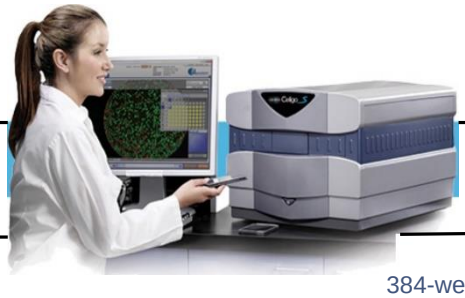
Celígo®

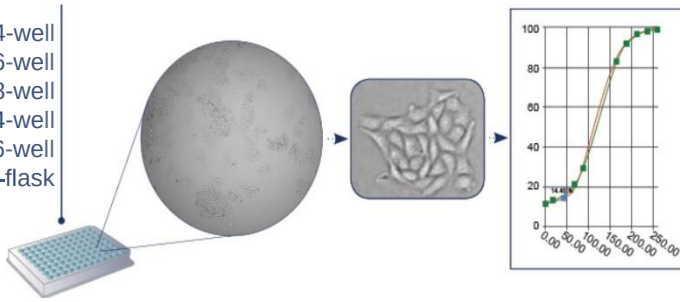
Quick Start Guide

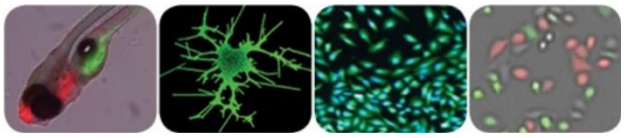


1. IMAGE ···▶ 2. ANALYZE ···▶ 3. RESULTS

whole well cell images **cell images** **data curves**

384-well
96-well
48-well
24-well
6-well
T-flask





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Nexcelom Bioscience

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Chapter 1: Celigo Training Programs Overview

Celigo Training Descriptions

Onsite Installation Training

Nexcelom's Celigo installation training program consists of a 2-day onsite training program and follow-up online support sessions (by request). The 2-day onsite training includes a 1-hour *In Classroom Seminar* and 2-hour *In Lab Hands-on* group/individual sessions.

1. **In Classroom Seminar:** Covers the description of commonly utilized Celigo applications, the technical aspects of the Celigo instrument, introduction to the Celigo software interface, and an overview of the application and technical support documentation.
2. **In Lab Hands-on:** Lab sessions will depend on the number of users (i.e., 2hr/group [2 to 3 people]) that require training. Users must sign-up in advance and pre-prepare their plates (see Chapter 2, plate map).
   - **Lab members will need to sign up prior to the training program.** This session will cover using the Celigo to perform brightfield proliferation (direct cell counting, confluence, growth tracking) (Module 1), fluorescence assays (Module 2), and viability assays in 3-channels with gating (Module 3), as well as learning how to manage data on Celigo. In this session, lab members will follow a provided instruction guide to prepare a plate of cells for brightfield analysis (see Chapter 2), and then use the same plate to stain with Hoechst 33342 and PI for fluorescence analysis.

Remote Online Training and Support

Online Support Sessions (by request): Follow-up online support sessions are available at no charge for those interested in specialized assays and are conducted by Celigo Nexcelom scientists remotely. Online support sessions need to be requested at least one week in advance and information provided about the assay setup and plate layout. Email support@nexcelom.com to schedule an online remote support session.

United States Email: support@nexcelom.com
Europe Email: support@nexcelom.com.uk
China Email: support@nexcelom.com.cn

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Chapter 2: Cell Plating Procedure and Plate Map Example

Example 96-Well Plate Preparation for Plating Cells

Cell Density Titration: This procedure is provided as a practice tool for observing cell densities and proliferation rates for your cell type. The cells are seeded in the wells as total cells/well indicated below (Part 1). Image the plate over multiple days for growth curves following Module 1. After 3-4 days, stain the cells with Hoechst and Propidium Iodide (Part 2) to get a viability measurement, following Modules 2 and 3.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	A
B	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	
C	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	B
D	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	
E	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	C
F	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	
G	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	D
H	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	

Condition/ Treatment (optional):

A Negative Control

B _____

C _____

D _____

Part 1 (Limiting Dilution of Cells)

1. Trypsinize cells in TC flask.
2. Count cells for cell/ mL concentration. You'll need at least $0.5x10^6$ cells.
3. In 15 mL tube labeled 40000, prepare 10 mL with cells at $2x10^5$ cells/ mL concentration (200 cells/µL)
4. Set up 11 more tubes with 5 mL media each
5. Serially dilute the 40000 into the 11 tubes using a dilution factor of 2 (adding 5 mL of cells, mix, etc.)
6. Pipet 200 µL of cell mixes into plate according to plate map provided.
7. Centrifuge plate (1000 rpm) to settle cells in bottom, balance with another plate containing same volumes (optional)
8. Incubate overnight or image and analyze on Celigo. Treatments can be added at this time or after overnight incubation.

Part 2 (Fluorescent Staining of Cells)

1. Prepare a 15 mL tube with 6 mL PBS for fluorescent staining
2. Add 24 µL Propidium Iodide, 12 µL Hoechst 33345
3. Mix the buffer and fluorescent stain well
4. Pipet 50 µL into wells containing 200 µL of cells
5. Incubate at 37ºC (98.6ºF) for 30 min
6. Image and analyze on Celigo

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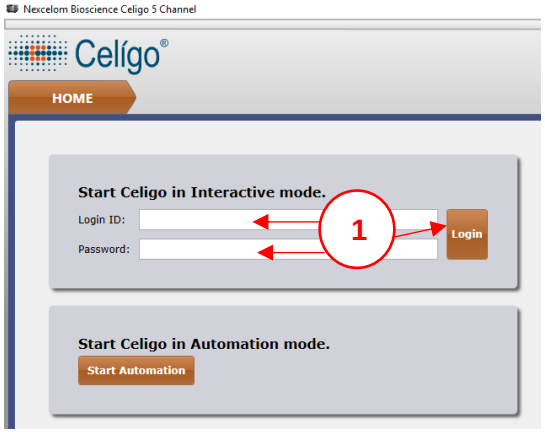
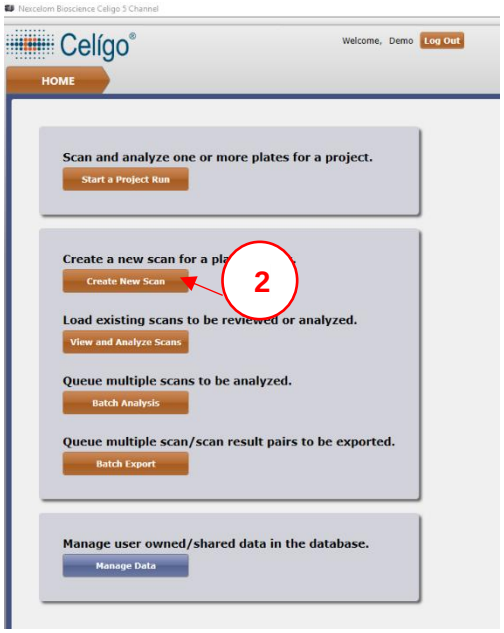
Chapter 3: Module 1 Cell Proliferation 1-Channel with Direct Cell Counts

HOME Tab

Login and Create New Scan:

1. Type in user **Login ID** and **password.** Click **Login**.
2. Click **Create New Scan**.





KNOWLEDGE:

Start a Project:
Uses a previously saved Project to scan analyze and export a plate scan.

Create a New Scan:
Scan and analyze a plate, entering in details at each tab.

View and Analyze Scans:
Load an unanalyzed scan or view a result of a scan and analysis.

Batch Analysis:
Using saved Analysis and Gating setting, analyzes multiple scans in a batch.

Batch Export:
Export multiple scan results and/or images in a batch.

Manage Data:
View all data files in folder structure format to organize, import, export, delete image scan files and data.

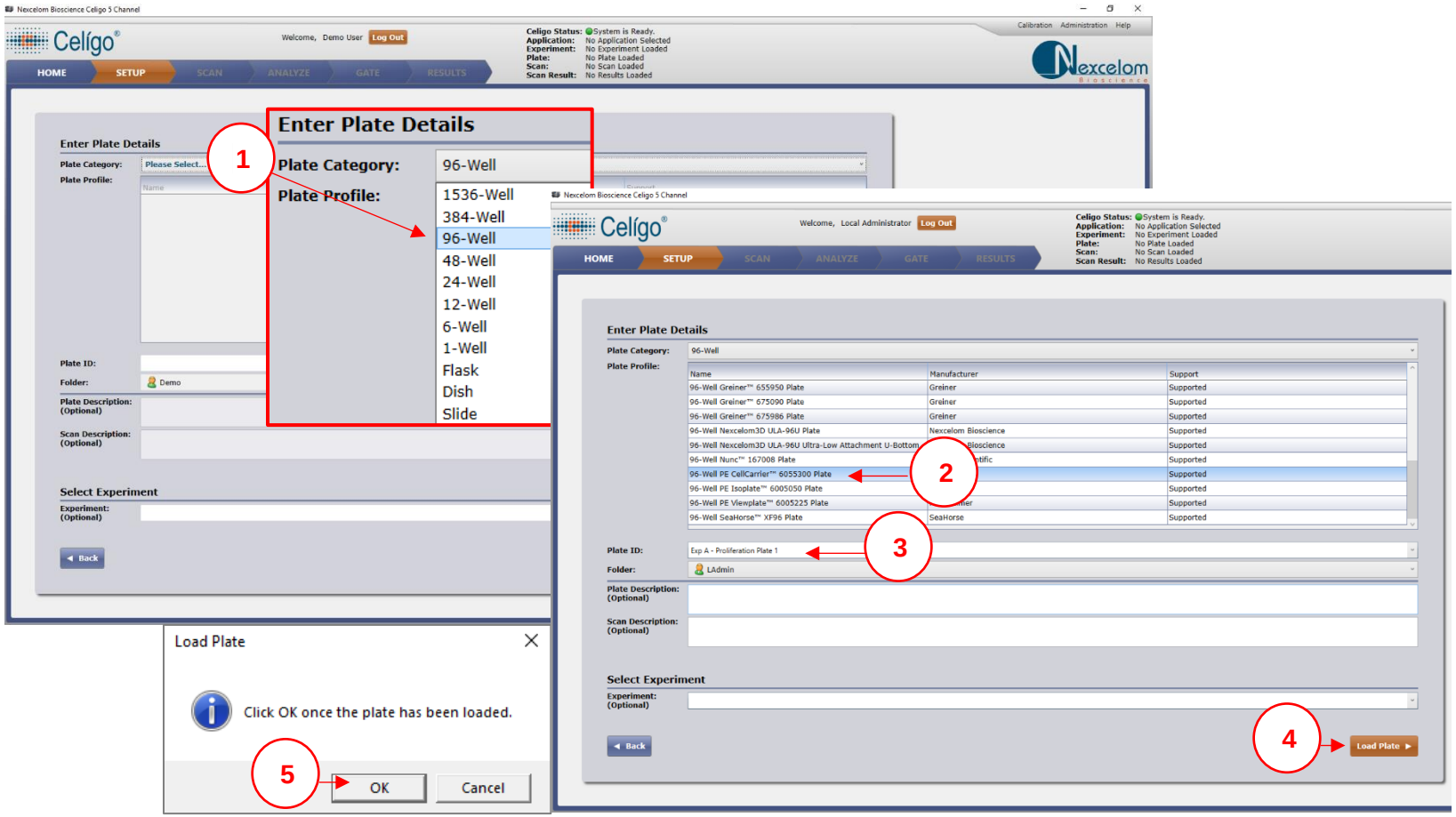
See User Guide for further details.

SETUP Tab

Setup Details of Plate:

1. Select Plate Category: **96-well**
2. Select Plate Profile: **Manufacturer and Catalog #**
3. Type in **Plate ID** or select a previous entered Plate ID
4. Click **Load Plate.**
5. Put plate on Stage and click **OK.**

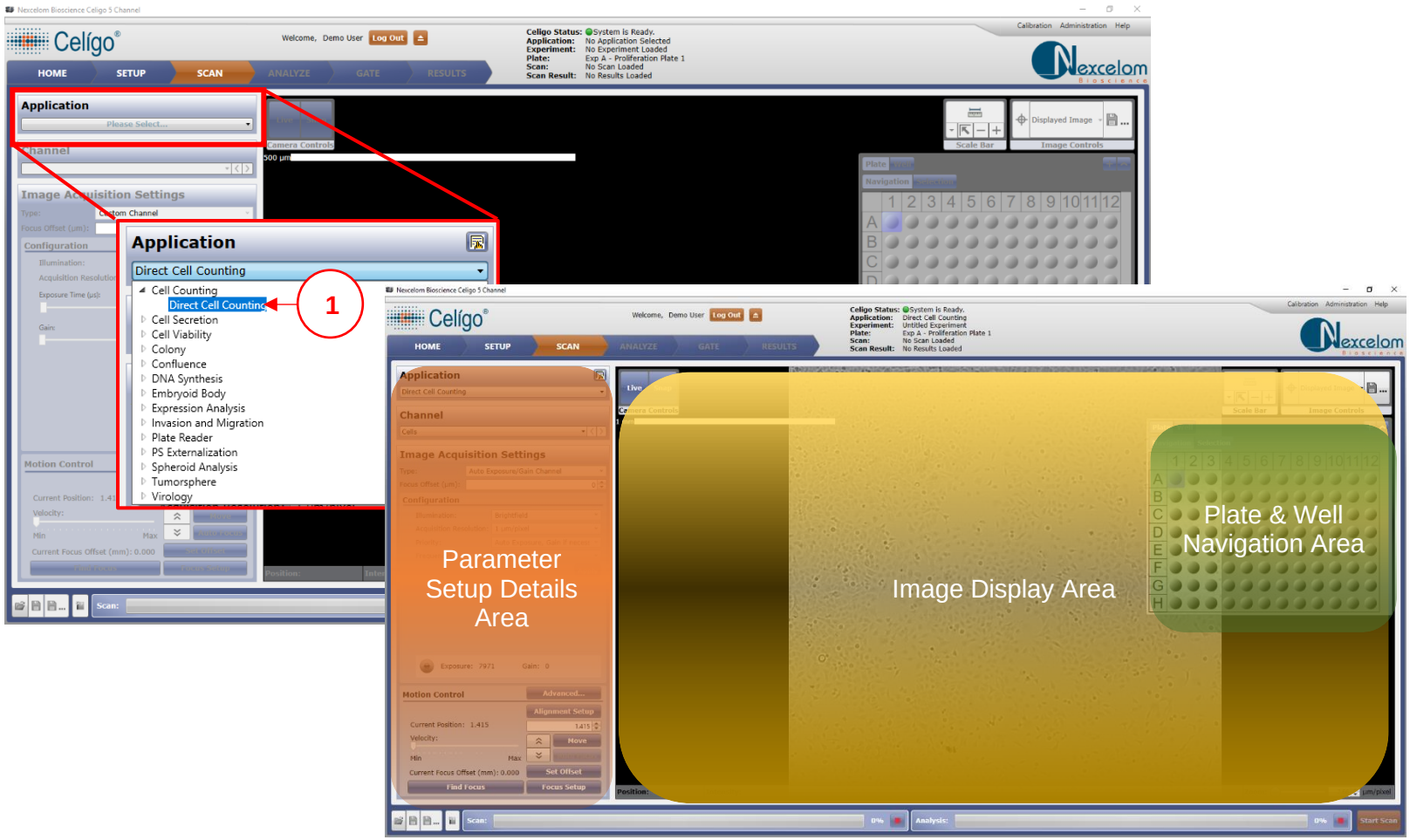
For a list of plate profiles provided please see Plate Profiles list document. See Nexcelom.com/Celigo Imaging Vessels
Contact support@nexcelom.com if a plate manufacture



SCAN Tab

Select Application:

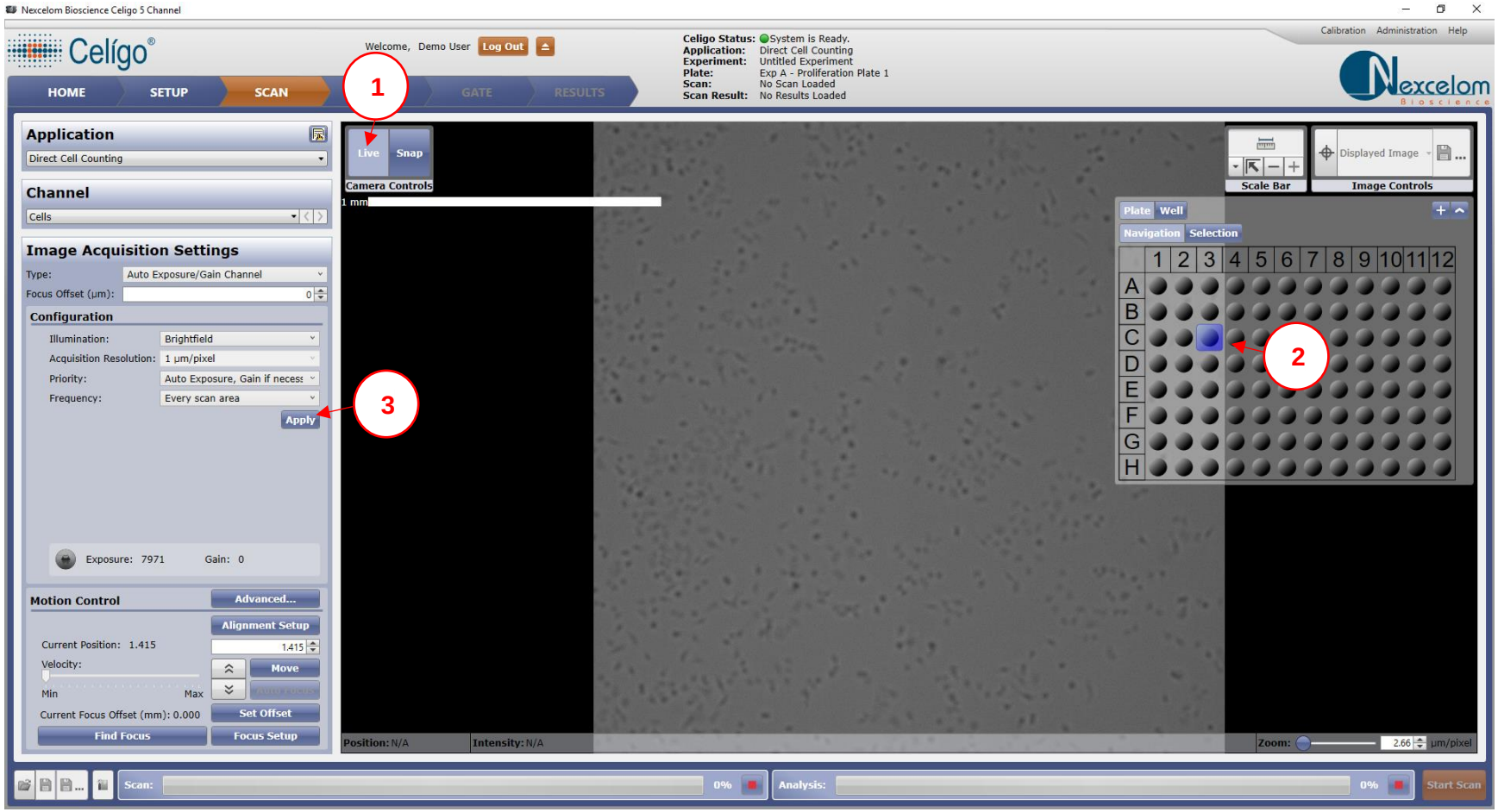
1. In Application Section, select **Cell Counting**>**Direct Cell Counting**



When first in SCAN tab, you must select an application. Then the system will display the appropriate setup parameters on the left panel area. The stage is located on Well A1 identifiable by the blue square on the Plate Navigation Area. An image snap is displayed in the Image Display Area.

Set Illumination and Exposure:

1. Click on **Live** Camera.
2. Select a well (any well that has cells in the center of the well) on the plate map in the Navigation area.
3. Click **Apply** for Auto Exposure in Brightfield Illumination.



The ideal background for brightfield images are pixel values in the middle of the camera range (0-255), therefore 125 to 130. When clicking **Apply**, the system will determine the correct exposure to produce this background grayscale image. It will also do this during the image acquisition, so if the volumes are different or media has color differences, the background will remain consistent.

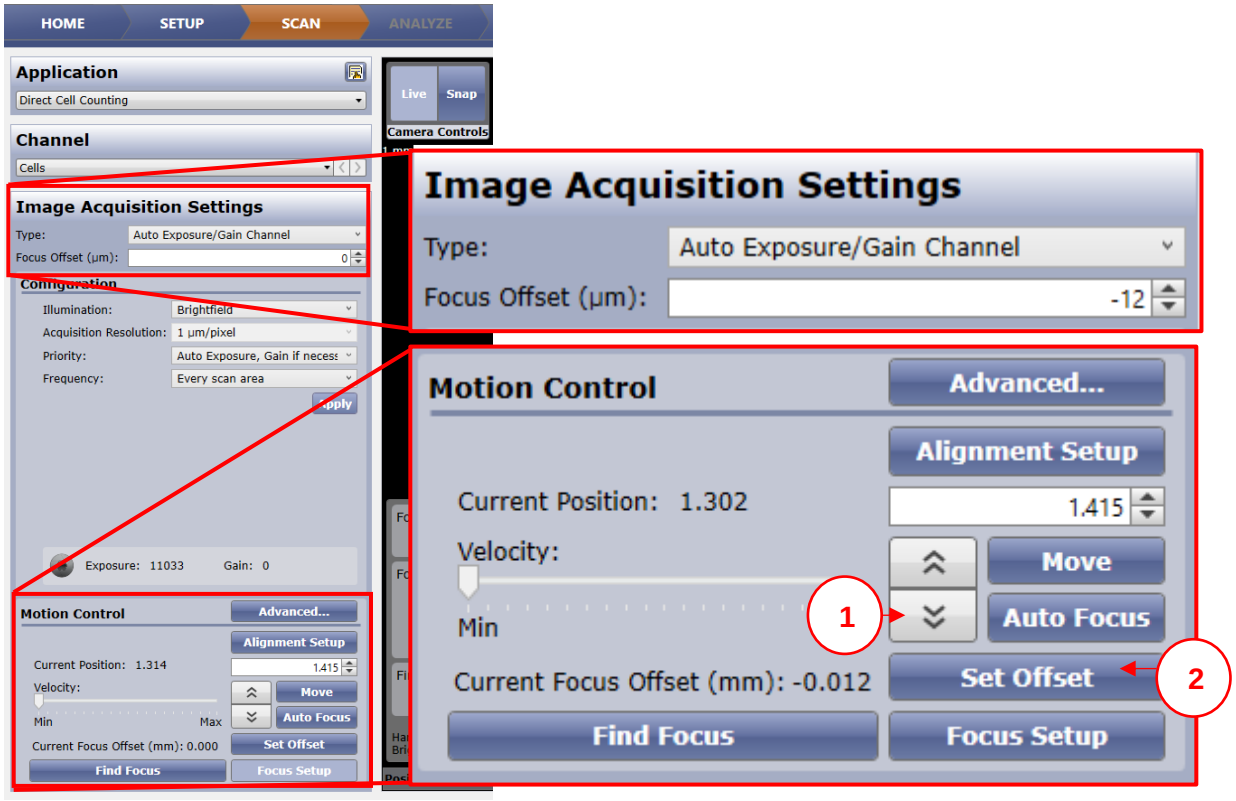
Register Focus:

1. Click on **Focus Setup**.
2. Click **Register Auto**.
3. Click **Focus Setup** again to close dialog box.

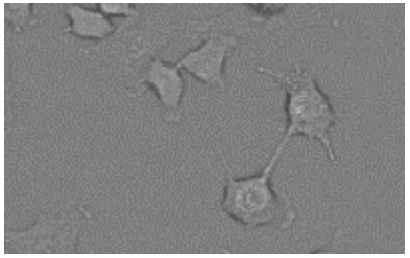
The Hardware Auto Focus (HWAF) mechanism uses two LED beams (not shown) on the bottom of the plate to measure the z-position of the plate after the cells are determined to be in focus. Once registered, the system will use this mechanism to move to the proper focus plane for subsequent wells. Proper plate selection on the SETUP tab and consistent plate thickness from well to well is essential for good HWAF performance. Alternative focus options are: Image-based Auto Focus or None (see Image-Based Auto Focus (IBAF) Focus Modes pg 79).

Set Focus Offset:

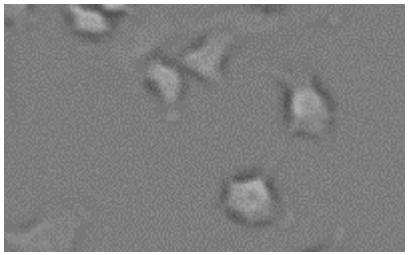
1. In Live camera mode, click on the **down double arrow button** to defocus cells. One click = 0.001 mm or 1 µm, see images below for appropriate focus of cells.
   Defocusing allows for the intentional loss of internal cellular objects highlighting the dark-outline/bright-center of the cells.
2. Click **Set Offset**. Number is displayed in Image Acquisition Settings Focus Offset text box.



After Hardware Auto Focus (HWAF) Registration
0 µm Offset



<u>Best</u> Focus for Analysis
Defocus -10 to -20 µm



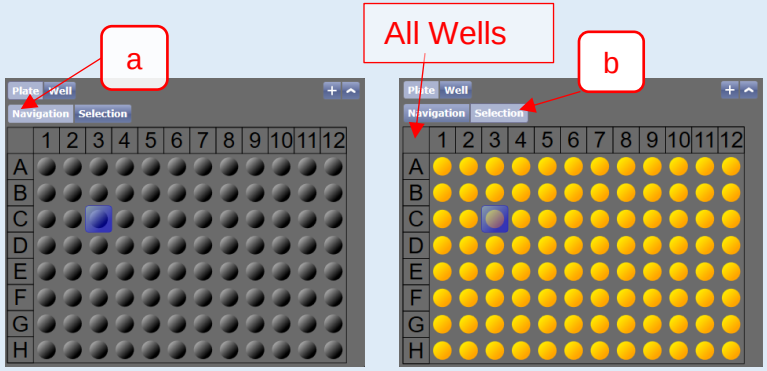
The correct focus contrast for cells in bright field is a dark membrane with a bright center; often -10 to -20 µm from the HWAF registration position. Clicking **<Set Offset>**, stores this value in the Focus offset parameter for this channel. The system is now programmed for hardware focus (HWAF) with this additional focus offset distance. You can test this by navigating to another well or click **Auto Focus** in current well. See Image Analysis Algorithms section for further explanation on how cell objects are detected in an image.

KNOWLEDGE: Plate and Well Navigation Areas in SCAN Tab

The Celigo cytometer's fast imaging speed is largely due to its proprietary optical pathway, which employs a large field-of-regard (FOR) F-theta lens with high-speed galvanometer mirrors to rapidly scan large areas without moving the sample. The large FOR allows the scanning mirrors to obtain a series of images (fields-of-view or FOV) with fewer movements of the plate carrier.

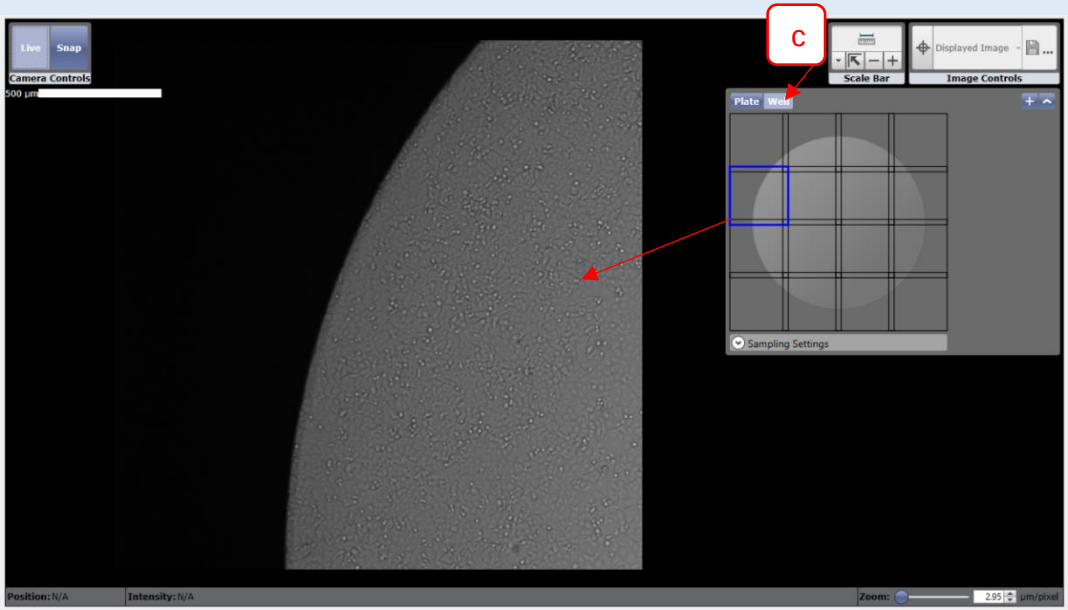
Plate Navigation Buttons

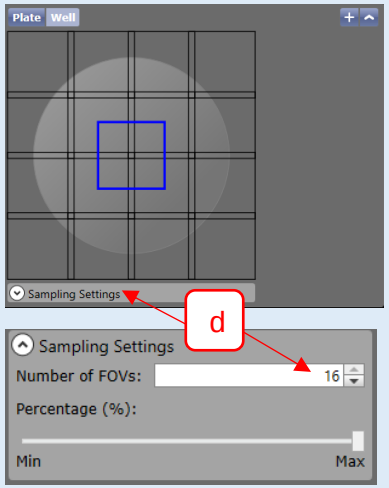
a. **Navigation:** Clicking on individual wells moves the stage to selected well.
b. **Selection:** Highlight wells, columns, rows or whole plate for image acquisition. Blank square highlights all wells.



Well Navigation Buttons

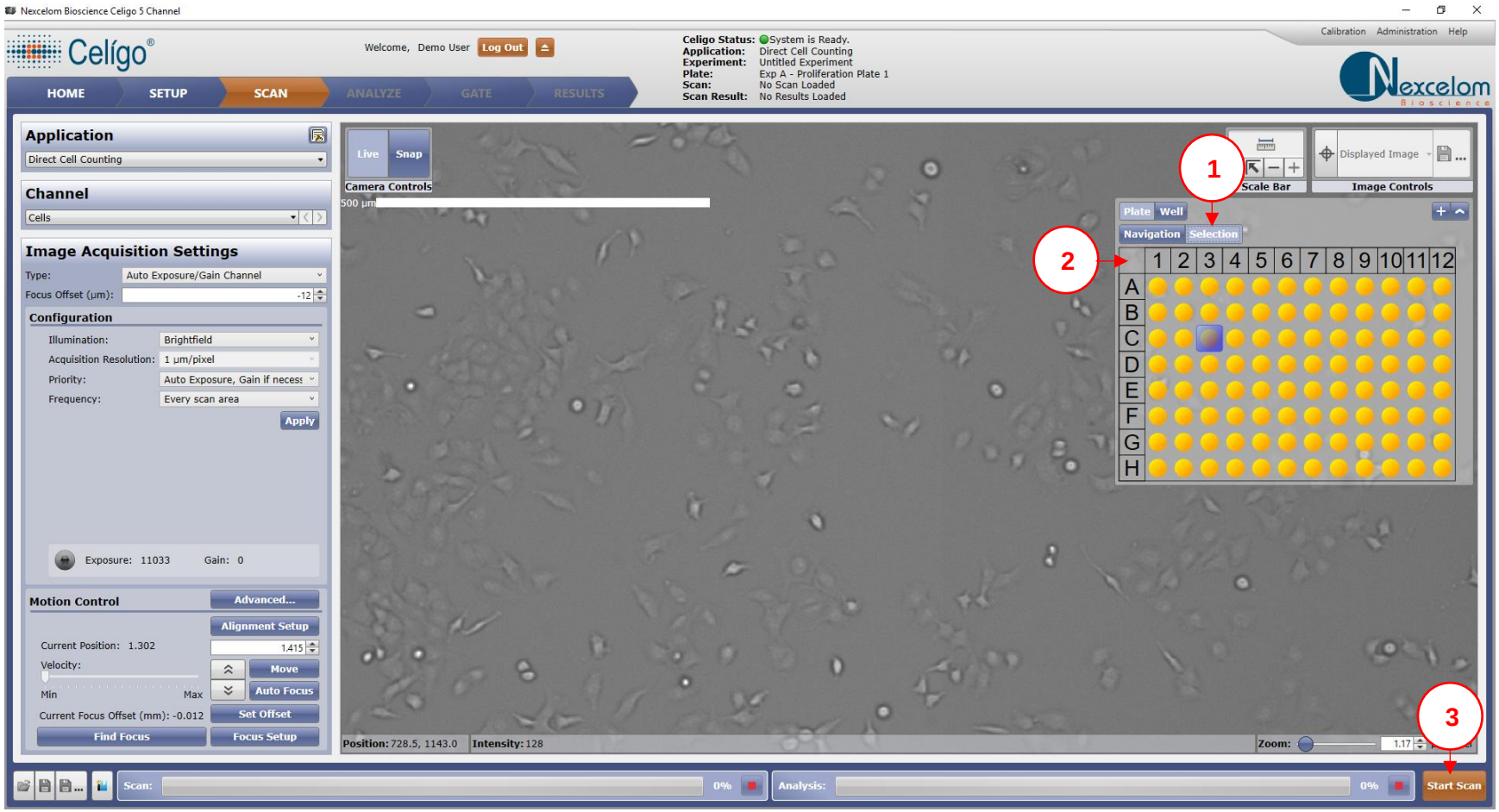
c. Click on **Well** to activate, then select an area of well for image display. Return to center of well by clicking on **Plate**, then select the same well.
d. Sampling Settings: Allows image acquisition to be less than a whole well. Example for 96-well, subsample for 4 or 1 image only per well.





Select Wells for Image Acquisition:

1. In Plate mode, click **Selection.**
2. Highlight wells for image acquisition.
3. Click **Start Scan** to start image acquisition, screen will proceed to ANALYSIS Tab.

