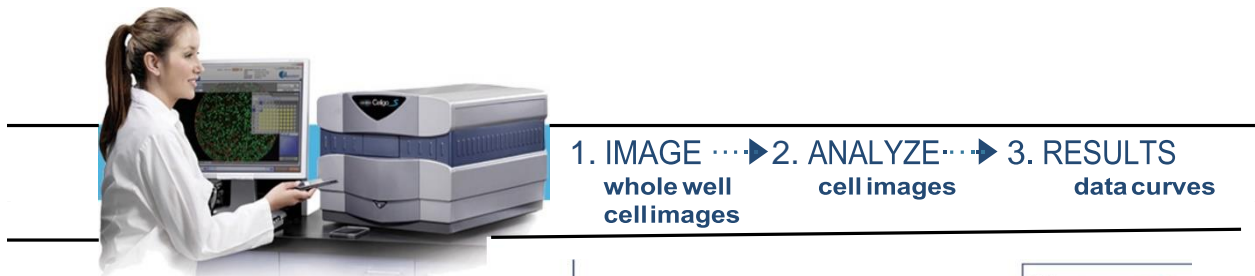
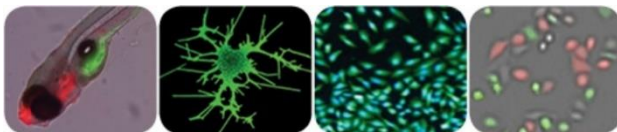
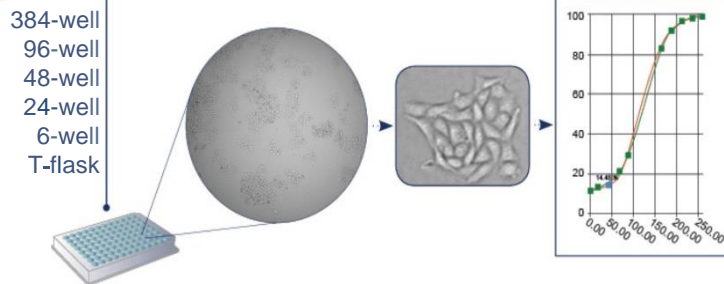




Quick Start Guide



1. IMAGE \cdots 2. ANALYZE \cdots 3. RESULTS
whole well cellimages cell images data curves



www.nexcelom.com



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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	B
C	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	C
D	40,000	20000	10000	5000	2500	1250	625	313	156	78	39	20	D
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	C
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	D

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 0.5×10^6 cells.
- 3 In 15 mL tube labeled 40000, prepare 10 mL with cells at 2×10^5 cells/ mL concentration (200 cells/ μ L)
- 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 μ L 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 μ L Propidium Iodide, 12 μ L Hoechst 33345
- 3 Mix the buffer and fluorescent stain well
- 4 Pipet 50 μ L into wells containing 200 μ L of cells
- 5 Incubate at 37°C (98.6°F) for 30 min
- 6 Image and analyze on Celigo

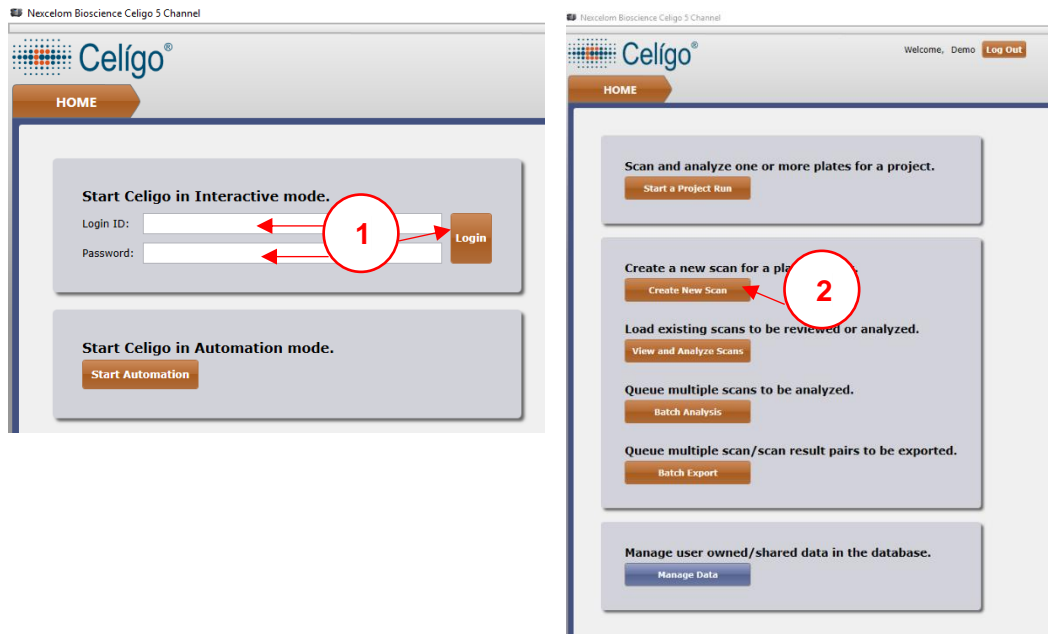
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Chapter 3: Module 1 Cell Proliferation 1-Channel with Direct Cell Counts

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

The screenshot shows the 'Enter Plate Details' workflow in the Celigo software. It consists of three main windows:

- Enter Plate Details (Top Left):** A form where the 'Plate Category' is set to '96-Well' (callout 1). A dropdown menu for 'Plate Profile' is open, showing options like '1536-Well', '384-Well', '96-Well', '48-Well', '24-Well', '12-Well', '6-Well', '1-Well', 'Flask', 'Dish', and 'Slide' (callout 2).
- Enter Plate Details (Top Right):** A table of plate profiles. The '96-Well PE CellCarrier™ 965200 Plate' is selected (callout 2). The 'Plate ID' is entered as 'Exp A - Proliferation Plate 1' (callout 3).
- Load Plate (Bottom Left):** A dialog box with an information icon and the text 'Click OK once the plate has been loaded.' The 'OK' button is highlighted with callout 5.
- Enter Plate Details (Bottom Right):** The 'Load Plate' button is highlighted with callout 4.

SCAN Tab

Select Application:

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

The image displays two screenshots of the Celigo software interface. The top screenshot shows the 'Application' dropdown menu with 'Direct Cell Counting' selected, indicated by a red circle and the number '1'. The bottom screenshot shows the 'SCAN' tab with the 'Application' dropdown set to 'Direct Cell Counting'. The interface is annotated with a yellow box for 'Parameter Setup Details Area', a large green box for 'Image Display Area', and a green circle for 'Plate & Well Navigation Area'.



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.

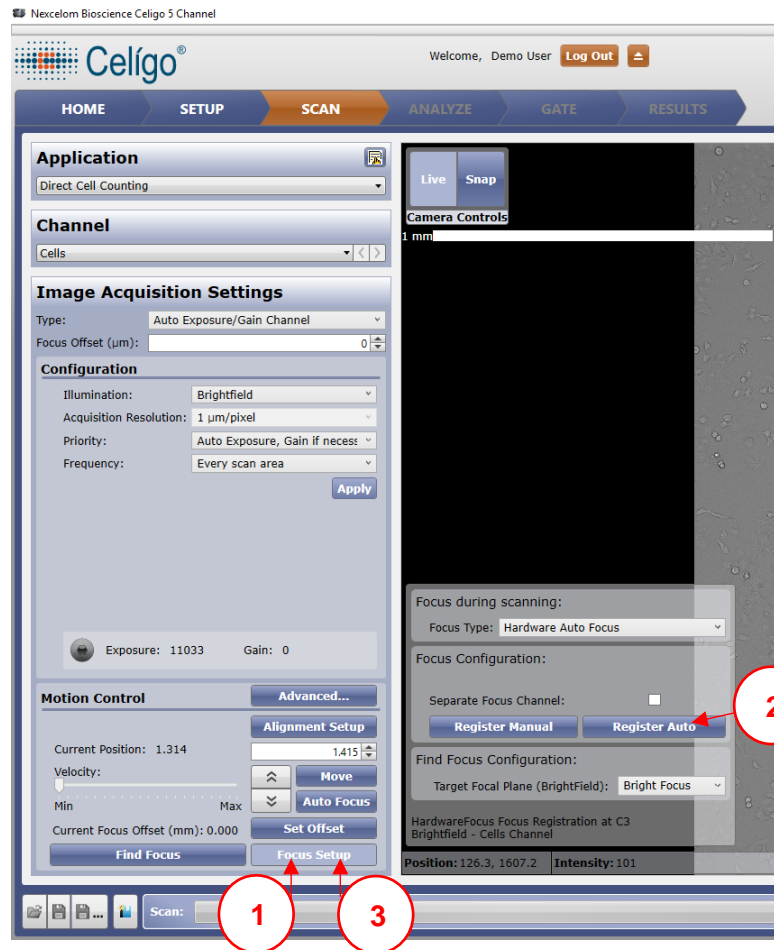
The screenshot shows the Celigo software interface. The 'SCAN' tab is selected in the top navigation bar. In the 'Image Acquisition Settings' panel on the left, the 'Illumination' is set to 'Brightfield' and the 'Apply' button is highlighted with a red circle and the number 3. In the main camera view, the 'Live' button is highlighted with a red circle and the number 1. In the 'Plate Map' on the right, well D4 is selected, highlighted with a blue square and a red circle and the number 2. The status bar at the bottom shows 'Scan: 0%' and 'Analysis: 0%'.



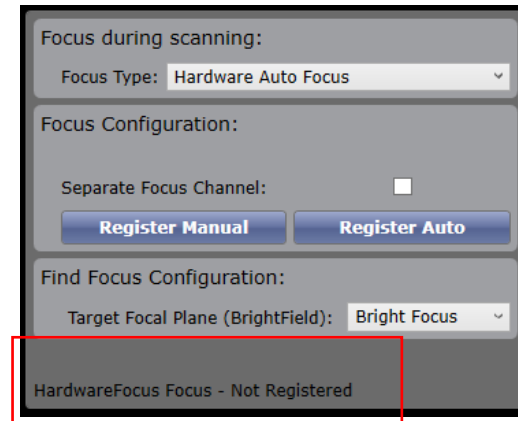
The ideal background for brightfield images are pixel values in the middle of the camera range (0-255), therefore 125 to 130. When clicking **Apply**, the system will determine the correct exposure to produce this background grayscale image. It will also do this during the image acquisition, so if the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

Register Focus:

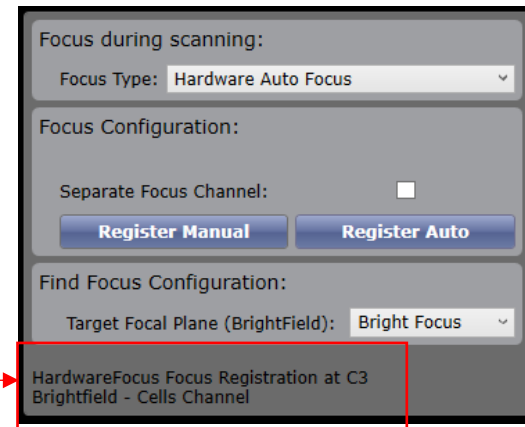
1. Click on **Focus Setup**.
2. Click **Register Auto**.
3. Click **Focus Setup** again to close dialog box.



Before
Registration



After
Registration



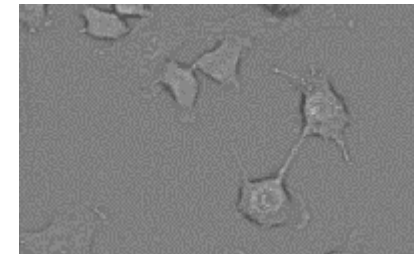
The Hardware Auto Focus (HWAFF) mechanism uses two LED beams (not shown) on the bottom of the plate to measure the z-position of the plate after the cells are determined to be in focus. Once registered, the system will use this mechanism to move to the proper focus plane for subsequent wells. Proper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

Set Focus Offset:

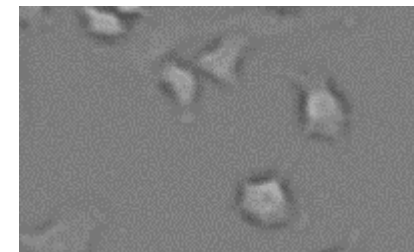
1. In Live camera mode, click on the **down double arrow button** to defocus cells. One click = 0.001 mm or 1 μm , see images below for appropriate focus of cells.
Defocusing allows for the intentional loss of internal cellular objects highlighting the dark-outline/bright-center of the cells.
2. Click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

The screenshot displays the software interface with two panels highlighted by red boxes. The top panel, 'Image Acquisition Settings', shows 'Type: Auto Exposure/Gain Channel' and 'Focus Offset (μm): -12'. The bottom panel, 'Motion Control', shows 'Current Position: 1.302', 'Velocity: 1.415', and 'Current Focus Offset (mm): -0.012'. A 'Set Offset' button is visible in the 'Motion Control' panel. Red circles with numbers '1' and '2' indicate the 'down double arrow button' and the 'Set Offset' button, respectively.

After Hardware Auto Focus (HWAF) Registration
0 μm Offset



Best 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

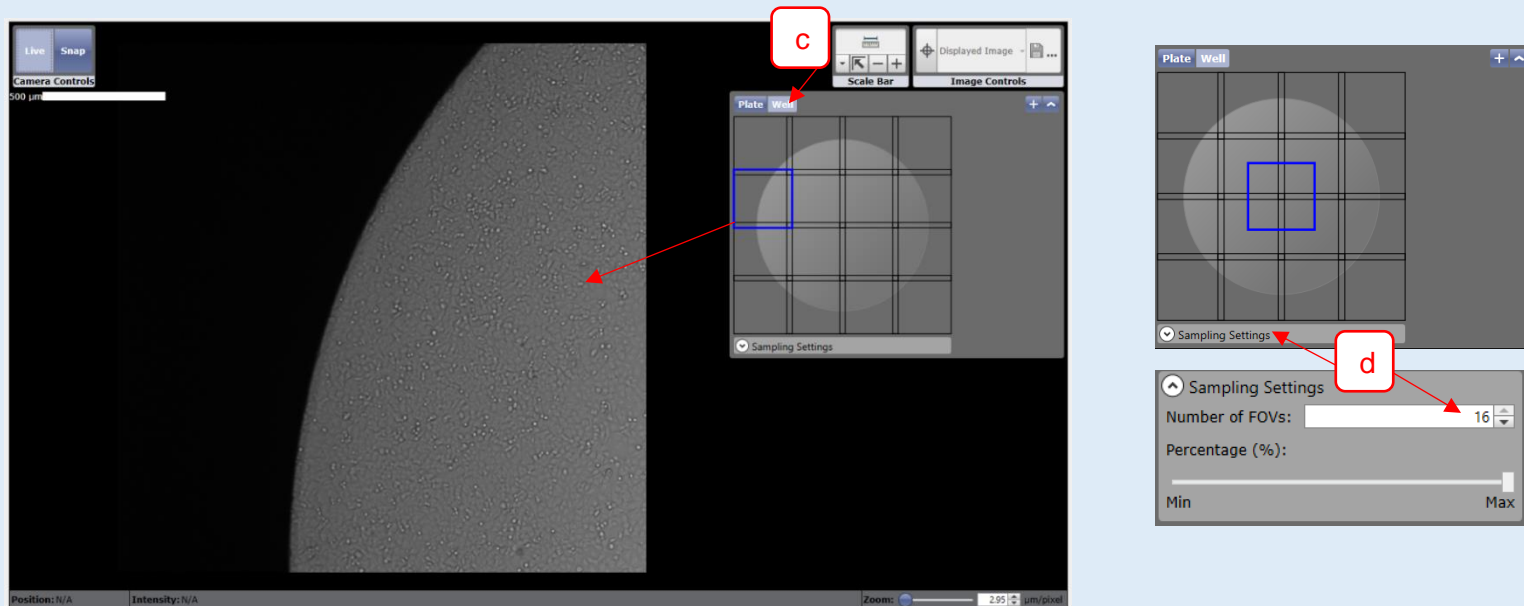
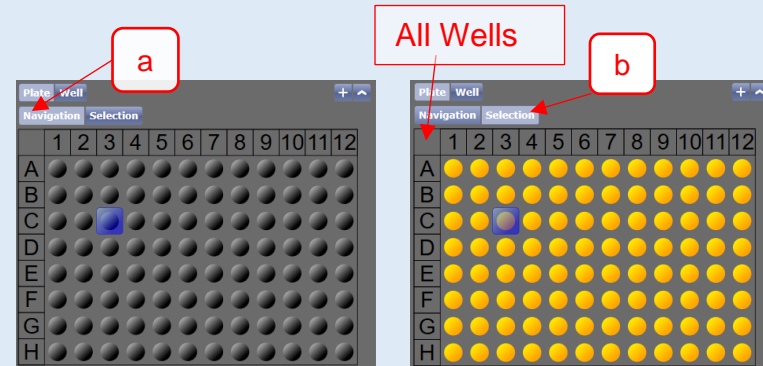
The Celigo cytometer's fast imaging speed is largely due to its proprietary optical pathway, which employs a large field-of-regard (FOR) F-theta lens with high-speed galvanometer mirrors to rapidly scan large areas without moving the sample. The large FOR allows the scanning mirrors to obtain a series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

Plate Navigation Buttons

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

Well Navigation Buttons

- 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.
- 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.

