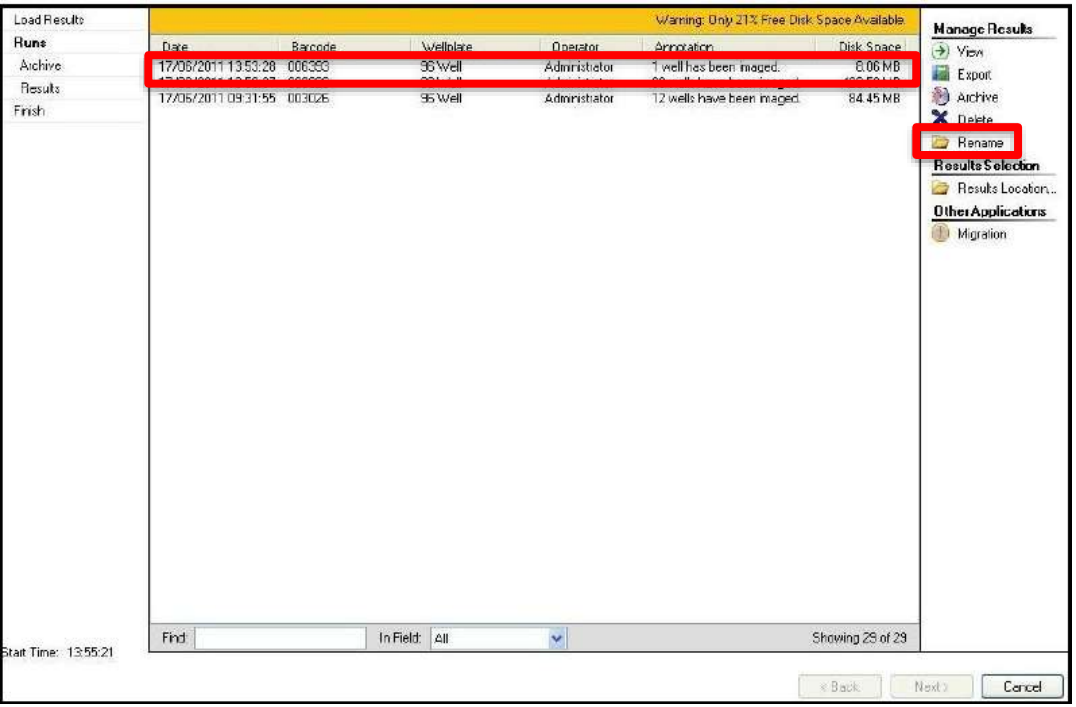
# Deleting Selected Results




- To delete selected **results**, **shift-click or control-click** to **highlight the data** set(s) of interest in the list and then click **Delete.**

# Renaming Selected Results




- To rename selected **results**, **highlight the data** set of interest in the list and then click **Rename.**
- **Barcodes/plate names** can be **re-named** in case of any **misnamed plates which will subsequently be excluded from growth curve data.**
- A **small dialogue box** will appear in place of the **barcode/name** to enable a **new barcode/name to be typed in its place.**

# 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

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