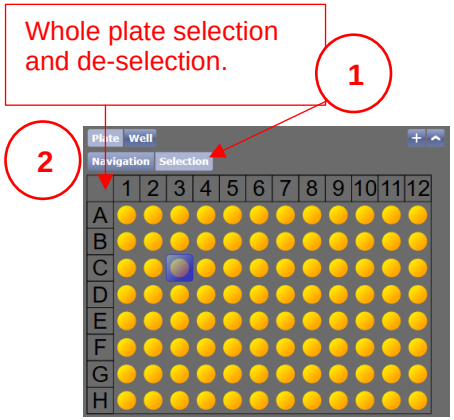


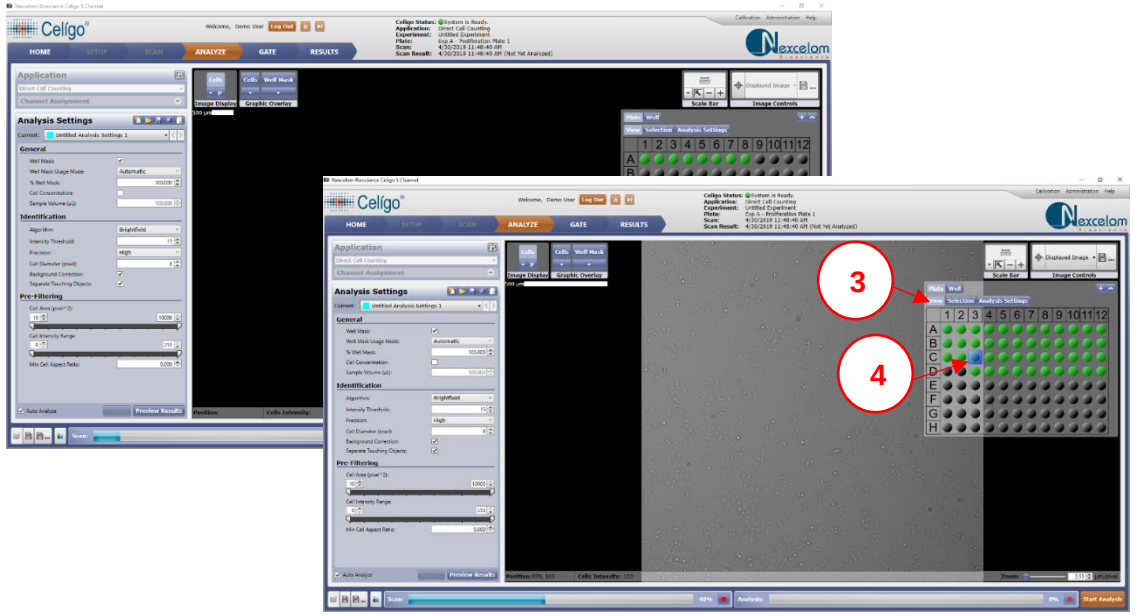
When selecting wells, user can either highlight the wells by dragging mouse over wells, click on columns or rows, or click the **Blank Square** between A and 1 to highlight all wells. De-selecting occurs with same process. User must select wells for Start Scan button to become active.

ANALYZE Tab

View Images in ANALYZE Tab:

1. Click **Selection** in the Plate Navigation area.
2. Click the **blank space** by well A1 to de-select wells that are highlighted yellow.
3. Click on **View** button.
4. Click on a green well in plate navigation area to view an image.

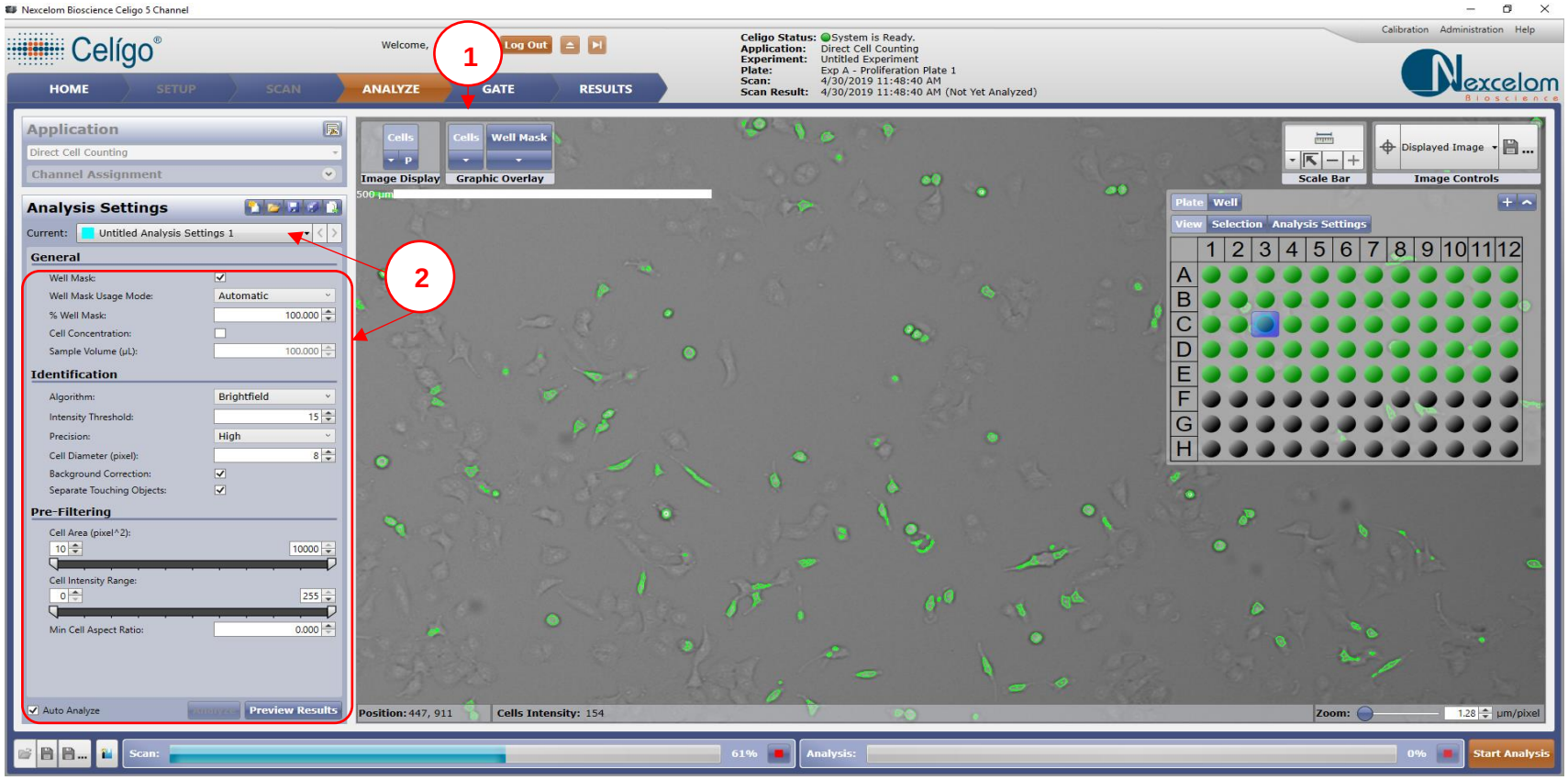




- Green wells are images that have been taken, automatically saved to database and are viewable in the ANALYZE and RESULTS tabs.
- Click on different green wells at any time and look for good focus and illumination.
- Use mouse to zoom in and out and move image around on display area.
- Click on **Well** button to navigate and look at well edge images.
- Acquisition can be stopped at any time ( ). Return back to SCAN tab by clicking **SCAN tab** to adjust acquisition parameters.
- Scanning and Analysis can occur at the same time. If only Scanning is desired, click on **RESULTS** to just view whole well images.

Adjust Analysis Parameters:

1. Click on **Cells** graphic overlay button to see green outline of cells.
2. Adjust analysis settings or load previously saved settings.

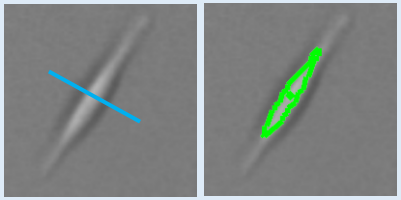


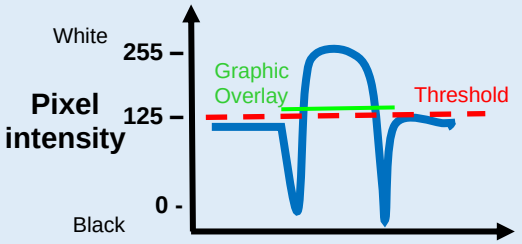
The default analysis settings may need adjustment. Low contrast cells will require a lower Intensity Threshold while high contrast cells may require a higher Intensity Threshold for proper segmentation. Previously saved settings can also be loaded. You can save the newly defined settings for future recall with the save icon next to the folder icon (Load settings). The next two sections provide knowledge on how objects are identified in the image (**Image Analysis Algorithms** and **Adjust Analysis Parameters**).

KNOWLEDGE: Image Analysis Algorithms

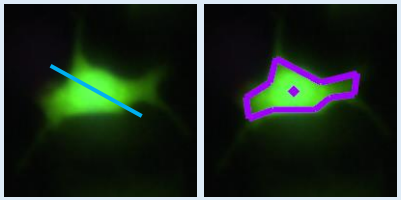
Image analysis looks at the pixels in the image, where each pixel has a value in the range of 0 = black to 255 = white. If an imaginary line was drawn over the object of interest and the pixels plotted according to their values, a graph similar to one displayed below would be seen. The intensity threshold is a value you set where any pixel above that value is identified as an object of interest. The value typed in does not correlate directly to the value of the pixel, but is relative to the algorithm formula.

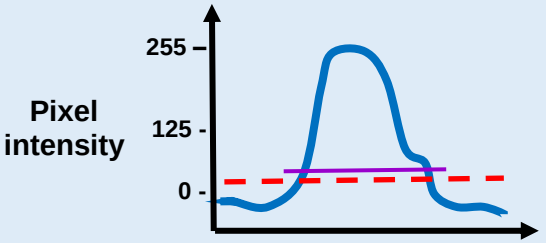
Brightfield – The algorithm looks for objects with a bright center and dark edges. Intensity Threshold and Cell Diameter work together to identify objects. Applications: Brightfield images looking at single cell identification, direct cell counts and expression analysis.



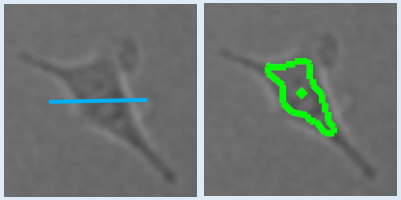


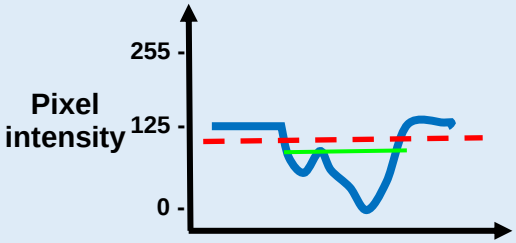
Fluorescence – The algorithm looks for fluorescent objects (bright pixels over darker background). Applications: All applications looking at fluorescently labelled cells.



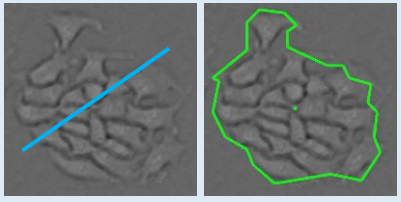


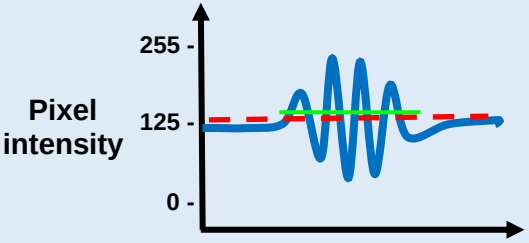
Dark Object – The algorithm looks for dark objects with no bright center in brightfield images. Applications: Direct cell counting





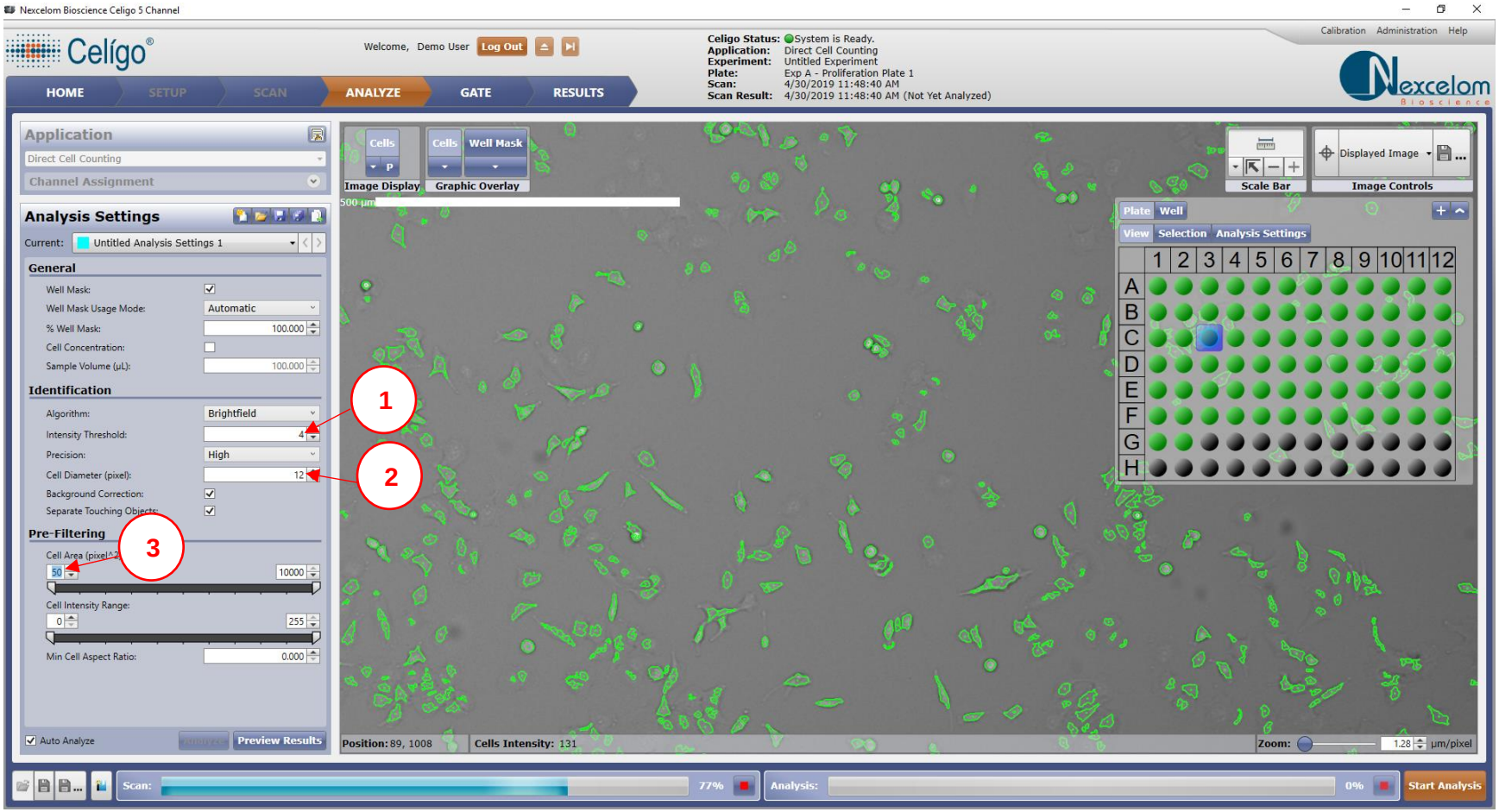
Texture – The algorithm looks for texture differences between the objects found and the background areas (Recommended for large areas or large objects). Applications: Confluence and colony





Adjust Analysis Parameters:

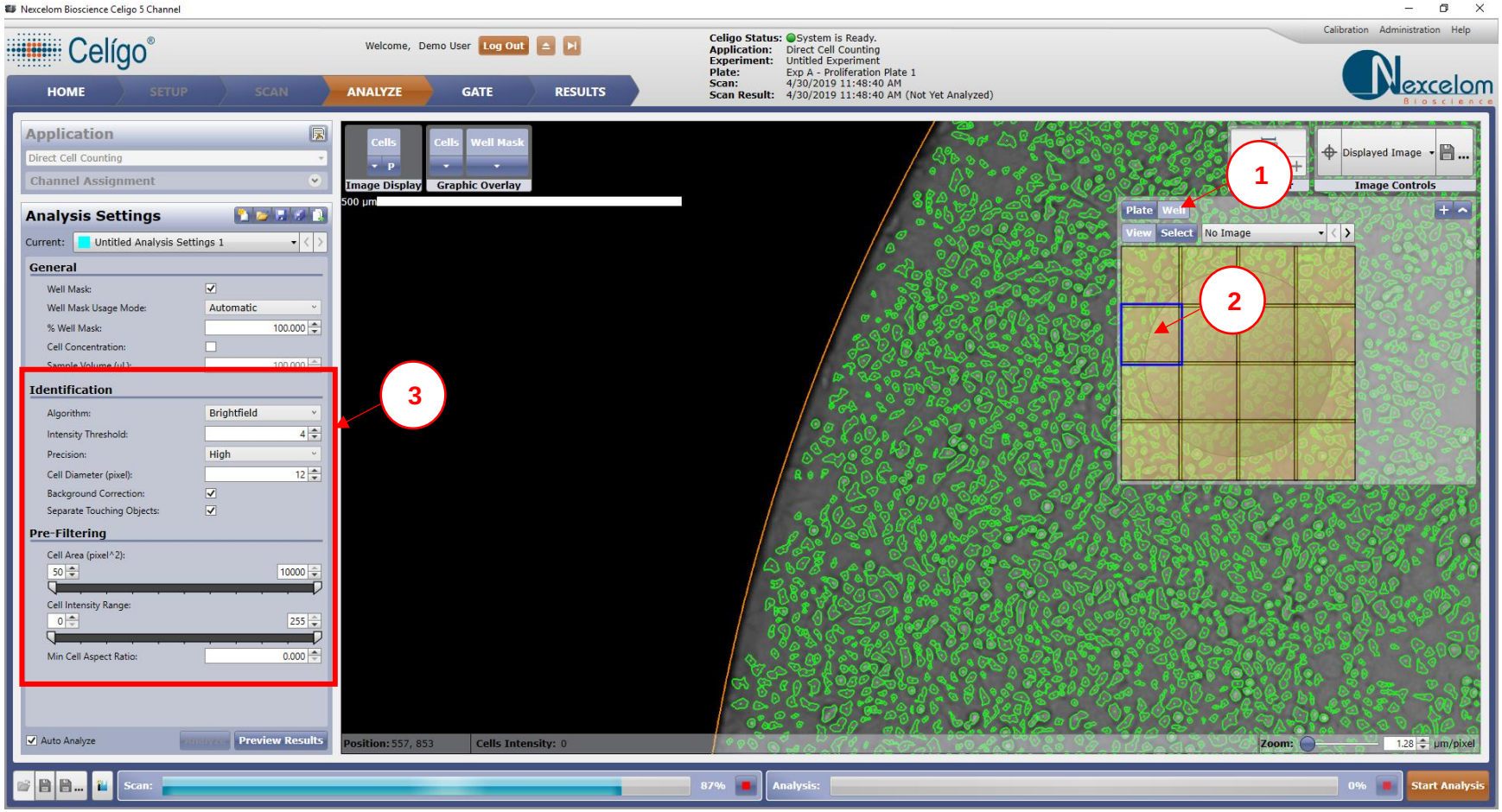
1. Set value for **Intensity Threshold** to detect all cell objects according to pixel intensity relative to background.
2. Set value for **Cell Diameter** to the average expected cell diameter.
3. Set value for **Minimum Cell Area** to exclude objects smaller than a cell.



Intensity Threshold: For adherent flat cells, lower the intensity threshold to values between 2-5, and for suspension cells use values between 10-20.
Cell Diameter: For adherent cells, use values between 10-15 and for suspension cells use values between 5-10.
Separate Touching Objects: Turn on for suspension cells, and On or Off for adherent cells.
For additional information on analysis parameters, see the User Guide in the Celigo Learning Center.

Observe Cell Segmentation (Outline) at Well Edge:

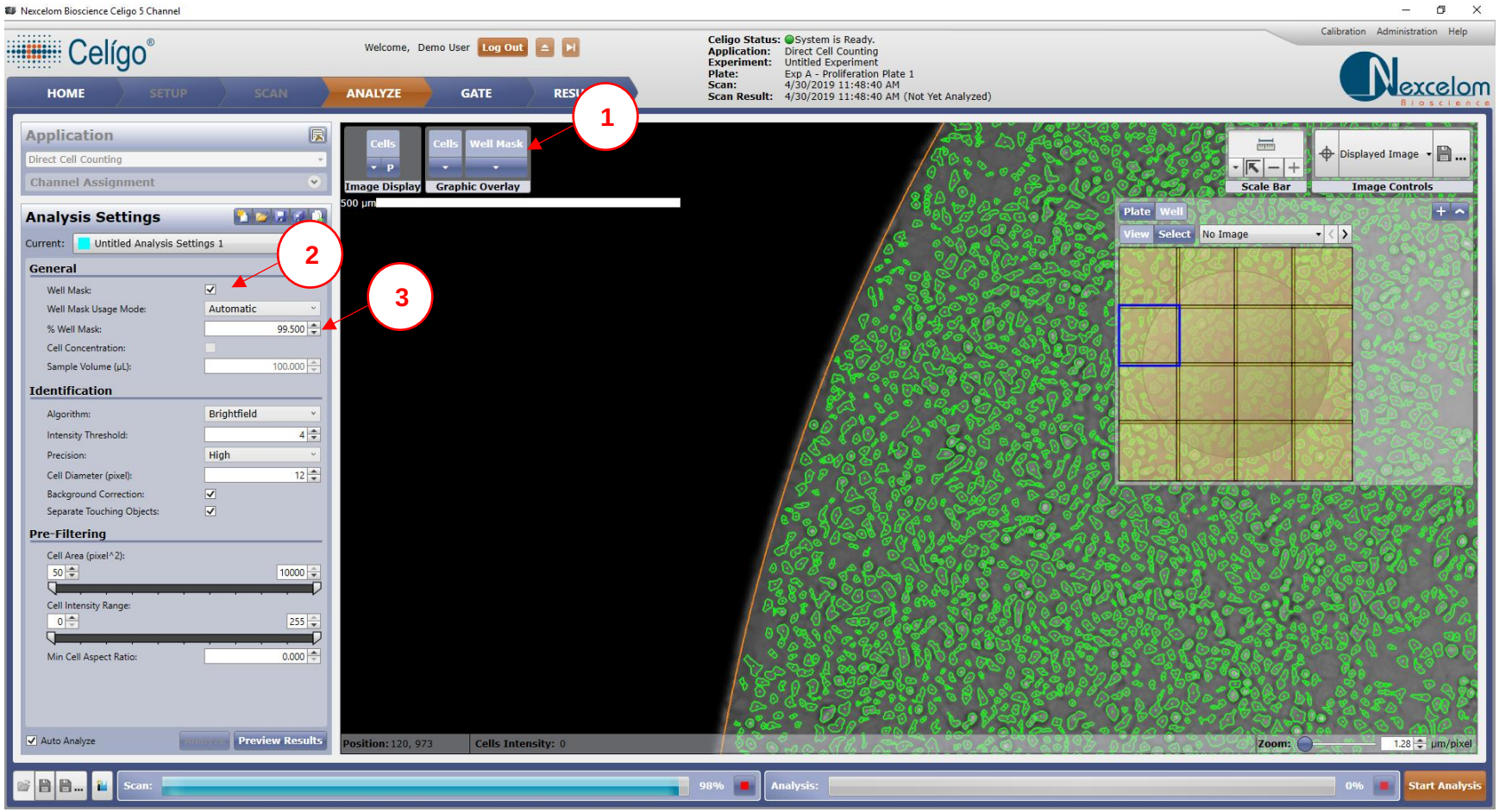
1. Click on **Well** Button
2. Click on a **Well Edge Image** and observe green graphic overlay identification of cells.
3. Make analysis settings adjustments as necessary.



For additional details on Plate and Well Navigation, Analysis Settings and Well please see the User Guide.

Well Mask Adjustment

1. Turn on **Well Mask** graphic overlay, if not already on.
2. Check **Well Mask** box, if not already checked.
3. Reduce **well mask** % to view cells that sit on well edge.

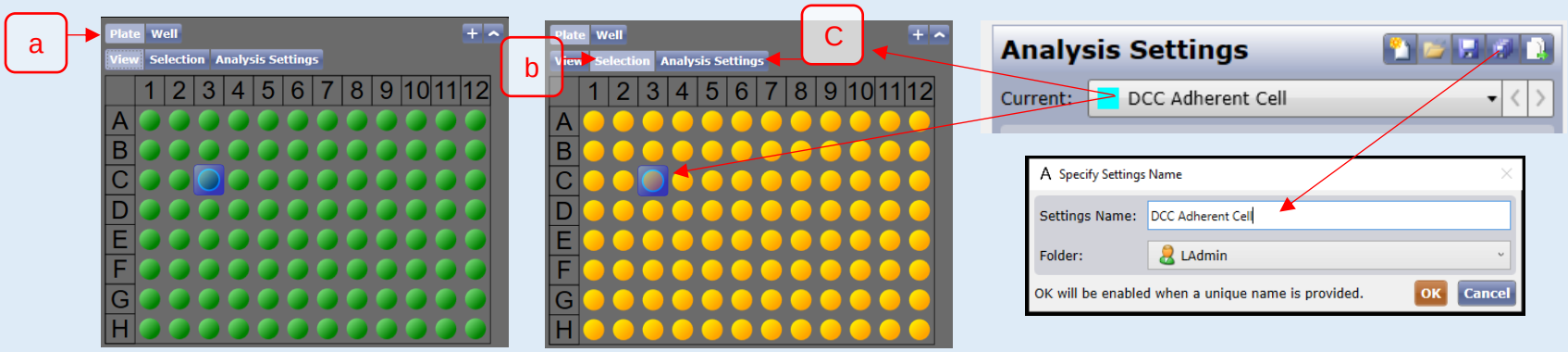


Well Mask: When well mask is turned on; the image analysis algorithm will only evaluate pixels inside the well mask and ignore the area outside it. The Original Well Mask is defined by the Plate Profile definition according to the manufacturer's measurements. Automatic Well Mask uses these values plus the image itself to better define a well mask. The Automatic setting only works for brightfield images, therefore fluorescent images default to Original even if Automatic is selected.

KNOWLEDGE: Plate and Well Navigation Areas in SCAN Tab:

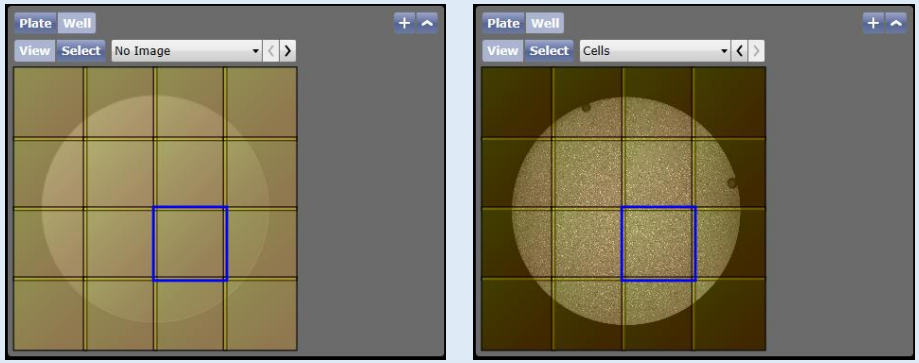
Plate Navigation Buttons

a. **Navigation**: Clicking on individual wells displays images of selected well. Wells turn green when images have been saved to database.
b. **Selection**: Highlight wells, columns, rows or whole plate for image acquisition. Wells turn yellow when selected.
c. **Analysis Setting**: Using Saved Analysis settings to highlight wells for specific analysis settings. Wells outline in color selected.



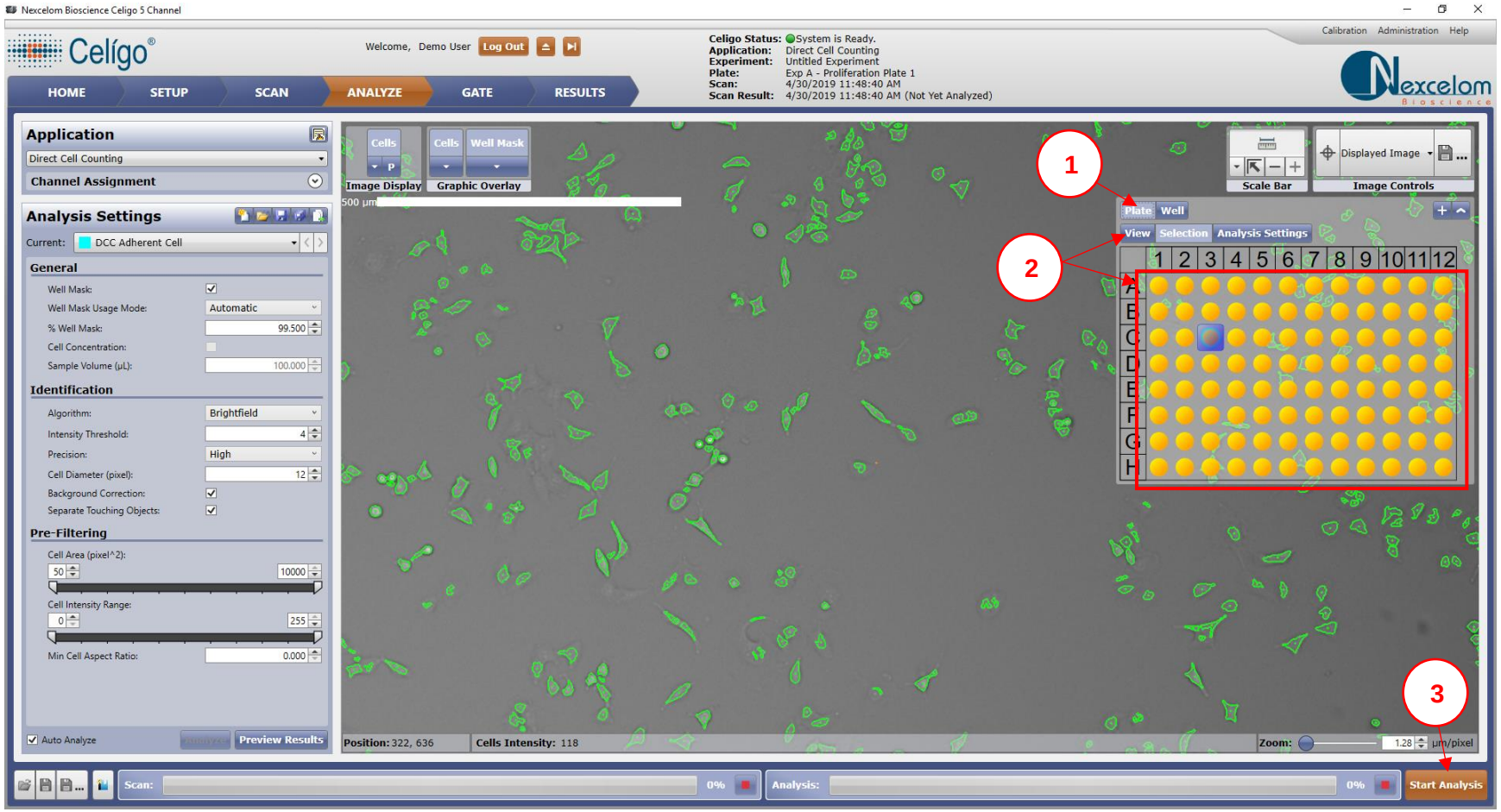
Well Navigation Buttons

a. Click on **Well** to activate
b. **View**: Select an area of well for image display
c. **Select**: Allows user to select less than the whole well area for image analysis. The same region that is selected is applied to all wells selected for analysis.



Start Analysis of Images:

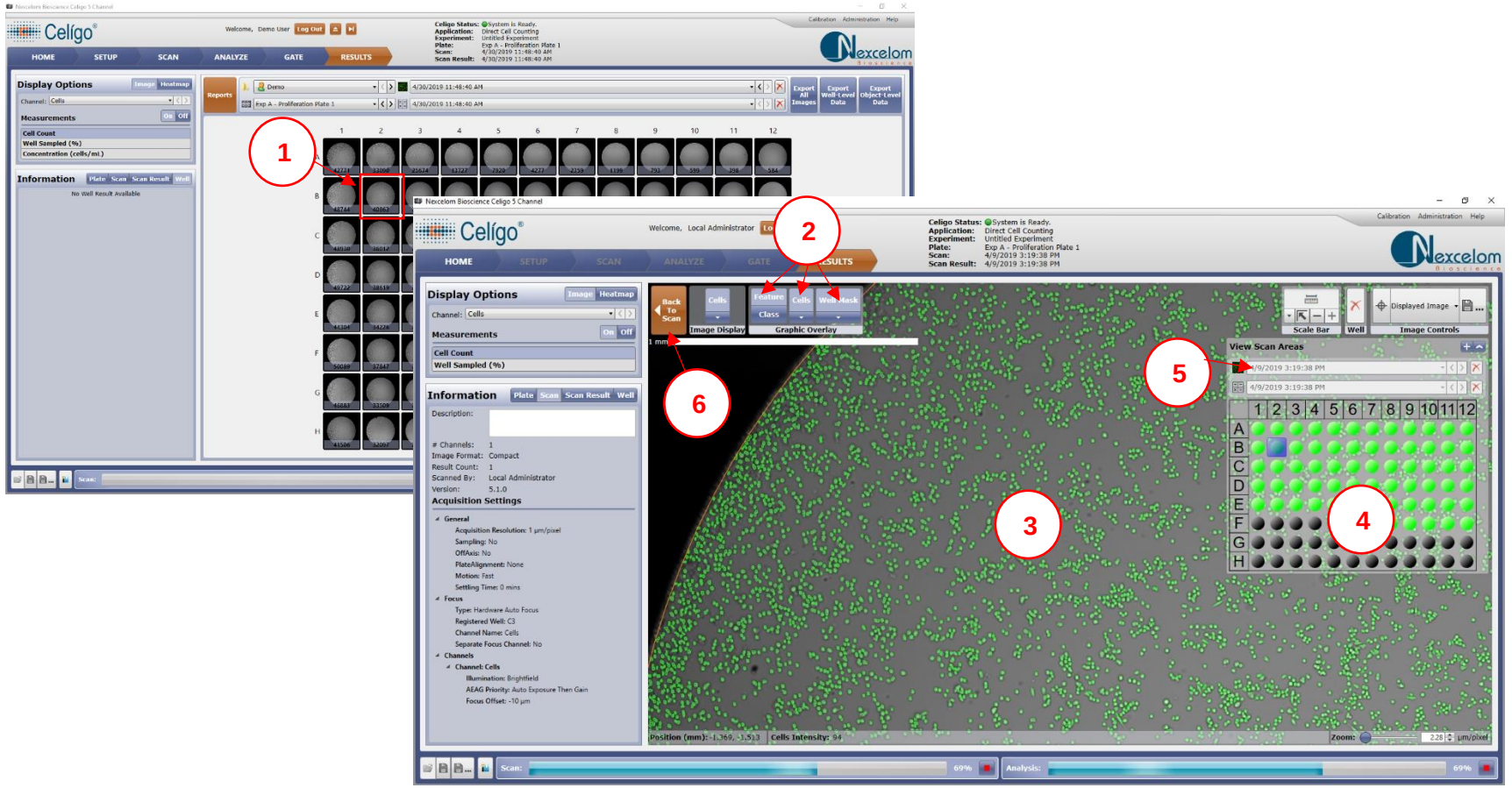
1. Click on **Plate** to return to Plate Navigation tool.
2. Click **View,** then Click on other wells to observe graphic overlay detection of cells in other wells.
3. Once satisfied with parameter settings, click **Start Analysis.** System proceeds to analyze the selected wells and progresses to the RESULTS tab.