The screenshot displays the Celigo software interface in the 'SCAN' tab. The main window shows a live camera view of a cell culture plate. A 'Plate' selection window is overlaid on the right, showing a grid of wells (A-H, 1-12) with a 'Selection' tab active. A blue square highlights well C3. Red circles with numbers 1, 2, and 3 point to the 'Selection' tab, the highlighted well, and the 'Start Scan' button at the bottom right, respectively. The bottom status bar shows 'Scan: 0%' and 'Analysis: 0%'.



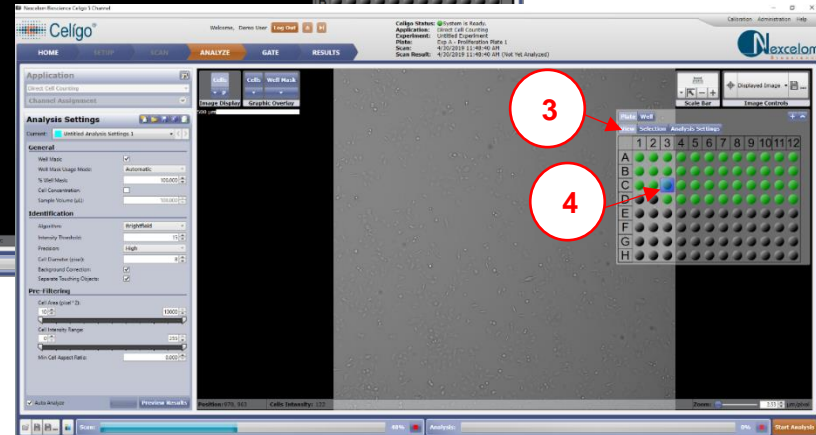
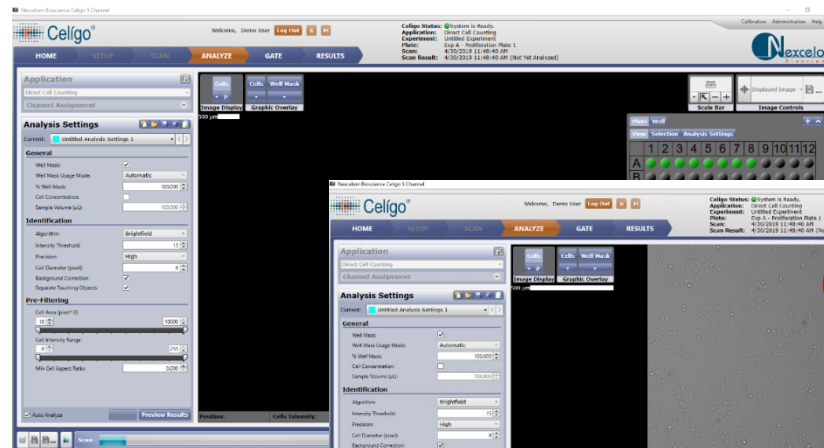
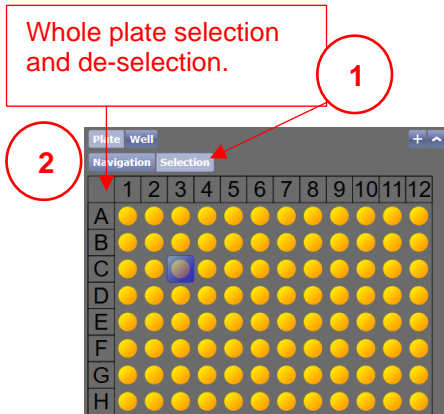
When selecting wells, user can either highlight the wells by dragging mouse over wells, click on columns or rows, or click the **Blank Square** between A and 1 to highlight all wells. De-selecting occurs with same process. User must select wells for Start Scan button to become active.


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.

Whole plate selection and de-selection.



- 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 screenshot displays the Celigo software interface. At the top, there is a navigation bar with tabs for HOME, SETUP, SCAN, ANALYZE, GATE, and RESULTS. The ANALYZE tab is active. On the left, there is a sidebar with 'Application' (Direct Cell Counting) and 'Analysis Settings' (Untitled Analysis Settings 1). The 'Analysis Settings' panel is expanded, showing sections for General, Identification, and Pre-Filtering. A red box highlights the 'Analysis Settings' panel, and a red circle with the number '2' points to it. In the center, there is a large image of a cell culture well with green outlines around the cells. A 'Cells' graphic overlay button is visible above the image, and a red circle with the number '1' points to it. On the right, there is a 'Plate' grid showing a 96-well plate layout with a blue square in well C3. The bottom status bar shows 'Scan: 61%' and 'Analysis: 0%'.



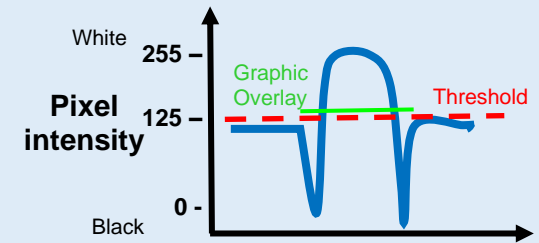
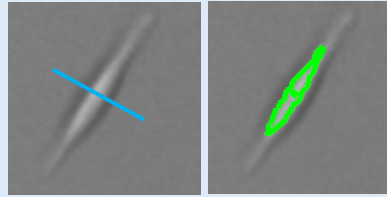
The default analysis settings may need adjustment. Low contrast cells will require a lower Intensity Threshold while high contrast cells may require a higher Intensity Threshold for proper segmentation. Previously saved settings can also be loaded. You can save the newly defined settings for future recall 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

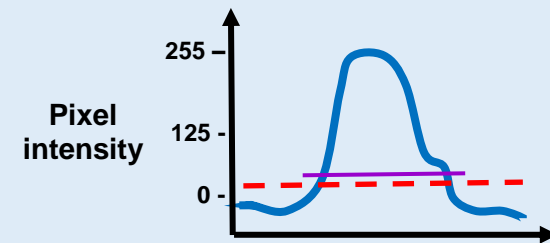
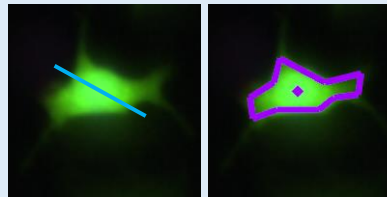
Image analysis looks at the pixels in the image, where each pixel has a value in the range of 0 = black to 255 = white. If an imaginary line was drawn over the object of interest and the pixels plotted according to their values, a graph similar to one displayed below would be seen. The intensity threshold 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.



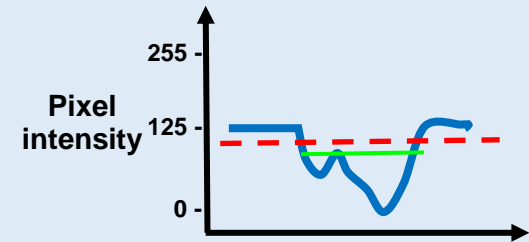
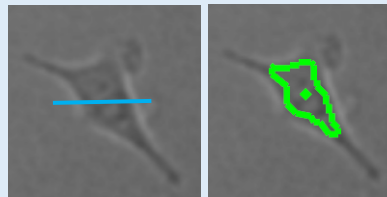
Brightfield – The algorithm looks for objects with a bright center and dark edges. Intensity Threshold and Cell Diameter work together to identify objects. Applications: Brightfield images looking at single cell identification, direct cell counts and expression analysis.



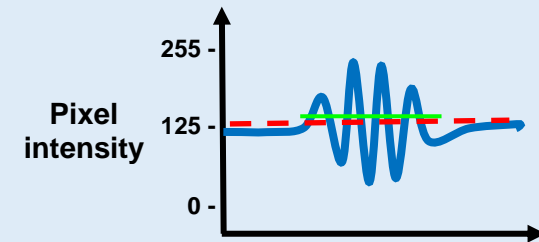
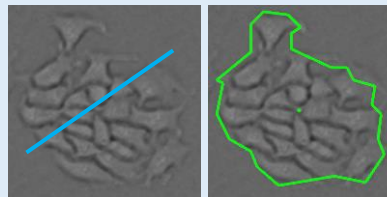
Fluorescence – The algorithm looks for fluorescent objects (bright pixels over darker background). Applications: All applications looking at fluorescently labelled cells.



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.

The screenshot displays the Celigo software interface. The top navigation bar includes HOME, SETUP, SCAN, ANALYZE (highlighted), GATE, and RESULTS. The left sidebar contains 'Application' (Direct Cell Counting), 'Channel Assignment', and 'Analysis Settings'. The 'Analysis Settings' panel is expanded to show 'General', 'Identification', and 'Pre-Filtering' sections. Three red circles with numbers 1, 2, and 3 are overlaid on the 'Intensity Threshold', 'Cell Diameter', and 'Cell Area' sliders, respectively. The main window shows a grayscale image of cells with green outlines. A 'Plate' grid is visible on the right, and a 'Zoom' slider is at the bottom right. The status bar at the bottom shows 'Scan: 77%' and 'Analysis: 0%'. A 'Start Analysis' button is located in the bottom right corner.

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.

The screenshot displays the Celigo software interface during the analysis phase. The main window shows a large image of a cell culture well with green outlines indicating segmented cells. A smaller inset window shows a zoomed-in view of a well edge, with a red circle '2' highlighting a specific cell. The 'Analysis Settings' panel on the left is highlighted with a red box and a red circle '3', showing the 'Identification' and 'Pre-Filtering' sections. The 'Identification' section includes settings for Algorithm (Brightfield), Intensity Threshold (4), Precision (High), Cell Diameter (12), Background Correction (checked), and Separate Touching Objects (checked). The 'Pre-Filtering' section includes settings for Cell Area (50 to 10000), Cell Intensity Range (0 to 255), and Min Cell Aspect Ratio (0.000). The top right corner shows system status: 'System is Ready', 'Direct Cell Counting', 'Untitled Experiment', 'Exp A - Proliferation Plate 1', '4/30/2019 11:48:40 AM', and '4/30/2019 11:48:40 AM (Not Yet Analyzed)'. The bottom status bar shows 'Scan: 87%' and 'Analysis: 0%' with a 'Start Analysis' button.



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.

The screenshot displays the Celigo software interface during the analysis phase. The 'ANALYZE' tab is selected, and the 'Graphic Overlay' button (1) is visible. In the 'Analysis Settings' panel, the 'Well Mask' checkbox (2) is checked, and the '% Well Mask' is set to 99.500 (3). The main image shows a grid of wells with green cell outlines. A zoomed-in view of a well is shown on the right. The bottom status bar shows 'Scan: 98%' and 'Analysis: 0%'.



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:

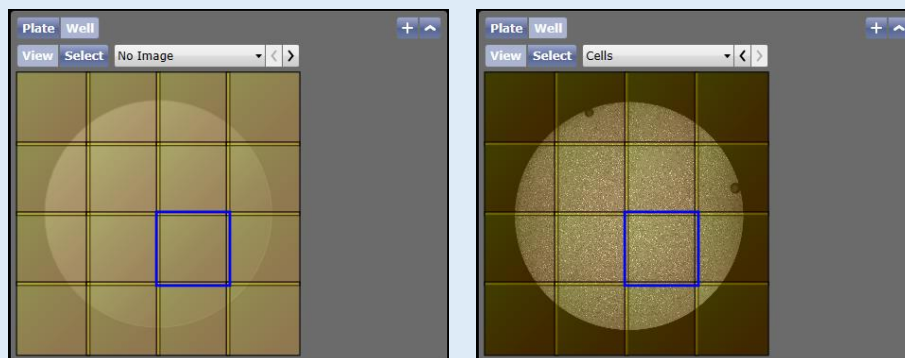
Plate Navigation Buttons

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

The screenshot shows the SCAN software interface. On the left, a grid of wells (A-H, 1-12) is displayed. The 'View' menu is open, showing 'Selection' and 'Analysis Settings'. A red box 'a' highlights the 'View' menu. A red box 'b' highlights the 'Selection' menu. A red box 'c' highlights the 'Analysis Settings' menu. To the right, the 'Analysis Settings' panel is shown, with 'Current:' set to 'DCC Adherent Cell'. Below this, a dialog box titled 'Specify Settings Name' is open, with 'Settings Name:' set to 'DCC Adherent Cell' and 'Folder:' set to 'LAdmin'. Red arrows point from the 'Analysis Settings' panel to the dialog box.

Well Navigation Buttons

- Click on **Well** to activate
- View:** Select an area of well for image display
- 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.

The screenshot displays the Celigo software interface in the ANALYZE tab. The top navigation bar includes tabs for HOME, SETUP, SCAN, ANALYZE, GATE, and RESULTS. The left sidebar contains the following sections:

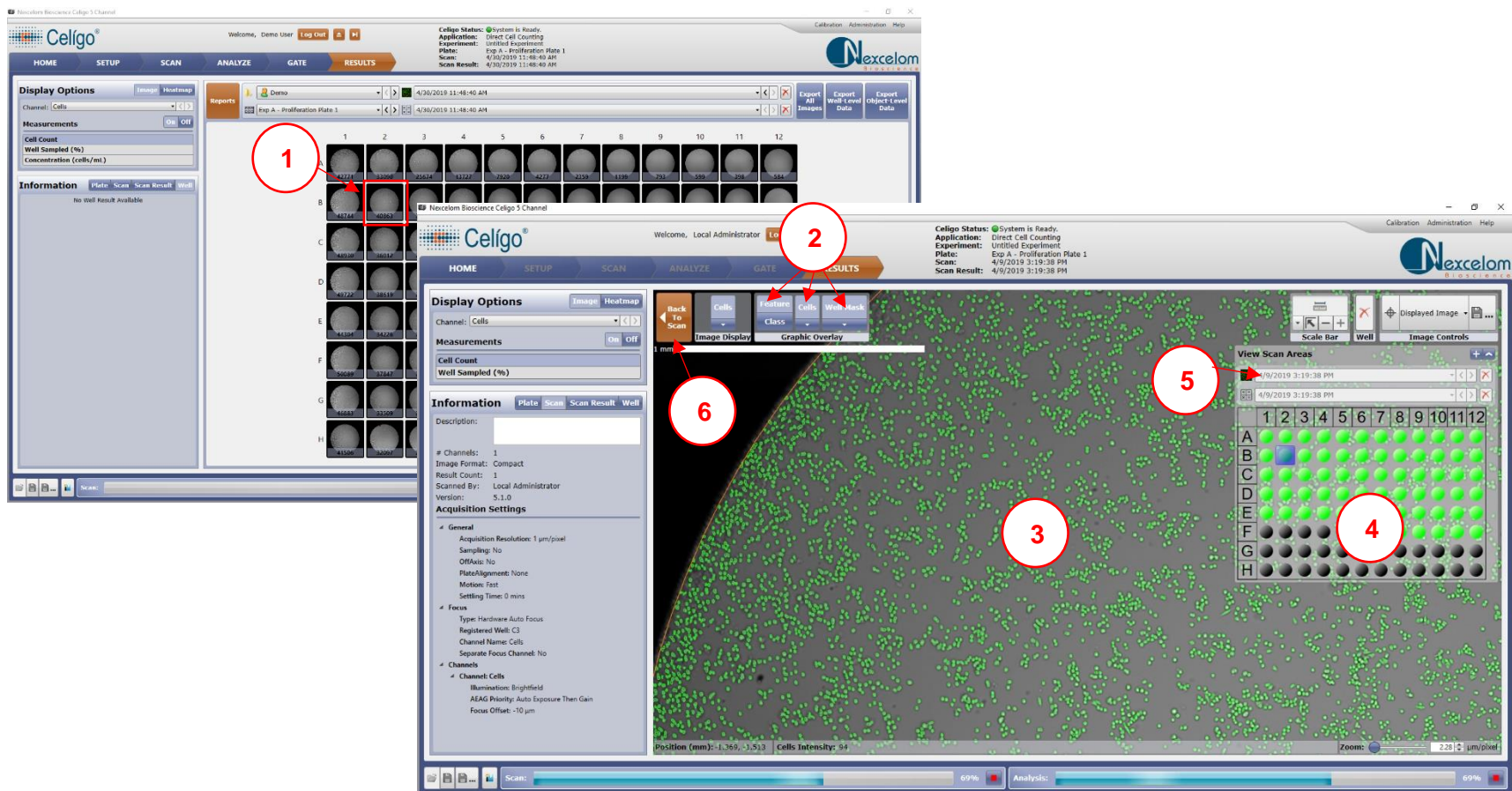
- Application:** Direct Cell Counting
- Channel Assignment:** (Dropdown menu)
- Analysis Settings:**
 - Current: DCC Adherent Cell
 - General:** Well Mask (checked), Well Mask Usage Mode: Automatic, % Well Mask: 99.500, Cell Concentration: (Slider), Sample Volume (μL): 100.000
 - Identification:** Algorithm: Brightfield, Intensity Threshold: 4, Precision: High, Cell Diameter (pixel): 12, Background Correction: (checked), Separate Touching Objects: (checked)
 - Pre-Filtering:** Cell Area (pixel²): 50 (Slider), Cell Intensity Range: 0 (Slider), Min Cell Aspect Ratio: 0.000
- Auto Analyze:** (checked)

The main image area shows a microscopic view of cells with a green overlay. A red box highlights a 12x12 well plate grid in the top right corner, with a blue square indicating the current well. Red circles with numbers 1, 2, and 3 point to the 'Plate' button, the 'View' button, and the 'Start Analysis' button respectively. The bottom status bar shows 'Position: 322, 636', 'Cells Intensity: 118', 'Zoom: 1.28 μm/pixel', and progress bars for 'Scan: 0%' and 'Analysis: 0%'. The Celigo logo and Nexcelom Bioscience logo are visible in the top right.