RESULTS Tab

View Well Level Images and Graphic Overlays:

1. Double click a well. The high-resolution image is displayed in the image display area.
2. Click on **Feature** then **Cell** and **Well Mask** to display the Graphic Overlay for the cells that were detected and counted and **well mask.**
3. Zoom in/out with mouse scroll button to zoom in/out on the image.
4. Click on wells in the Navigation Area to view different wells at same zoomed in level.
5. Toggle to different time points on a specific well to observe specific cell proliferations over time.
6. Click **Back to Scan** to return to the Plate Thumbnail view.



KNOWLEDGE: Overview of RESULTS Tab Features

a. Work Flow Tabs: The Highlighted Orange is the current location, Tabs with Blue+White text can be navigated to, Tabs with Blue+Gray text are not currently available.

b. Display Area: Plate thumbnail or well view display area is shown there. Double click on a well to view individual well in high resolution.

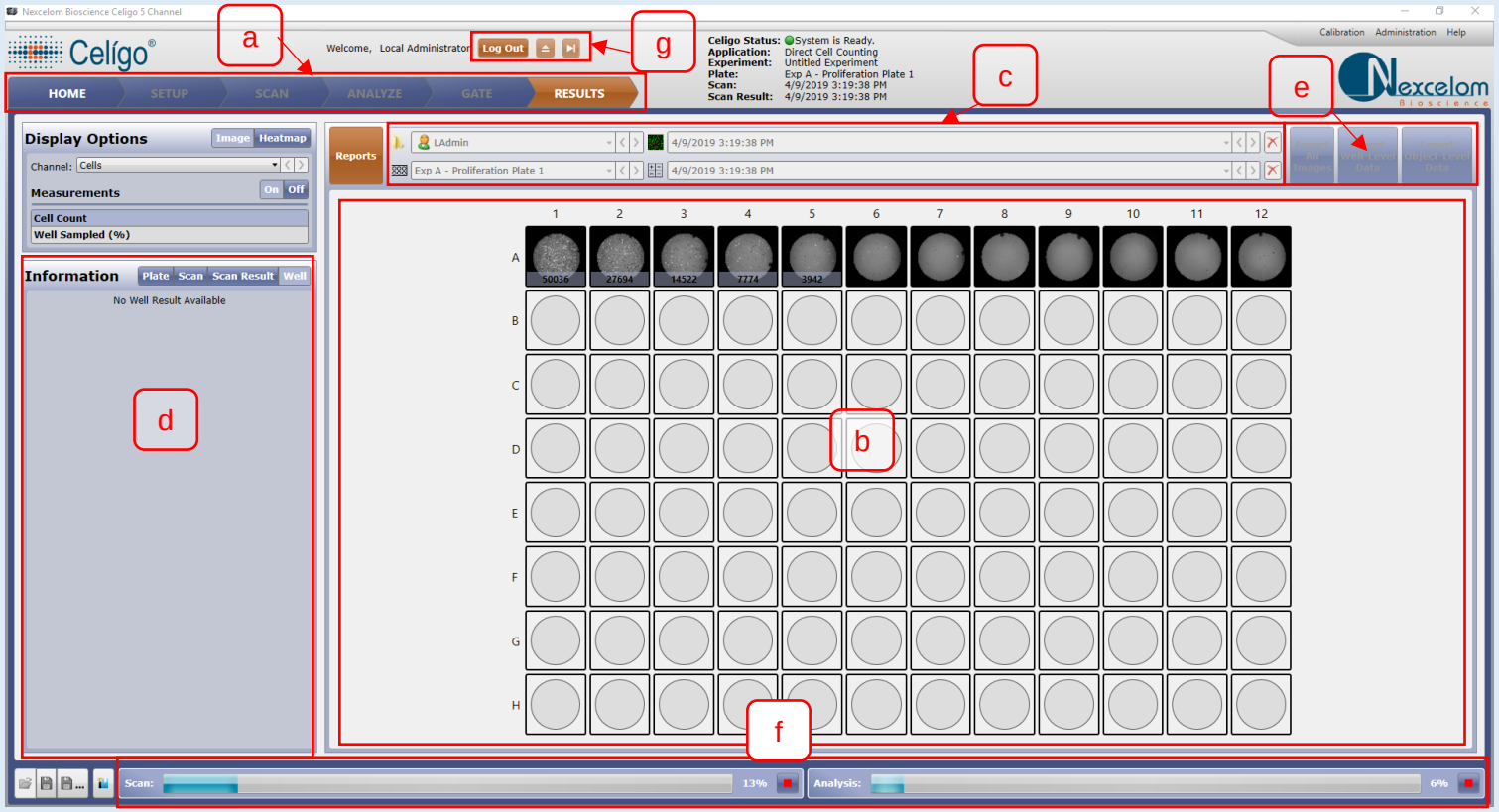
c. User Folder, Plate ID, Scan and Analysis Time Display: Current data is displayed, can select other time points after scan and analysis are complete

d. Information Area: Information displayed is dependent on selected button. See next page for more details.

e. Export Options: Data export options for Well-Level, Object-Level and Images

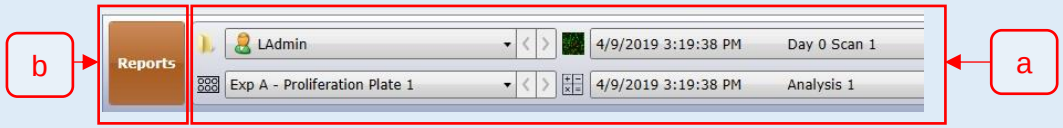
f. Scan (Image Acquisition) **and Analysis Progress bars**: May run simultaneously. Can be stopped using red square button at any time.

g. User Log Out, Plate Eject, Next Plate: Once scan and analysis are complete, these buttons become available.



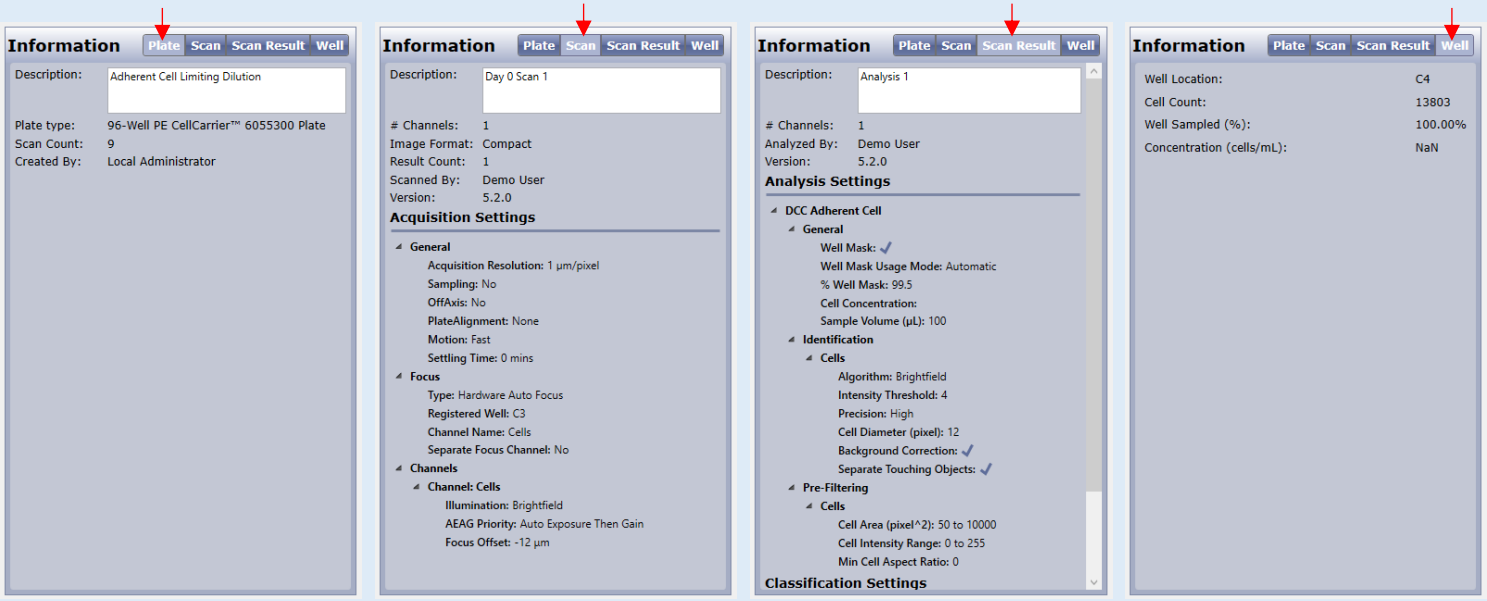
KNOWLEDGE: Overview of RESULTS Tab Features (continued)

a. Data Display Toggle: Once scan and analysis are complete, user can toggle to a different Plate ID, Scan Time or Analysis time and thumbnails and data will be displayed in Display area. **Reports** switches to panel to select data for growth tracking curves.



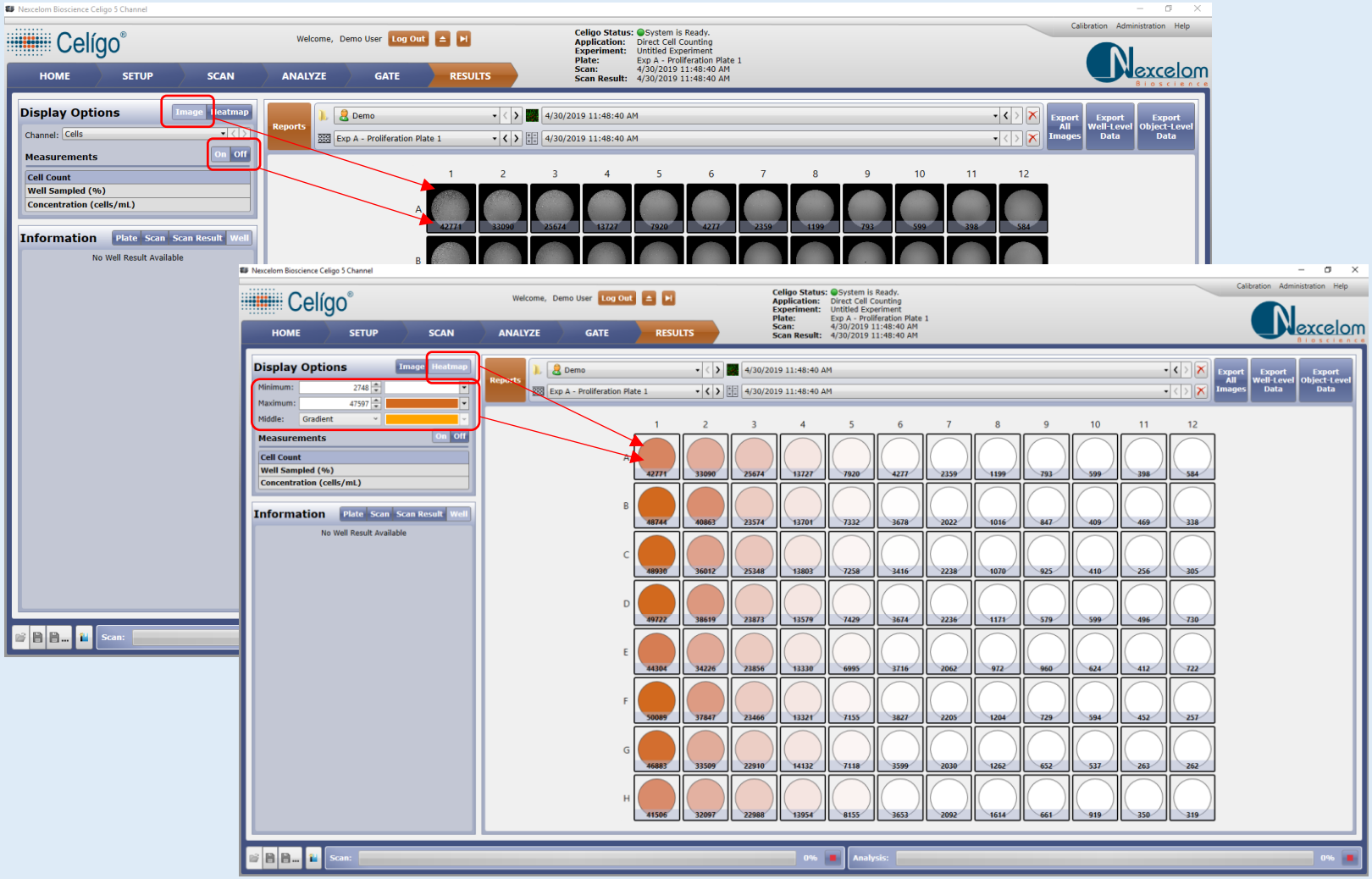
b. Reports: When more than one scan has been imaged with the same Plate ID, the data can be graphed in time scatter plot to show Proliferation graphs.

c. Informations Display Toggle: Enter **Description** details for each tab. Each tab refers to a specific level of content. **Plate** refers to Plate ID level (e.g. a plate with specific cells plated and treated with a compound). **Scan** refers to the image scan time, showing details of scan acquisition. **Scan Result** refers to the Analysis and Gating details applied to images and displayed as numerical data. **Well** refers to numerical data specifically found in the analysis of the selected well.



KNOWLEDGE: Plate Level Thumbnail Views:

Image shows thumbnail images of current channel (swappable if additional channels imaged) with numeric data measurement displayed on top, (toggle-able On/Off). **Heatmap** color codes the well to shade of color in numeric range selected for minimum and maximum data values.

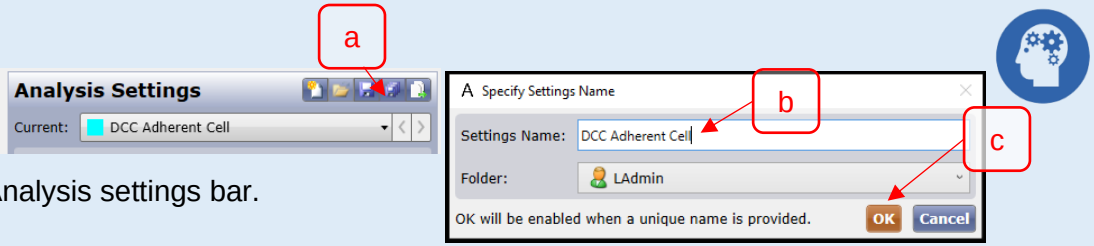


KNOWLEDGE: Save Features for Future Recall

Save Analysis Setting

Saves Analysis parameters on Analysis Tab

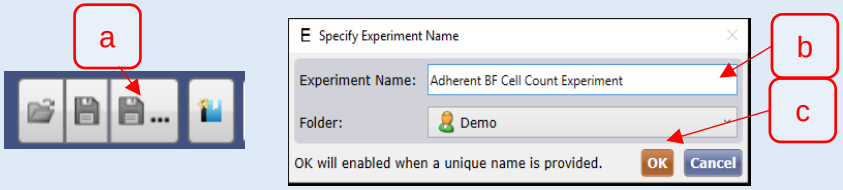
a. Click on **Save Analysis** icon.
b. Type in a desired settings name.
c. Click **OK**. Observe name updated in Current Analysis settings bar.



Save Experiment Setting

Saves settings from Scan, Analysis and Gating tab.

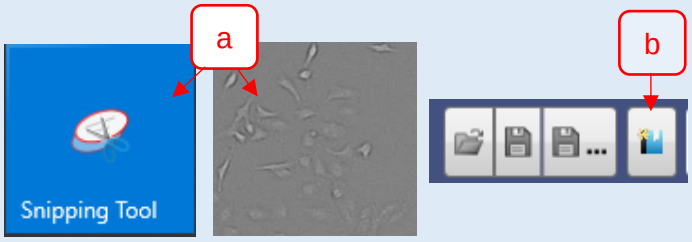
a. Click on **Save As Experiment** icon.
b. Type in desired name.
c. Click **OK**.

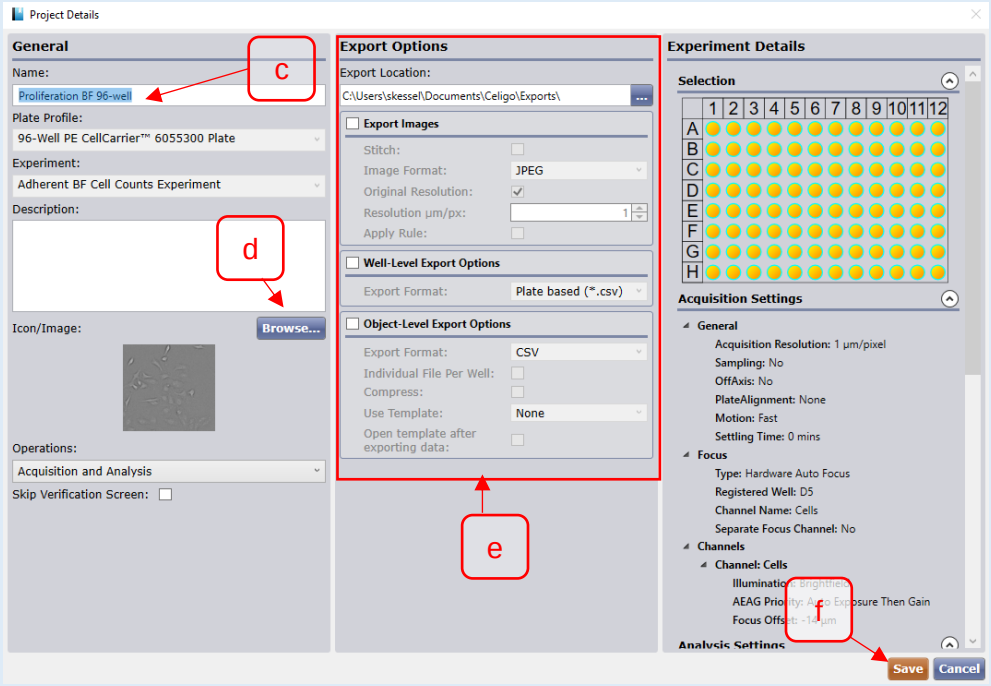


Save Project

Saves Experiment settings, plus Plate Profile selection and Export Options. Uses the least number of clicks start to finish.

a. Using screen **Snipping Tool** from Windows Accessories folder, take a **screen shot of cells** and save to a folder.
b. Click on **Project** icon.
c. Enter in **Project Name**
d. Select **Browse** and select the **Saved Picture**.
e. Select **Export Options** and folder location.
f. Click **Save**.

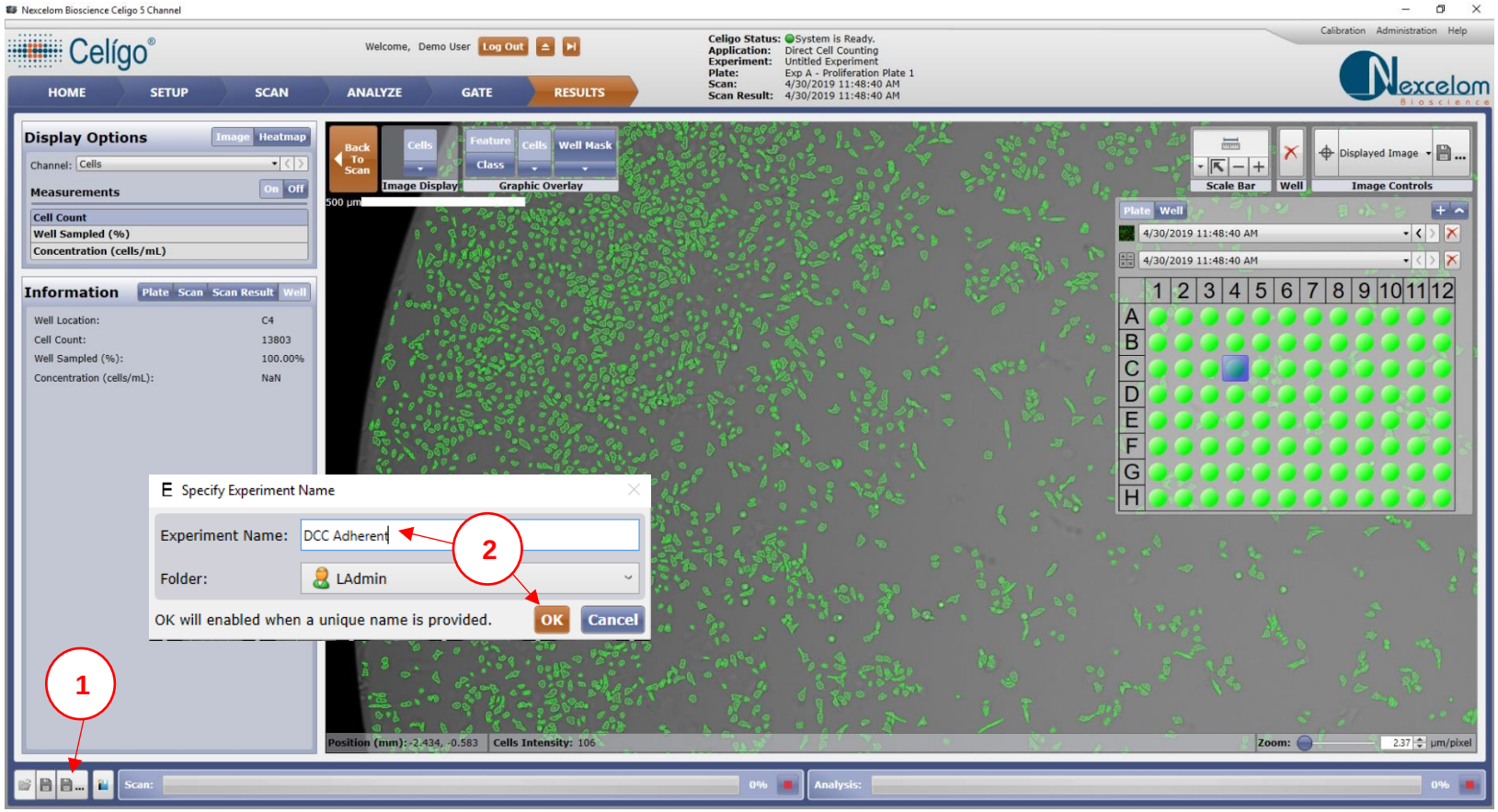




Save Experiment Setting and Project

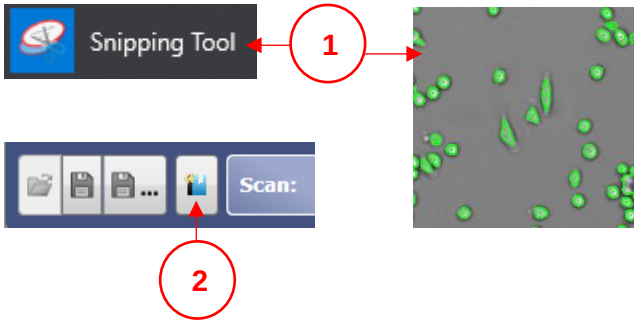
Save Experiment:

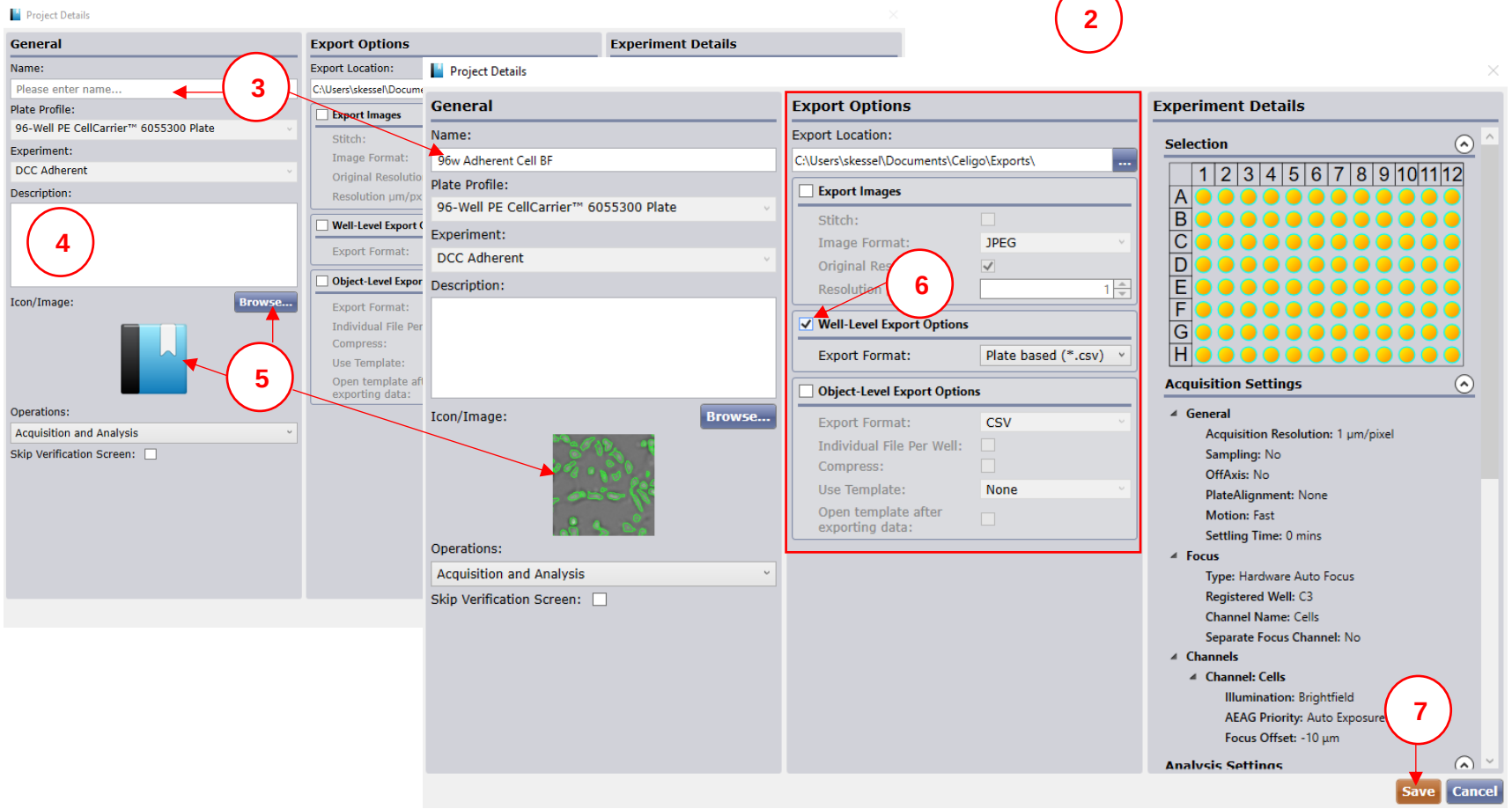
1. Click on **Save Experiment** Icon.
2. Enter name for future recall. Click **OK**.



Save a process as a Project

1. Save a screen snippet of cells using the **Window Snipping Tool.**
2. Click on **Project Icon.**
3. Enter a **Project name.**
4. Add a **Description** (optional).
5. Click **Browse**, Select **Saved Image**, click **OK**.
6. Check appropriate **Export Option** and **Folder Location**.
7. Click **Save**.

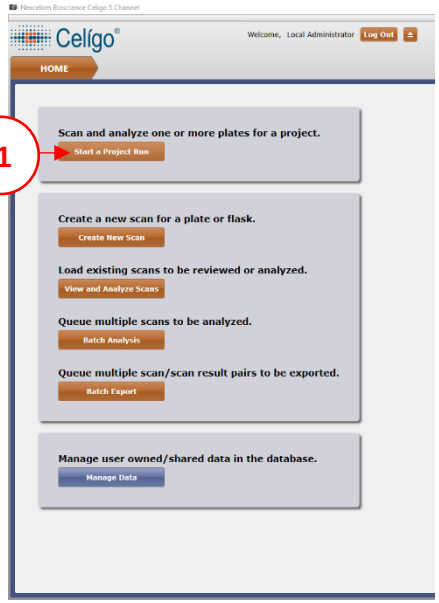


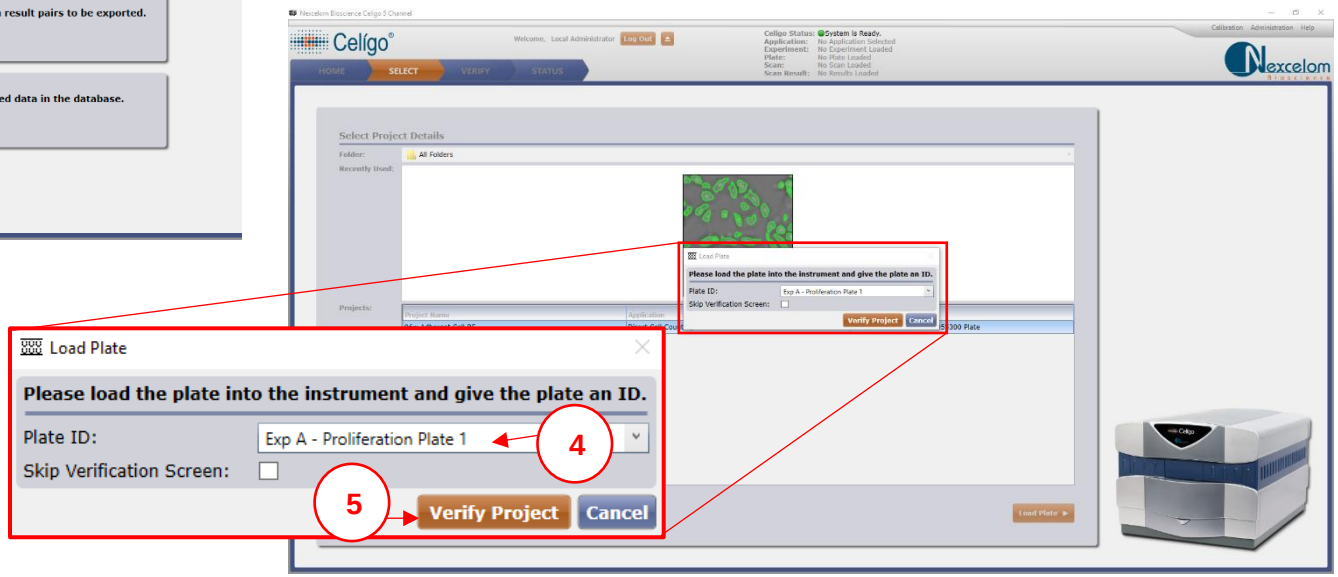


HOME Tab

Load a plate with Project Mode

1. Click **Start a Project Run.**
2. Select **Project.**
3. Click **Load Plate.**
4. Enter **Plate ID.**
5. Click **Verify Project.**

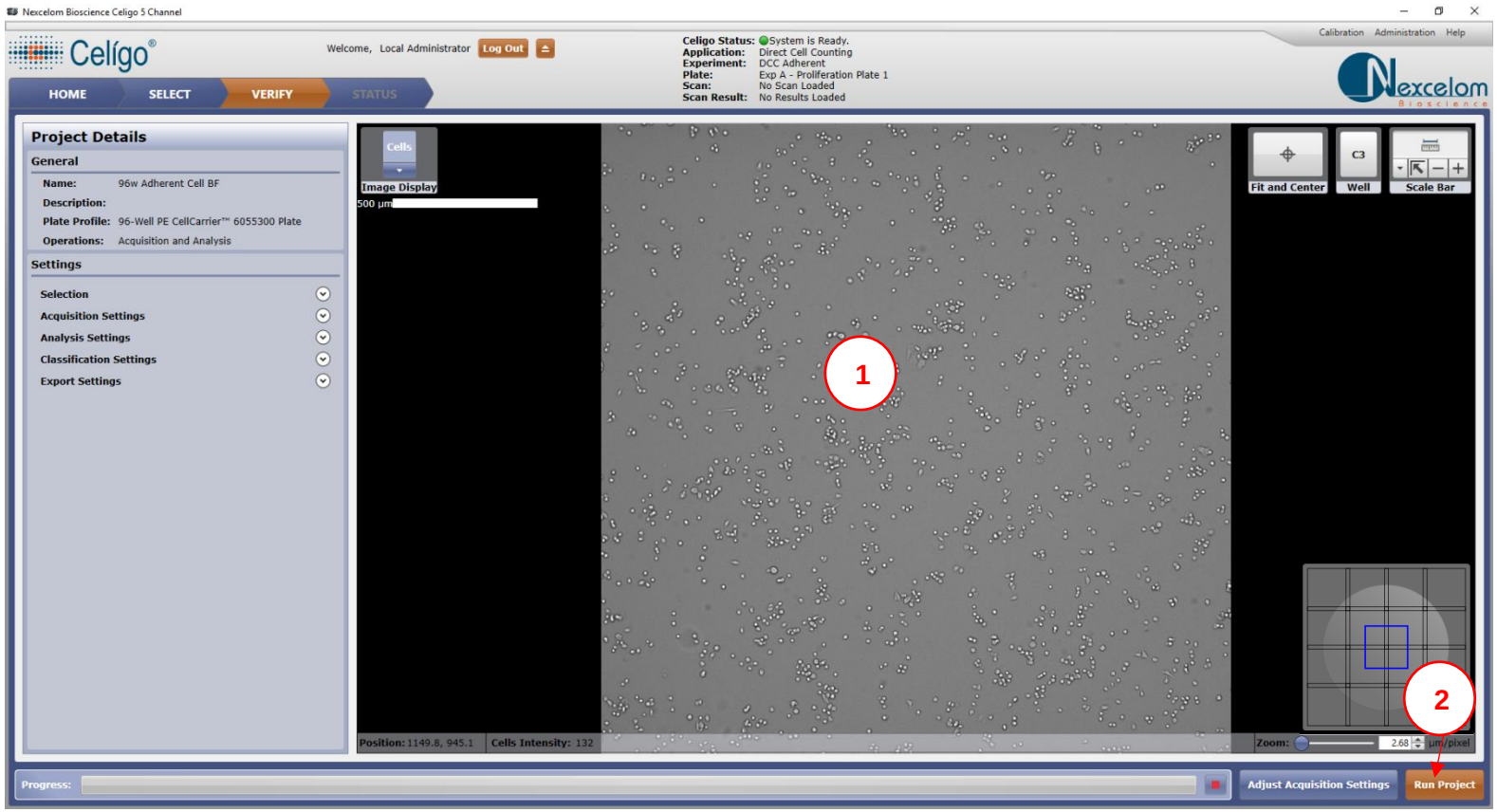




VERIFY Tab

Verify Focus and Illumination

1. Observe focus and illumination is good.
2. Click **Run Project.**

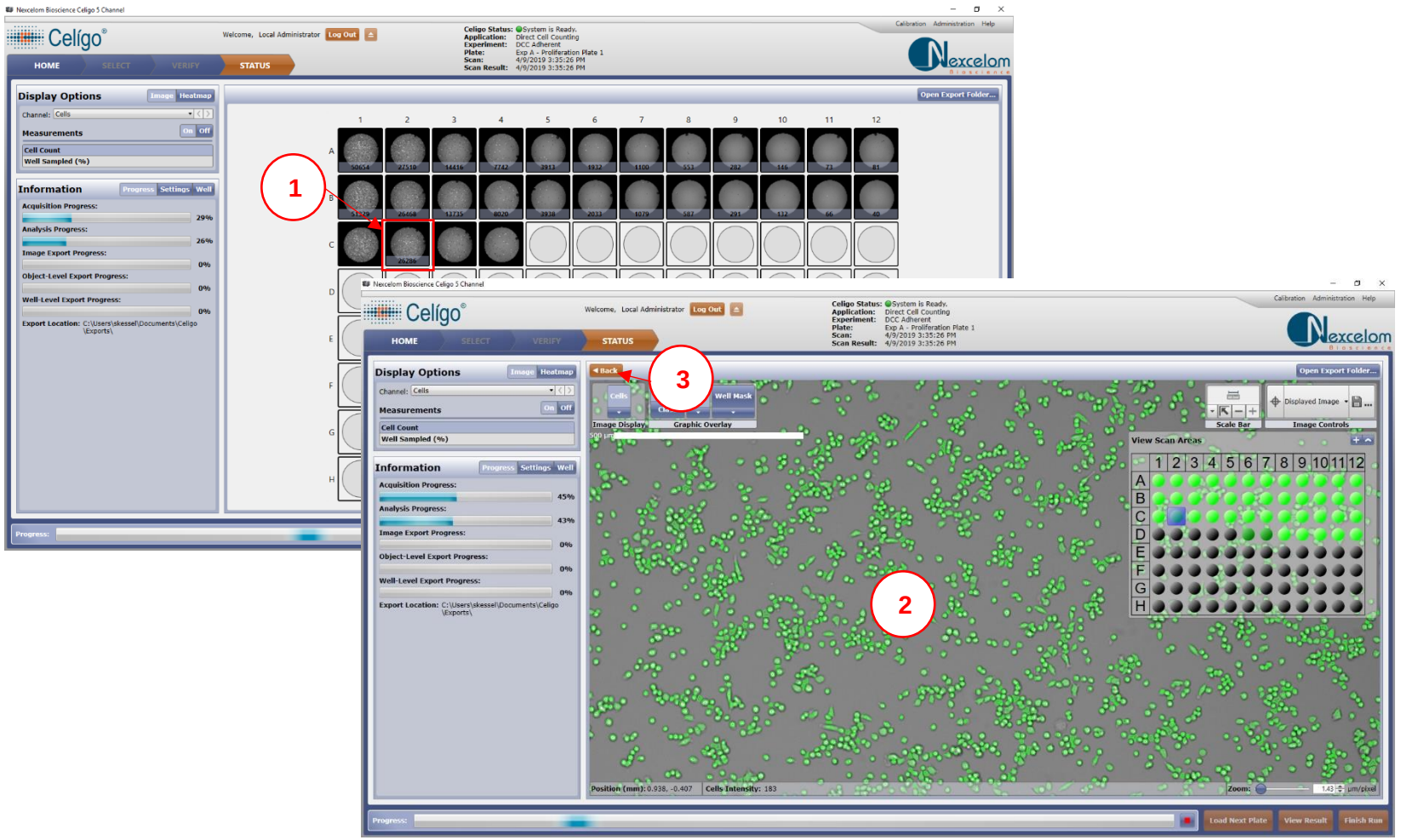


Adjust Acquisition Settings: If settings need adjusting, click **Adjust Acquisition Settings** button. System will switch into **Standard Mode**, where focus, illumination and well selection can be adjusted. Please see User Guide for further details.