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

- 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.
- Display Area:** Plate thumbnail or well view display area is shown there. Double click on a well to view individual well in high resolution.
- User Folder, Plate ID, Scan and Analysis Time Display:** Current data is displayed, can select other time points after scan and analysis are complete
- Information Area:** Information displayed is dependent on selected button. See next page for more details.
- Export Options:** Data export options for Well-Level, Object-Level and Images
- Scan (Image Acquisition) and Analysis Progress bars:** May run simultaneously. Can be stopped using red square button at any time.
- User Log Out, Plate Eject, Next Plate:** Once scan and analysis are complete, these buttons become available.

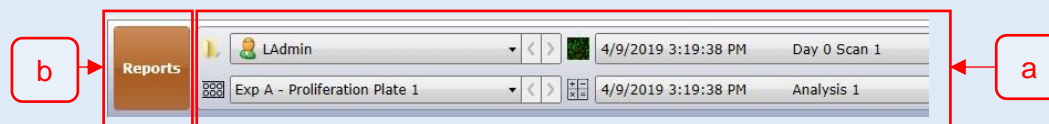
The screenshot displays the Celigo software interface with the following features highlighted:

- a:** The **RESULTS** tab in the top navigation bar.
- b:** A well in the grid (row D, column 5).
- c:** The **Reports** section in the top right.
- d:** The **Information** panel on the left side.
- e:** The **Export Options** buttons in the top right.
- f:** The **Scan** and **Analysis** progress bars at the bottom.
- g:** The **Log Out** button in the top right.

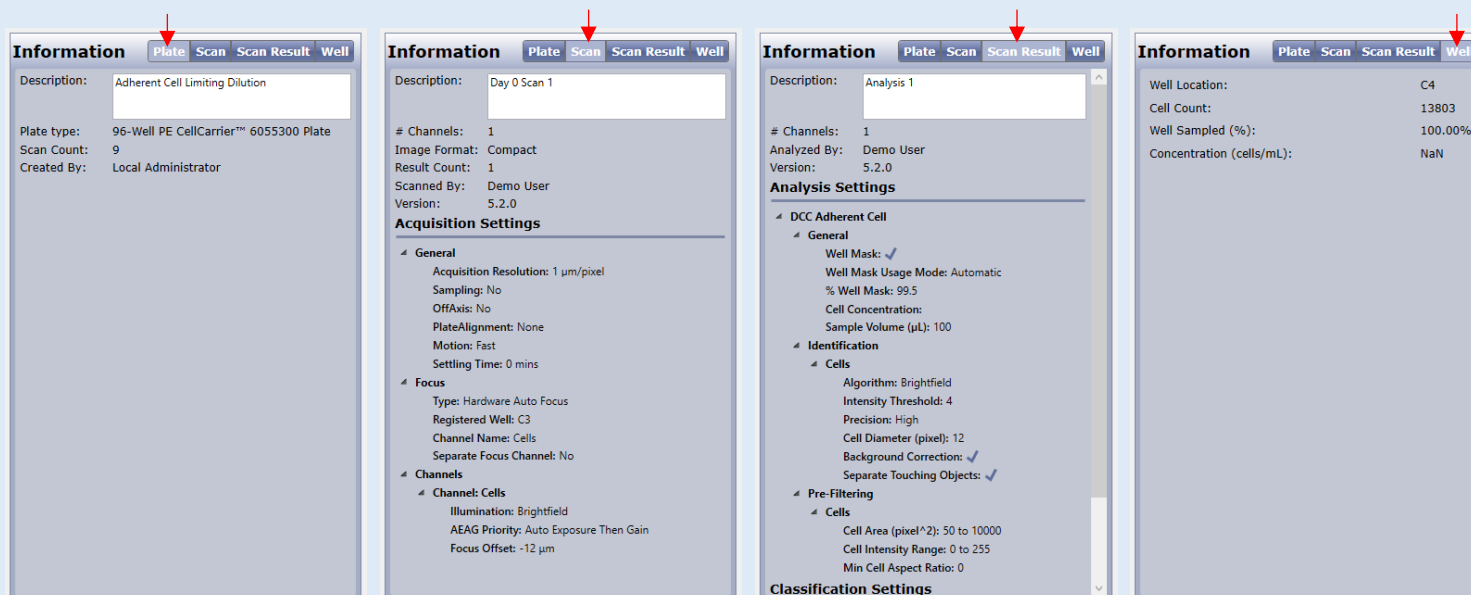


KNOWLEDGE: Overview of RESULTS Tab Features (continued)

- 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.



The top screenshot shows the 'Image' view of the Celigo software. The 'Display Options' panel has 'Image' selected and 'Heatmap' unselected. The 'On/Off' toggle for the image view is set to 'On'. The main display area shows a 2x12 grid of thumbnail images of wells, with numerical values displayed above each image. The values for the first two rows are: Row A: 42771, 33090, 25674, 13727, 7920, 4277, 2359, 1199, 793, 599, 398, 584; Row B: 48744, 40863, 23574, 13701, 7332, 3678, 2022, 1016, 847, 409, 469, 338.

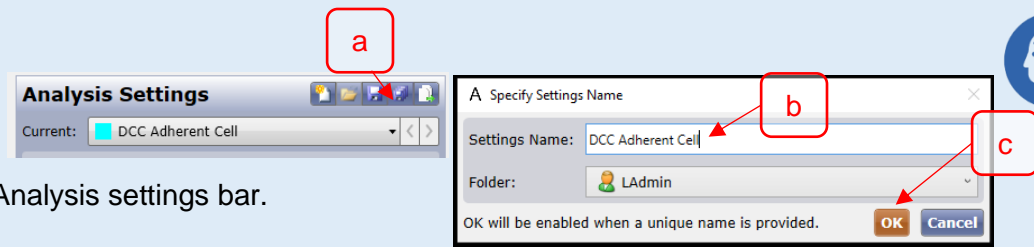
The bottom screenshot shows the 'Heatmap' view. The 'Display Options' panel has 'Heatmap' selected and 'Image' unselected. The 'On/Off' toggle for the heatmap view is set to 'Off'. The 'Minimum' value is set to 2748 and the 'Maximum' value is set to 47597. The main display area shows a 2x12 grid of wells colored according to their numerical values. The values for the first two rows are: Row A: 42771, 33090, 25674, 13727, 7920, 4277, 2359, 1199, 793, 599, 398, 584; Row B: 48744, 40863, 23574, 13701, 7332, 3678, 2022, 1016, 847, 409, 469, 338.

KNOWLEDGE: Save Features for Future Recall

Save Analysis Setting

Saves Analysis parameters on Analysis Tab

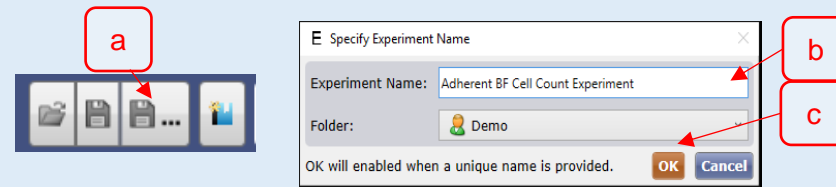
- Click on **Save Analysis** icon.
- Type in a desired settings name.
- Click **OK**. Observe name updated in Current Analysis settings bar.



Save Experiment Setting

Saves settings from Scan, Analysis and Gating tab.

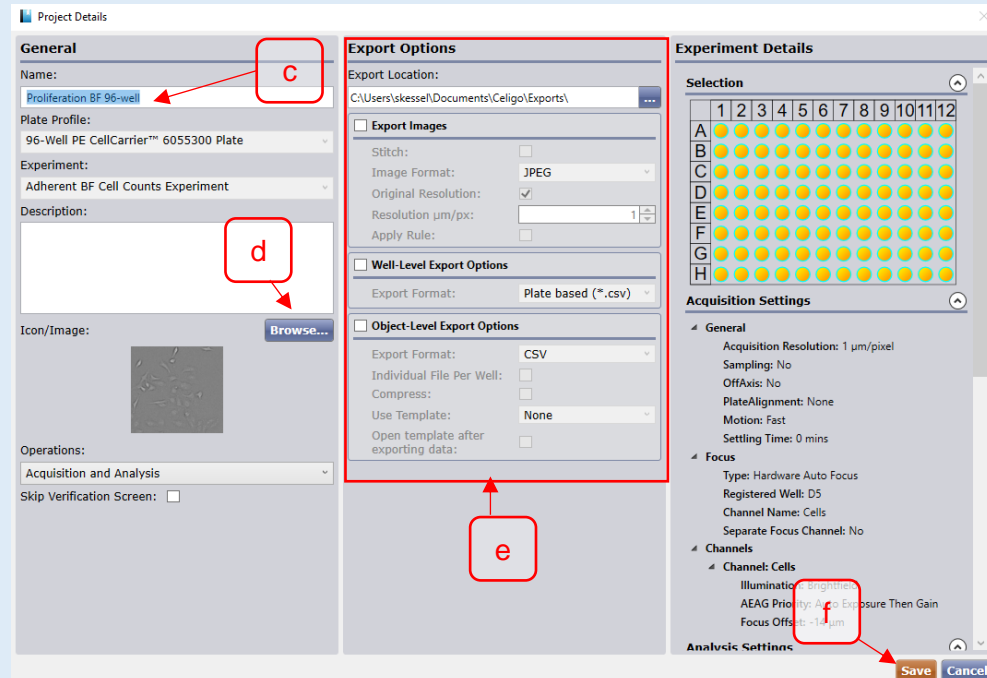
- Click on **Save As Experiment** icon.
- Type in desired name.
- Click **OK**.



Save Project

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

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



Save Experiment Setting and Project

Save Experiment:

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

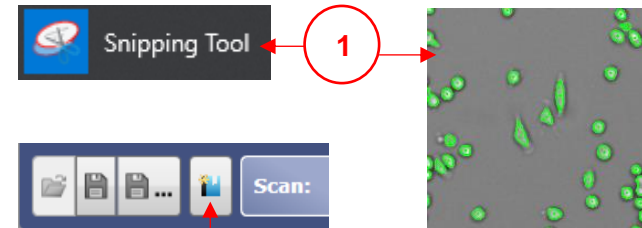
The screenshot shows the Celigo software interface. The top navigation bar includes HOME, SETUP, SCAN, ANALYZE, GATE, and RESULTS. The main display area shows a cell counting image with a 500 µm scale bar. A dialog box titled "Specify Experiment Name" is open, with the following fields:

- Experiment Name: DCC Adherent
- Folder: LAdmin

Buttons for OK and Cancel are visible. A red circle '1' points to the 'Save Experiment' icon in the bottom toolbar, and a red circle '2' points to the 'Experiment Name' input field.

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**.



Project Details

General

Name: (3)

Plate Profile: 96-Well PE CellCarrier™ 6055300 Plate

Experiment: DCC Adherent

Description: (4)

Icon/Image: (5)

Operations: Acquisition and Analysis

Skip Verification Screen:

Export Options

Export Location: C:\Users\skessel\Documents\Project Details

Export Images

Stitch:

Image Format: JPEG

Original Resolution:

Resolution $\mu\text{m}/\text{px}$: 1

Well-Level Export Options (6)

Export Format: Plate based (*.csv)

Object-Level Export Options

Export Format: CSV

Individual File Per Well:

Compress:

Use Template: None

Open template after exporting data:

Icon/Image: (5)

Operations: Acquisition and Analysis

Skip Verification Screen:

Experiment Details

Selection

	1	2	3	4	5	6	7	8	9	10	11	12
A	●	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	●	●	●	●	●	●	●	●	●	●	●	●
E	●	●	●	●	●	●	●	●	●	●	●	●
F	●	●	●	●	●	●	●	●	●	●	●	●
G	●	●	●	●	●	●	●	●	●	●	●	●
H	●	●	●	●	●	●	●	●	●	●	●	●

Acquisition Settings

General

- Acquisition Resolution: 1 $\mu\text{m}/\text{pixel}$
- Sampling: No
- OffAxis: No
- PlateAlignment: None
- Motion: Fast
- Settling Time: 0 mins

Focus

- Type: Hardware Auto Focus
- Registered Well: C3
- Channel Name: Cells
- Separate Focus Channel: No

Channels

- Channel: Cells**
 - Illumination: Brightfield
 - AEAG Priority: Auto Exposure
 - Focus Offset: -10 μm

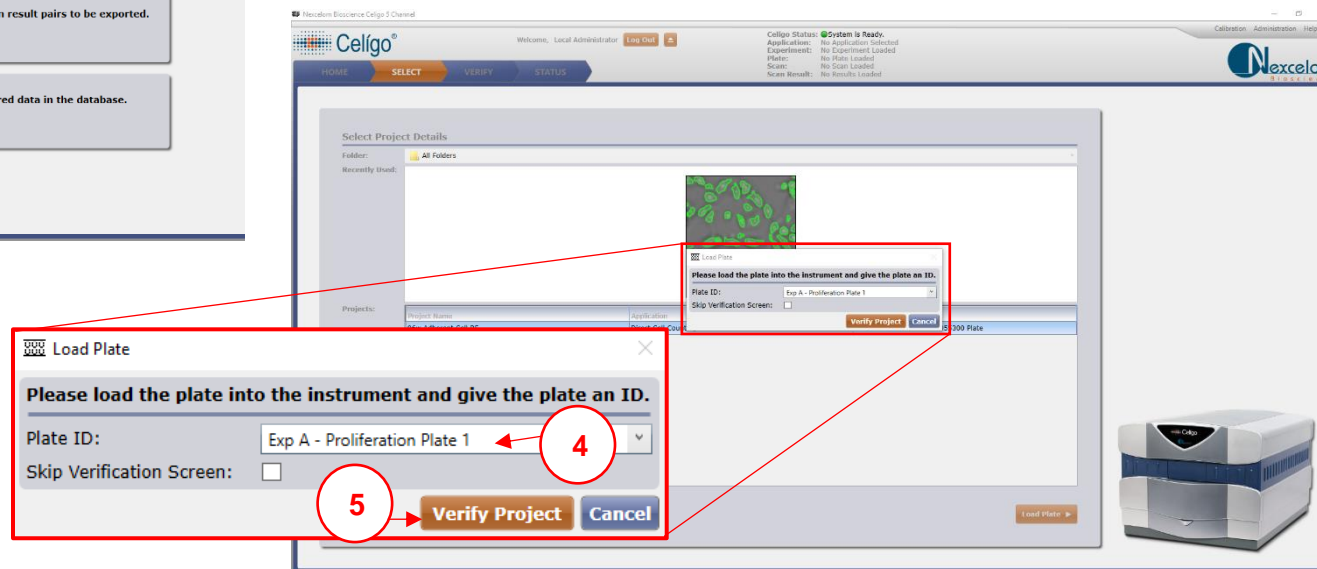
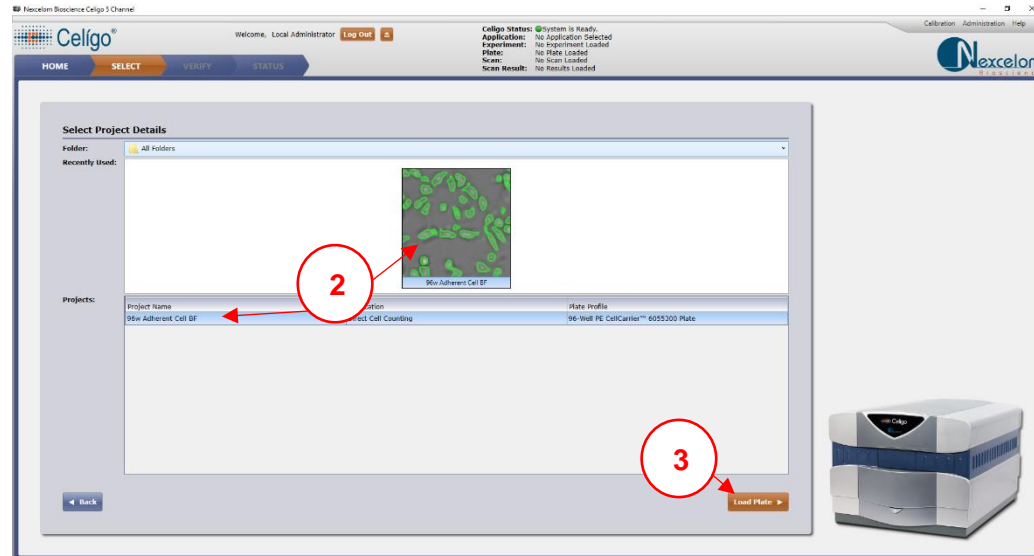
Analysis Settings

(7)

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**.

The screenshot displays the Celigo software interface in the VERIFY tab. The main window shows a large image of cells with a red circle labeled '1' over it. On the left, there are 'Project Details' and 'Settings' panels. On the right, there are control buttons for 'Fit and Center', 'Well', and 'Scale Bar'. At the bottom right, there are buttons for 'Adjust Acquisition Settings' and 'Run Project', with a red circle labeled '2' over the 'Run Project' button. The status bar at the bottom shows 'Position: 1149.8, 945.1' and 'Cells Intensity: 132'.

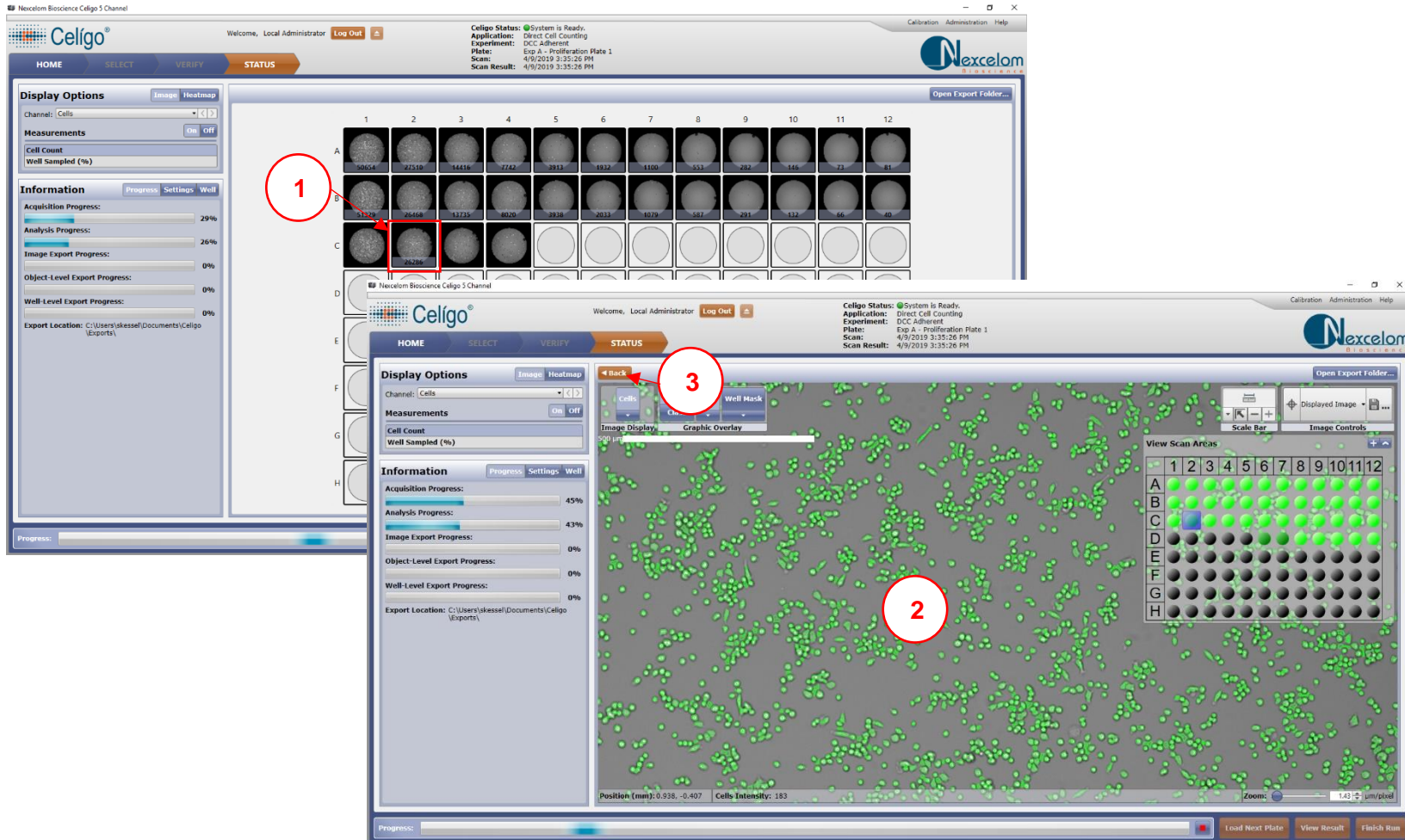


Adjust Acquisition Settings: If settings need adjusting, click **Adjust Acquisition Settings** button. System will switch into **Standard Mode**, where focus, illumination and well selection can be adjusted. Please see User Guide for further details.

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.



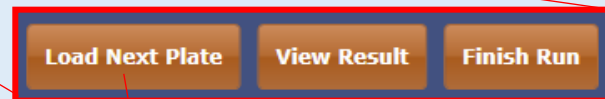
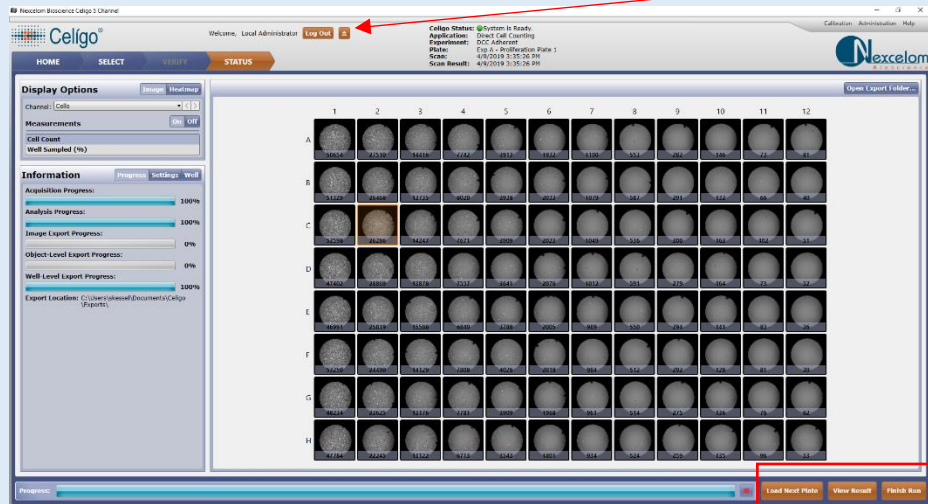


KNOWLEDGE: Options in STATUS tab

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.



Load Plate ✕

Please load the plate into the instrument and give the plate an ID.

Plate ID:

Skip Verification Screen:

Verify Project **Cancel**

This Page Left Intentionally Blank

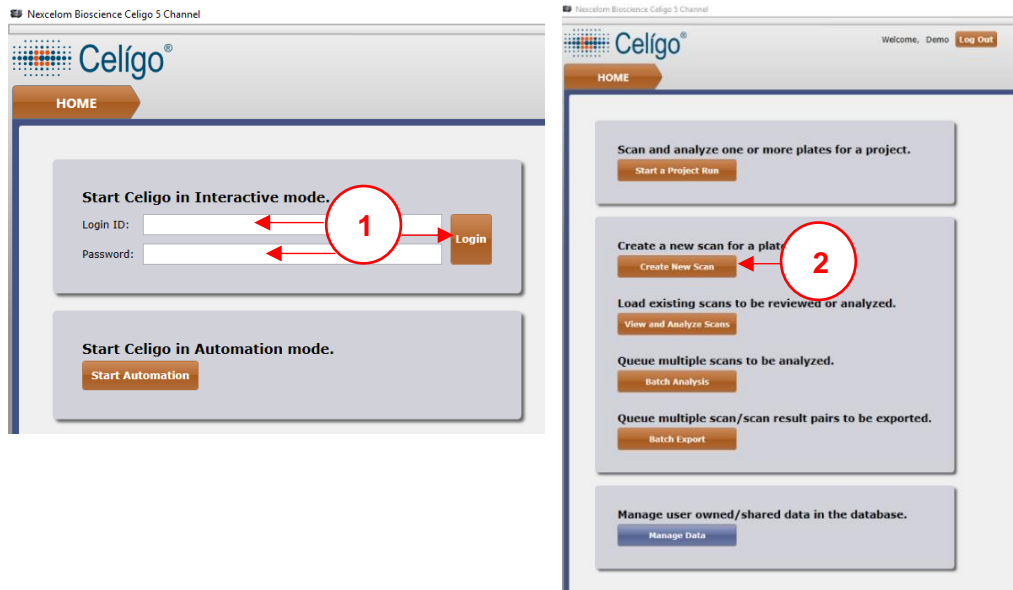
Chapter 4: Module 2 Cell Viability 2-Channel Dead and Total

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**.

Enter Plate Details

Plate Category: 96-Well

Plate Profile: 1536-Well, 384-Well, 96-Well, 48-Well, 24-Well, 12-Well, 6-Well, 1-Well, Flask, Dish, Slide

Plate ID: Exp 8 - Viability Plate 1

Folder: Demo

Plate Description: (Optional)

Scan Description: (Optional)

Select Experiment

Experiment: (Optional)

Load Plate

Click OK once the plate has been loaded.

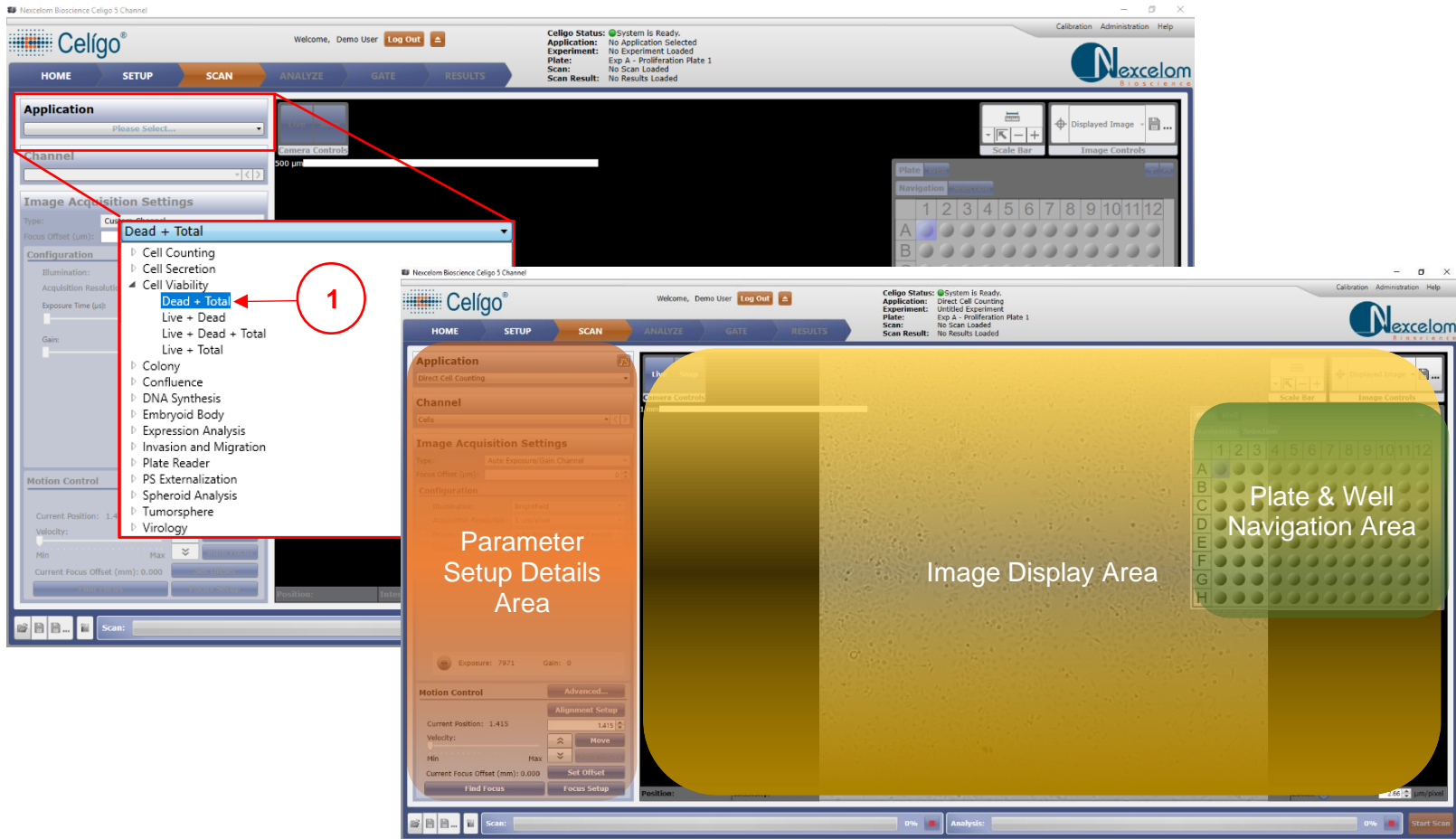
OK Cancel


Name	Manufacturer	Support
96-Well Nexcelom3D ULA-96U Plate	Nexcelom Bioscience	Supported
96-Well Nexcelom3D ULA-96U_Lot60939020_2017-3-29	Nexcelom Bioscience	Supported
96-Well Nuncl™ 167008 Plate	Corning	Supported
96-Well Oris™ Plate 6.35 mm	Corning	Unsupported
96-Well PE CellCarrier™ 6055300 Plate	Corning	Supported
96-Well PE CellCarrier™ 6055300 Plate_257	Corning	Supported
96-Well PE CellCarrier™ 6055300 Plate_387	Corning	Supported
96-Well PE Isoplate™ 6005050 Plate	PerkinElmer	Supported
96-Well PE Viewplate™ 6005225 Plate	PerkinElmer	Supported
96-Well Seahorse™ XF96 Plate	Seahorse	Supported

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 μ second.
4. Click **Focus Setup**.
5. Click **Register Auto**.
6. Click **Focus Setup**. Adjust exposure if necessary.

The screenshot shows the Celigo software interface with the following callouts:

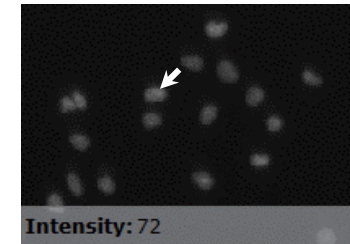
- 1:** Points to the Channel dropdown menu, which is currently set to 'Dead' and needs to be changed to 'Total'.
- 2:** Points to the Plate map navigation area, which is a grid of wells (A-H, 1-12) used for selecting a well.
- 3:** Points to the Exposure Time (µs) field in the Image Acquisition Settings, which is currently set to 50000.
- 4:** Points to the Focus Setup button in the Motion Control section.
- 5:** Points to the Register Auto button in the Focus during scanning dialog box.
- 6:** Points to the Focus Setup button in the Motion Control section.