STATUS Tab

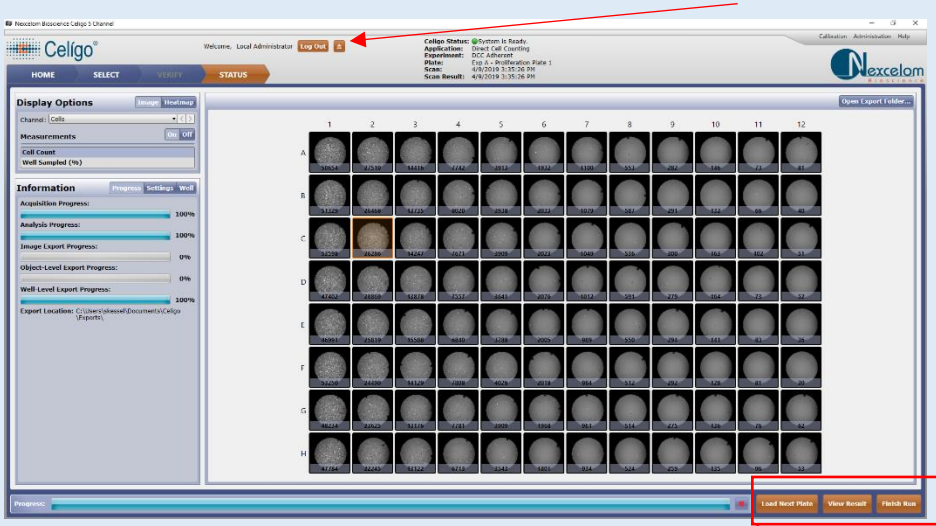
View a well image

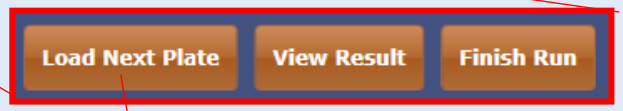
1. Double click on a well.
2. Scroll in/out with mouse scroll wheel.
3. Click **Back** to return to plate level view.

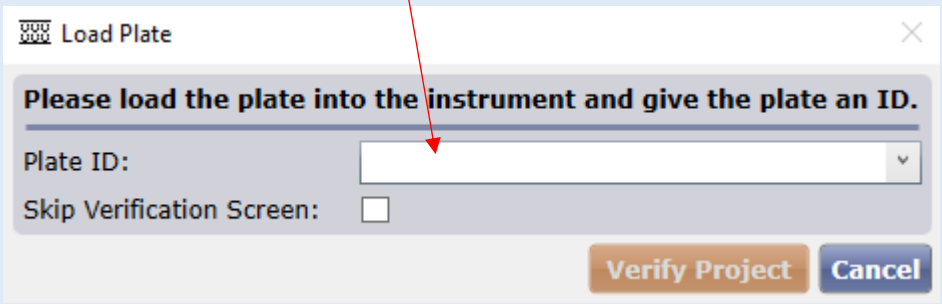


KNOWLEDGE: Options in STATUS tab

Load Next Plate: Image another plate with same Project. Enter next Plate ID in dialog box.
View Result: Switches view to Standard Mode in RESULTS tab.
Finish Run: Returns to HOME tab. Does not eject plate. Use Eject button to remove plate from instrument.







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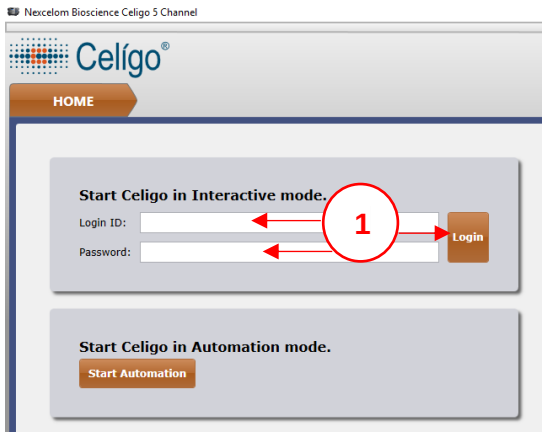
Chapter 4: Module 2 Cell Viability 2-Channel Dead and Total

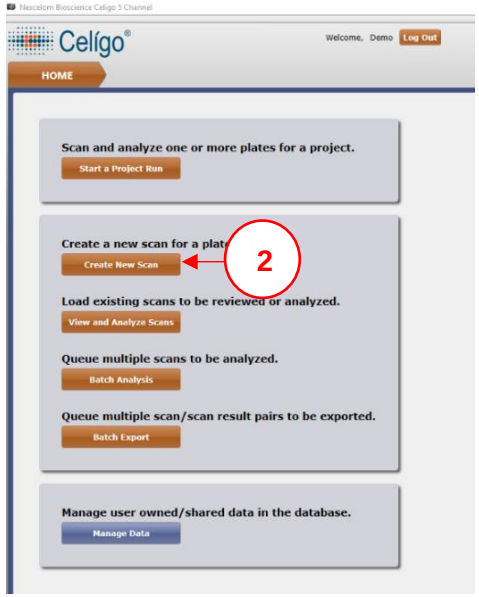
Outline of step-by-step instructions for Hoechst and PI stained cells:

HOME Tab

Login and Create New Scan in HOME Tab

1. Type in user **Login ID** and **password.** Click **Login**.
2. Click **Create New Scan**.





KNOWLEDGE:

Start a Project:
Uses a previously saved Project to scan analyze and export a plate scan.

Create a New Scan:
Scan and analyze a plate, entering in details at each tab.

View and Analyze Scans:
Load an unanalyzed scan or view a result of a scan and analysis.

Batch Analysis:
Using saved Analysis and Gating setting, analyzes multiple scans in a batch.

Batch Export:
Export multiple scan results and/or images in a batch.

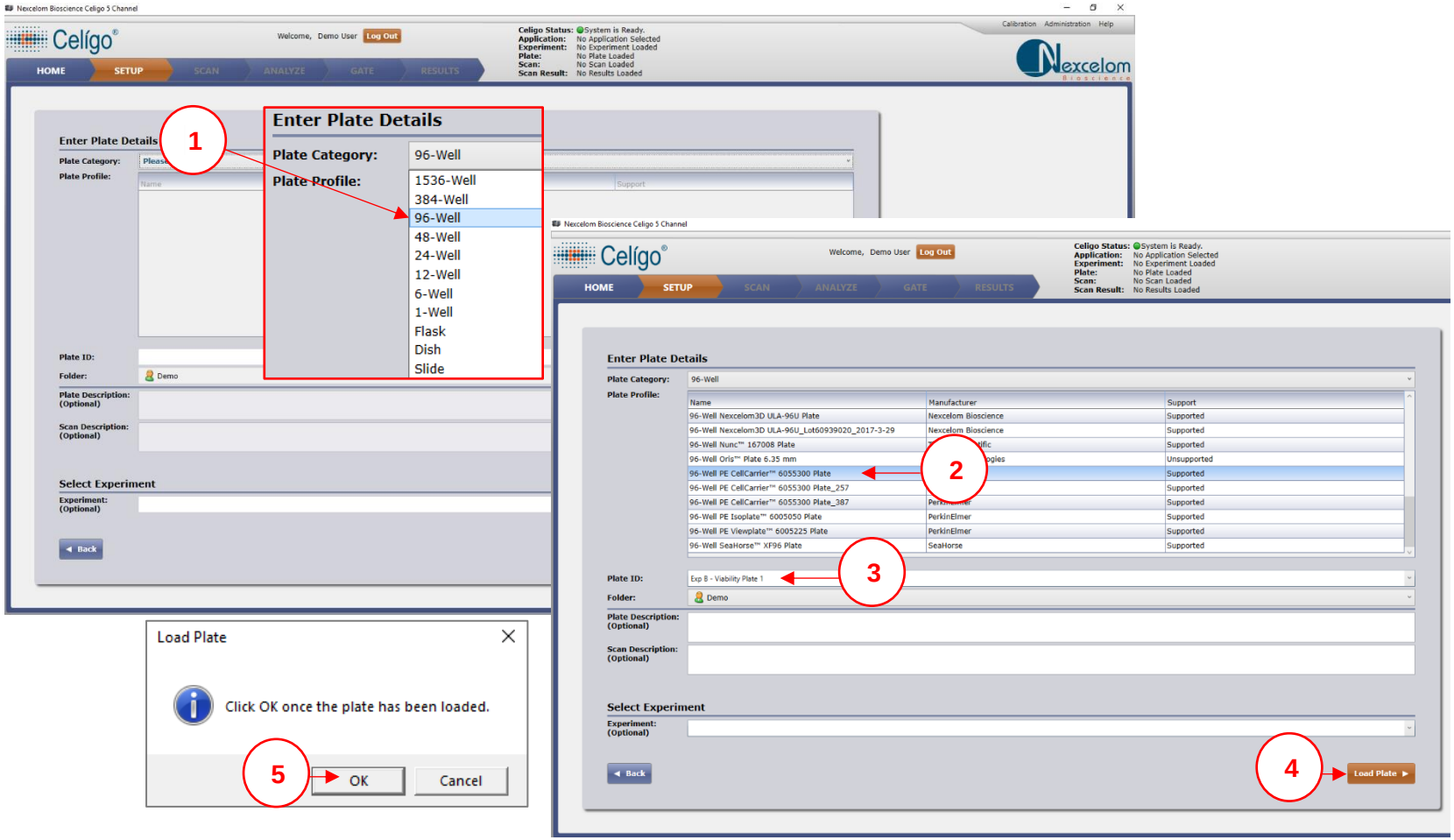
Manage Data:
View all data files in folder structure format to organize, import, export, delete image scan files and data.

See User Guide for further details.

SETUP Tab

Setup Details of Plate:

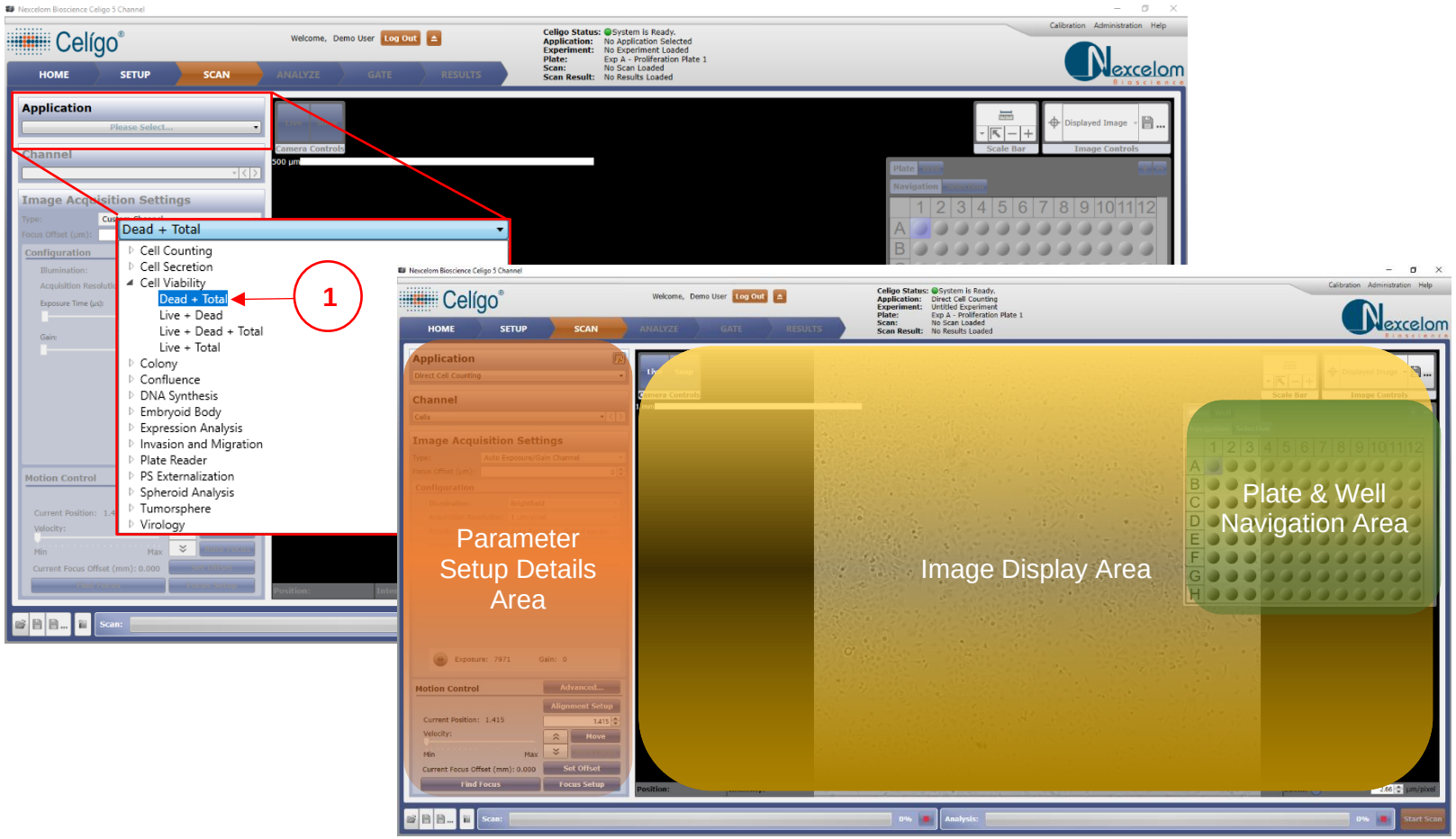
1. Select Plate Category: **96-well**
2. Select Plate Profile: **Manufacturer and Catalog #**
3. Type in a unique **Plate ID** or select a previous entered Plate ID
4. Click **Load Plate.**
5. Put plate on Stage and click **OK.**



SCAN Tab

Select Application: Dead + Total

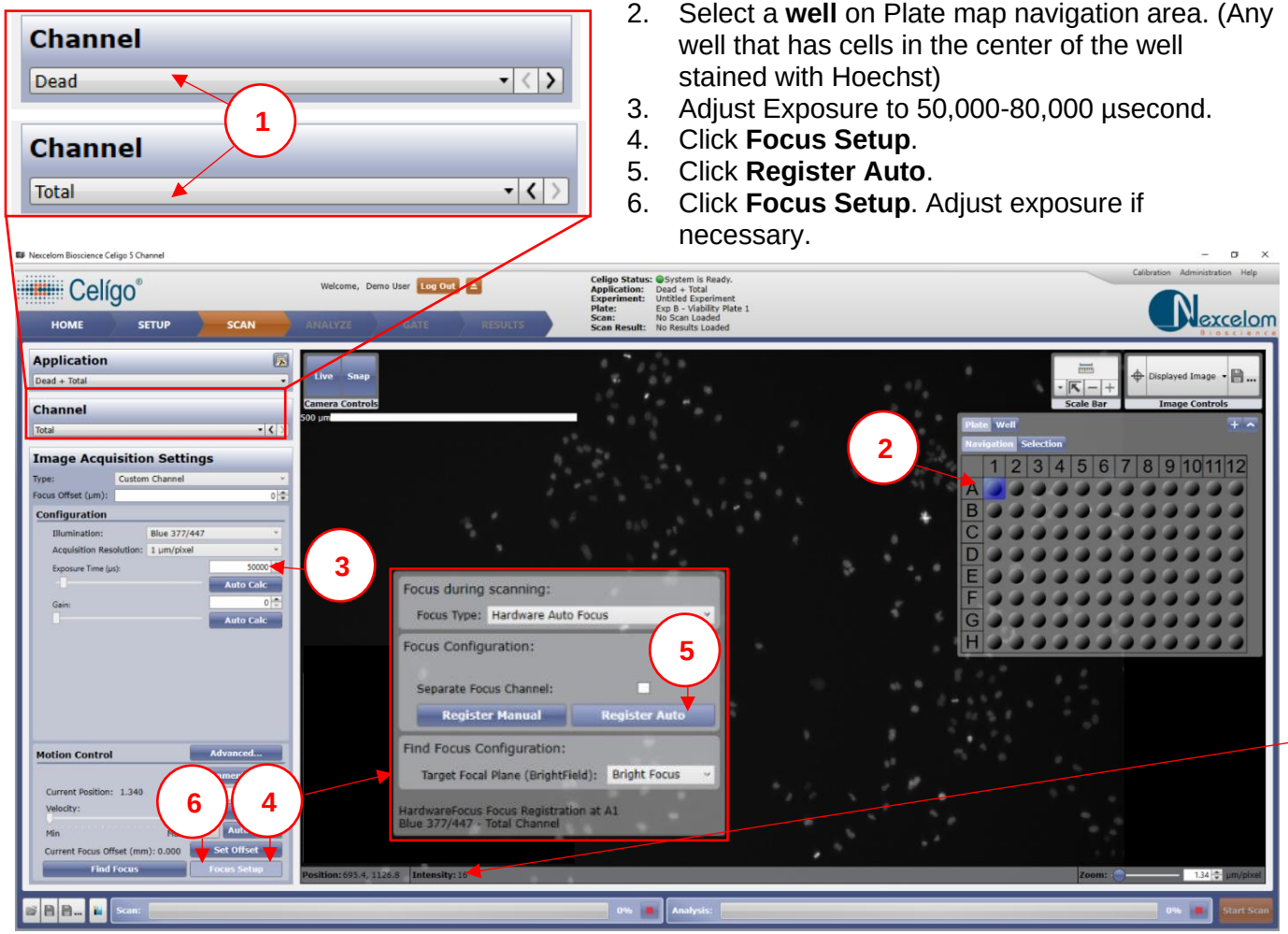
1. In Application Section, select **Cell Viability** > **Dead + Total**



When first in SCAN tab, you must select an application. Then the system will display the appropriate setup parameters on the left panel area. The stage is located on Well A1 identifiable by the blue square on the Plate Navigation Area. An image snap is displayed in the Image Display Area.

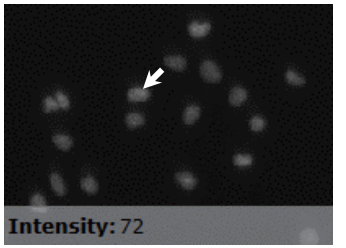
Total Channel Setup for Illumination, Exposure, Focus Registration:

1. Change Channel from Dead to **Total.**
2. Select a **well** on Plate map navigation area. (Any well that has cells in the center of the well stained with Hoechst)
3. Adjust Exposure to 50,000-80,000 µsecond.
4. Click **Focus Setup**.
5. Click **Register Auto**.
6. Click **Focus Setup**. Adjust exposure if necessary.

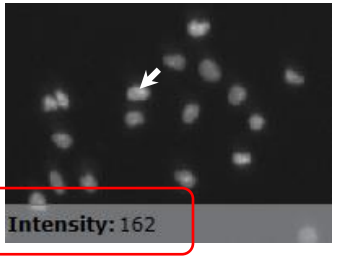


KNOWLEDGE: Hover mouse over objects to see pixel intensity. Adjust Exposure and Gain to obtain object pixel (signal) and background pixel (noise) intensity ratio to be greater than 2.0.

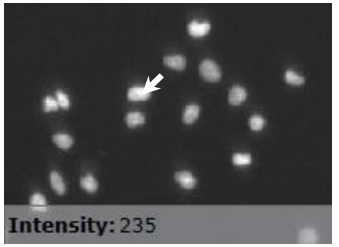
Exposure: 50,000 us, Gain 0
Object / background (72 / 18)



Exposure: 80,000 us, Gain 0
Object/ background (162 / 29)

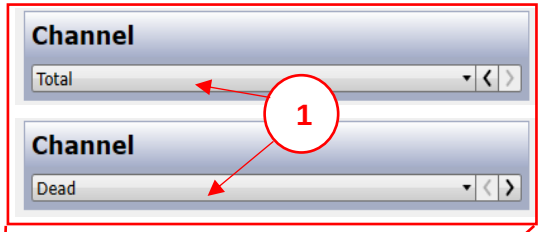


Exposure: 80,000 us, Gain 100
Object/ background (235 / 43)

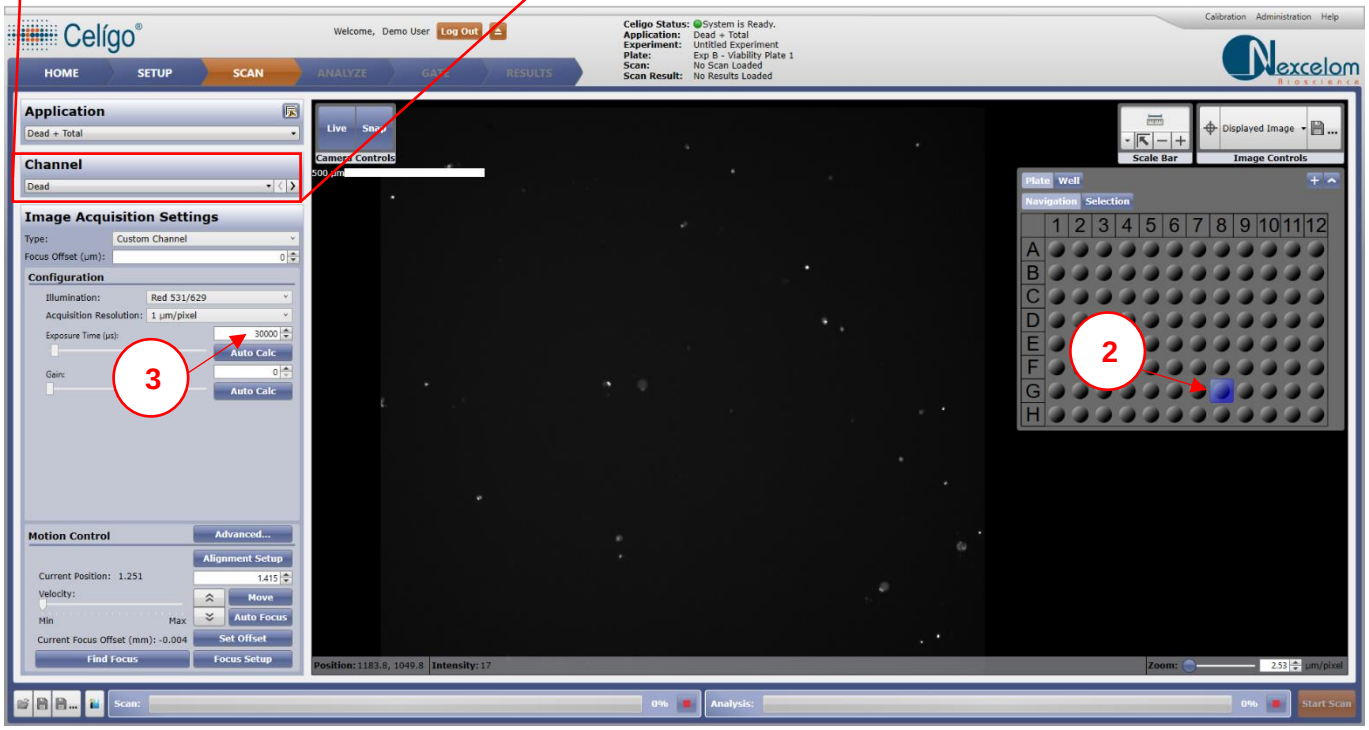


Check pixel intensities after focus registration by hovering mouse over objects and background. The ideal fluorescent images have object pixel values with signal to background ratio above 2.0 and in the upper end of the camera range (0-255). Therefore a 150 to 200 object intensity with background pixel values below 75.

Dead Channel Setup for Illumination, Exposure, Focus Registration:

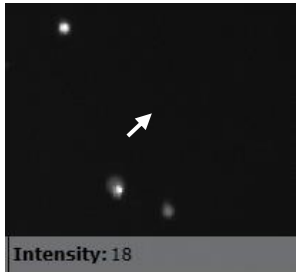


1. Change Channel from Total to **Dead.**
2. Select a **well** on Plate map navigation area.
   (Any well that has dead cells in the center of the well stained with Propidium Iodide (PI), it doesn't have to be the same well as focus registration well.)
3. Adjust Exposure to 30,000-50,000 µsecond



KNOWLEDGE: Dead cells can vary in intensity and amount of cellular decay. PI stains DNA and RNA of cells with compromised membranes.

Background: 18

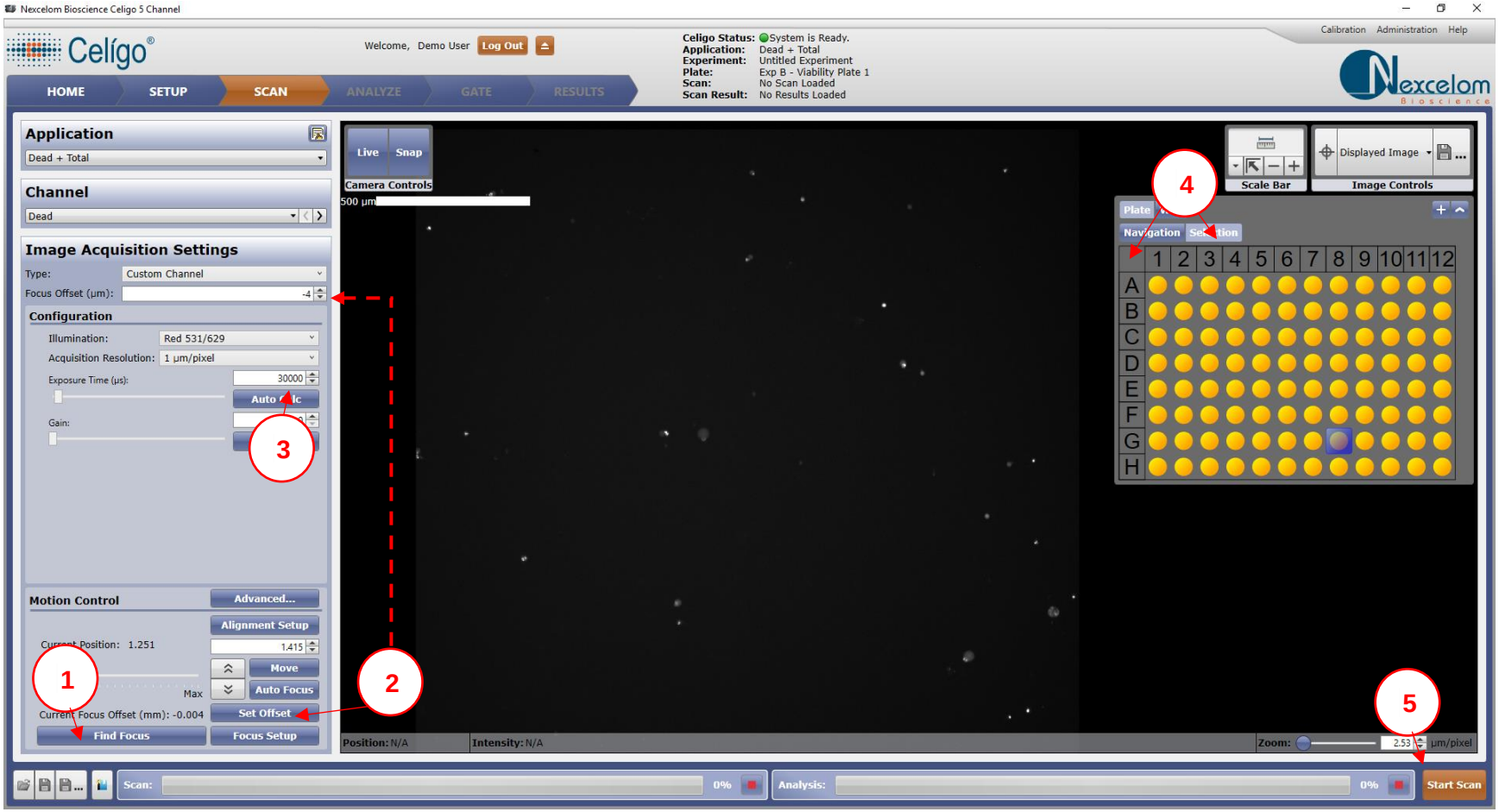


Dimmest Cell: 92

Bright cell signal: 244

Dead Channel Focus Offset and Well Selection:

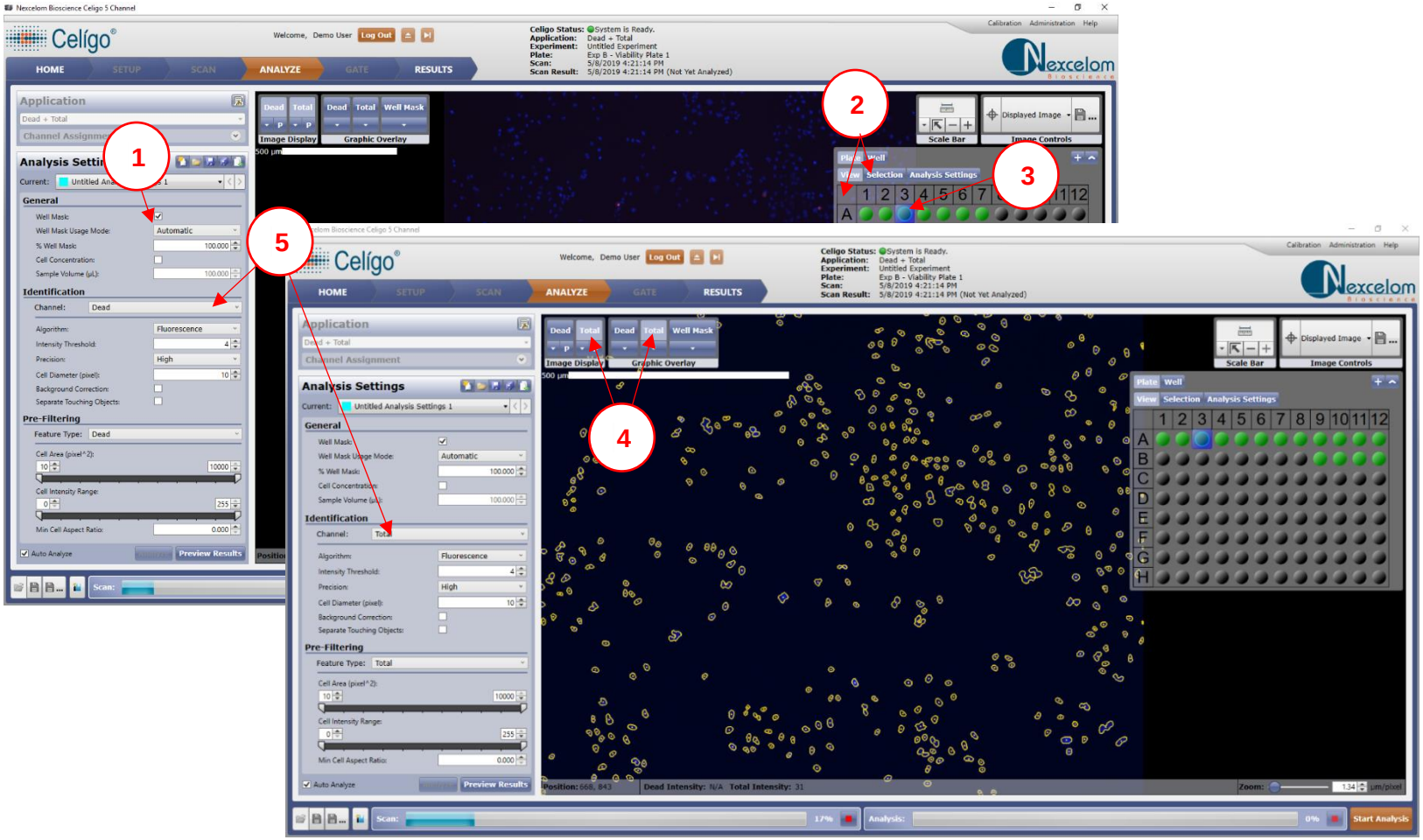
1. Click **Find Focus.**
2. Click **Set Offset**. (A focus offset populates in Focus Offset text box.)
3. Adjust exposure, if necessary. Ideal object pixel intensity between 150-200.
4. Click **Selection** and **Highlight the wells** (yellow) on Plate Area for imaging.
5. Click **Start Scan**.