The focus registration dialog box shows the following settings:

- Focus Type: Hardware Auto Focus
- Focus Configuration: Separate Focus Channel:
- Buttons: Register Manual, Register Auto
- Find Focus Configuration: Target Focal Plane (BrightField): Bright Focus
- HardwareFocus Focus Registration at A1 Blue 377/447 - Total Channel

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)

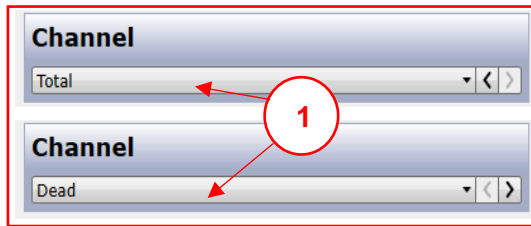


Exposure: 80,000 us, Gain 100
Object/ background (235 / 43)

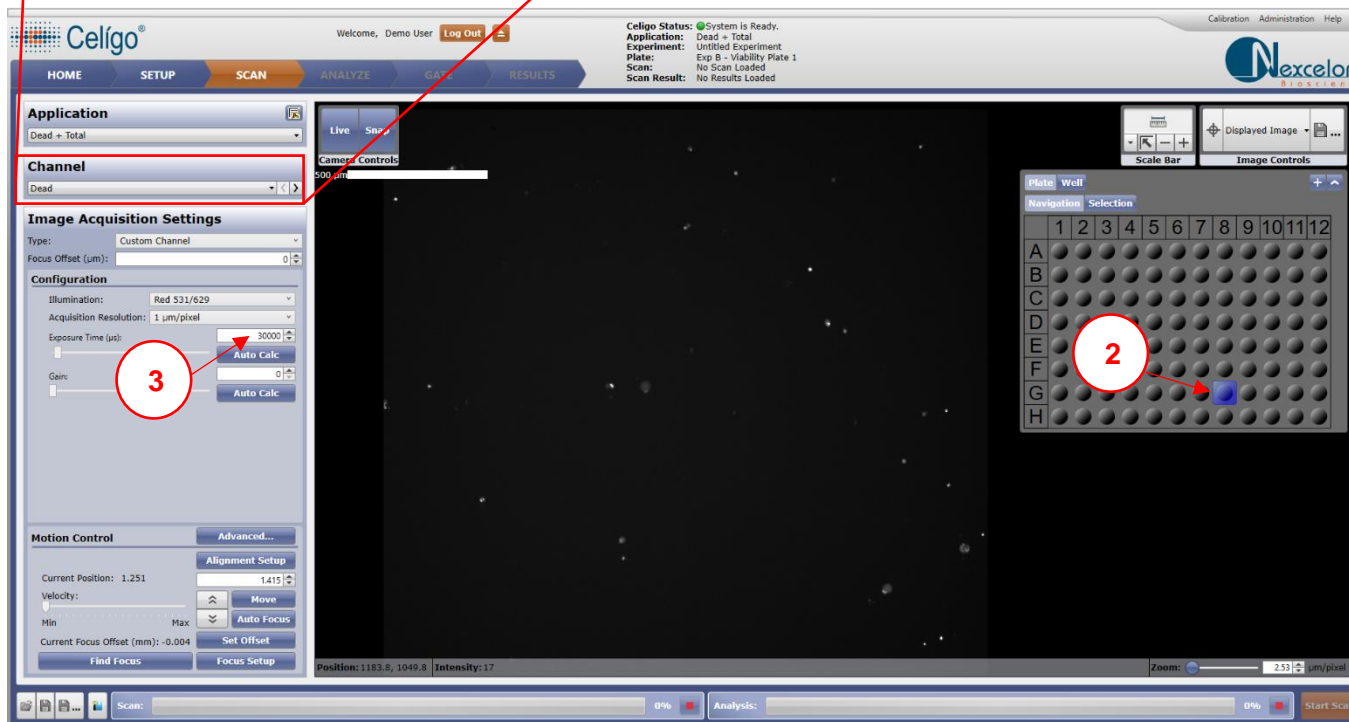


Check pixel intensities after focus registration by hovering mouse over objects and background. The ideal fluorescent images have object pixel values with signal to background ratio above 2.0 and in the upper end of the camera range (0-255). Therefore a 150 to 200 object intensity with background pixel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

Dead Channel Setup for Illumination, Exposure, Focus Registration:

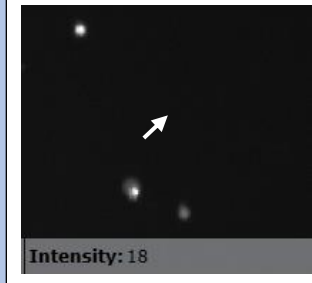


1. Change Channel from Total to **Dead**.
2. Select a **well** on Plate map navigation area.
(Any well that has dead cells in the center of the well stained with Propidium Iodide (PI), it doesn't have to be the same well as focus registration well.)
3. Adjust Exposure to 30,000-50,000 μ second



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



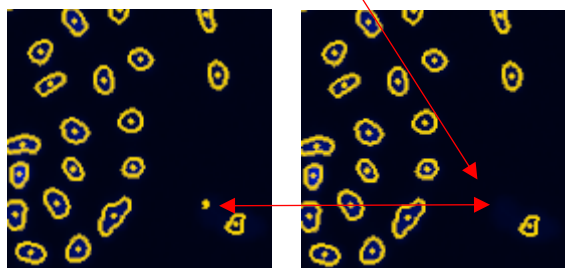
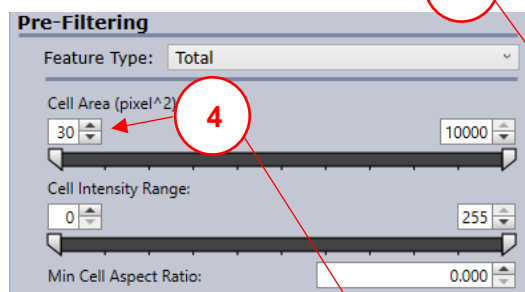
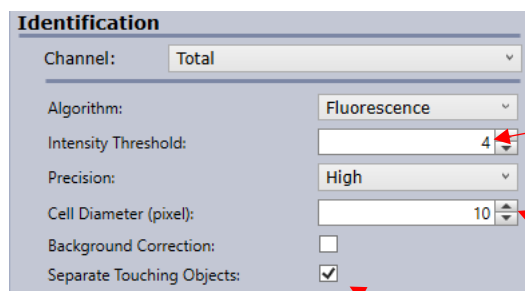
Dead Channel Focus Offset and Well Selection:

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**.



Adjust Analysis Parameter for Total Channel Objects

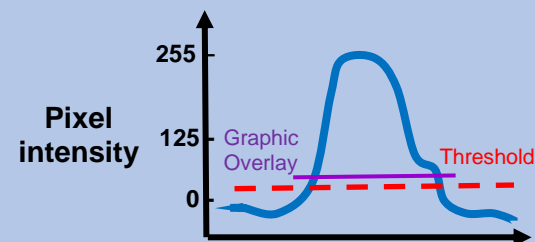
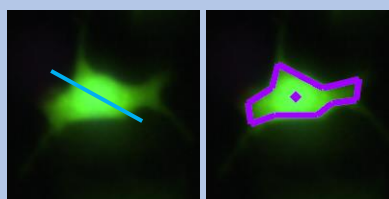
1. Adjust **Intensity Threshold**
2. Adjust **Cell Diameter**
3. Check **Separate Touching Objects**
4. Increase **Minimum Cell Area** to eliminate small objects



KNOWLEDGE: Identification Section



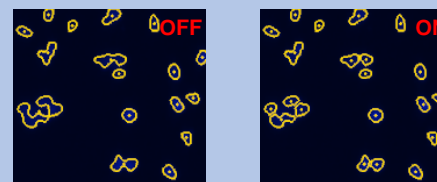
Intensity Threshold: When signal to noise ratio is 2.0 or greater the default settings will be a good starting point. If signal is lower or background is high, lower the intensity threshold.



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**.

The screenshot displays the Celigo software interface during the 'ANALYZE' phase. The top navigation bar includes 'HOME', 'SETUP', 'SCAN', 'ANALYZE', 'GATE', and 'RESULTS'. The 'Celigo Status' bar at the top right indicates 'System is Ready' and provides details for the current application: 'Dead + Total', 'Untitled Experiment', 'Exp B - Viability Plate 1', and a scan date of '5/8/2019 4:21:14 PM'. The left sidebar contains the 'Application' and 'Analysis Settings' panels. The 'Application' panel shows 'Dead + Total' and 'Channel Assignment'. The 'Analysis Settings' panel is divided into 'General', 'Identification', and 'Pre-Filtering' sections. The 'Identification' section shows 'Channel: Dead'. The main workspace displays a 'Dead Image' with a 'Graphic Overlay' (blue circles) and a 'Well Mask' grid. Red circles with numbers 1, 2, and 3 point to the 'Dead' button, the 'Well' button, and the 'Channel' dropdown, respectively. The bottom status bar shows 'Position: 8, 1186', 'Dead Intensity: 17', 'Total Intensity: N/A', and 'Zoom: 1.22 um/pixel'.

Adjust Analysis Parameter for Dead Channel Objects

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

Identification

Channel:

Algorithm:

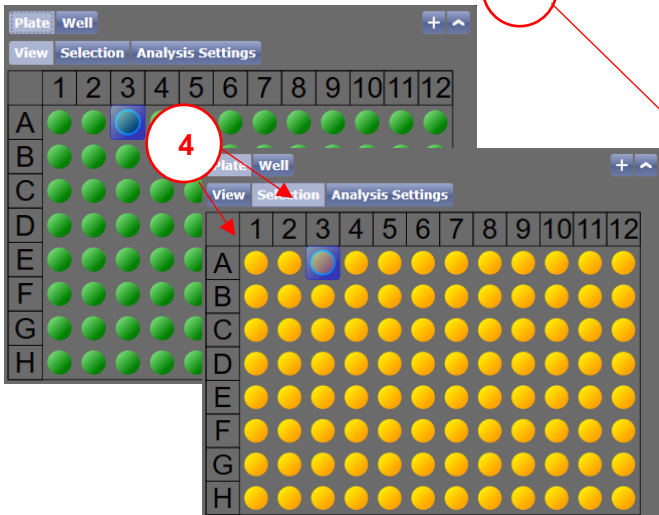
Intensity Threshold:

Precision:

Cell Diameter (pixel):

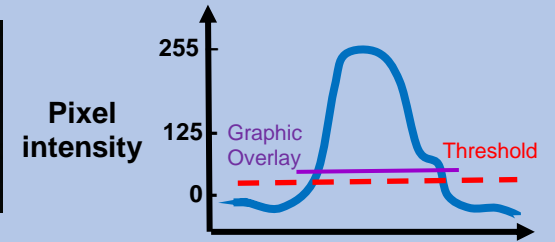
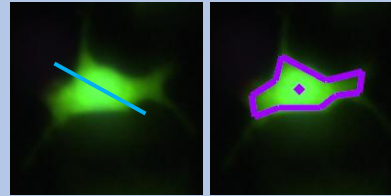
Background Correction:

Separate Touching Objects:

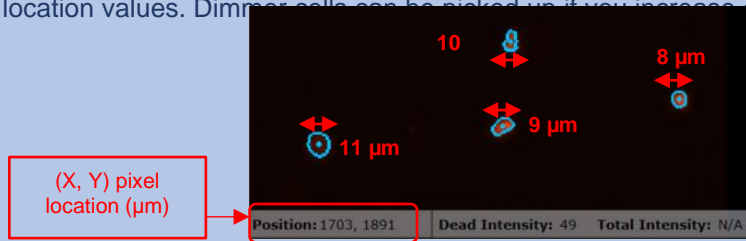


KNOWLEDGE:

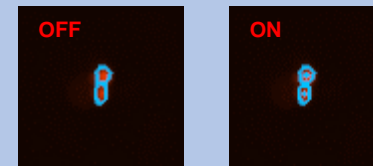
Intensity Threshold: When signal to noise ratio is 2.0 or greater the default settings will be a good starting point. If signal is lower or background is high, lower the intensity threshold.



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.

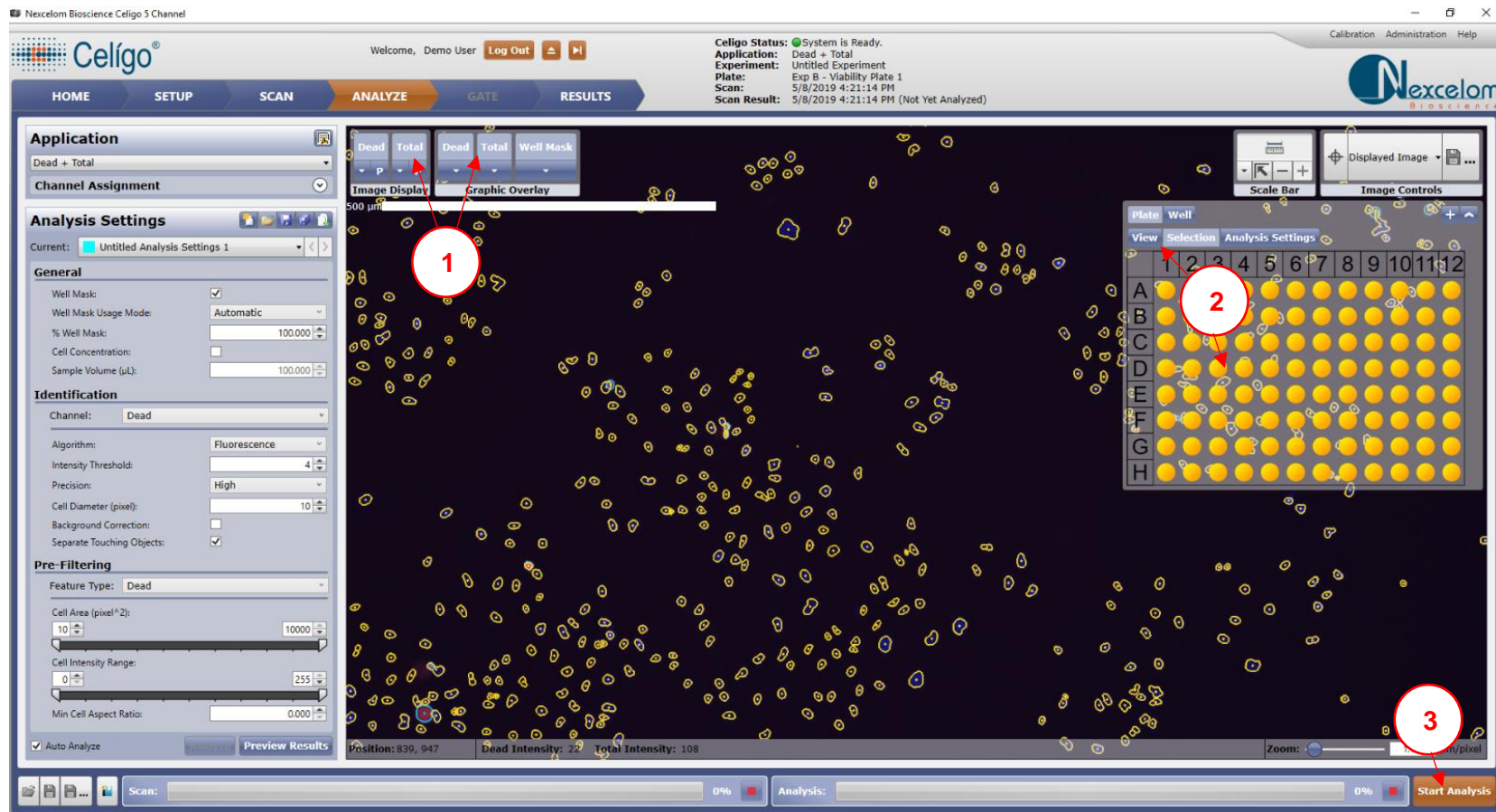


Separate Touching Objects will separate multiple objects that are close together identified as one into separate objects.