ANALYZE Tab

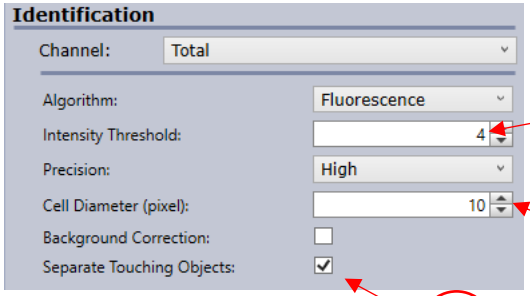
Setup Analysis Settings for Total Image and Graphic Overlay

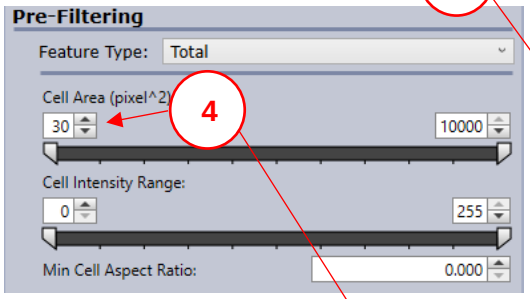
1. In General Section, check Well Mask.
2. Click **Selection** and Un-highlight wells yellow
3. Select a **green well** to view
4. Select **Total image** and graphic overlay ON. Turn OFF **Dead Image** and **Graphic Overlay**
5. Change **Channel** to **Total**.

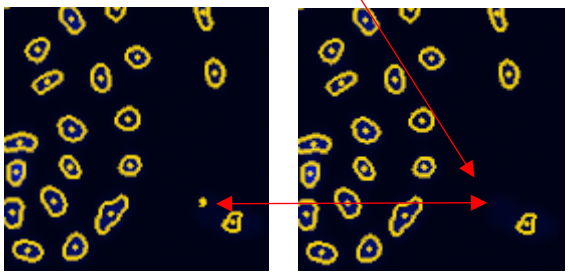


Adjust Analysis Parameter for Total Channel Objects

1. Adjust **Intensity Threshold**
2. Adjust **Cell Diameter**
3. Check **Separate Touch Objects**
4. Increase **Minimum Cell Area** to eliminate small objects

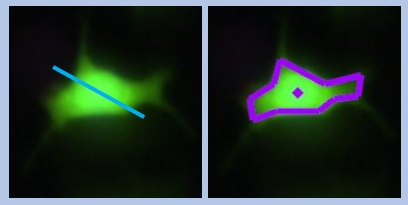




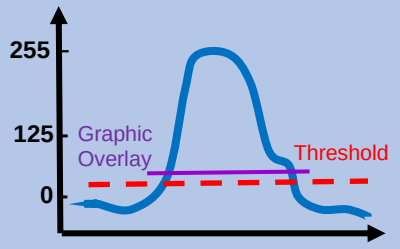


KNOWLEDGE: Identification Section

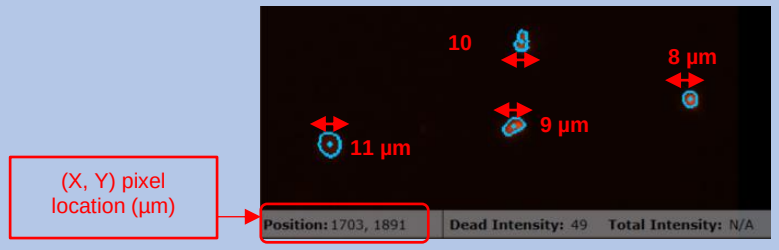
Intensity Threshold: When signal to noise ratio is 2.0 or greater the default settings will be a good starting point. If signal is lower or background is high, lower the intensity threshold.



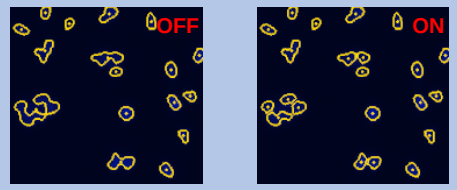
Pixel intensity



Cell Diameter is the average expected diameter of the majority of the objects. Hover mouse over objects left and right side and observe diameter of object from pixel location values. Dimmer cells can be picked up if you increase diameter.



Separate Touching Objects will separate multiple objects that are close together identified as one into separate objects.

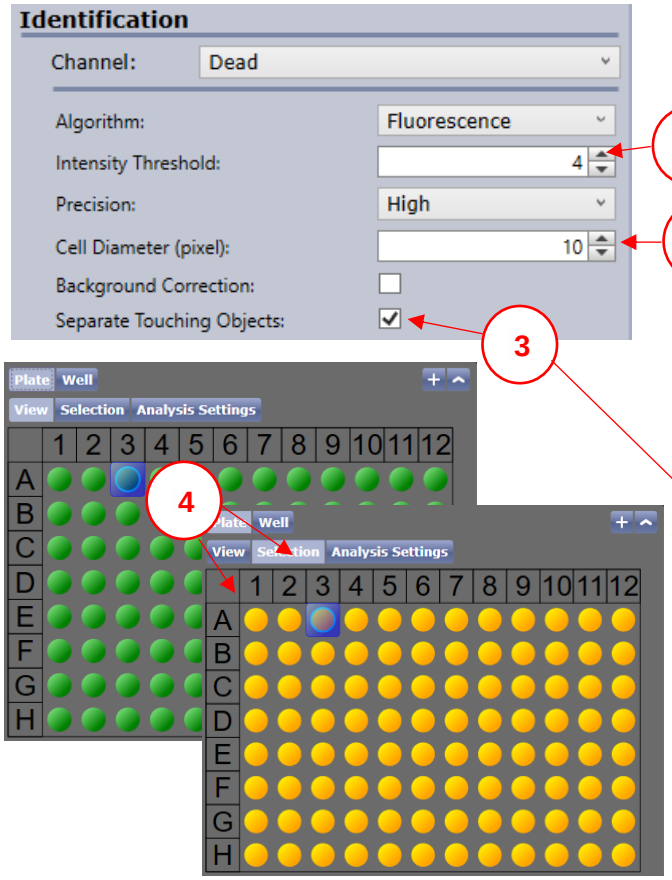


View Dead Image and Graphic Overlay

1. Select **Dead** Image and turn Graphic Overlay ON. Turn OFF **Total Image** and **Graphic Overlay**
2. Switch to **Well view** to navigate around well to find more dead stain cells.
3. Change Channel to **Dead**.

Adjust Analysis Parameter for Dead Channel Objects

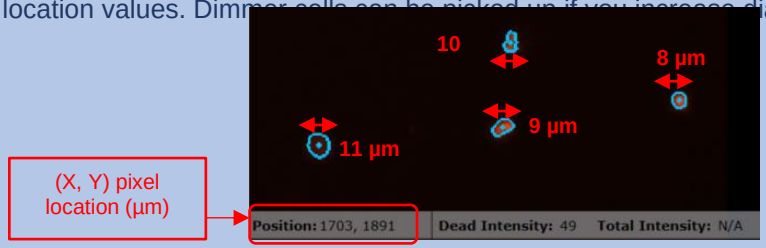
1. Adjust **Intensity Threshold**
2. Adjust **Cell Diameter**
3. Check **Separate Touch Objects**
4. Click **Selection**, highlight wells for analysis



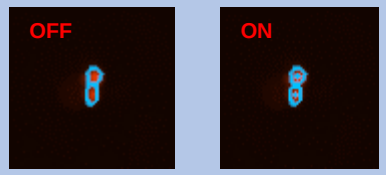
KNOWLEDGE:

Intensity Threshold: When signal to noise ratio is 2.0 or greater the default settings will be a good starting point. If signal is lower or background is high, lower the intensity threshold.

Cell Diameter is the average expected diameter of the majority of the objects. Hover mouse over objects left and right side and observe diameter of object from pixel location values. Dimmer cells can be picked up if you increase diameter.

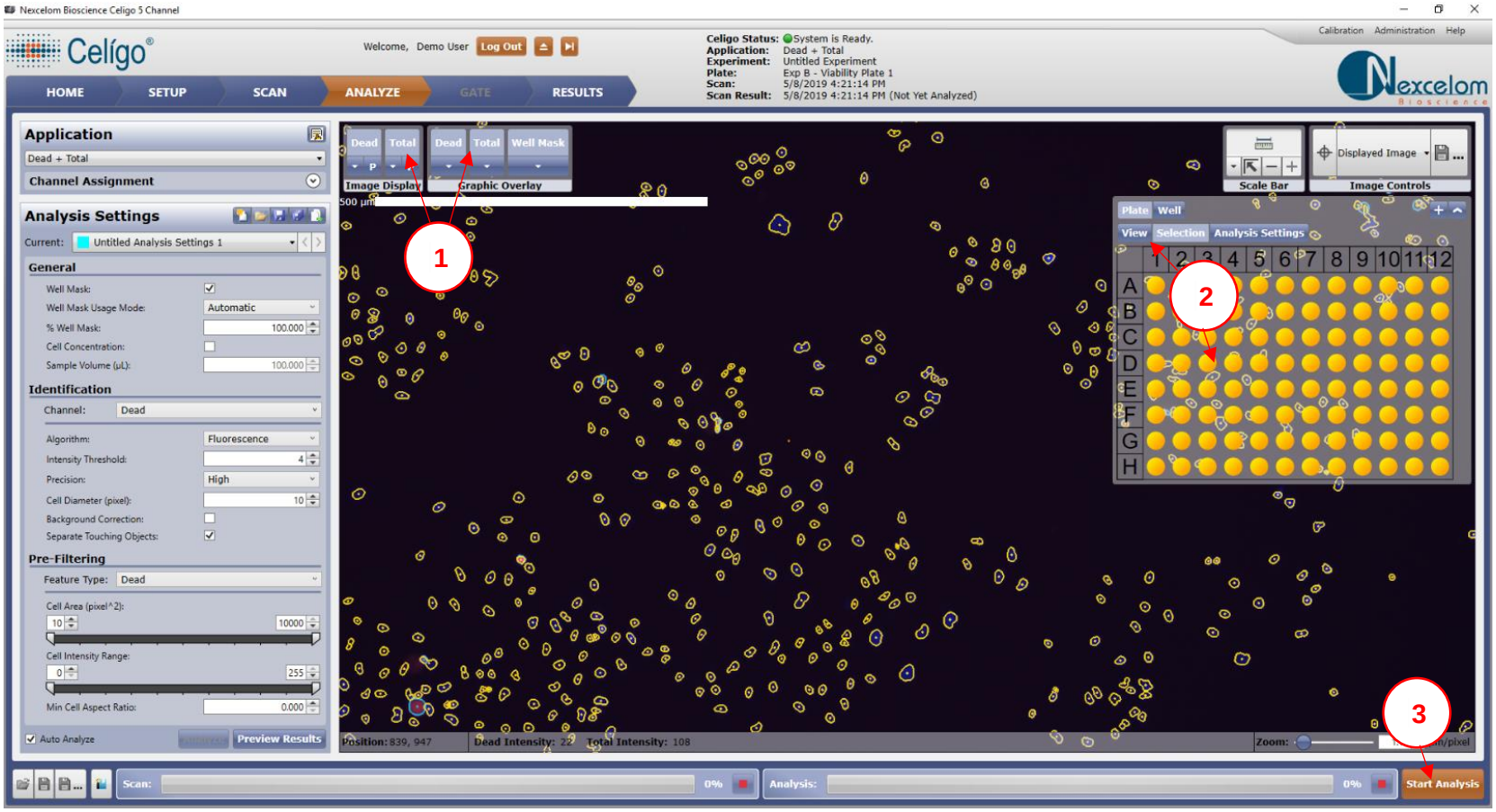


Separate Touching Objects will separate multiple objects that are close together identified as one into separate objects.



View Dead and Total Images and Graphic Overlays

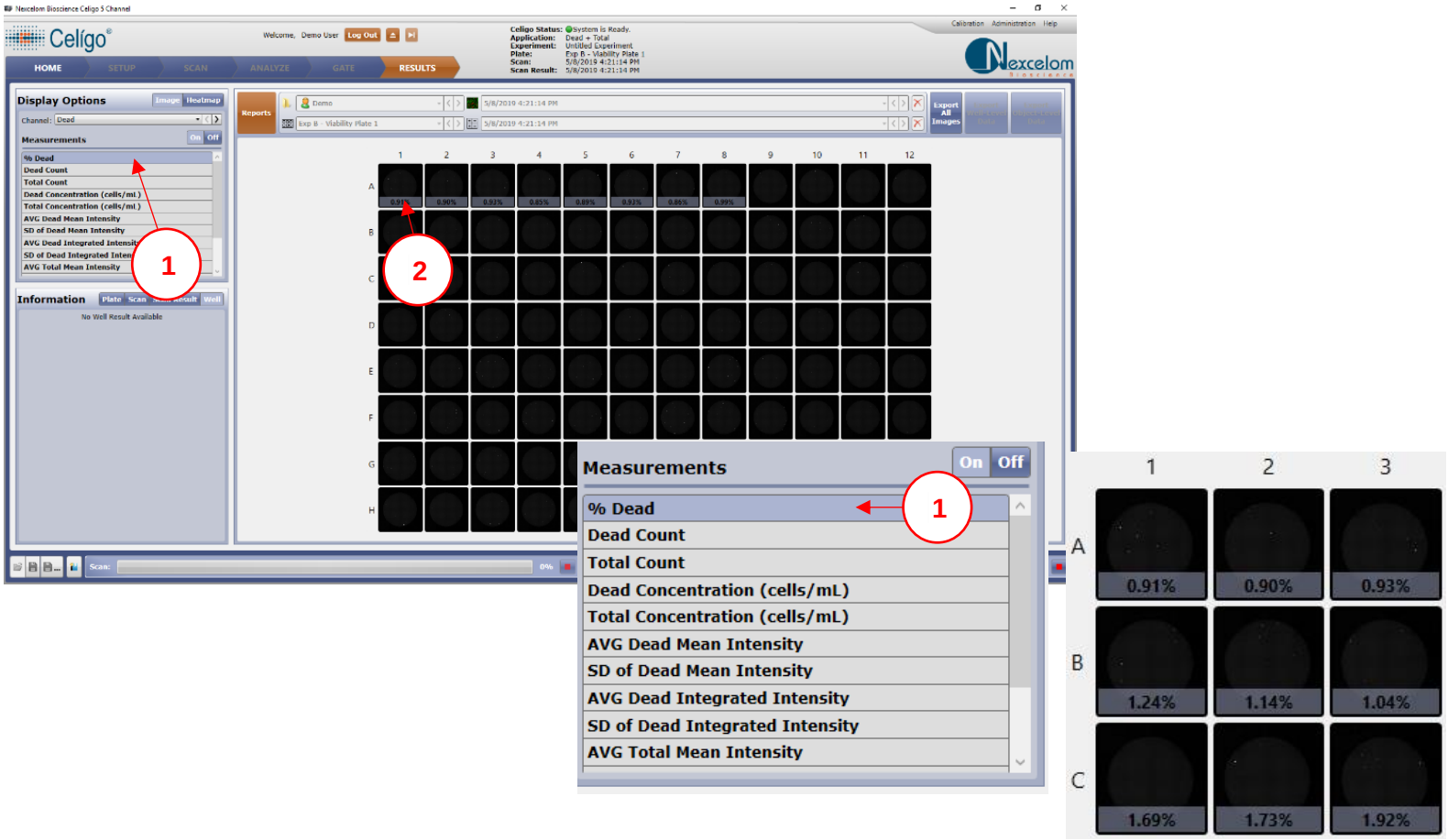
1. Select **Dead** and **Total Image** and **Graphic Overlay** buttons ON.
2. Click **View** to view other wells by clicking on wells in navigation area.
3. Click **Start Analysis**.



RESULTS Tab

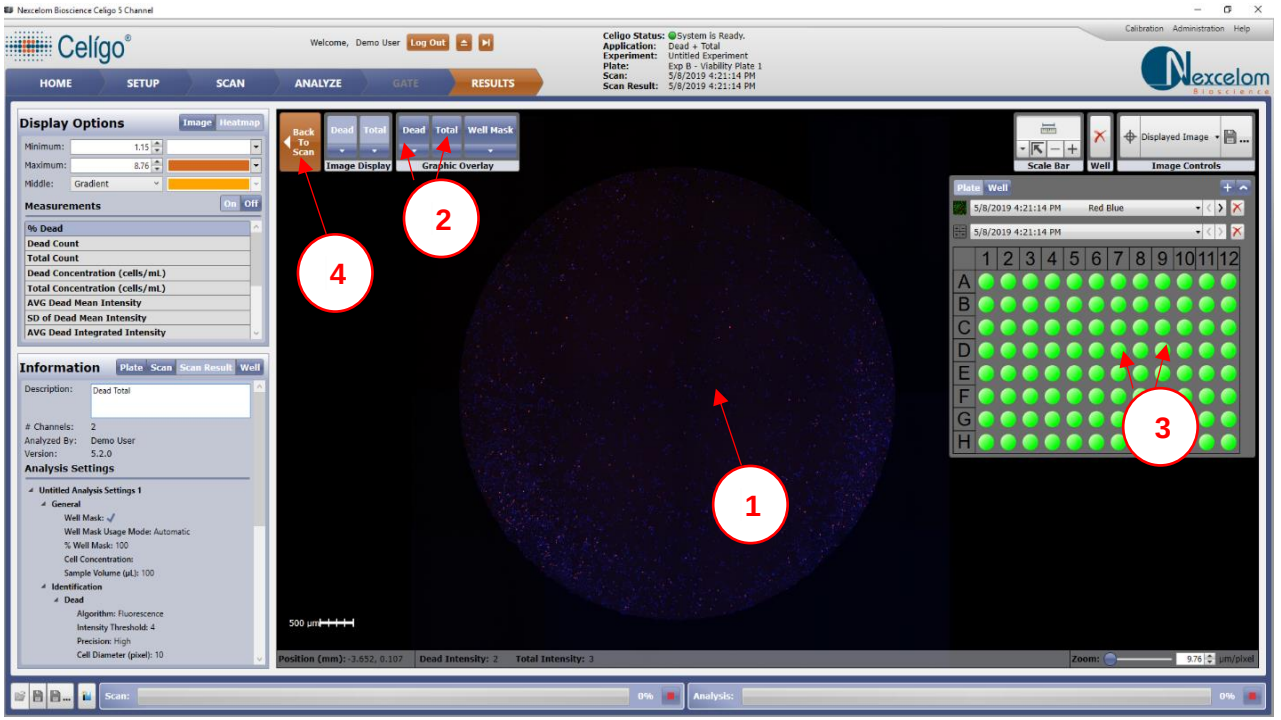
Review Images and Data Output Measurements

1. Select a different data Measurement. Data output displays on thumbnail image.
2. Double click a well to see high resolution image and graphic overlay of counted objects.

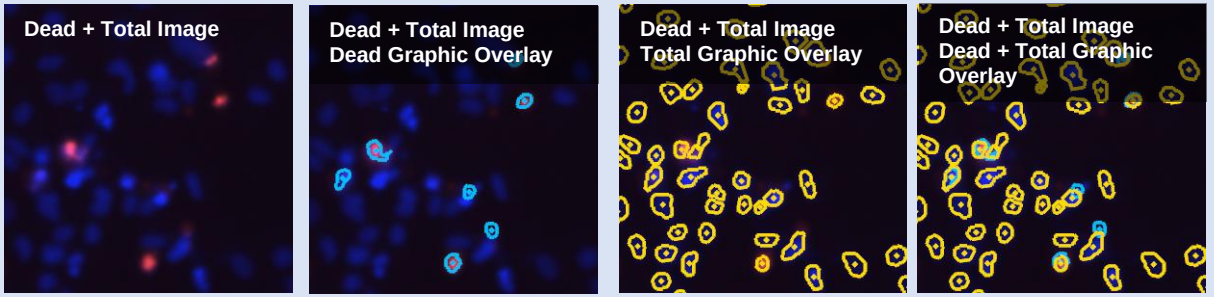


Review Images and Data Output Measurements

1. Zoom in with mouse scroll button.
2. Turn On/Off graphic overlays to see detected objects.
3. Click on wells in plate map to see other well images.
4. Click on **Back to Scan** to return to plate level thumbnails.

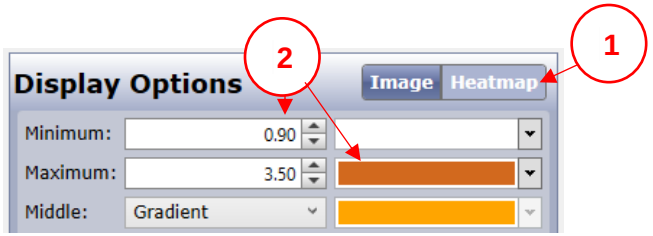


KNOWLEDGE: Example images with and without corresponding graphic overlay



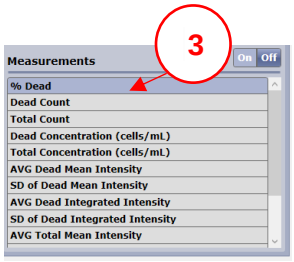
View Data Output Measurements with Heatmap

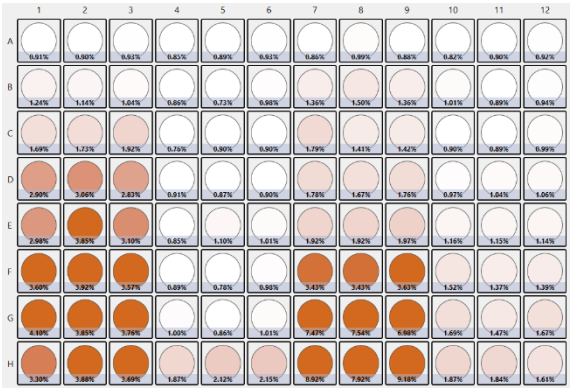
1. Click **Heatmap**.
2. Adjust Minimum and Maximum values and colors.
3. Select different Measurements to display.



Example drug dilution plated in

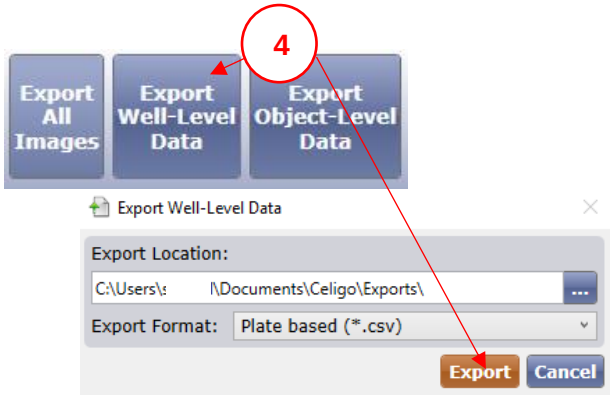
	1	2	3	4	5	6	7	8	9	10	11	12
A	0			0			0			0		
B	0.014			0.014			0.014			0.014		
C	0.041			0.041			0.041			0.041		
D	0.123			0.123			0.123			0.123		
E	0.370			0.370			0.370			0.370		
F	1.111			1.111			1.111			1.111		
G	3.333			3.333			3.333			3.333		
H	10.000			10.000			10.000			10.000		
	Drug A			**Drug B**			**Drug C**			**Drug D**		





Export Data Output Measurements

4. Click **Export Well-Level Data** to export into CSV file. Then **Export**.



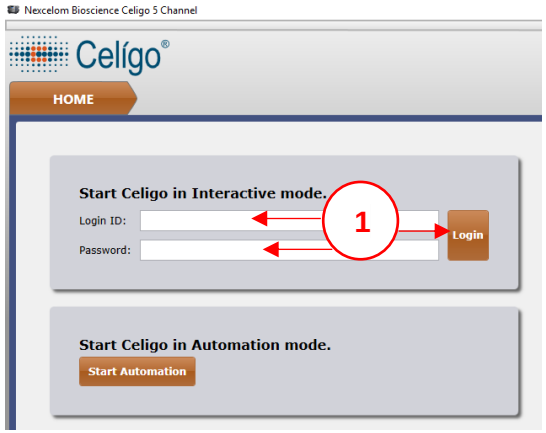
Chapter 5: Module 3 Cell Viability 3-Channel with Gating and Brightfield

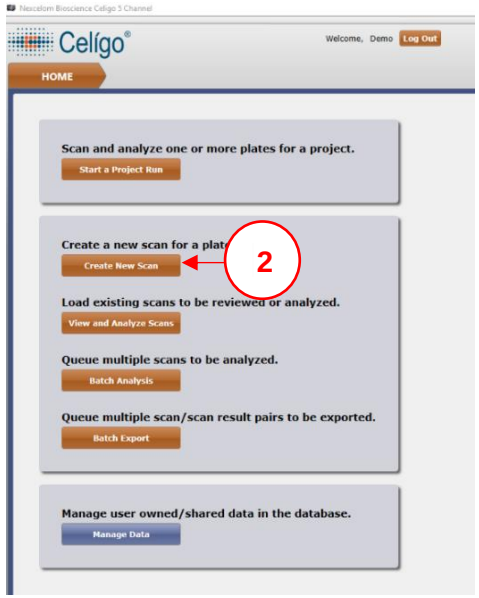
Outline of step-by-step instructions for Hoechst and PI stained cells, additionally capture a brightfield channel and use gating tab:

HOME Tab

Login and Create New Scan in HOME Tab

1. Type in user **Login ID** and **password.** Click **Login**.
2. Click **Create New Scan**.





KNOWLEDGE:

Start a Project:
Uses a previously saved Project to scan analyze and export a plate scan.

Create a New Scan:
Scan and analyze a plate, entering in details at each tab.

View and Analyze Scans:
Load an unanalyzed scan or view a result of a scan and analysis.

Batch Analysis:
Using saved Analysis and Gating setting, analyzes multiple scans in a batch.

Batch Export:
Export multiple scan results and/or images in a batch.

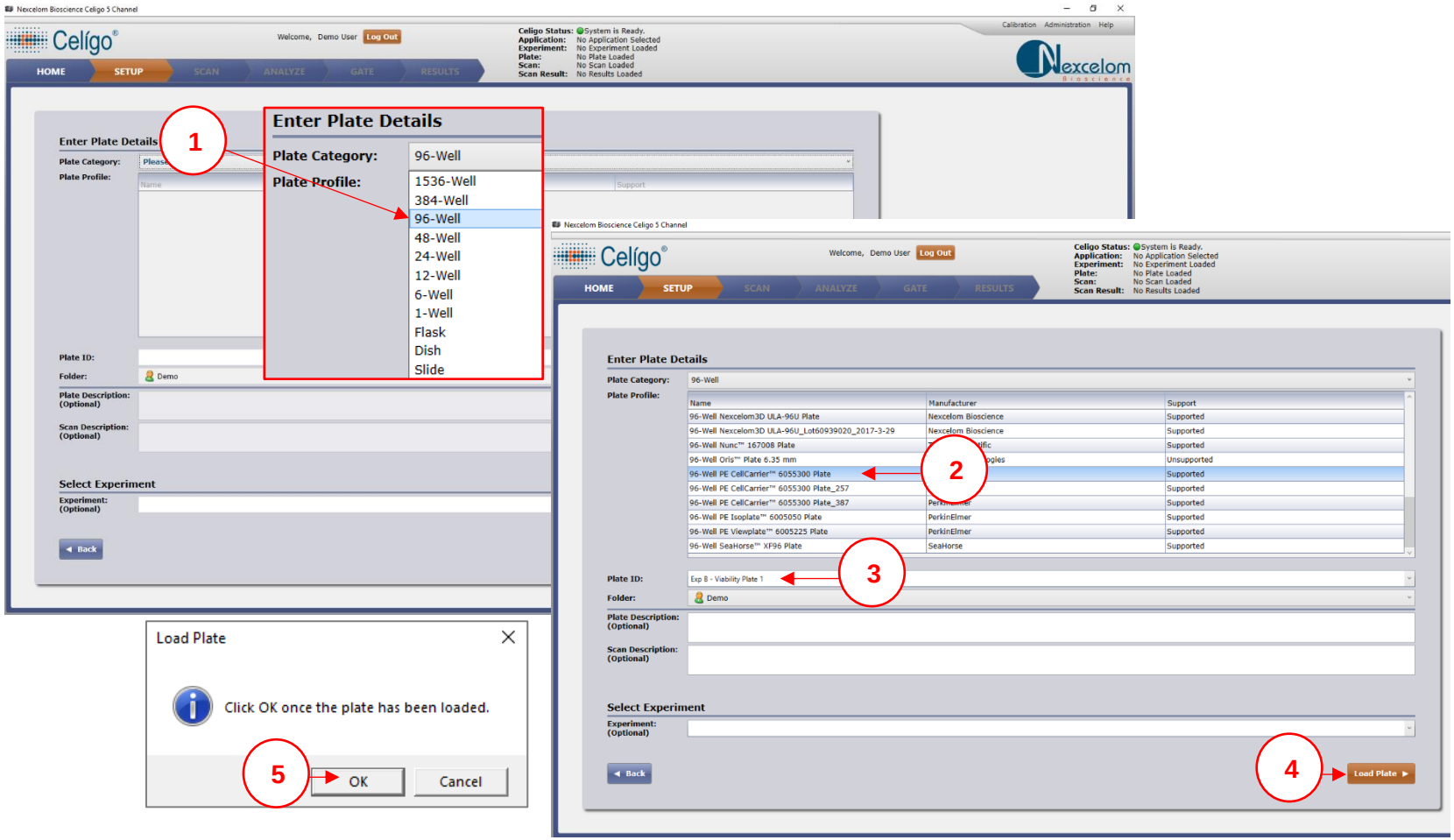
Manage Data:
View all data files in folder structure format to organize, import, export, delete image scan files and data.

See User Guide for further details.

SETUP Tab

Setup Details of Plate:

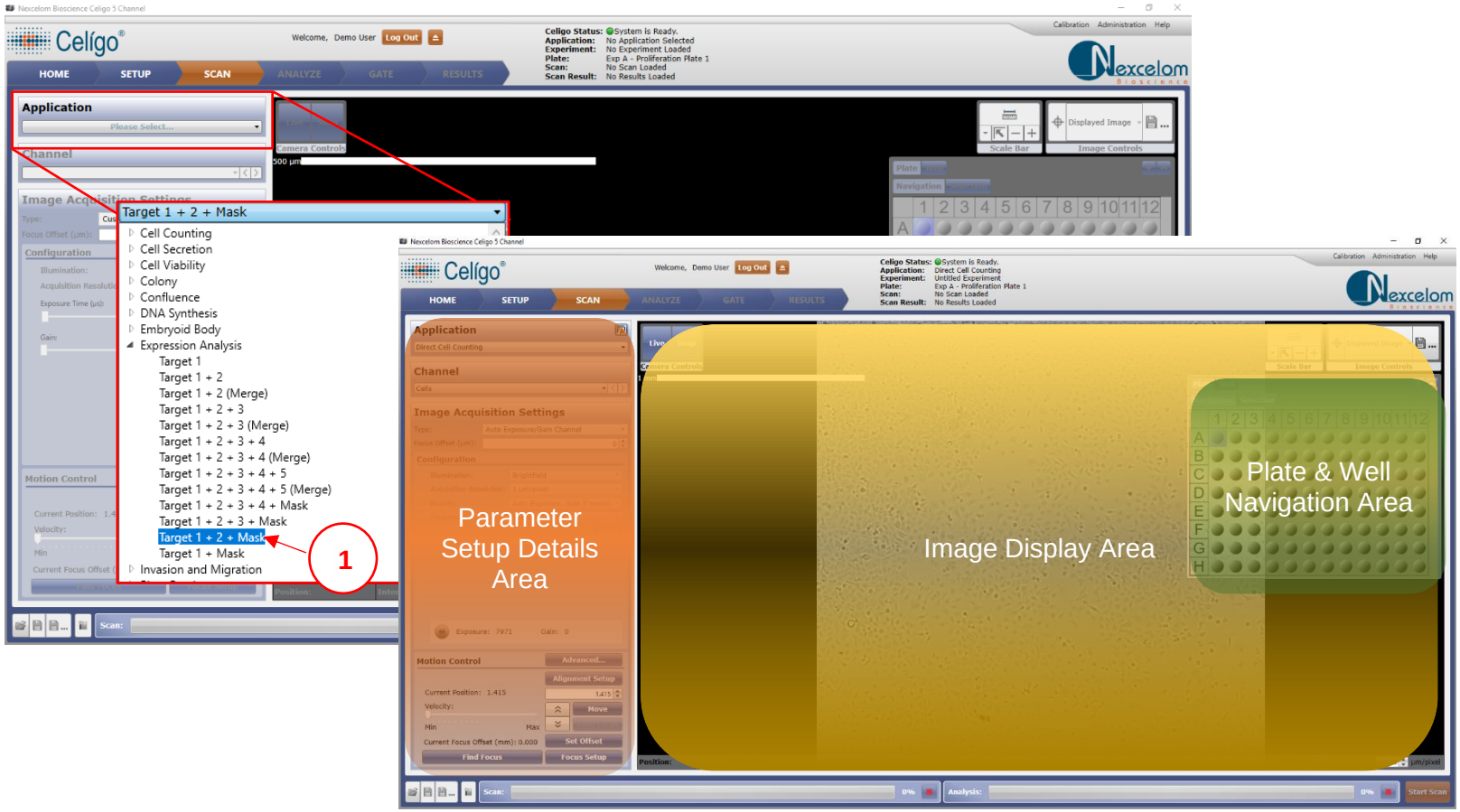
1. Select Plate Category: **96-well**
2. Select Plate Profile: **Manufacturer and Catalog #**
3. Type in **Plate ID** or select a previous entered Plate ID
4. Click **Load Plate.**
5. Put plate on Stage and click **OK.**



SCAN Tab

Select Application: Expression Analysis → Target 1 + 2 + Mask

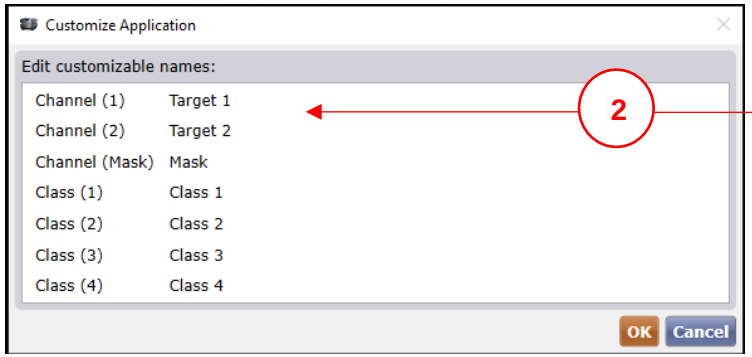
1. In Application Section, select **Expression Analysis → Target 1 + 2 + Mask**

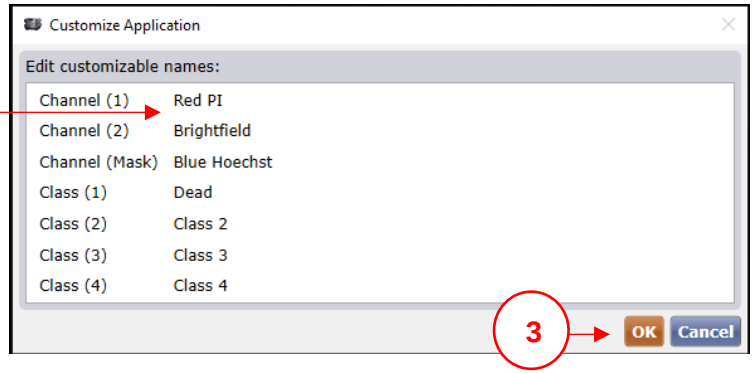


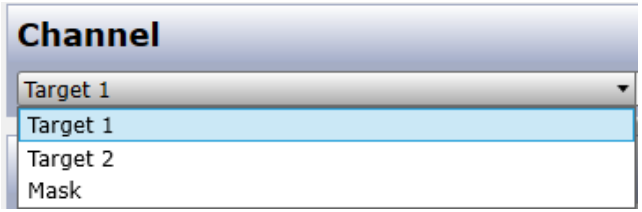
When first in SCAN tab, you must select an application. Then the system will display the appropriate setup parameters on the left panel area. The stage is located on Well A1 identifiable by the blue square on the Plate Navigation Area. An image snap is displayed in the Image Display Area.