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

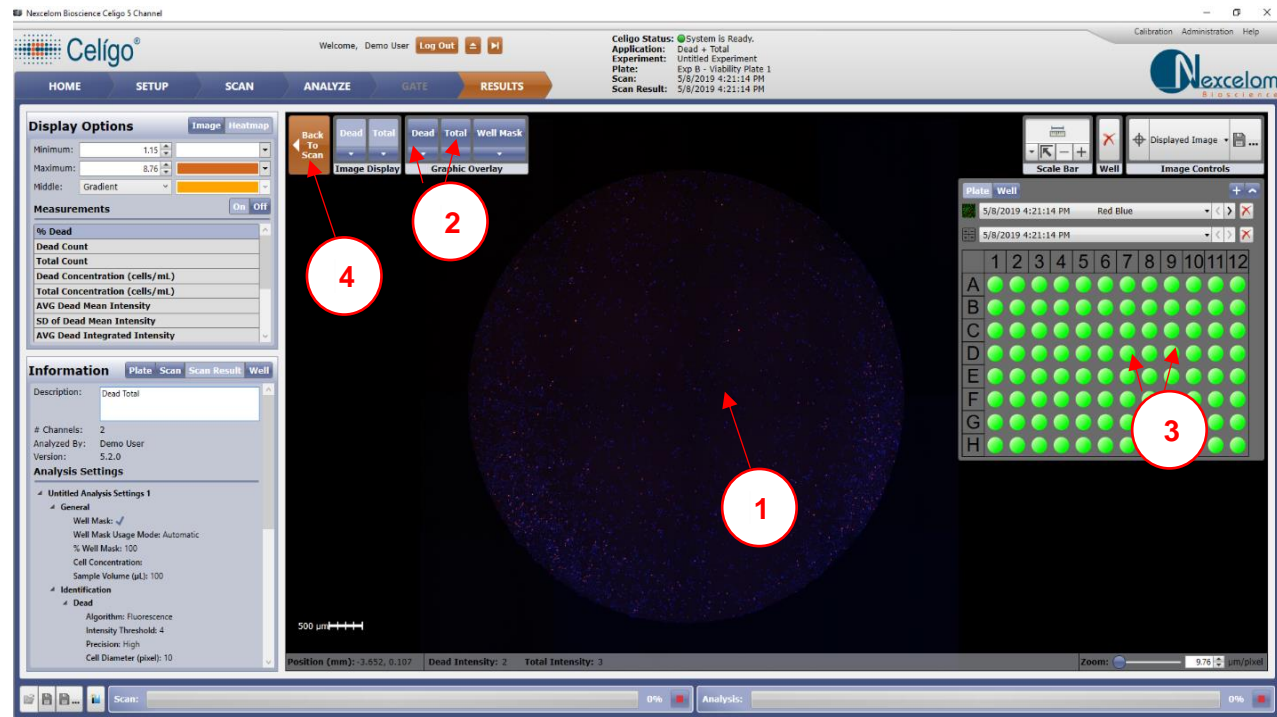
1. Select a different data Measurement. Data output displays on thumbnail image.
2. Double click a well to see high resolution image and graphic overlay of counted objects.

The screenshot displays the Celigo software interface in the RESULTS tab. The main window shows a grid of 96 wells (8 rows by 12 columns) with data overlays. The 'Display Options' panel on the left has a 'Measurements' list with a red circle '1' highlighting it. A 'Measurements' dropdown menu is open at the bottom, also with a red circle '1' highlighting it. A red circle '2' highlights a well in the grid. To the right, a zoomed-in view of a 3x3 grid of wells shows data overlays for rows A, B, and C.

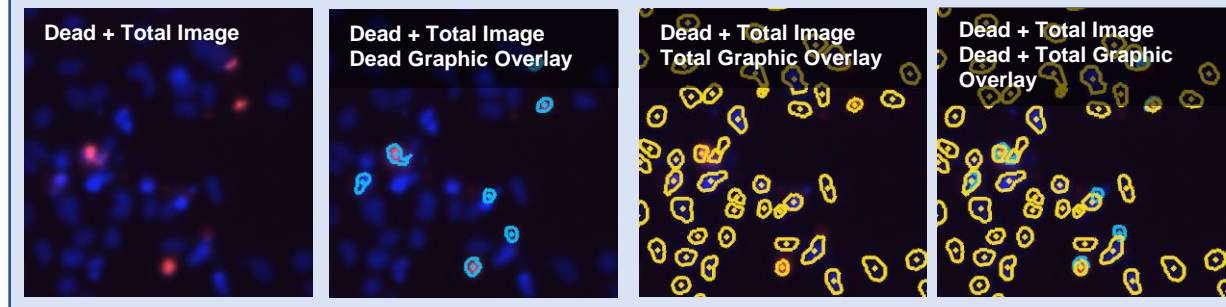
	1	2	3
A	0.91%	0.90%	0.93%
B	1.24%	1.14%	1.04%
C	1.69%	1.73%	1.92%

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	0	0	0	0	0	0	0	0
B	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014
C	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.041
D	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.123
E	0.370	0.370	0.370	0.370	0.370	0.370	0.370	0.370	0.370	0.370	0.370	0.370
F	1.111	1.111	1.111	1.111	1.111	1.111	1.111	1.111	1.111	1.111	1.111	1.111
G	3.333	3.333	3.333	3.333	3.333	3.333	3.333	3.333	3.333	3.333	3.333	3.333
H	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
	Drug A			Drug B			Drug C			Drug D		

The screenshot shows the 'Display Options' dialog box with the 'Heatmap' tab selected. The 'Minimum' value is set to 0.90 and the 'Maximum' value is set to 3.50. The 'Middle' option is set to 'Gradient'. Below this, the 'Measurements' panel is visible, showing a list of measurement types such as '% Dead', 'Dead Count', and 'Total Count'. A red circle labeled '3' points to this list. To the right, a heatmap grid is displayed with numerical values ranging from 0.00% to 10.00% across 12 columns and 8 rows (A-H).

Export Data Output Measurements

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

The screenshot shows the 'Export Well-Level Data' dialog box. The 'Export Location' is set to 'C:\Users\... \Documents\Celigo\Exports\'. The 'Export Format' is set to 'Plate based (*.csv)'. The 'Export' button is highlighted with a red circle and arrow labeled '4'.

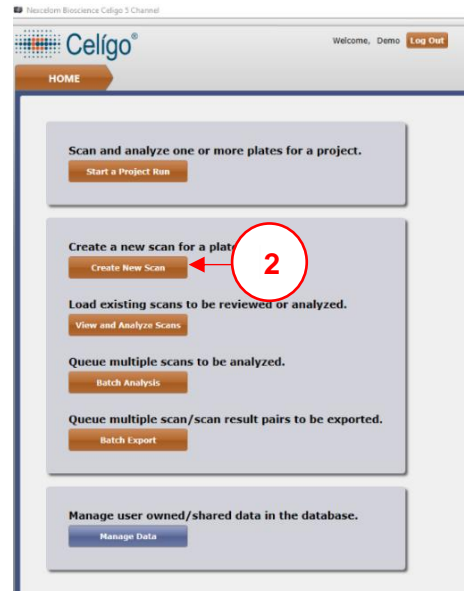
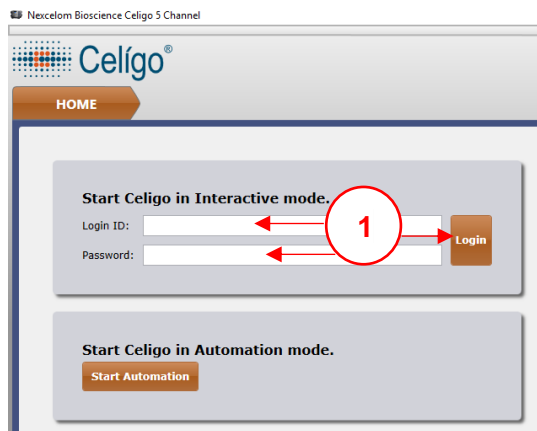
Chapter 5: Module 3 Cell Viability 3-Channel with Gating and Brightfield

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**.

Enter Plate Details

Plate Category: **96-Well**

Plate Profile:

Name	Manufacturer	Support
96-Well Nexcelom3D ULA-96U Plate	Nexcelom Bioscience	Supported
96-Well Nexcelom3D ULA-96U_Lot60939020_2017-3-29	Nexcelom Bioscience	Supported
96-Well Nunclon™ 167008 Plate	Nunc	Supported
96-Well Oris™ Plate 6.35 mm	Corning	Unsupported
96-Well PE CellCarrier™ 6055300 Plate	Corning	Supported
96-Well PE CellCarrier™ 6055300 Plate_257	Corning	Supported
96-Well PE CellCarrier™ 6055300 Plate_387	PerkinElmer	Supported
96-Well PE Isoplate™ 6005050 Plate	PerkinElmer	Supported
96-Well PE Viewplate™ 6005225 Plate	PerkinElmer	Supported
96-Well Seahorse™ XF96 Plate	Seahorse	Supported

Plate ID: **Exp 8 - Viability Plate 1**

Folder: Demo

Plate Description: (Optional)

Scan Description: (Optional)

Select Experiment

Experiment: (Optional)

Load Plate

Click OK once the plate has been loaded.

OK Cancel

SCAN Tab

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

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

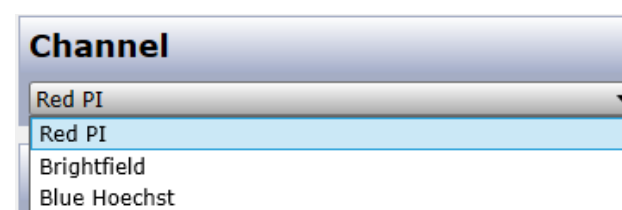
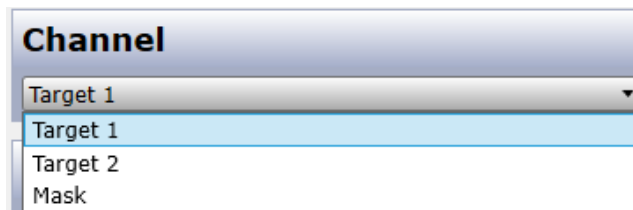
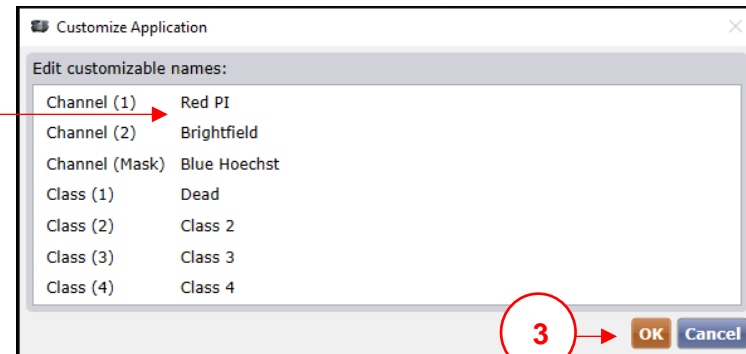
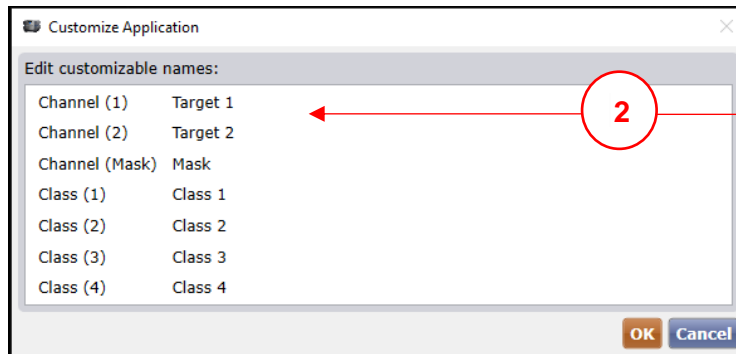
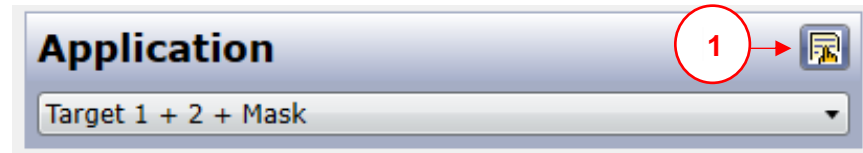
The image displays two screenshots of the Celigo software interface. The top screenshot shows the 'SCAN' tab with the 'Application' dropdown menu open, highlighting 'Target 1 + 2 + Mask'. A red circle with the number '1' is next to this option. The bottom screenshot shows the same interface with the 'Target 1 + 2 + Mask' application selected, displaying setup parameters on the left and a live image of a cell culture plate in the center. A green callout box labeled 'Plate & Well Navigation Area' points to a grid of wells on the image, with well A1 highlighted in blue.



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

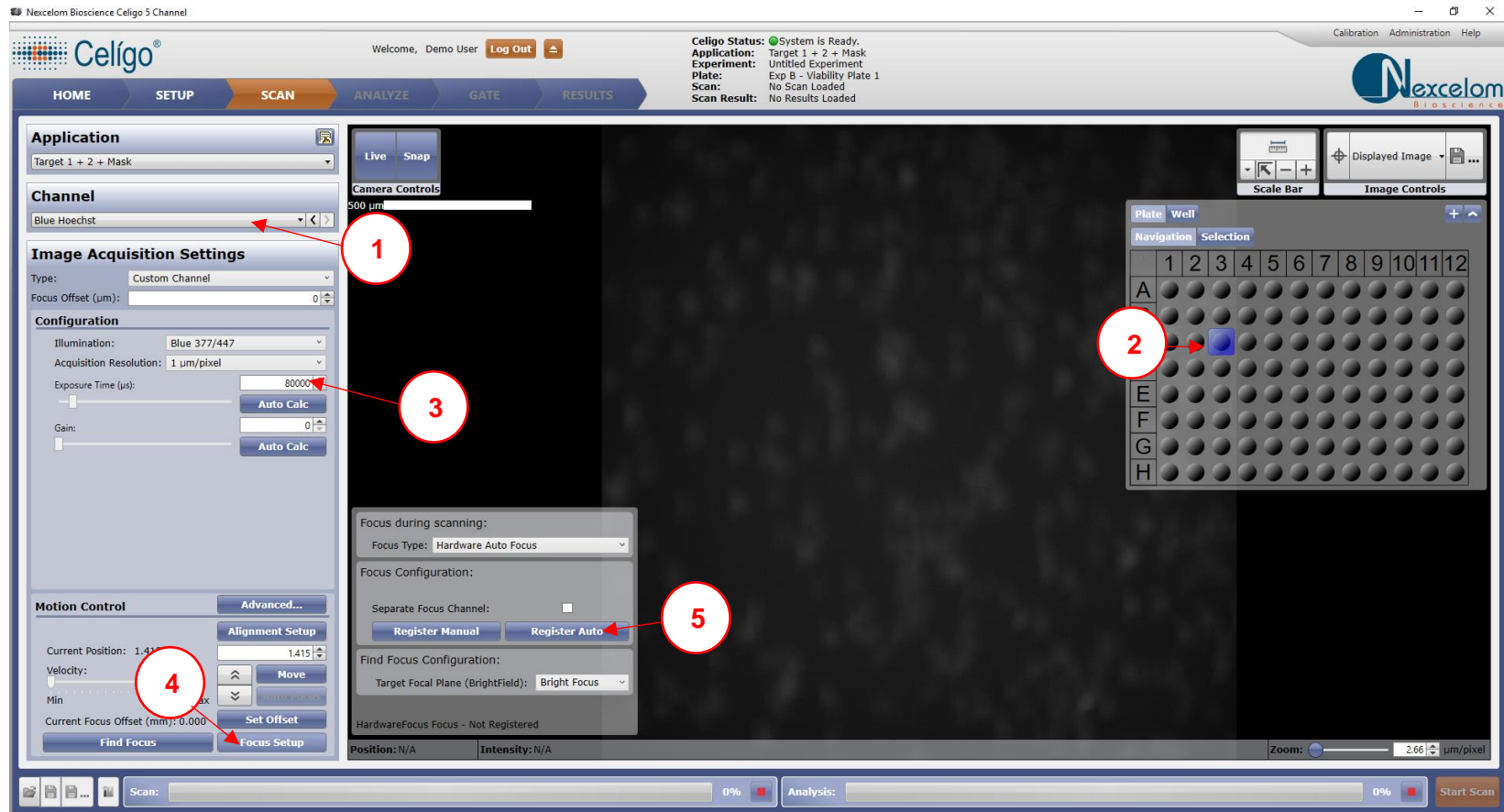
1. Click on  Icon in the Application section.
2. Change name of Channels Target 1, Target 2, Mask and Class 1 to Red PI, Brightfield, Blue Hoechst and Dead.
3. Click **OK**.