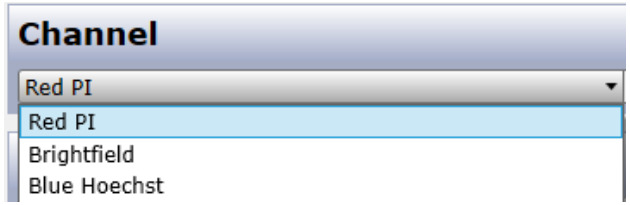
Rename Channels and Classes

1. Click on  Icon in the Application section.
2. Change name of Channels Target 1, Target 2, Mask and Class 1 to Red PI, Brightfield, Blue Hoechst and Dead.
3. Click **OK**.





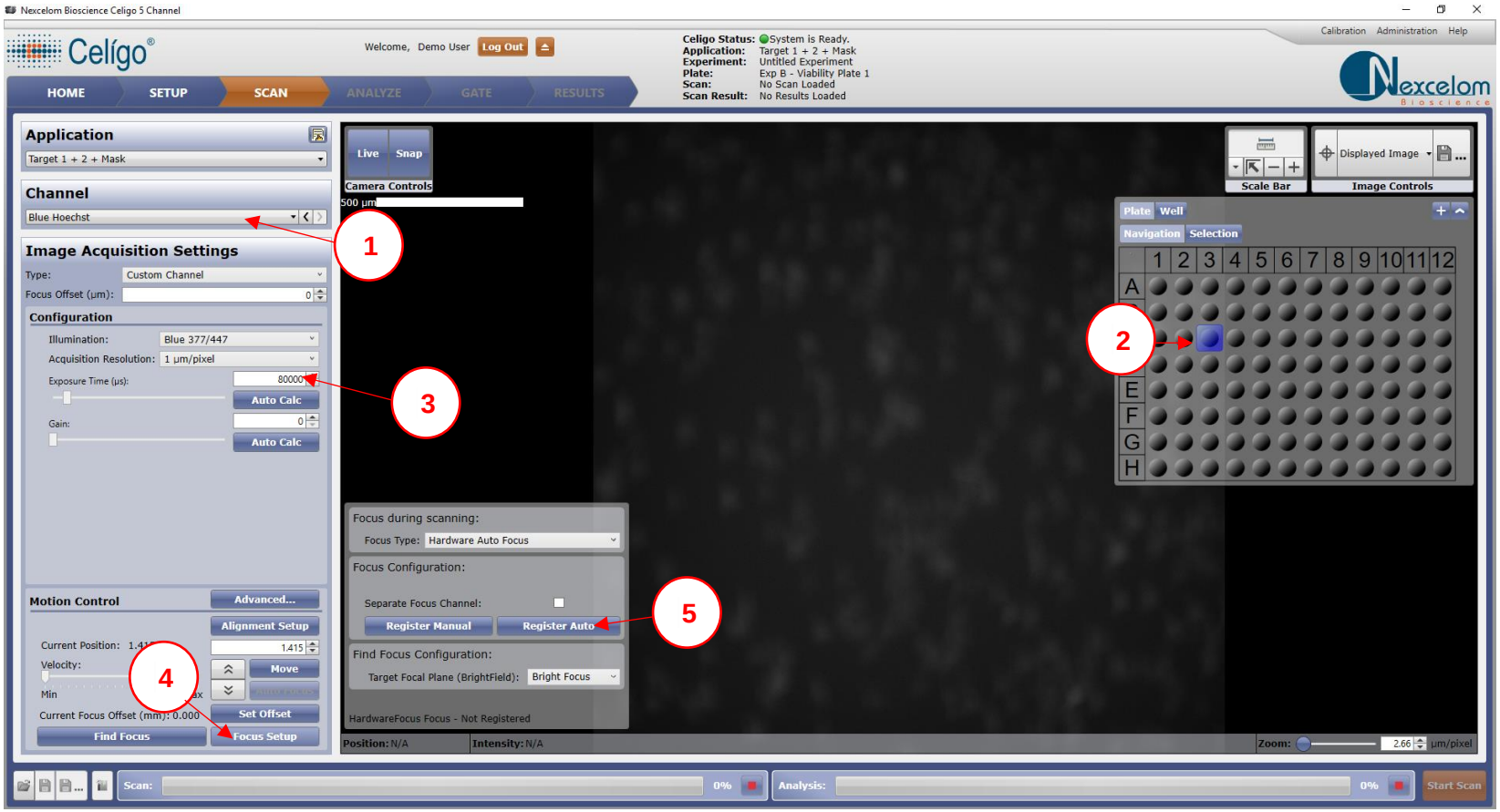




Renaming Channels and classes can be done from any tab, SCAN, ANALYZE, GATE, except the RESULTS tab.

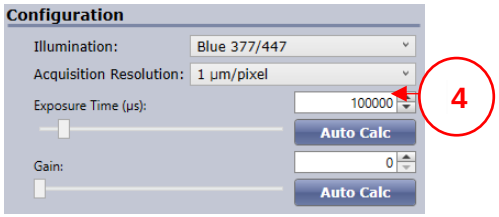
Setup Blue Hoechst Channel for Focus and Illumination

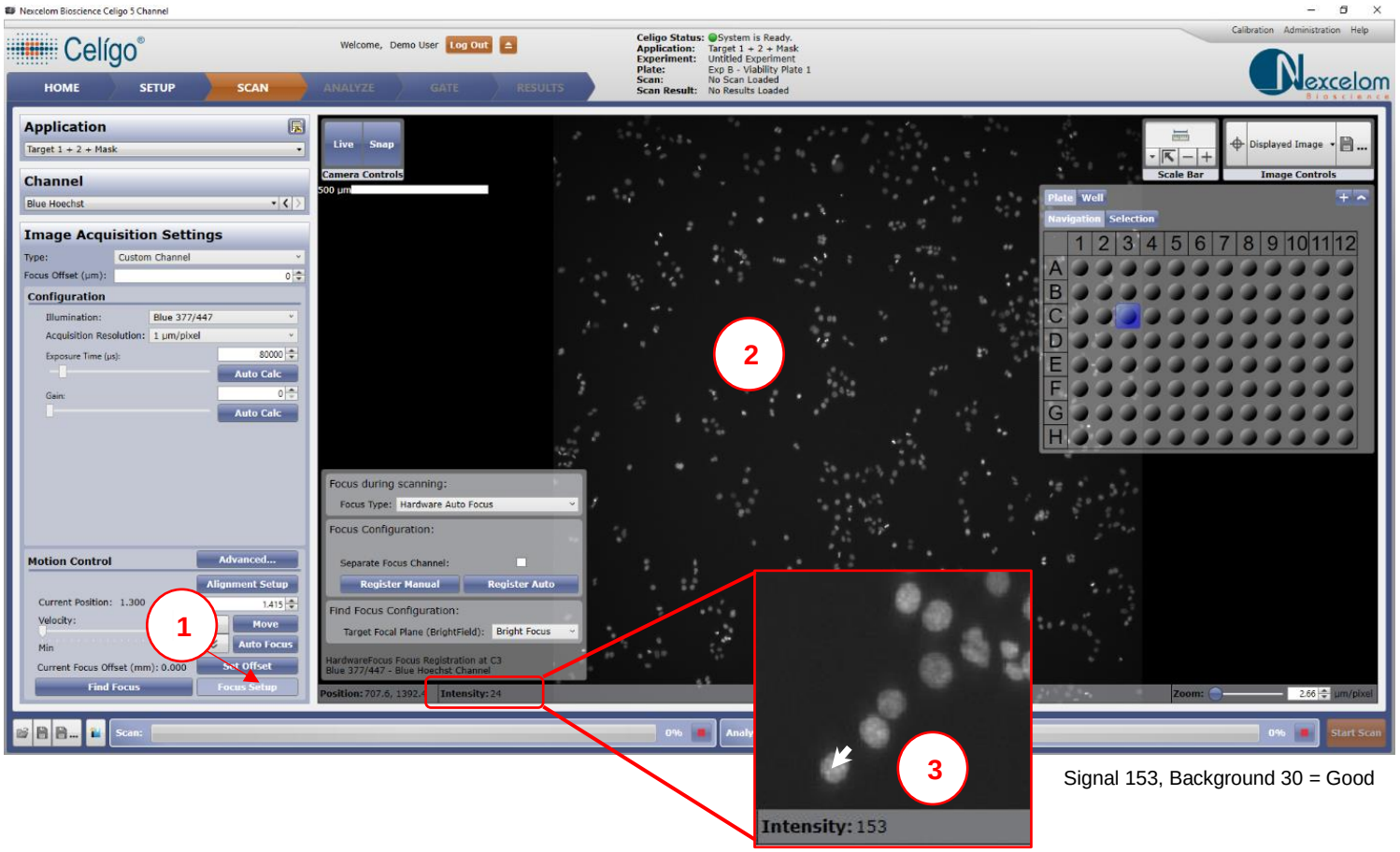
1. Change channels to **Blue Hoechst.**
2. Click on a **well** that has Hoechst stained cells.
3. Change Exposure to **80,000** µseconds.
4. Click on **Focus Setup.**
5. Click **Register Auto.**



Setup Exposure for Blue Hoechst Channel.

1. Click **Focus Setup** to close dialog box.
2. Zoom in on cells and hover mouse over nuclei stained object.
3. Observe pixel intensity of nuclei and background noise.
4. Adjust exposure to ensure Object Signal is above 150 and background below 75.

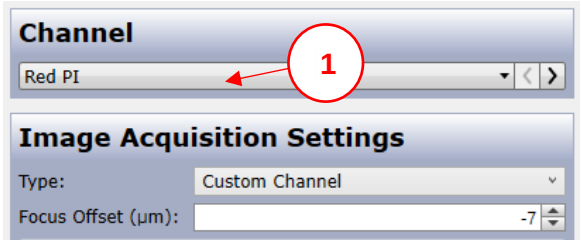


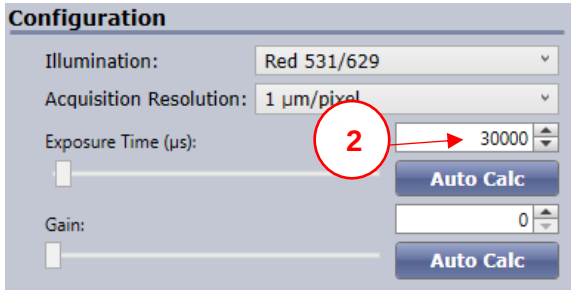


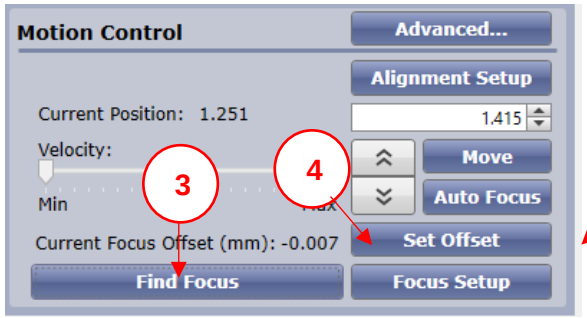
Signal 153, Background 30 = Good

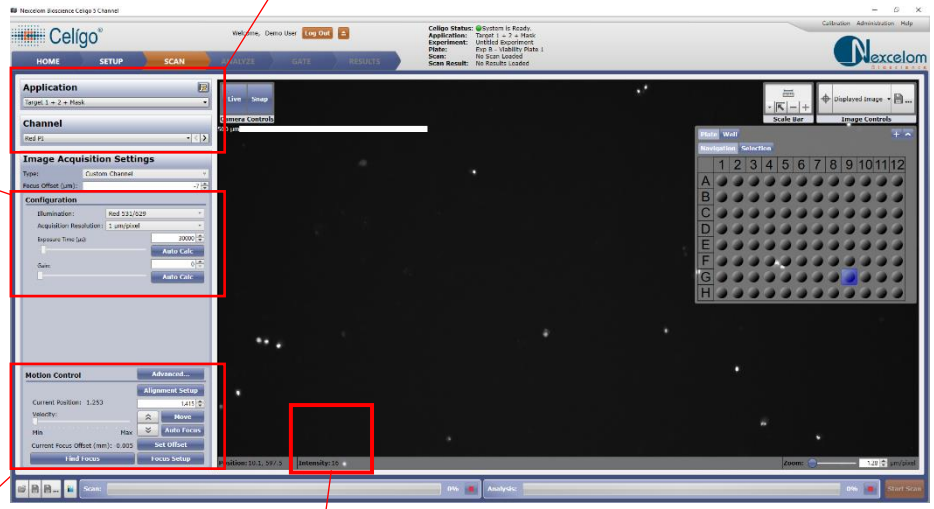
Setup Proper Focus and Illumination for Red PI channel

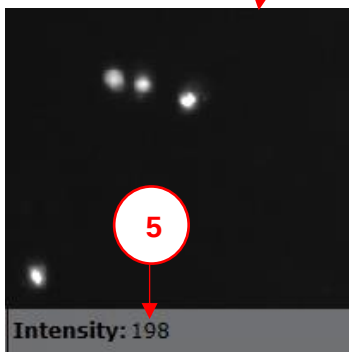
1. Switch Channels to **Red PI**.
2. Adjust Exposure to **30000** µseconds.
3. Click **Find Focus**.
4. Click **Set Offset**.
5. Observe Dead cell pixel intensity is above 150, adjust Exposure if necessary.





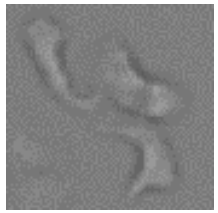


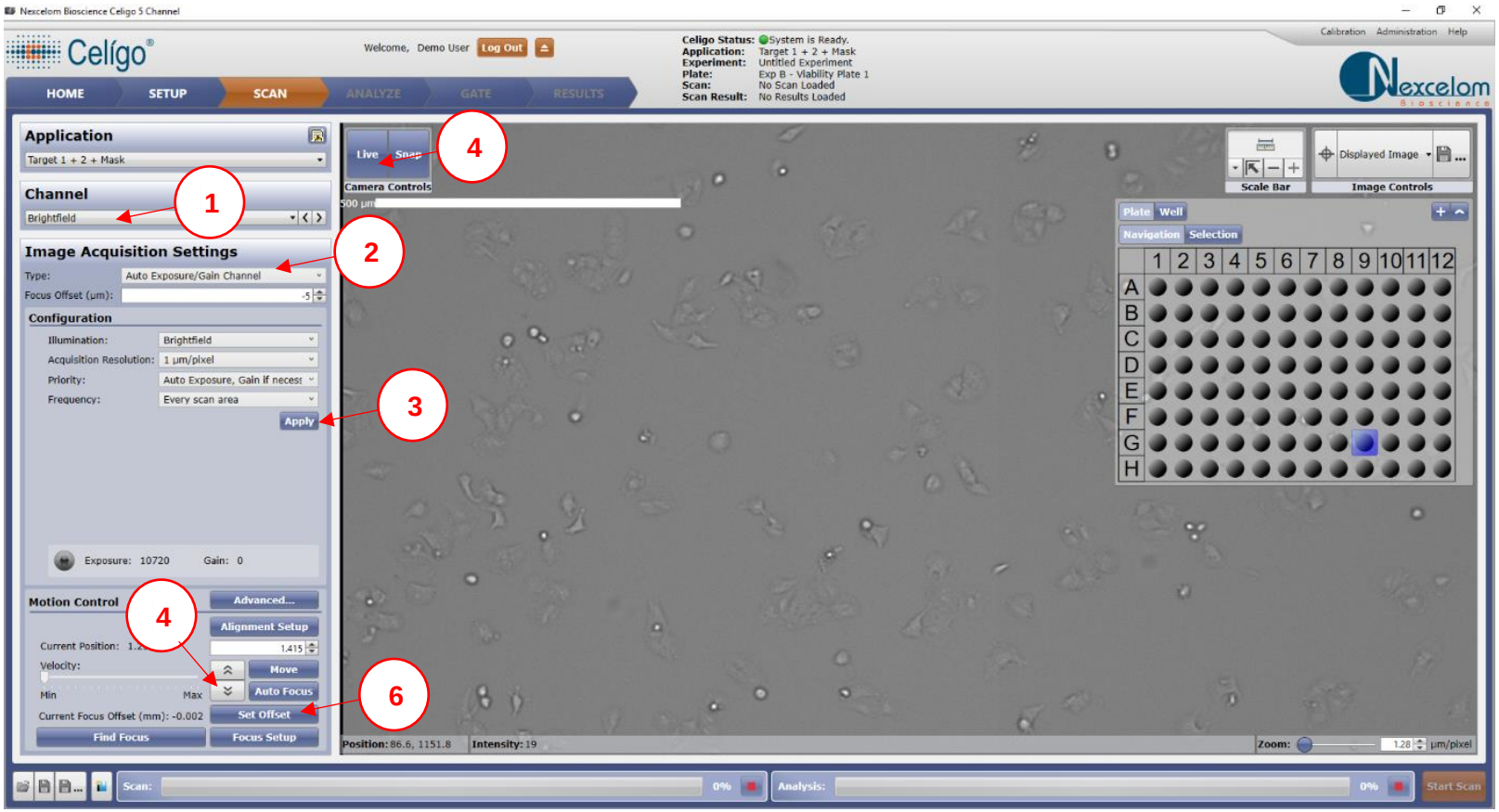




Setup Brightfield Channel for Illumination

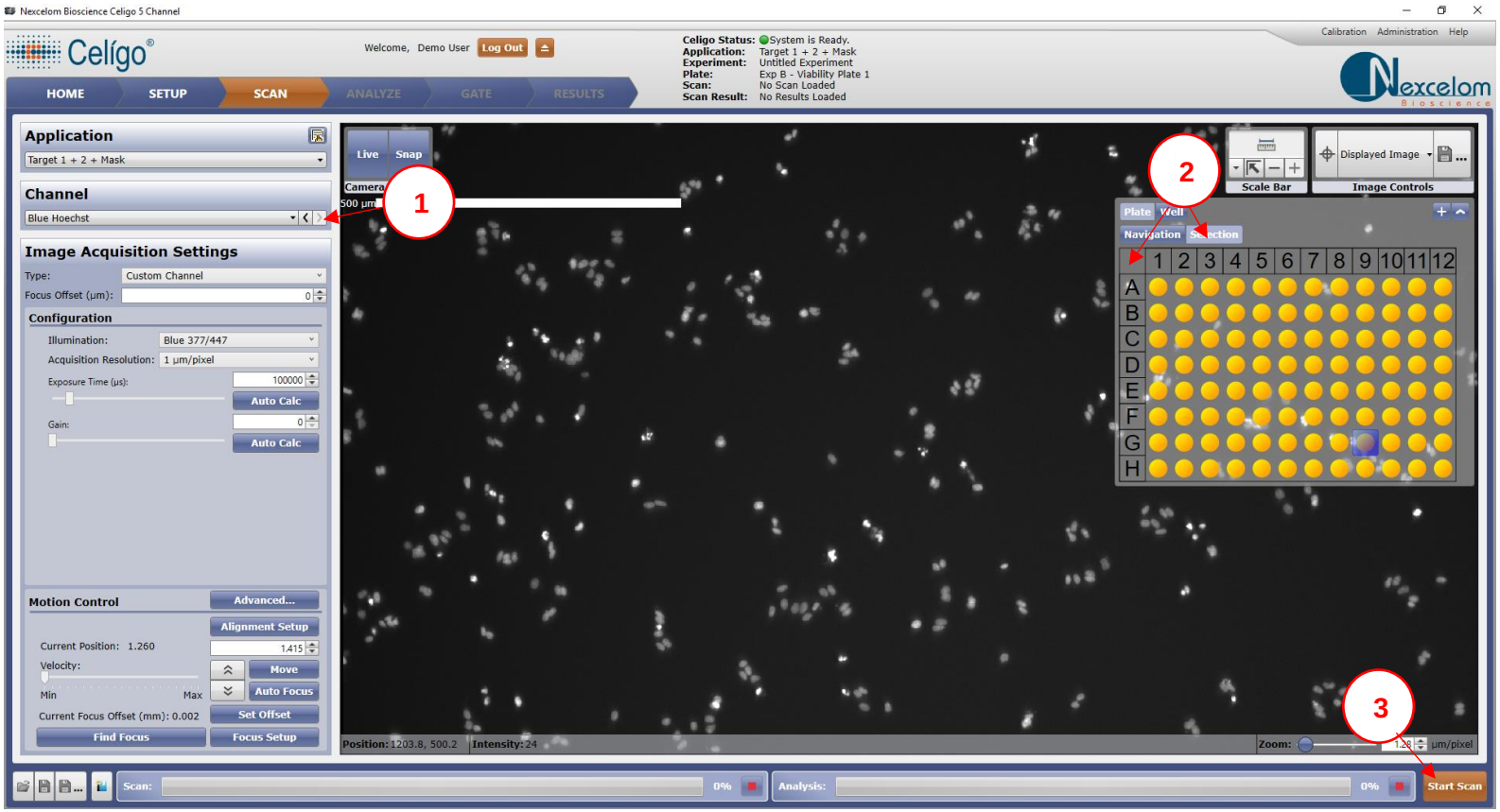
1. Switch Channel to **Brightfield**
2. Switch Type to **Auto Exposure/Gain Channel** if not already done.
3. Click **Apply**.
4. Turn on **Live** Camera
5. Adjust Z position (**down arrow**) for the cells to be slightly defocused. Bright center, black edge around cell.
6. Click **Set Offset**.





Well Selection, Channel Review and Start Scan

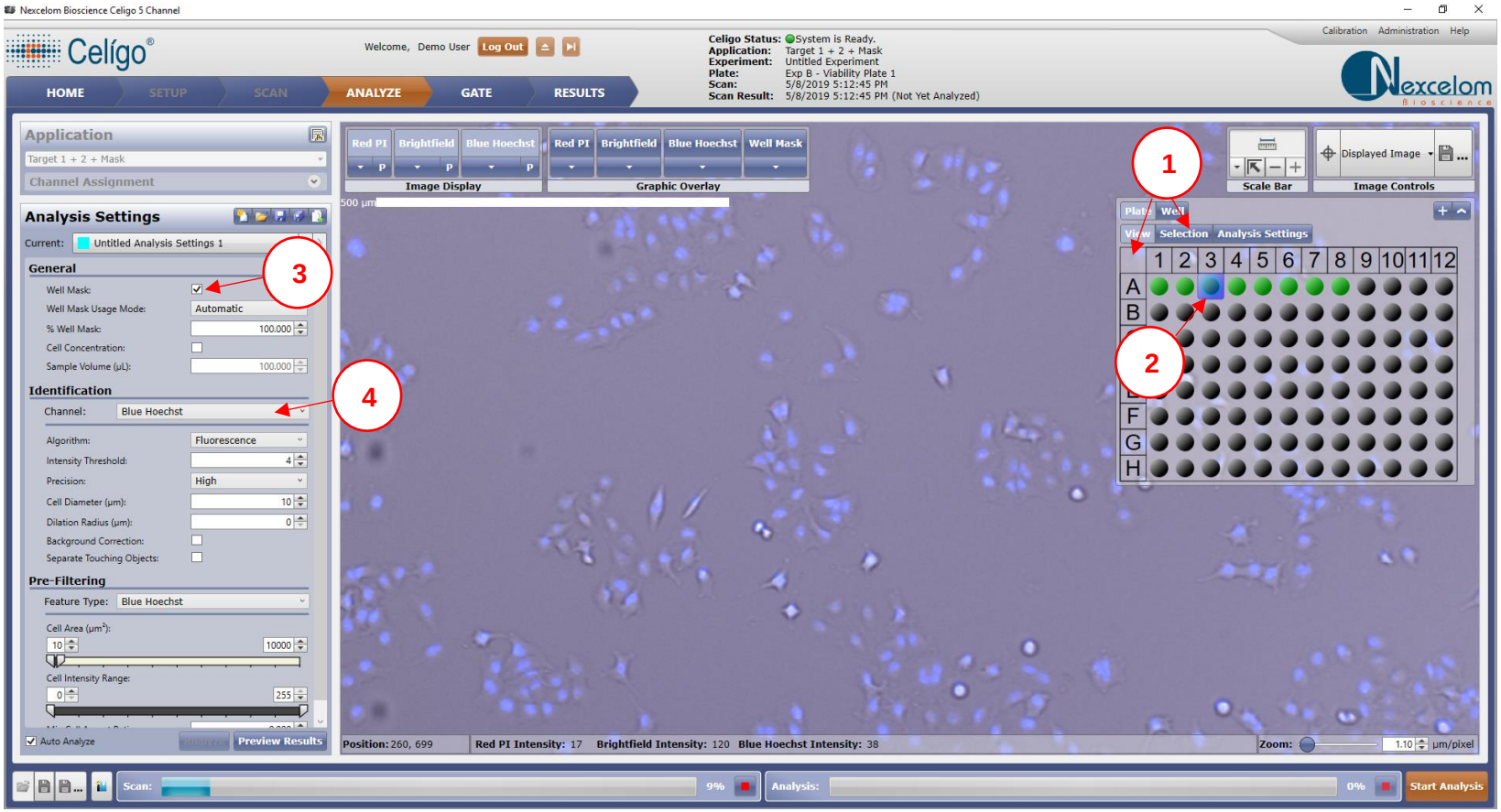
1. Switch Channels to review proper illumination and focus are occurring in all channels.
2. Click **Selection** and highlight wells for image acquisition.
3. Click **Start Scan**.



ANALYZE Tab

Setup Analysis Settings for Well Mask

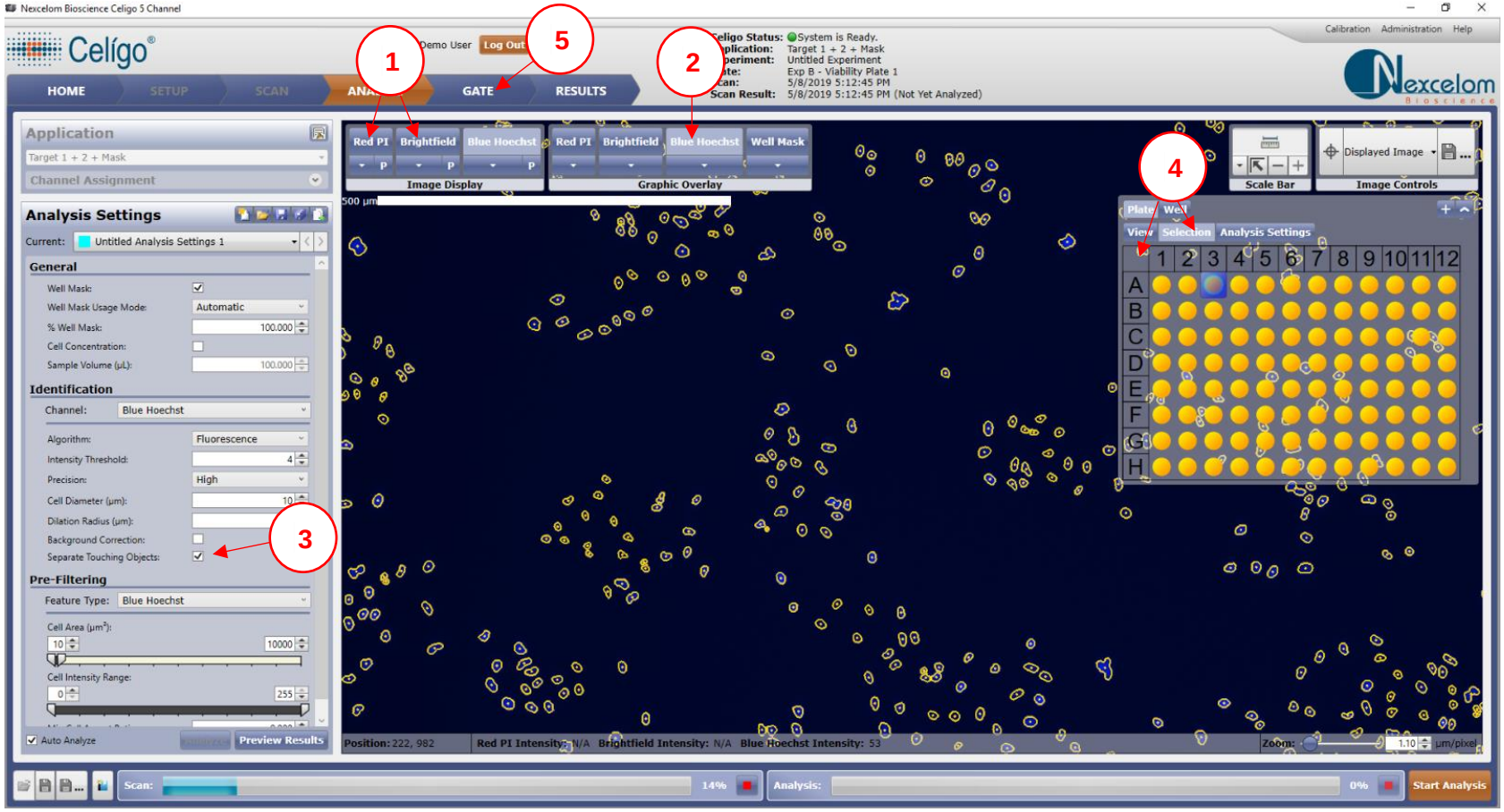
1. In Plate Navigation, click **Selection** and unselect the wells (no yellow) to see the green wells (images saved to database)
2. Select **View** and click a green well to view images in display area.
3. In General Section, check **Well Mask**.
4. Change Channel selection to **Blue Hoechst**.



When wells are green, the images have been automatically saved in database. Click on green wells to view and set up analysis parameters while remainder of wells are acquired.

Adjust Blue Hoechst Mask Analysis Parameters

1. Turn OFF image display of **Red PI** and **Brightfield** channels.
2. Turn ON graphic overlay for **Blue Hoechst**.
3. Check ON **Separate Touching Objects**. Observe nuclei are properly outlined.
4. Click **Selection** and highlight yellow the wells to be analyzed.
5. Click on **GATE** tab.

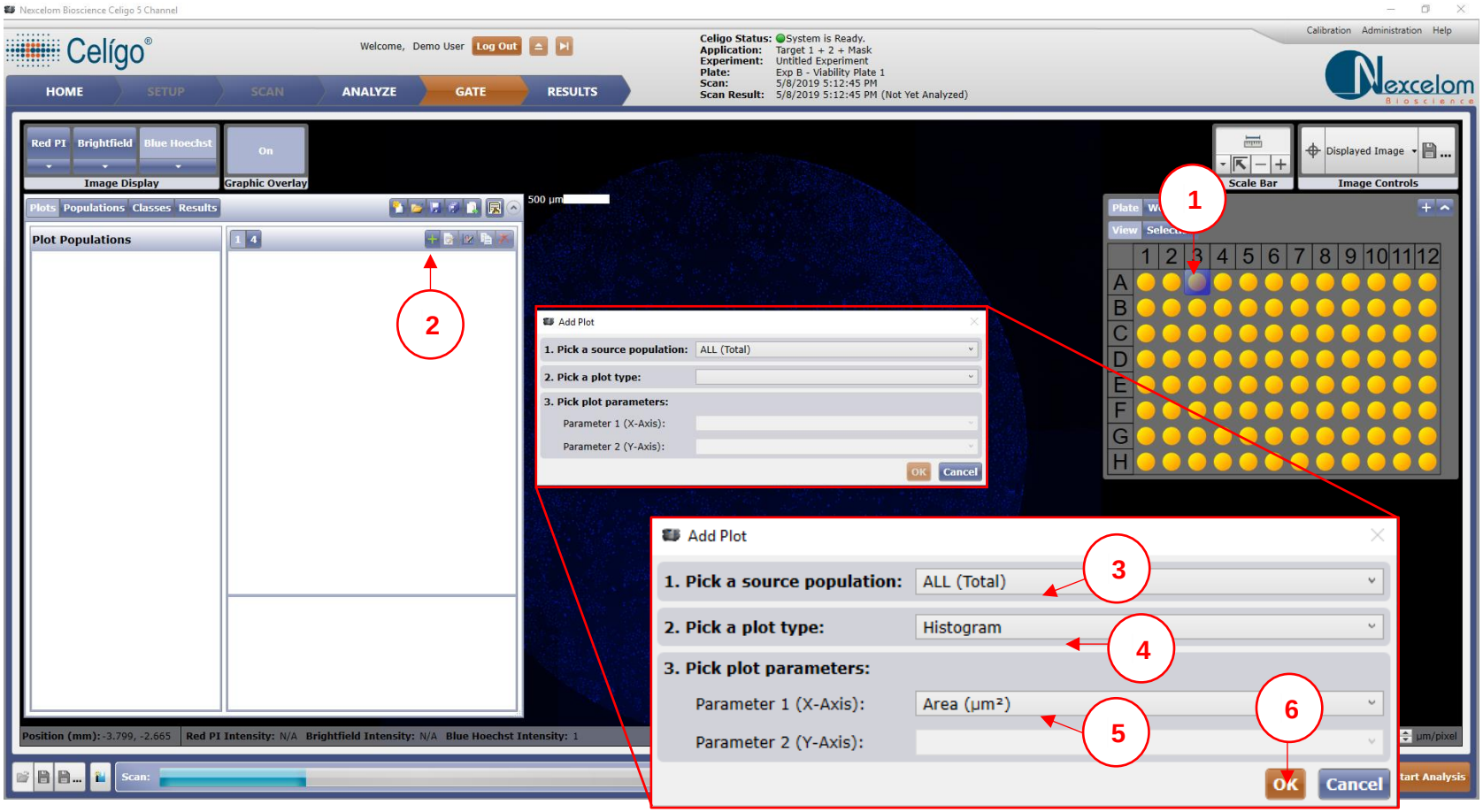


When using a Mask application, in this case Target 1 + 2 + Mask, the analysis settings only need to be setup for the Mask channel. The Mask outline in the Blue Hoechst channel is the area (mask) that will be observed for signal in the other channels, Target 1 + 2.

GATE Tab

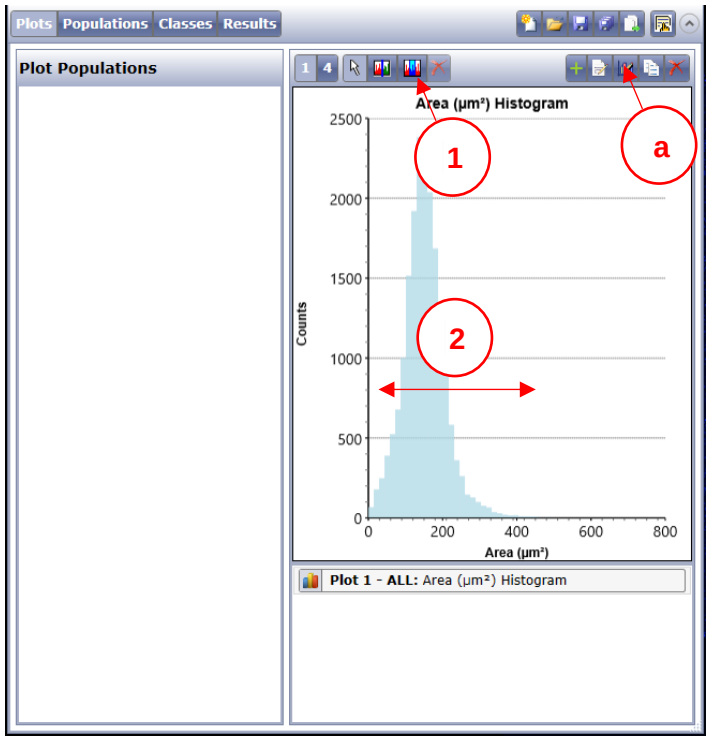
Create Area Histogram Plot

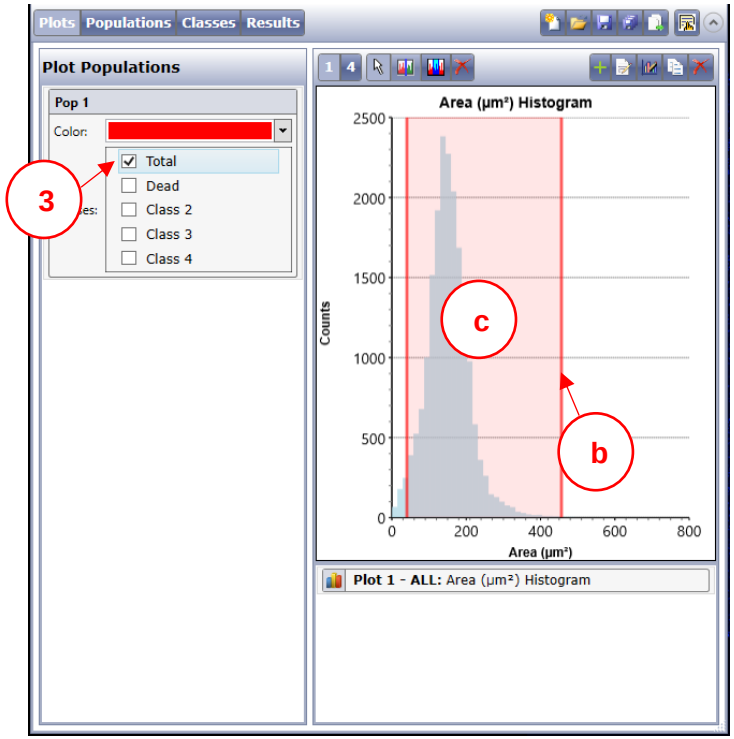
1. Select a **Control well** to view images in image display area.
2. Click on **green plus (+)** to create a new plot.
3. Select **ALL (Total)** for a source population.
4. Select **Histogram** for a plot type.
5. Select **Area (µm²)** for parameter 1 (X-Axis).
6. Click **OK**.



Add a Minimum and Maximum Range Gate

1. Select **Min/Max Range** gate.
2. Click and hold left mouse on left side of data peak, then drag to right and release on right side of data peak.
3. Check **Total** box.

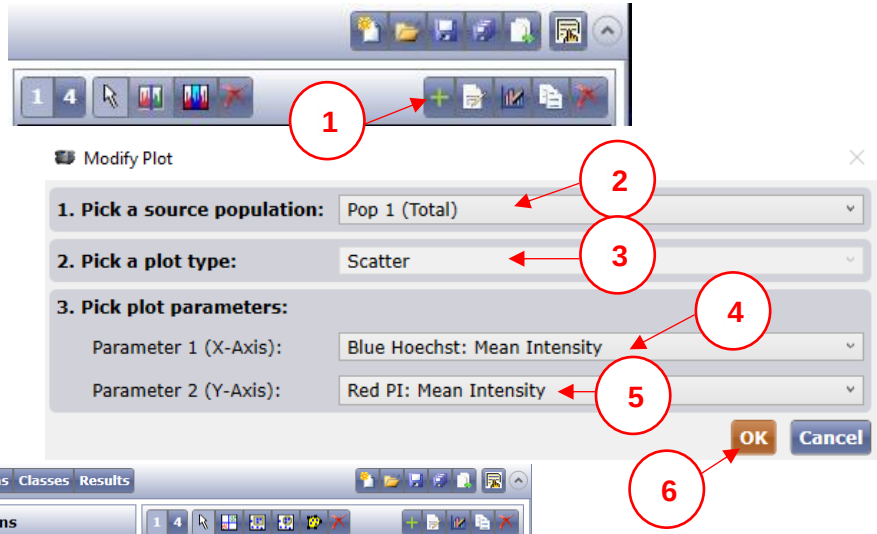


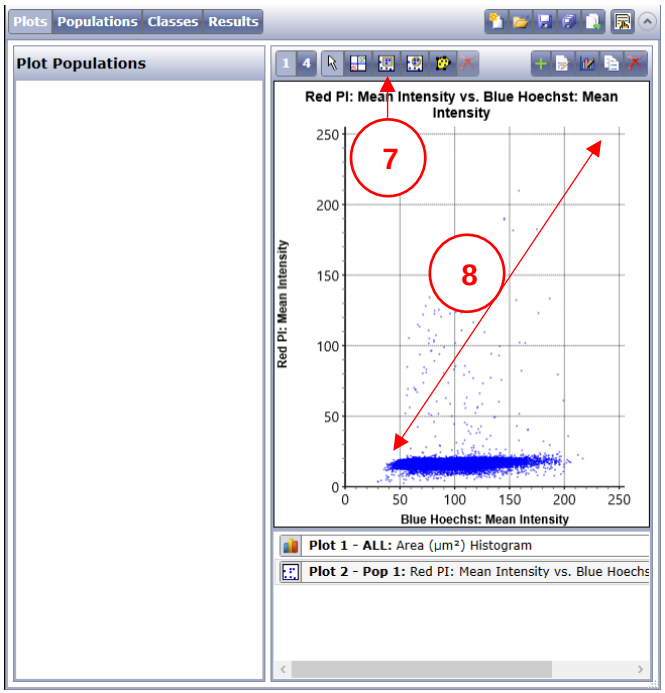


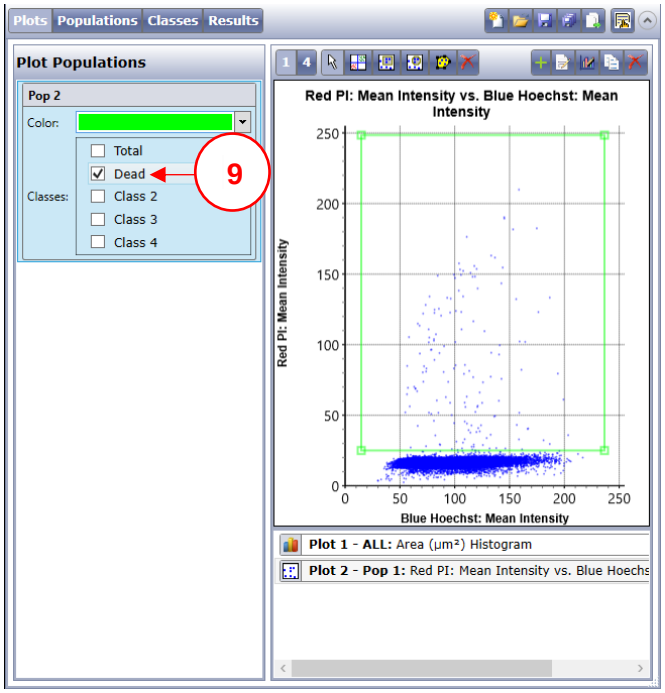
The plot axis's can be adjusted manually with edit plot icon (a) or scroll with mouse. The gate minimum and maximum (b) lines can be adjusted by clicking on red line and moving left or right. Click and hold on center of min/max gate (c), then slide whole gate left and right.

Create a Scatterplot

1. Click on **green plus** (+) to create a new plot.
2. Select **Pop 1 (Total)** for a source population.
3. Select **Scatter** for a plot type.
4. Select **Blue Hoechst: Mean Intensity** for parameter 1 (X-Axis).
5. Select **Red PI: Mean Intensity** for parameter 2 (Y-axis).
6. Click **OK.**
7. Select the **rectangle gate** tool.
8. Draw a diagonal across area.
9. Check the **Dead** box.



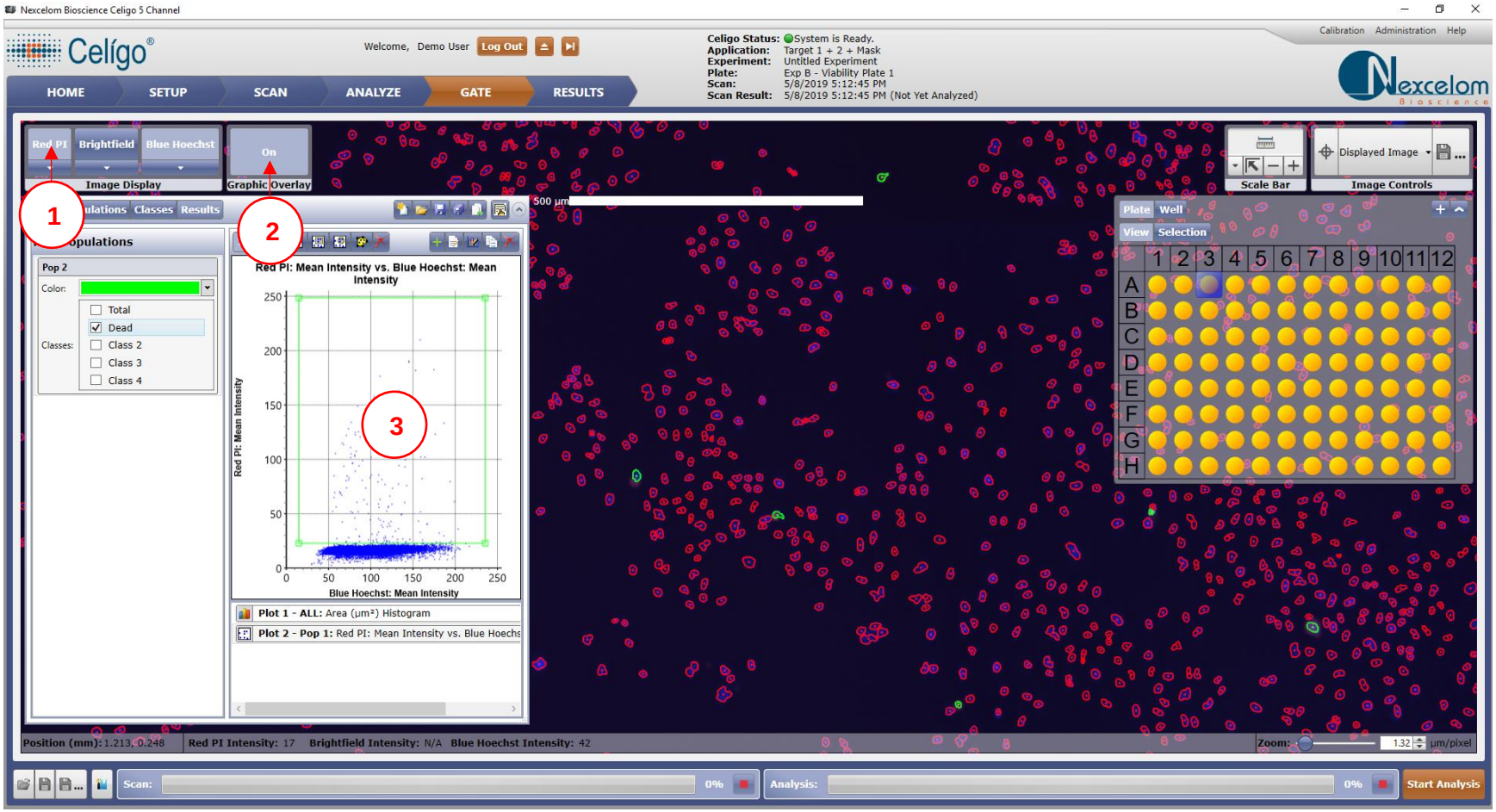




The plot XY axis's can be adjusted manually or scroll in/out with mouse. The gate size can be adjusted by left clicking on gate squares and moving. Left-click and hold on center of gate to move whole gate around. Click and hold scroll button on the plot to move entire plot and with gate around.

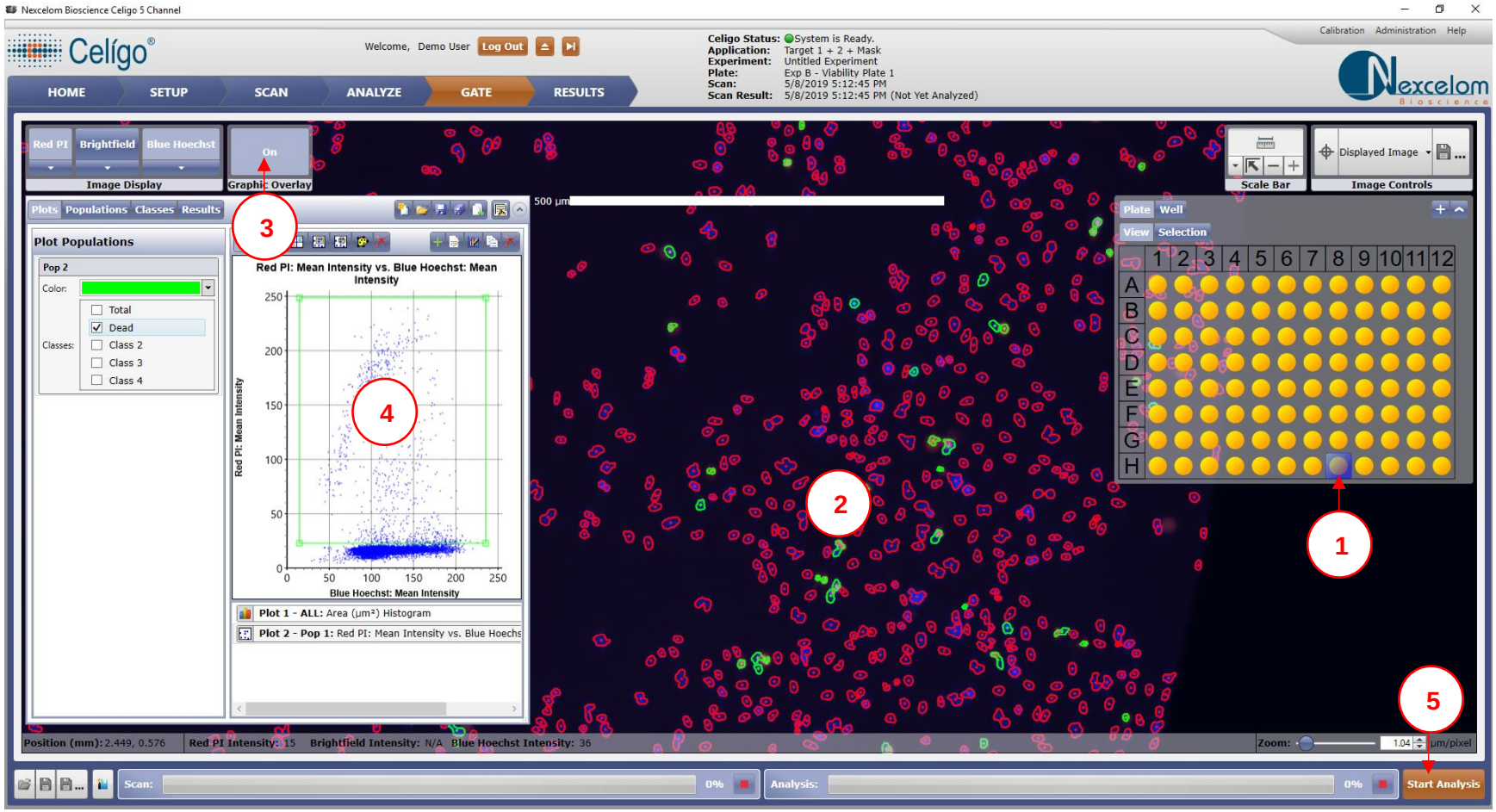
Observe Control Well for Proper Gate Placement

1. Turn ON **Red PI** Image display.
2. Turn ON and OFF graphic overlay to observe green graphic overlay is detecting Red PI stained cells.
3. Make adjustments to gate if necessary.



Observe Treated Well for Proper Gate Placement

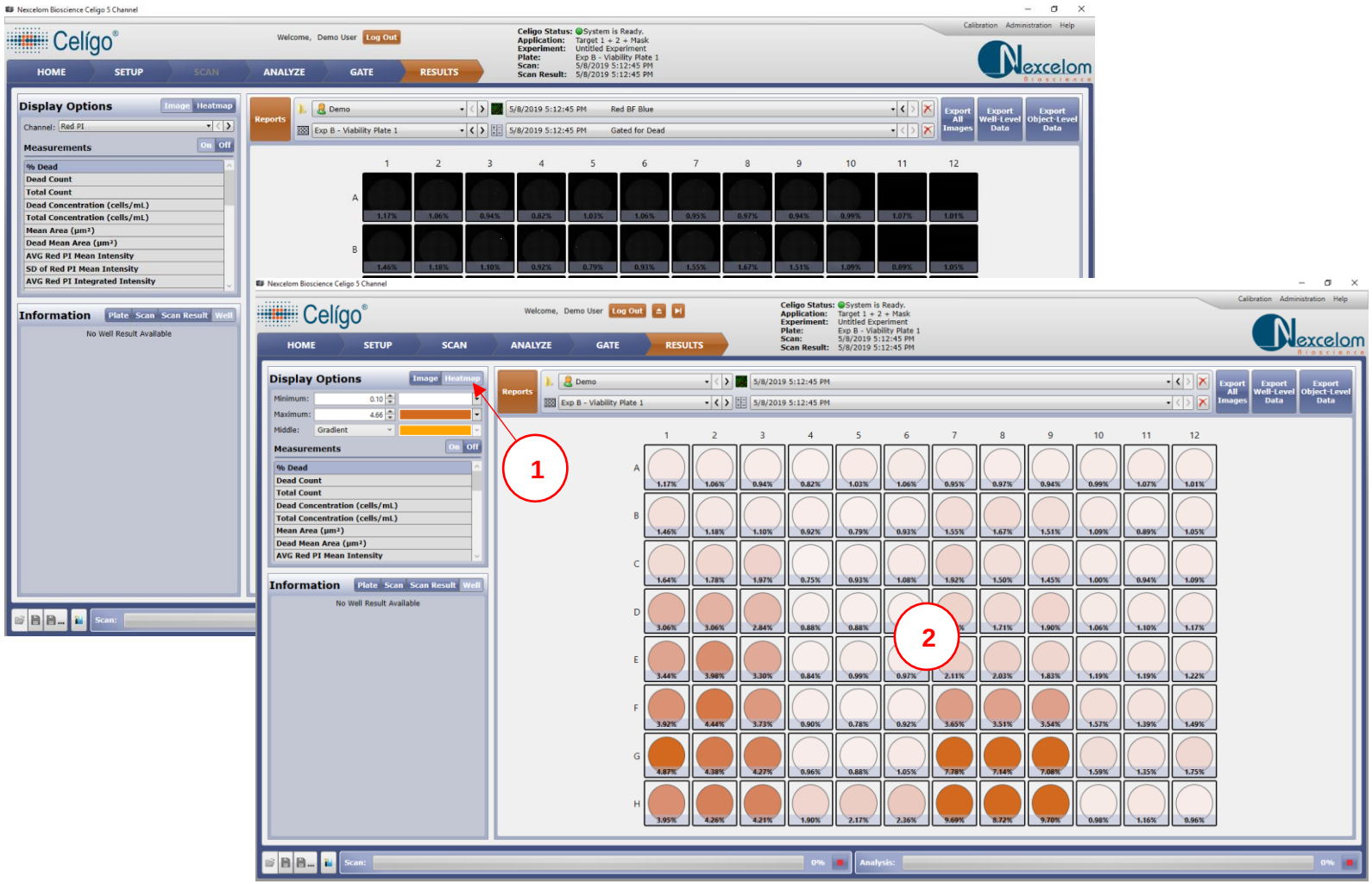
1. Click on a **treated well**.
2. Zoom image in and out, move image around display are to observe dead cells.
3. Turn ON and OFF graphic overlay to observe green graphic overlay is detecting Red PI stained cells.
4. Make adjustments to gate if necessary.
5. Click **Start Analysis**.



RESULTS Tab

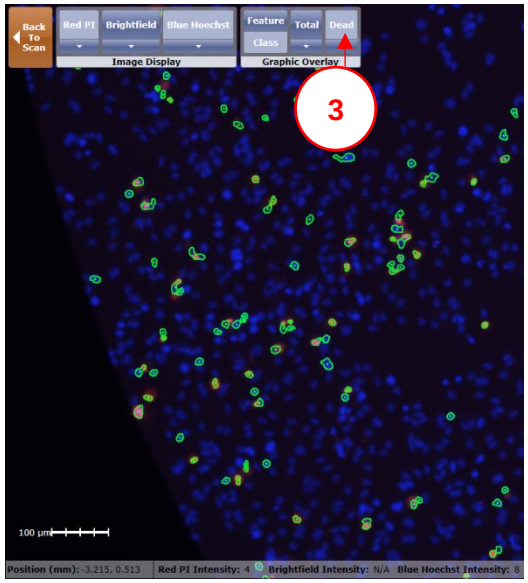
Observe Plate Level Data Measurements with Heatmap

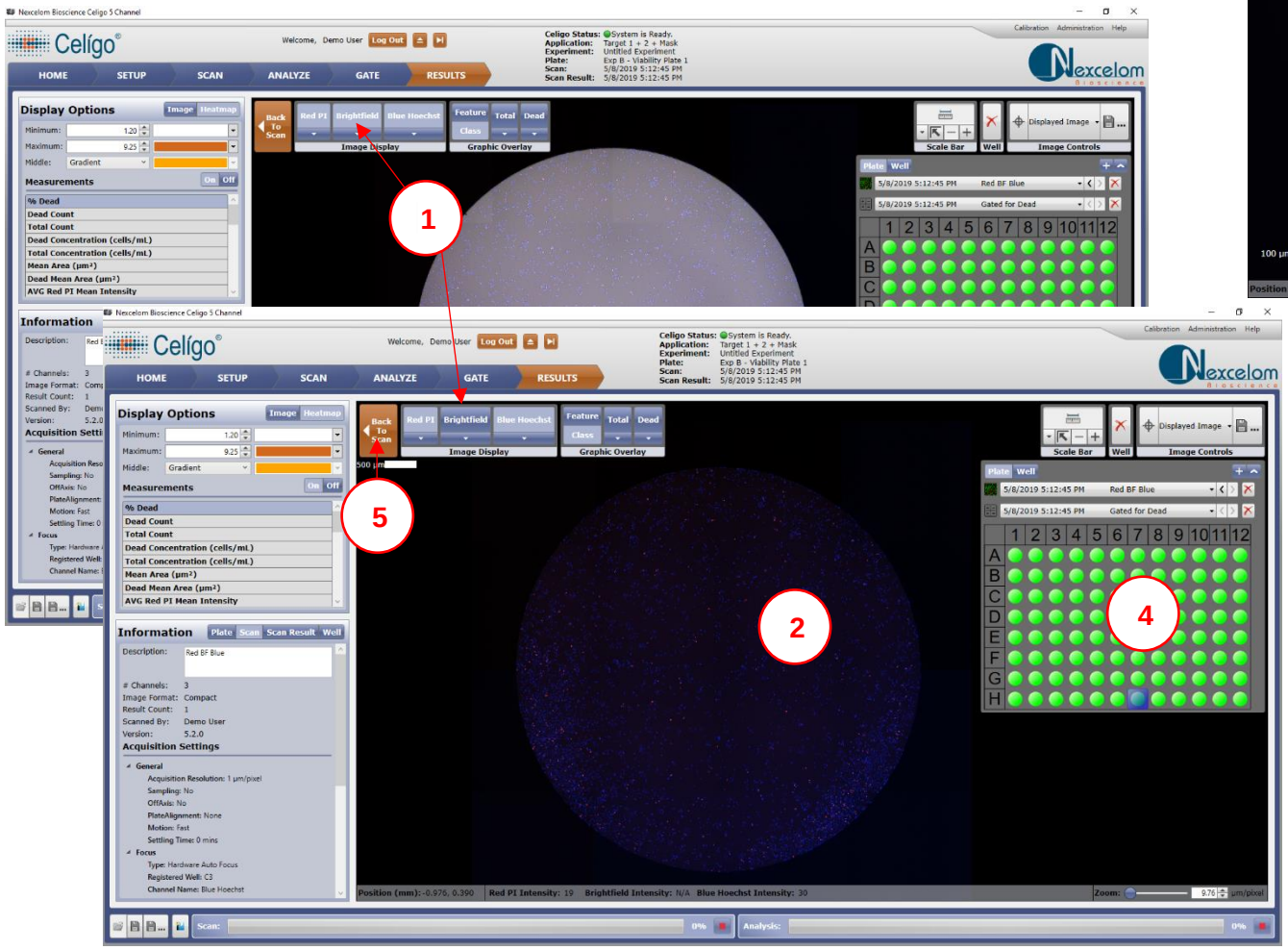
1. Click **Heatmap**.
2. Double click a well to view high resolution images. Single click a well to just view data in lower left display area, Information panel.



Observe Well Level Images

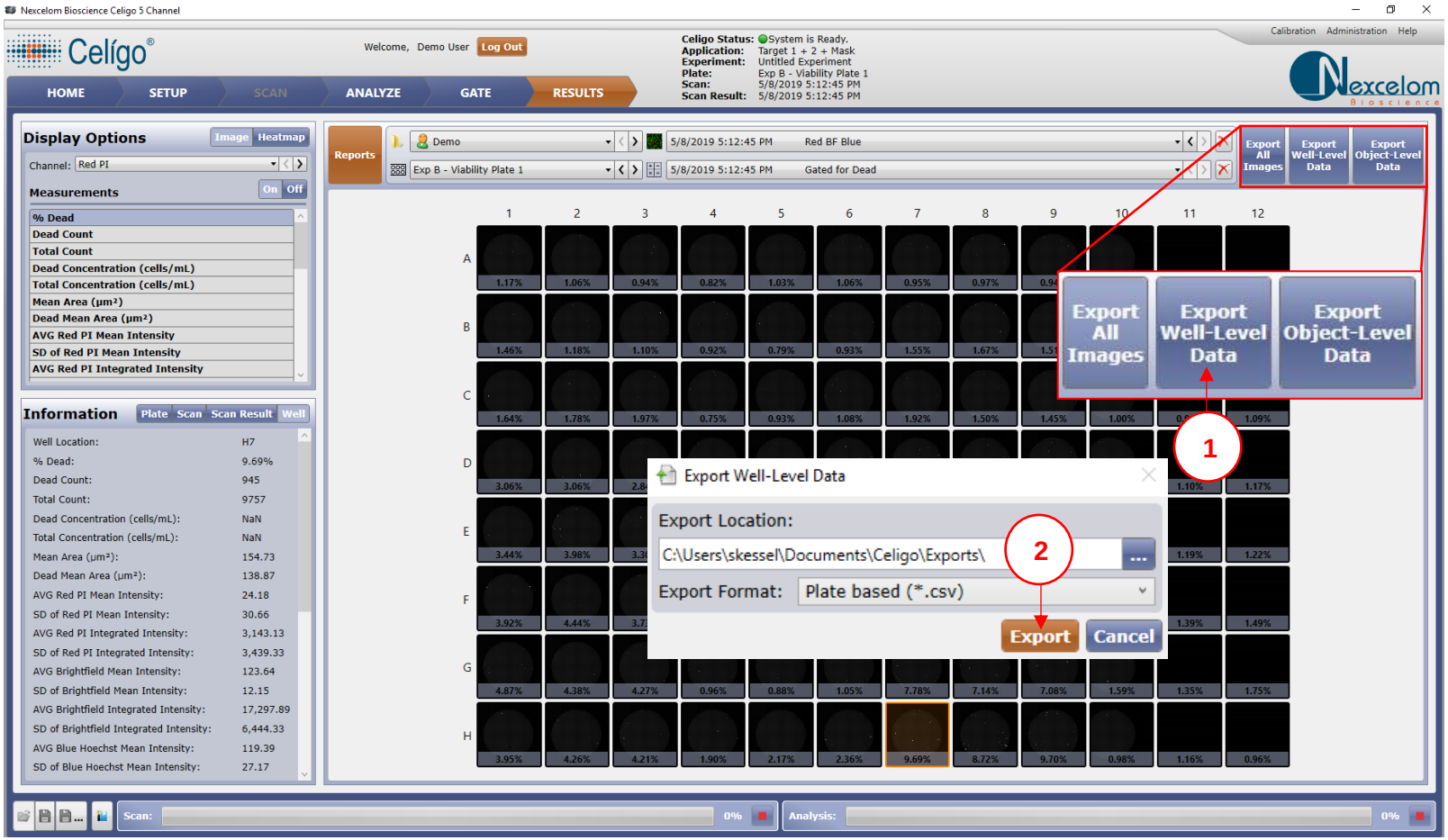
1. Click **Brightfield** to turn OFF Brightfield image display.
2. Zoom in with mouse scroll.
3. Click on **Dead** graphic overlay.
4. Click on another **well** to view.
5. Click **Back To Scan** to return to Plate Level View.





Export Plate Level Data Measurements

1. Click on **Export Well-Level Data**.
2. Use default folder location or select another. Click **Export**.



Well-Level Data exports a csv file with data displayed in plate layout format or tabular. **Object Level Data** exports data for every object found in the well. The format is optional (CSV, ICE, FCS). **Image Export** is all images in raw pixel formal in black and white format. Celigo software pseudo-colors the images but the images are in gray-scale. Each channel is exported separate as its own file. For example, for a 3-channel scan, there will be three images per well. If you right-click on a well, image and object level data is exportable for that selected well.

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Chapter 6: Plate Profiles Provided on Celigo

See Nexcelom.com for the latest version of the Plate Profiles.