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

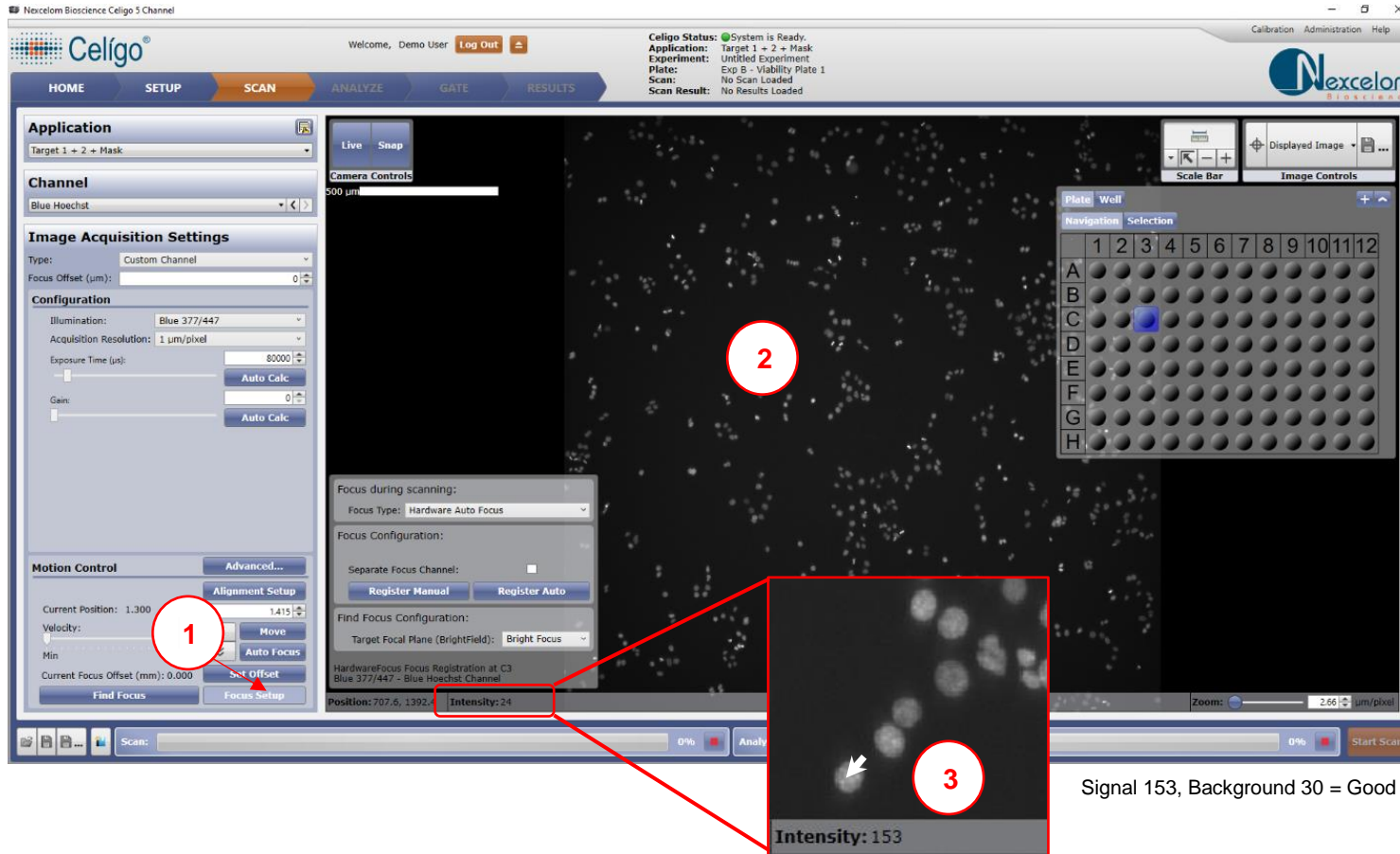
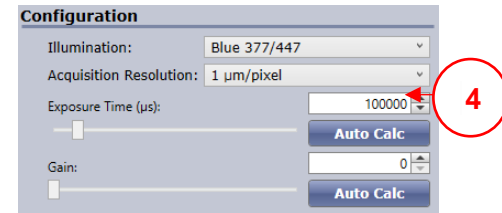
Setup Blue Hoechst Channel for Focus and Illumination

1. Change channels to **Blue Hoechst**.
2. Click on a **well** that has Hoechst stained cells.
3. Change Exposure to **80,000** μ seconds.
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.



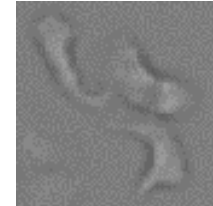
Setup Proper Focus and Illumination for Red PI channel

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

The image illustrates the steps for setting up the Red PI channel. It shows the 'Configuration' panel where the channel is set to 'Red PI' (1), exposure time is set to 30000 μ s (2), and the 'Find Focus' button is highlighted (3). The 'Motion Control' panel shows the 'Set Offset' button being clicked (4). The main software interface shows the 'Image Acquisition Settings' and 'Configuration' panels, with a red circle and arrow pointing to the 'Intensity: 198' value (5) at the bottom of the image, indicating that the dead cell pixel intensity is above 150.

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**.



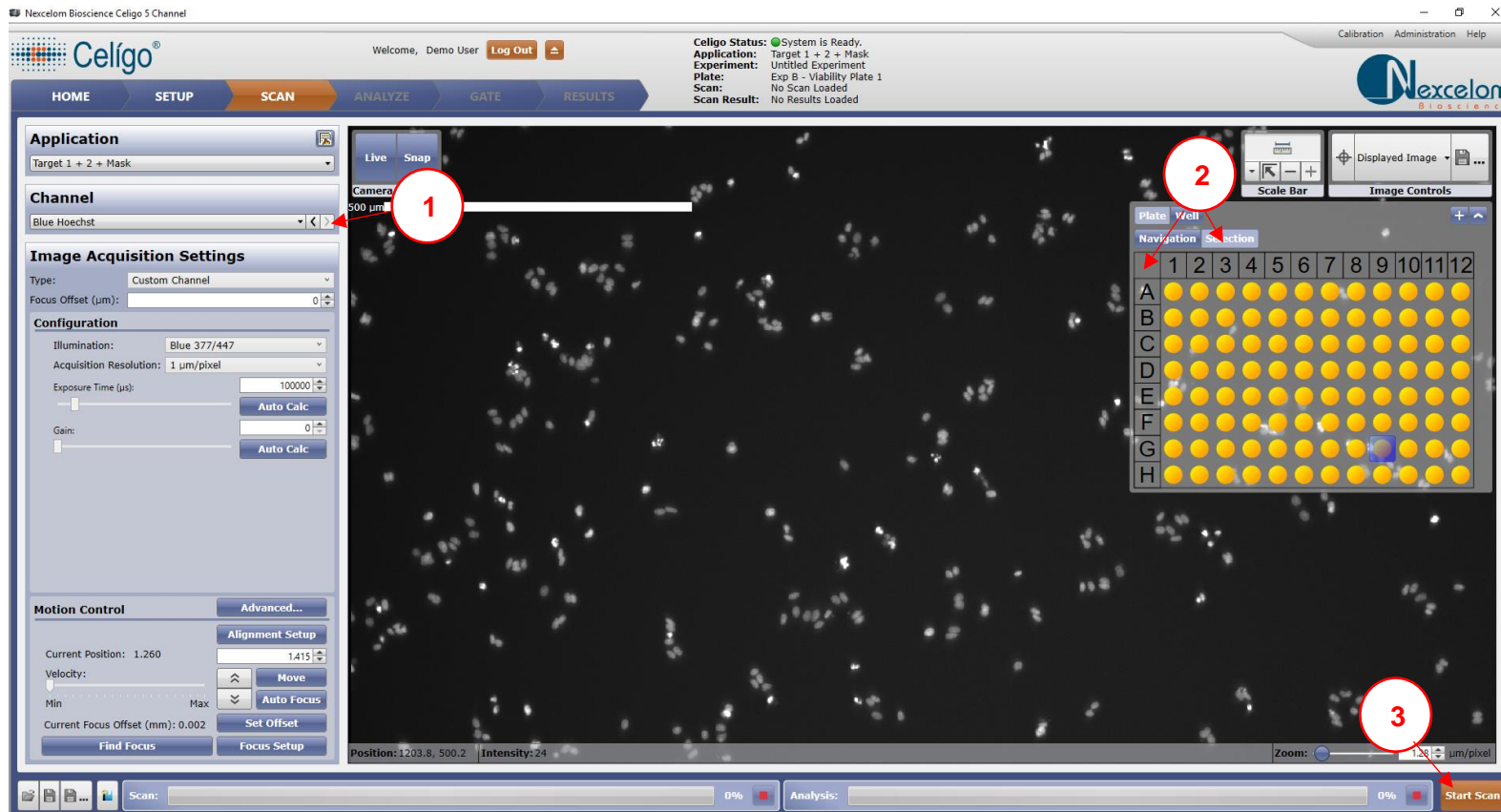
The screenshot shows the Celigo software interface with the following settings and actions highlighted:

- Channel:** Brightfield (Step 1)
- Image Acquisition Settings:** Type: Auto Exposure/Gain Channel (Step 2)
- Configuration:** Illumination: Brightfield, Acquisition Resolution: 1 µm/pixel, Priority: Auto Exposure, Gain if neces, Frequency: Every scan area. **Apply** button (Step 3)
- Camera Controls:** **Live** button (Step 4)
- Motion Control:** **Set Offset** button (Step 6)

Other visible elements include the Celigo logo, user information (Welcome, Demo User), system status (System is Ready), application details (Target 1 + 2 + Mask), and a plate grid (A-H, 1-12) on the right side of the main image area.

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**.

The screenshot displays the Celigo software interface in the ANALYZE tab. The main window shows a fluorescence image of cells. On the left, the 'Analysis Settings' panel is visible, with the 'Well Mask' checkbox checked and the 'Channel' set to 'Blue Hoechst'. On the right, the 'Plate Navigation' window shows a 12x8 grid of wells, with the 'Selection' tab active. The wells are colored green or grey. Red circles with numbers 1, 2, 3, and 4 highlight the 'Selection' tab, a green well, the 'Well Mask' checkbox, and the 'Channel' dropdown menu, respectively. The bottom of the interface shows a progress bar for 'Scan' (9%) and 'Analysis' (0%), along with a 'Start Analysis' button.



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.

The screenshot displays the Celigo software interface. The top navigation bar includes HOME, SETUP, SCAN, ANALYSIS, GATE, and RESULTS. The ANALYSIS tab is active. On the left, the 'Analysis Settings' panel is open, showing 'General' and 'Identification' sections. The 'Separate Touching Objects' checkbox is checked. The main image area shows a cell image with a yellow mask overlay. A 'Well Mask' panel is visible on the right, showing a grid of wells with one well highlighted in yellow. Red circles and arrows point to specific UI elements: 1 points to the 'Red PI' and 'Brightfield' channel selection buttons; 2 points to the 'Blue Hoechst' channel selection button; 3 points to the 'Separate Touching Objects' checkbox; 4 points to the 'Selection' button in the Well Mask panel; and 5 points to the 'GATE' tab in the top navigation bar.



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 (μm^2)** for parameter 1 (X-Axis).
6. Click **OK**.

The screenshot shows the Celigo software interface in the GATE tab. The main window displays a 96-well plate with a grid of yellow wells. A red circle labeled '1' points to well A3. A red circle labeled '2' points to the green plus icon in the 'Plots' panel. A red circle labeled '3' points to the 'ALL (Total)' dropdown in the 'Add Plot' dialog. A red circle labeled '4' points to the 'Histogram' dropdown. A red circle labeled '5' points to the 'Area (μm^2)' dropdown. A red circle labeled '6' points to the 'OK' button.

Celligo
Welcome, Demo User Log Out

Celligo Status: System is Ready.
Application: Target 1 + 2 + Mask
Experiment: Untitled Experiment
Plate: Exp B - Viability Plate 1
Scan: 5/8/2019 5:12:45 PM
Scan Result: 5/8/2019 5:12:45 PM (Not Yet Analyzed)

HOME SETUP SCAN ANALYZE **GATE** RESULTS

Red PI Brightfield Blue Hoechst On
Image Display Graphic Overlay

Scale Bar Image Controls

Plots Populations Classes Results

Plot Populations

1 4

1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H

1. Pick a source population: ALL (Total)
2. Pick a plot type:
3. Pick plot parameters:
Parameter 1 (X-Axis):
Parameter 2 (Y-Axis):

OK Cancel

1. Pick a source population: ALL (Total)
2. Pick a plot type: Histogram
3. Pick plot parameters:
Parameter 1 (X-Axis): Area (μm^2)
Parameter 2 (Y-Axis):

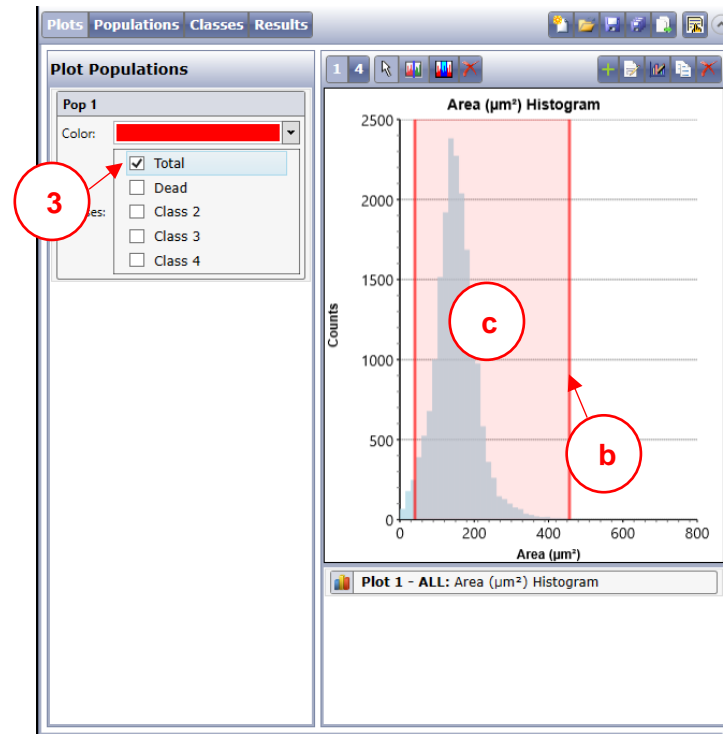
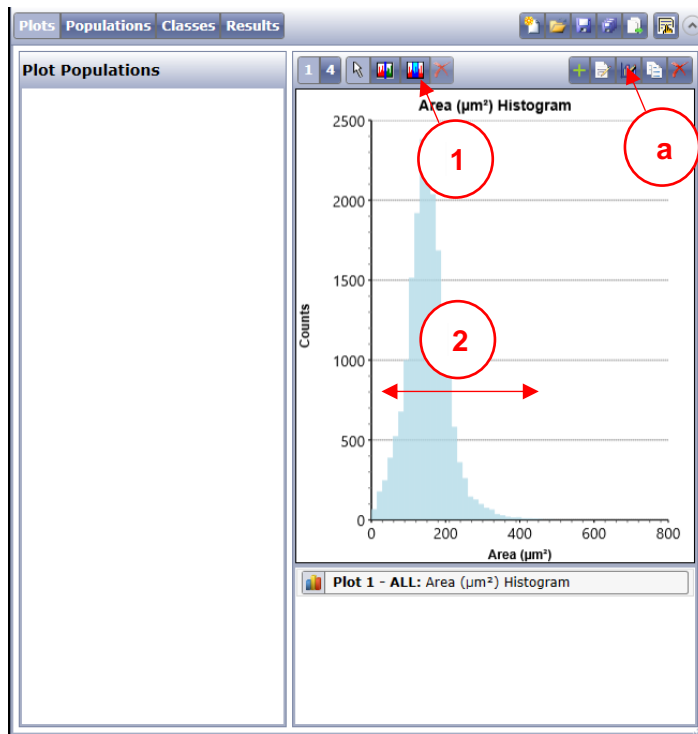
OK Cancel

Position (mm): -3.799, -2.665 Red PI Intensity: N/A Brightfield Intensity: N/A Blue Hoechst Intensity: 1