Plate Name	Manufacturer	Well Type	Compatibles
6-Well BD Falcon 353046 Plate	Corning	Clear	353224, 353934, 353846, 351146, 353502
6-Well Corning 3516 Plate	Corning	Clear	3471, 3506, 3335
6-Well CytoOne CC7682-7506 Plate	CytoOne	Clear	---
6-Well Greiner 657160 Plate	Greiner	Clear	657185, 657165
6-Well Nunc 140675 Plate	Thermo	Clear	---
12-Well BD Falcon 353043 Plate	Corning	Clear	353224, 351143, 353503
12-Well Corning 3513 Plate	Corning	Clear	3336, 3512
12-Well CytoOne CC7682-7512 Plate	CytoOne	Clear	---
24-Well BD Falcon 353047 Plate	Corning	Clear	353226, 353935, 353847, 351147, 358115,354723, 356723, 354775, 356775, 353504
24-Well Corning 3524 Plate	Corning	Clear	3337, 3526, 3527, 3573
24-Well CytoOne CC7682-7524 Plate	CytoOne	Clear	---
24-Well Greiner 662160 Plate	Greiner	Clear	662102, 622165
24-Well PE Visiplate 1450606 Plate	Perkin Elmer	Black	---
24-Well Seahorse XF24 Plate	Seahorse Biosciences	Clear	---
48-Well Corning 3548 Plate	Corning	Clear	---
48-Well Greiner 677180 Plate	Greiner	Clear	677102, 677165
96-Well BD Falcon 353219 Plate	Corning	Black, White	353377
96-Well BD Falcon 354640 Plate	Corning	Black, White	354650, 356650, 354651, 356651, 356701, 356693, 354649, 356649, 356640, 356700, 356692, 356717
96-Well BD Falcon 356717 Plate	Corning	Black	354717
96-Well BD Falcon 351177 U-Bottom Plate	Corning	Clear, Round Bottom ULA	---

Plate Name	Manufacturer	Well Type	Compatibles
96-Well BD Falcon 353219 Plate	Corning	Black, White	353377
96-Well BD Falcon 353072 Plate	Corning	Clear	351172, 353075, 354407, 354429, 353916, 353936, 354409, 354410, 354461, 354516, 354596, 354607, 354657, 354670, 354689, 356461, 356516, 356689, 356690, 356698
96-Well BD Falcon 354640 Plate	Corning	Black, White	354650, 354651, 356640, 356650, 356651, 354649, 356649, 356692, 356693, 356700, 356701, 356717
96-Well BD Falcon 356717 Plate	Corning	Black	354717
96-Well BD Falcon 351177 U-Bottom Plate	Corning	Clear, Round Bottom ULA	---
96-Well Corning 3596 Plate	Corning	Clear	3300, 3474, 3595, 3598, 3599, 3585, 3595, 3628, 3841
96-Well Corning 3603 Plate	Corning	Black, White	3340, 3601, 3604, 3610, 3631, 3632, 3635, 3651, 3843, 3842, 3903, 3904
96-Well Corning 3696 Plate	Corning	Half Area, Black	3686, 3688, 3690, 3693, 3694, 3695, 3696, 3697
96-Well Corning 7007 U-Bottom Plate	Corning	Clear, Round Bottom ULA	---
96-Well Greiner 655090 Plate	Greiner	Black, White	655087, 655094, 655097, 655098, 655936, 655944, 655946, 655948, 655956
96-Well Greiner 655087 Plate	Greiner	Black	655088
96-Well Greiner 655161 Plate	Greiner	Clear	655101, 655192
96-Well Greiner 655180 Plate	Greiner	Clear, chimney	655182, 655185, 655930, 655940, 655950
96-Well Greiner 675986 Plate	Greiner	Half Area, Black	67509x
96-Well Greiner 650185 U-Bottom Plate	Greiner	Clear, Round Bottom ULA	---
96-well Nexcelom ULA-96U Plate	Nexcelom Bioscience	Clear, Round Bottom, ULA	---
96-Well Nunc 167008 Plate	Thermo	Clear	---
96-Well PE Viewplate 6005225 Plate	Perkin Elmer	Black	---
96-Well PE Isoplate 6005050 Plate	Perkin Elmer	Black	---
96-Well Seahorse FX96 Plate	Seahorse Bioscience	Black	---

Plate Name	Manufacturer	Well Type	Compatibles
384-well Nexcelom ULA-384U Plate	Nexcelom Bioscience	Clear, Round Bottom, ULA	---
384-Well BD Falcon 353962 Plate	Corning	Clear	---
384-Well Corning 3542 Plate	Corning	Low volume, Black	3540
384-Well Corning 3680 Plate	Corning	Clear	3640, 3844, 3700, 3701, 3702, 3844
384-Well Corning 3712 Plate	Corning	Black, White	3653, 3655, 3846, 3845, 3706, 3707, 3711, 3683, 3845, 3846
384-Well Corning 3827 Plate	Corning	Low attach	---
384-Well Greiner 781182 Plate	Greiner	Clear	781185, 781186, 781061, 781940, 781930, 781950
384-Well Greiner 781091 Plate	Greiner	Black	781098, 781095, 781094, 781944, 781090, 781096, 781097, 781946, 781948, 781936, 781956
1536-Well Corning 3838 Plate	Corning	Black, White	3833, 3836, 3893
1536-Well BD Falcon 356771 Plate	Corning	Black	---
1536-Well Greiner 789866 Plate	Greiner	Black	789896
1-Well Nunc Omnitray	Thermo Fisher	Clear	242811
T25 Greiner 690175 Flask	Greiner	Clear	---
T25 Greiner 690175 Flask - Single View	Greiner	Clear	---
T25 BD Falcon 353014 Flask	Corning	Clear	---
T25 BD Falcon 353014 Flask - Single View	Corning	Clear	---
T75 BD Falcon 353136 Flask	Corning	Clear	---
T75 BD Falcon 353136 Flask - Single View	Corning	Clear	---
10cm Dish BD Falcon 353003 Dish	Corning	Clear	353803
1-Slide Holder (2/3 cover slip)	Nexcelom Bioscience	Clear	---
1-Slide Holder (square cover slip)	Nexcelom Bioscience	Clear	---
4-Slide Holder (2/3 cover slip)	Nexcelom Bioscience	Clear	---
4-Slide Holder (square cover slip)	Nexcelom Bioscience	Clear	---

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Chapter 7: Liquid Volume and Cell Density Recommendations

When plating cells, it's important to identify the number of cells initially plated. Too many cells in a well will make cell identification difficult and cell growth may stop if over confluent. The initial cell number plated will be depend on the number of growth days of the experiment, cell growth rate and cell morphology. The table below is a general guideline for plating cells (minimum to maximum).

Plate Type	Recommended Final Volume (µL)	Cell Density Range
1536 Well	8	1 - 1,536
384 Well - Low Volume	20	1 - 2,000
384 Well - High Volume	50	1 - 4,000
96 Well - Full Area	175	1 - 20,000
96-well - Half Area	100	1 - 10,000
48 Well	250	20 - 50,000
24 Well	400	50 - 100,000
12 Well	1000	50 - 200,000
6 Well	2000	50 - 1,000,000

Special notes:

- The meniscus with a well can create a lens like effect in brightfield images, causing ghosts and shadows of cell near the edge. Follow recommended plating volumes to avoid meniscus effect.
- Starting cell densities for initial plating will ensure cells have enough spacing and not overlap.
- For multiple day experiments, estimate the number of cell doubling by the number of days by the cell number initially plated. The sum should not exceed the recommended maximum cell density.
- If you are plating few cells per well, for example for single cells imaging, it is recommended to **reserve one well in each plate for focus registration**. In this well, plate a sufficient number of cells in that well for proper focus evaluation.

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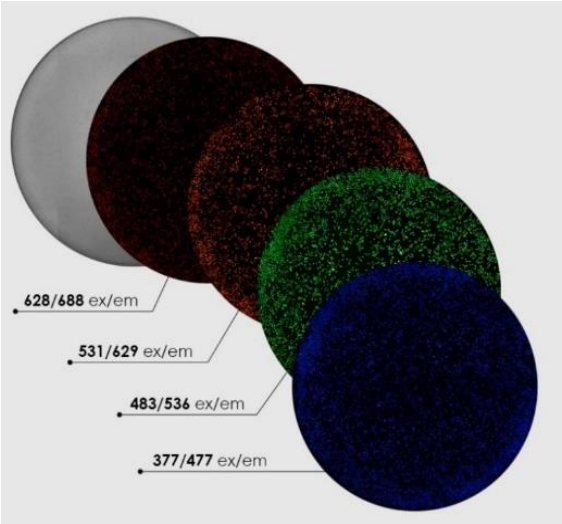
Chapter 8: Fluorescent Filter Reference for Multiplexed Reagent Assays



Fluorescent Filter Reference

Channel	Excitation (nm)	Dichroic (nm)	Emission (nm)	Example Dyes
Brightfield	N/A	N/A	N/A	N/A
Blue	377/50	409	470/22	Hoechst, DAPI
Green	483/32	506	536/40	FITC, Calcein, GFP, Alexa Fluor 488
Red	531/40	593	629/53	R-PE, PI, Texas Red, Alex Fluor 568
Far Red	628/40	660	688/31	DRAQ5, DRAQ7, Alexa Fluor 647

When using a new fluorescent dye on Celigo, check that it is compatible with the filters for the respective channel.

Reagent Kits Available at Nexcelom.com

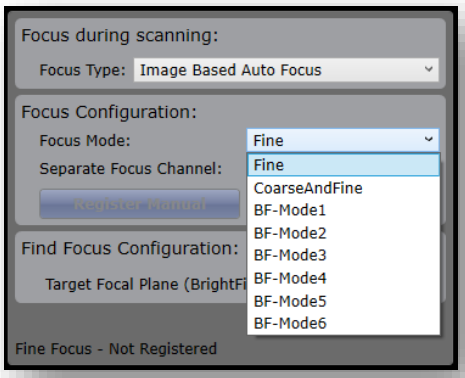
Reagent Name	Catalog #	Channel Color			
		Blue	Green	Red	Far Red
ViaStain™ AOPI Staining Solution	CS2-0106-5 mL		•	•	
ViaStain™ Hoechst / PI	CSK-V0005-1	•		•	
ViaStain™ Calcein AM / PI / Hoechst Cell Viability Kit	CSK-V0006-1	•	•	•	
ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture	CS1-V0002-1		•		
ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture with Hoechst	CSK-V0003-1	•	•		
Apoptosis Annexin V-FITC/PI/Hoechst Kit	CSK-V0007-1	•	•	•	
ViaStain™ Total Cell Nuclear Blue	CS1-V0011-1	•			
ViaStain™ Total Cell Nuclear Green	CS1-V0008-1		•		
ViaStain™ Total Cell Nuclear Red	CS1-V0009-1			•	
ViaStain™ Total Cell Nuclear Far Red	CS1-V0010-1				•
ViaStain™ Dead Cell Nuclear Blue	CS1-V0015-1	•			
ViaStain™ Dead Cell Nuclear Green	CS1-V0012-1		•		
ViaStain™ Dead Cell Nuclear Red	CS1-V0013-1			•	
ViaStain™ Dead Cell Nuclear Far Red	CS1-V0014-1				•
ViaStain™ Cell Cycle PI RNase	CS1-V0004-1			•	
MAR Hypoxia Reagent	CSK-V0016-1		•		
ViaStain™ CFSE	CS1-P0002-1		•		
ViaStain™ Tracer Blue	CS1-P0003-1	•			
ViaStain™ CMFDA	CS1-P0001-1		•		

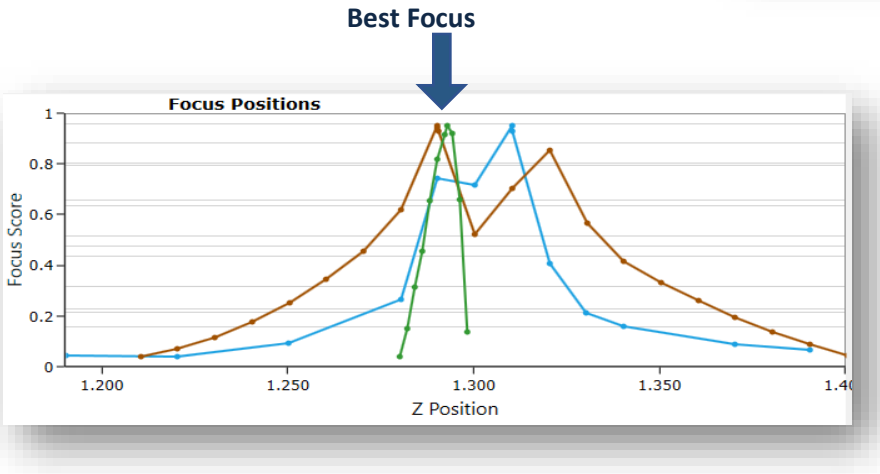
Chapter 9: Image-Based Auto Focus (IBAF) Focus Modes

Beginning in Celigo software version 5.2, additional Image-Based Auto Focus (IBAF) modes were made available to improve focus performance during image scans. Prior versions only have Fine and CoarseAndFine.

The eight selection modes are various combinations of 4 focus algorithms. See table below for details.

Users should evaluate which mode works best for their plate content when they chose IBAF focus type.





The focus algorithms look at the images in different z-focal planes and calculates and evaluates image contrast. The higher the focus score the better contrast. Each focus algorithm varies in z-step range and number of steps.

TABLE: Focus Modes with corresponding number of algorithms, time, Z-axis range and focus positions.

Focus Mode	# Algorithms	Time (seconds)	Max Z Range (µm)	Total # focus positions
Fine	1	3	77 µm	9
CoarseAndFine	2	9	200 µm	22
BF-Mode1	1	5	190 µm	22
BF-Mode2	2	8	190 µm	33
BF-Mode3	1	3	18 µm	11
BF-Mode4	3	14	200 µm	46
BF-Mode5	2	11	200 µm	35
BF-Mode6	1	6	200 µm	13

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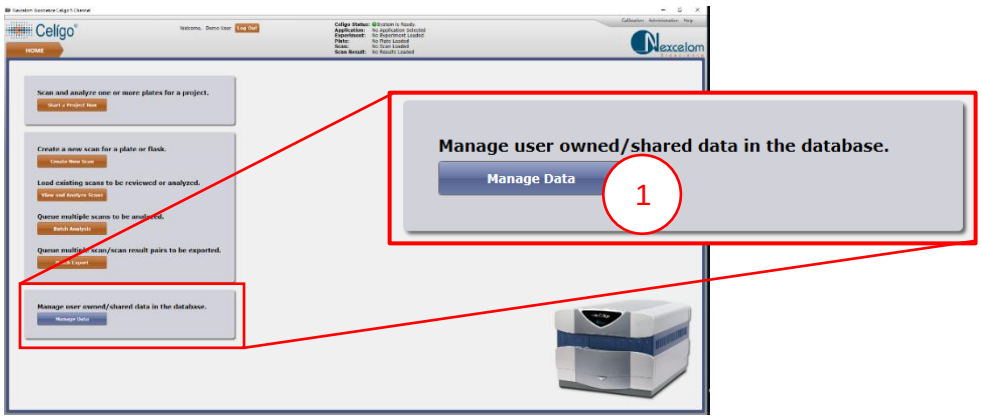
Chapter 10: Data Management

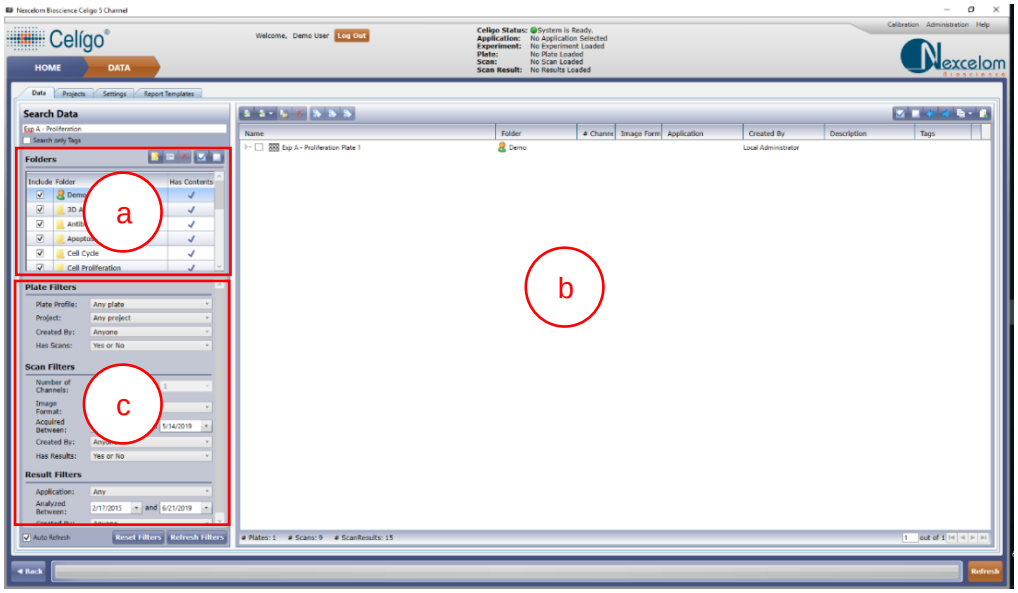
This is a quick start guide for Manage Data tab to show how to export, import and delete data. Settings can be imported, exported and deleted from this tab as well. For additional details, please see User and Administrator Guides.

Navigate to Manage Data Page

1. On the HOME tab, click the **Manage Data** button.

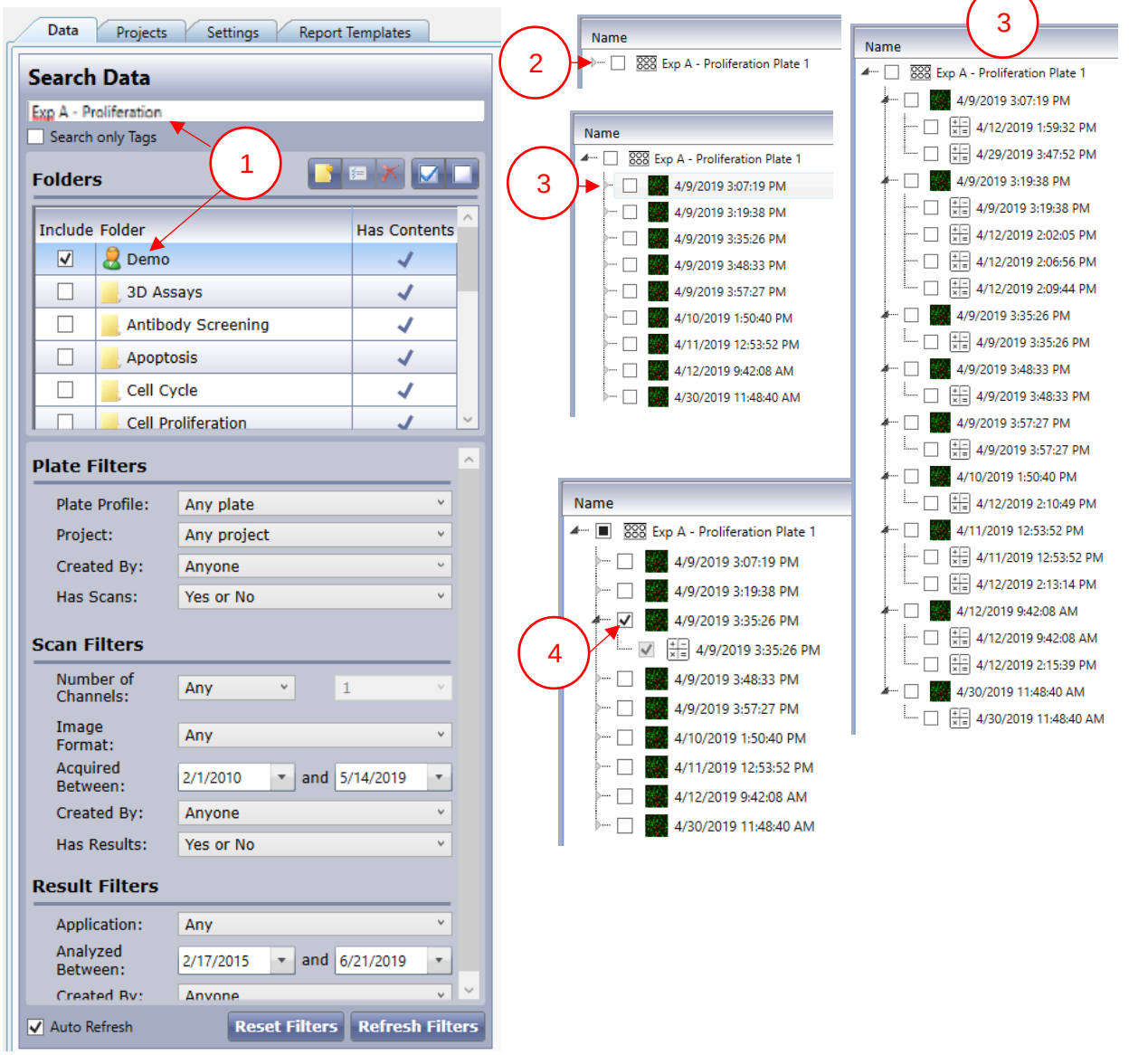


System moves to the DATA tab. User can click on individual user or folders that are shared with them (a). Data files are displayed in right panel area (b). Left panel area has searchable parameters to filter data (c).



Search and Select Data

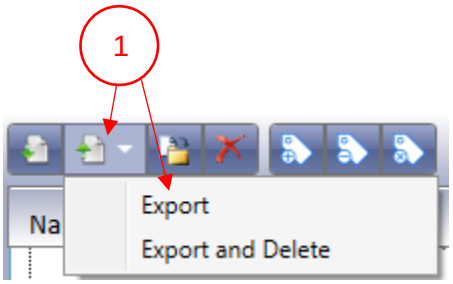
1. Enter in Plate ID or word in Plate ID or Description, or double click on **User** or **Folder** to display data file or files.
2. Click on **arrow next to plate ID**, expands data to show scan level.
3. Click on **arrow at Scan level** to expand Scan Result Level. Or click on the **Expand All** button.
4. Click on **Check Box** next to Scan desired. Scan Result is automatically selected.

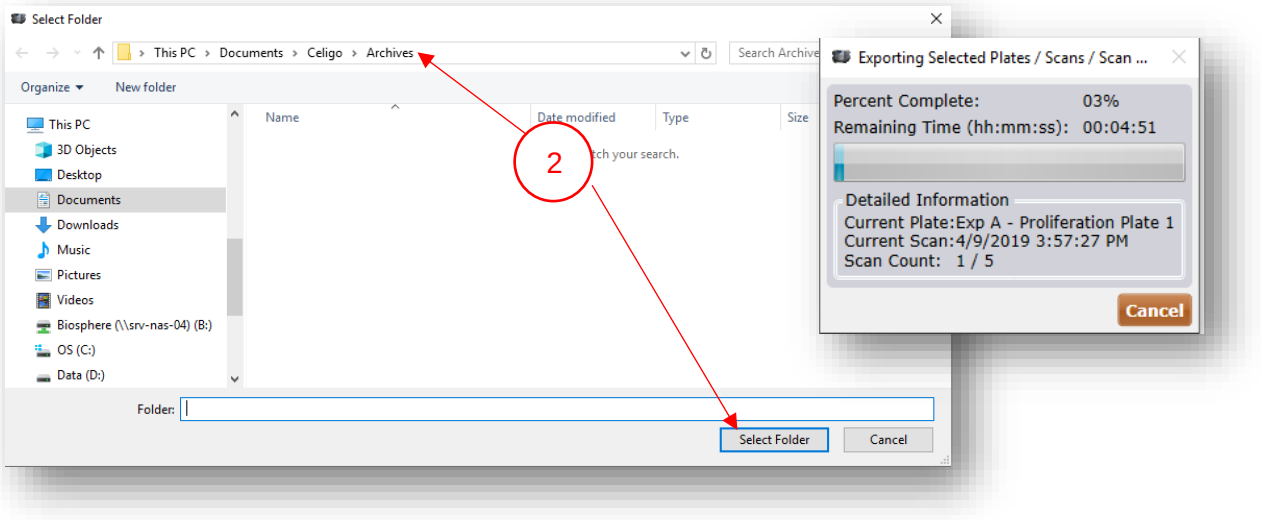


Export Scans and Results

1. Once one or more scans are selected, click on **Export** button at top of data file display area, then **Export**. (Export and Delete will export data first then delete it from the database.)
2. Select **File Location**, click **Select Folder**.

System will present a progress bar for data file export completed.

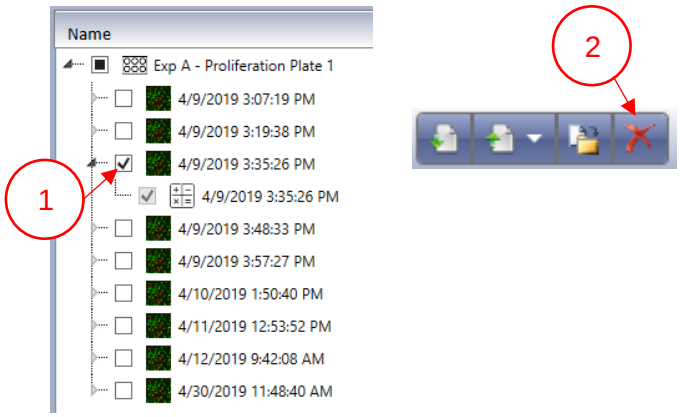




Delete Scans and Results

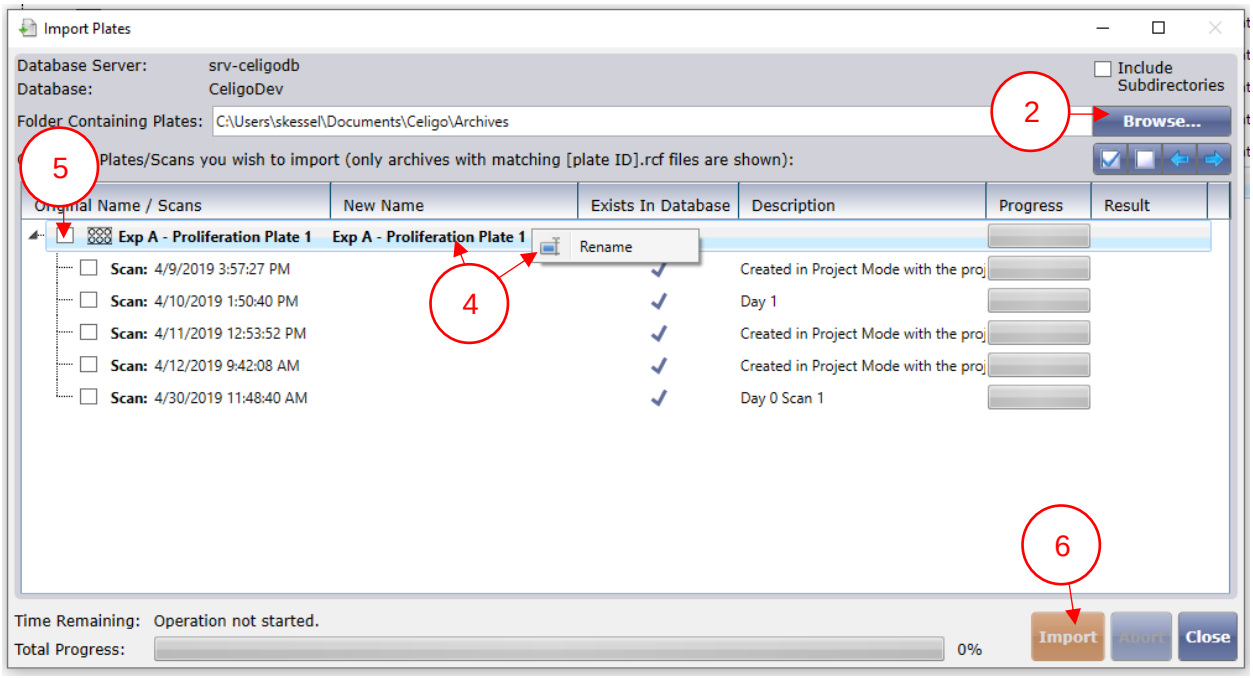
1. Click **Check Box** on Scan(s) and/or Scan Result(s) to be deleted
2. Click on **Delete** icon (red X)

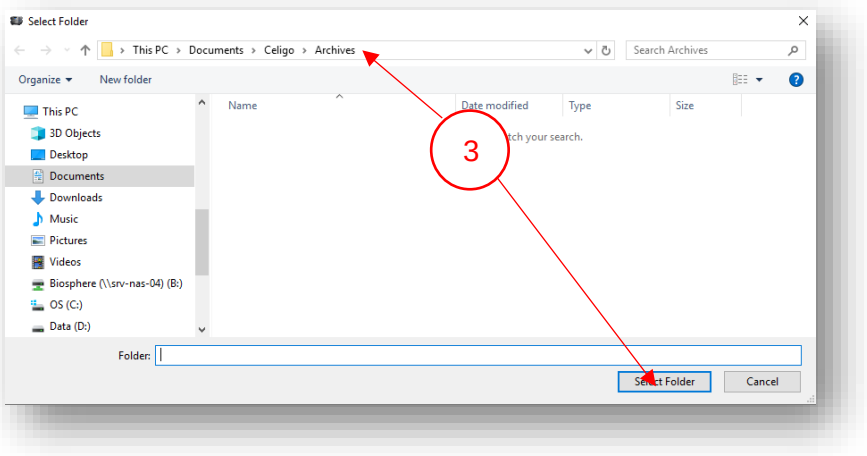
System will delete the data and show a progress bar for percent completed.



Import Scans and Results

1. Click on the **Import** icon above the data file display area.
2. Click on the **Browse…** button.
3. Select the **Folder** where the data is located. Click **Select Folder**.
4. (Optional) Right-click on the **Plate ID name** in the New Name column, click **Rename** and enter the new name. Click away from area to enter new name.
5. If the data doesn't exist in the database, scans and results will automatically be check for import. Ensure boxes are checked (not shown in image here) for the data to be imported.
6. Click **Import**.

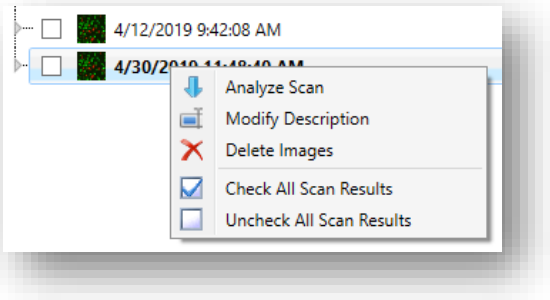




View Data from Manage Data Page

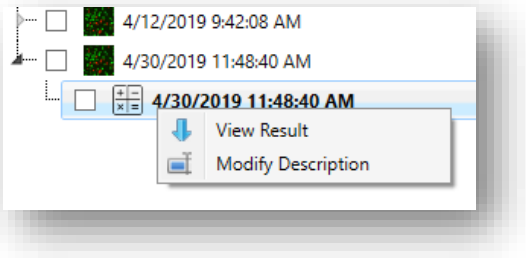
Load a Scan for Analysis

1. Right-click **Scan** time
2. Click on **Analyze Scan**. System will load Scan in ANALYZE tab.



Load a Scan and Result Scan for Review

1. Right-click **Scan Result** time
2. Click on **View Result**. System will load Scan and Scan Result in RESULT tab.



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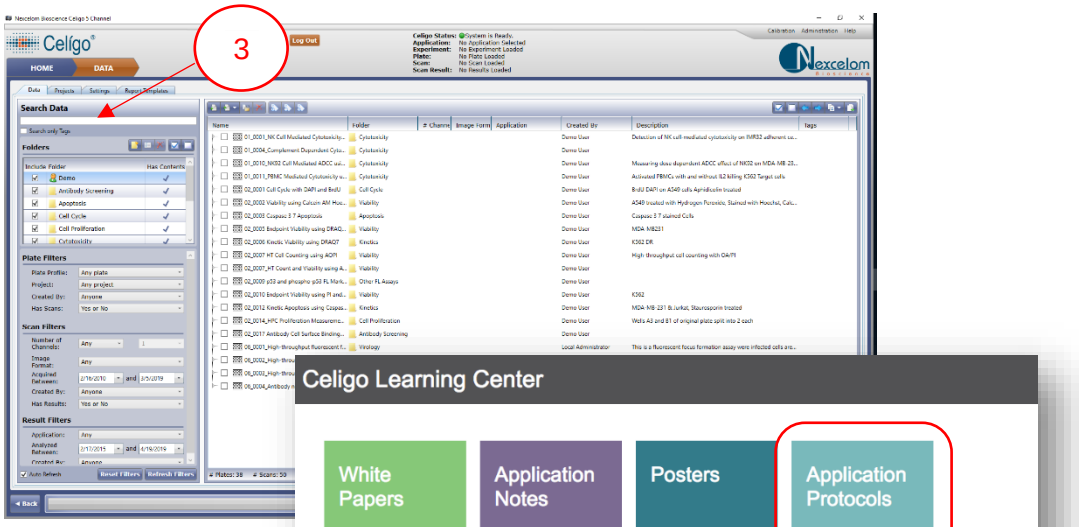
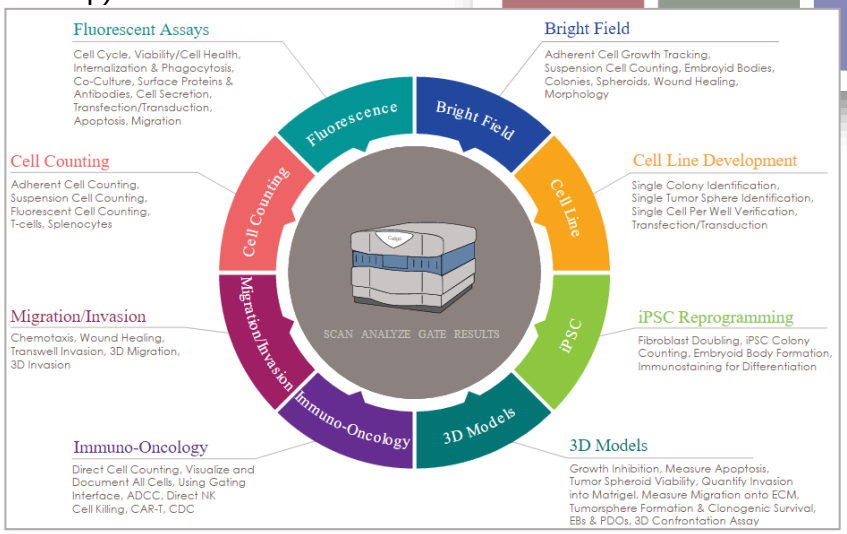
Chapter 11: Demo Data Scans for Review

Scans are preloaded on the Celigo Database for review at any time. If you are interested in learning about trying new application or assay, load the applicable scan that showcases how the images and analysis look for that application.

1. In the **HOME** tab, Login as **Demo**. No password required.
2. Click on the **Manage Data** button.
3. Click on the **Search Data** line and double-click **Enter**. Scan data appears in the data display area.
4. Load the data by right-clicking the scan result, then **View Result**. Data will load in the RESULTS tab.



The name of the Plate ID matches the **Application Protocols** that are provided in the **Celigo Learning Center** (a short cut is located on the desktop).



The Application Protocols provide the steps to perform the wet lab, image acquisition and analysis of the assay.

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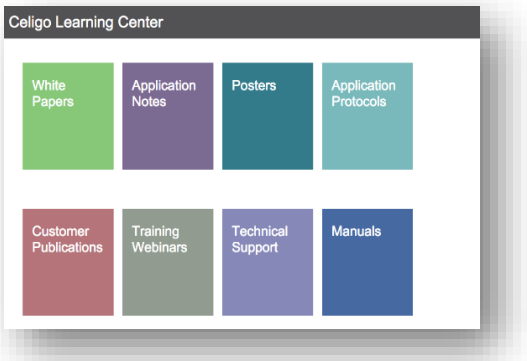
Chapter 12: Additional Resources

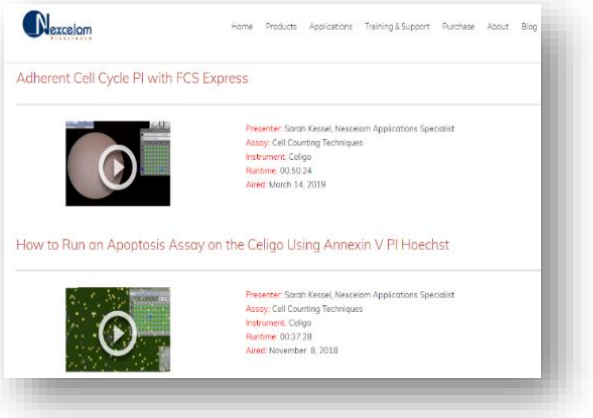
Celigo Learning Center:

(short cut on Celigo computer desktop)



Celigo Training Videos and Webinars:

(https://www.nexcelom.com/training-and-support)



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