Scan: Start Analysis

Add a Minimum and Maximum Range Gate

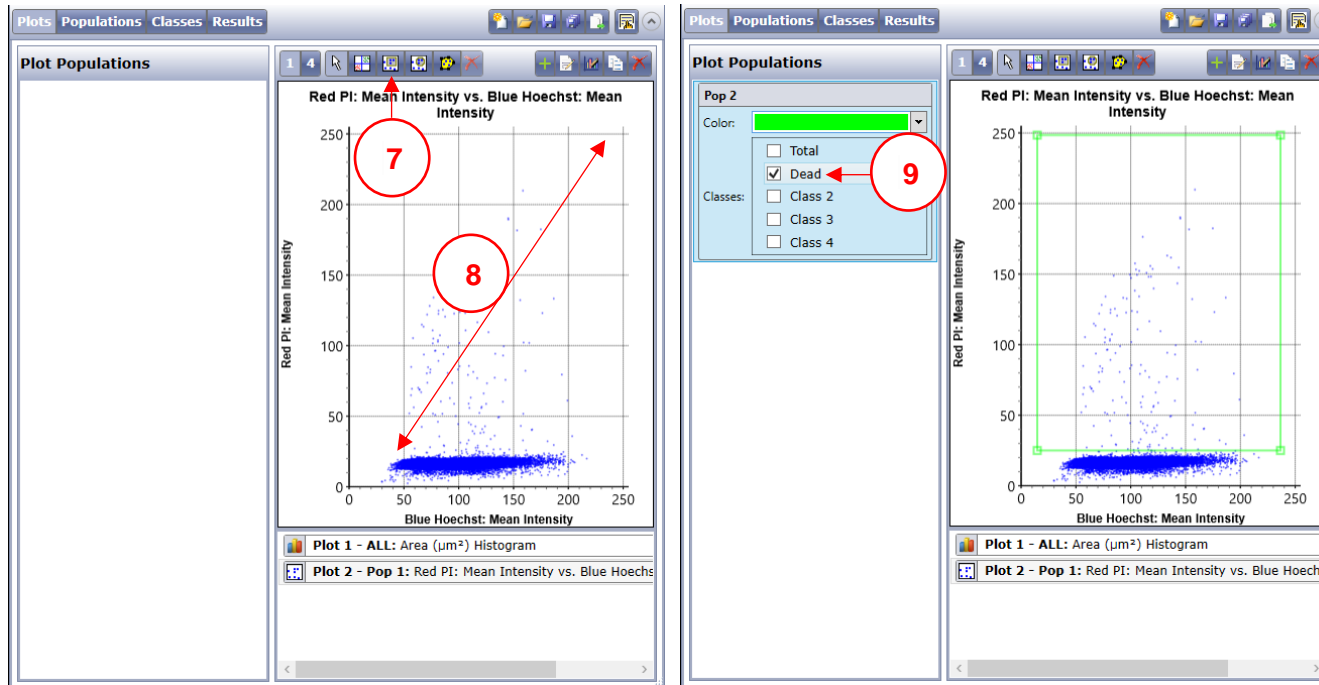
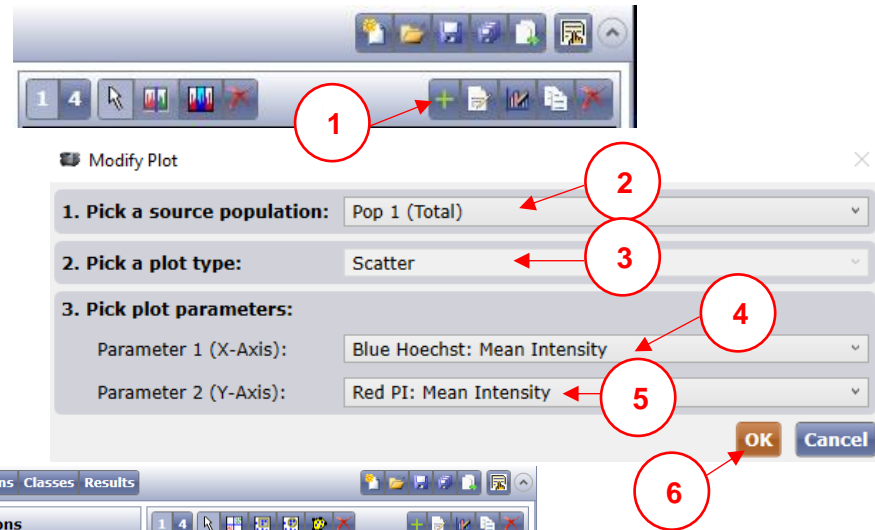
1. Select **Min/Max Range** gate.
2. Click and hold left mouse on left side of data peak, then drag to right and release on right side of data peak.
3. Check **Total** box.



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.

The screenshot shows the Celigo software interface. At the top, there is a navigation bar with tabs: HOME, SETUP, SCAN, ANALYZE, GATE, and RESULTS. The 'GATE' tab is currently selected. In the top right corner, there is a status bar with the following information: Celigo Status: System is Ready; Application: Target 1 + 2 + Mask; Experiment: Untitled Experiment; Plate: Exp B - Viability Plate 1; Scan: 5/8/2019 5:12:45 PM; Scan Result: 5/8/2019 5:12:45 PM (Not Yet Analyzed). The main workspace is divided into several sections. On the left, there is a 'Populations' panel with a dropdown menu for 'Pop 2' and checkboxes for 'Total', 'Dead', 'Class 2', 'Class 3', and 'Class 4'. The 'Dead' checkbox is checked. In the center, there is a scatter plot titled 'Red PI: Mean Intensity vs. Blue Hoechst: Mean Intensity'. The x-axis is 'Blue Hoechst: Mean Intensity' (0 to 250) and the y-axis is 'Red PI: Mean Intensity' (0 to 250). A green gate is drawn around the data points. On the right, there is a 'Plate' panel with a grid of wells (A-H, 1-12). The grid shows a pattern of yellow and blue wells. At the bottom, there is a status bar with the following information: Position (mm): 1.213, 0.248; Red PI Intensity: 17; Brightfield Intensity: N/A; Blue Hoechst Intensity: 42; Zoom: 1.32 $\mu\text{m}/\text{pixel}$. There are also buttons for 'Scan' (0%), 'Analysis' (0%), and 'Start Analysis'.

Observe Treated Well for Proper Gate Placement

1. Click on a **treated well**.
2. Zoom image in and out, move image around display area 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**.

The screenshot displays the Celigo software interface. At the top, the status bar shows 'Celigo Status: System is Ready' and 'Application: Target 1 + 2 + Mask'. The main interface is divided into several sections:

- Image Display:** Shows a cell image with a graphic overlay. A red circle labeled '3' points to the 'Graphic: Overlay' toggle, which is currently set to 'On'.
- Plots:** A scatter plot titled 'Red PI: Mean Intensity vs. Blue Hoechst: Mean Intensity' is shown. A red circle labeled '4' points to a green gate box on the plot. Below the plot, there are options for 'Plot 1 - ALL: Area (µm²) Histogram' and 'Plot 2 - Pop 1: Red PI: Mean Intensity vs. Blue Hoechst'.
- Plate Grid:** A grid of wells is shown, with a red circle labeled '1' pointing to a well in the grid.
- Image Controls:** Includes a 'Scale Bar' and 'Image Controls' section.
- Bottom Bar:** Shows 'Position (mm): 2.449, 0.576', 'Red PI Intensity: 15', 'Brightfield Intensity: N/A', 'Blue Hoechst Intensity: 36', and a 'Zoom: 1.04 µm/pixel' slider. A red circle labeled '5' points to the 'Start Analysis' button.

RESULTS Tab

Observe Plate Level Data Measurements with Heatmap

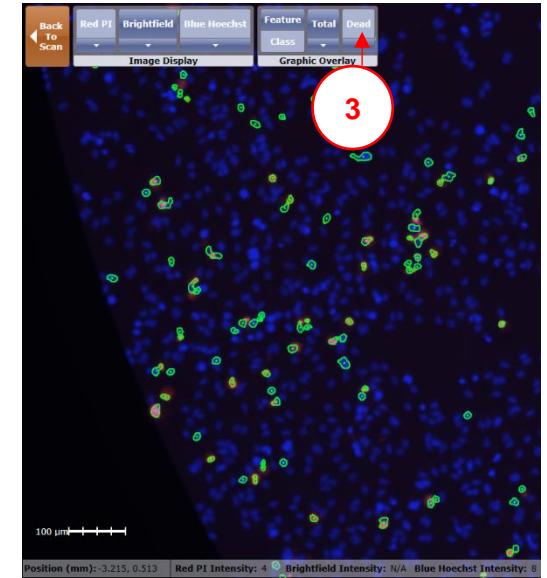
1. Click **Heatmap**.
2. Double click a well to view high resolution images. Single click a well to just view data in lower left display area, Information panel.

The screenshot displays the Celigo software interface in the Results tab. The top navigation bar includes HOME, SETUP, SCAN, ANALYZE, GATE, and RESULTS. The main area shows a heatmap of a 12-well plate with data values for each well. A red circle labeled '1' points to the 'Heatmap' button in the 'Display Options' panel. A second red circle labeled '2' points to a well in the heatmap grid.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.17%	1.06%	0.94%	0.82%	1.03%	1.06%	0.95%	0.97%	0.94%	0.99%	1.07%	1.01%
B	1.46%	1.18%	1.10%	0.92%	0.79%	0.83%	1.53%	1.67%	1.51%	1.00%	0.89%	1.05%
C	1.64%	1.78%	1.97%	0.75%	0.81%	1.08%	1.92%	1.50%	1.45%	1.00%	0.94%	1.09%
D	3.06%	3.06%	2.84%	0.88%	0.88%	1.05%	1.71%	1.90%	1.06%	1.10%	1.10%	1.17%
E	3.44%	3.98%	3.30%	0.84%	0.99%	0.97%	2.11%	2.03%	1.83%	1.19%	1.19%	1.22%
F	3.82%	4.44%	3.73%	0.90%	0.78%	0.92%	3.65%	3.51%	3.54%	1.57%	1.39%	1.48%
G	4.87%	4.38%	4.27%	0.96%	0.88%	1.05%	7.78%	7.14%	7.08%	1.59%	1.35%	1.75%
H	3.95%	4.26%	4.21%	1.90%	2.17%	2.36%	9.69%	8.72%	9.70%	0.98%	1.16%	0.96%

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**.

The screenshot shows the Celigo software interface. The top navigation bar includes HOME, SETUP, SCAN, ANALYZE, GATE, and RESULTS. The RESULTS tab is active, displaying a 12-well plate grid. The grid contains numerical data for each well, such as 1.17%, 1.06%, 0.94%, etc. A red box highlights the 'Export All Images', 'Export Well-Level Data', and 'Export Object-Level Data' buttons. A red circle with the number '1' points to the 'Export Well-Level Data' button. A dialog box titled 'Export Well-Level Data' is open, showing the 'Export Location' field with the path 'C:\Users\skesse\Documents\Celigo\Exports\' and the 'Export Format' set to 'Plate based (*.csv)'. A red circle with the number '2' points to the 'Export' button in the dialog box.



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 format 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 Nunc ULA-384U Plate	Nunc 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)	Nunc Bioscience	Clear	---
1-Slide Holder (square cover slip)	Nunc Bioscience	Clear	---
4-Slide Holder (2/3 cover slip)	Nunc Bioscience	Clear	---
4-Slide Holder (square cover slip)	Nunc 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 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).

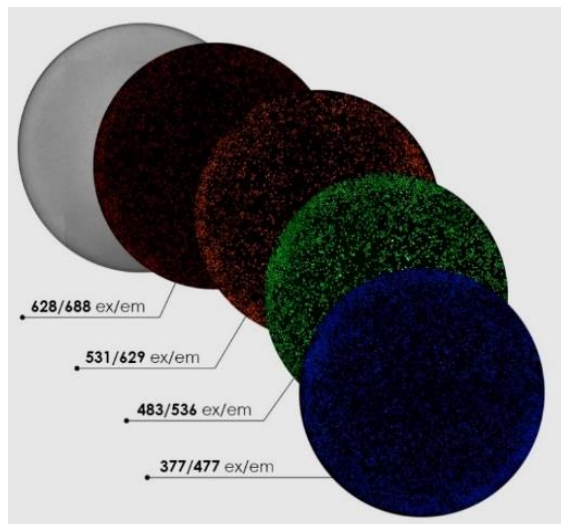
Plate Type	Recommended Final Volume (μL)	Cell Density Range
1536 Well	8	1 - 1,536
384 Well - Low Volume	20	1 - 2,000
384 Well - High Volume	50	1 - 4,000
96 Well - Full Area	175	1 - 20,000
96-well - Half Area	100	1 - 10,000
48 Well	250	20 - 50,000
24 Well	400	50 - 100,000
12 Well	1000	50 - 200,000
6 Well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

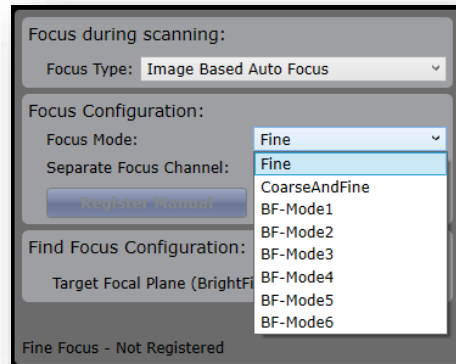
Reagent Name	Catalog #	Channel Color			
		Blue	Green	Red	Far Red
ViaStain™ AOPI Staining Solution	CS2-0106-5 mL		•	•	
ViaStain™ Hoechst / PI	CSK-V0005-1	•		•	
ViaStain™ Calcein AM / PI / Hoechst Cell Viability Kit	CSK-V0006-1	•	•	•	
ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture	CS1-V0002-1		•		
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

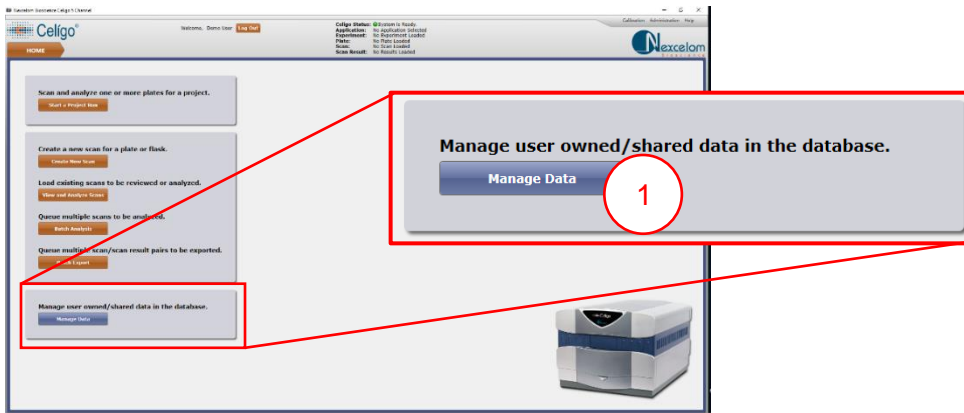
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Chapter 10: Data Management

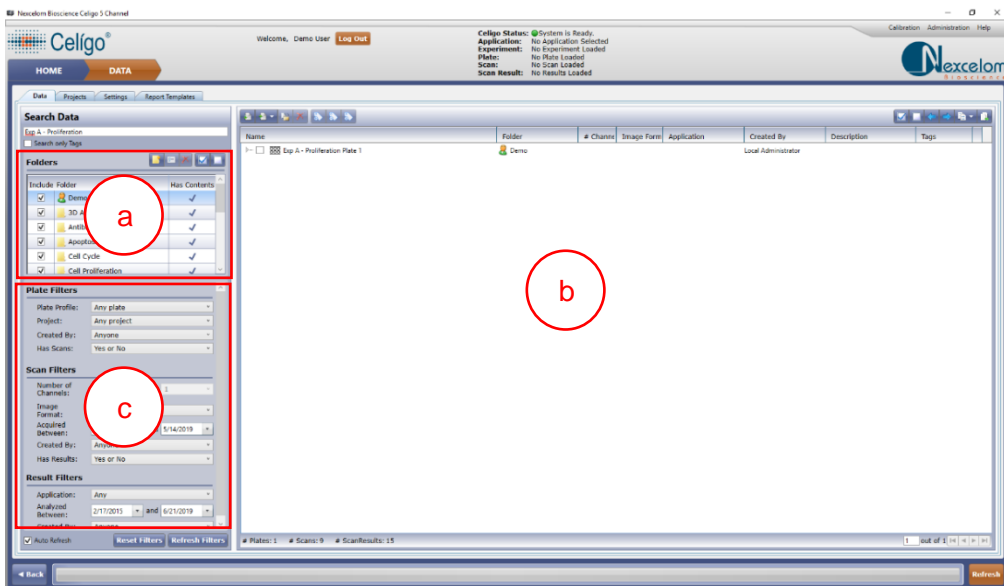
This is a quick start guide for Manage Data tab to show how to export, import and delete data. Settings can be imported, exported and deleted from this tab as well. For additional details, please see User and Administrator Guides.

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.

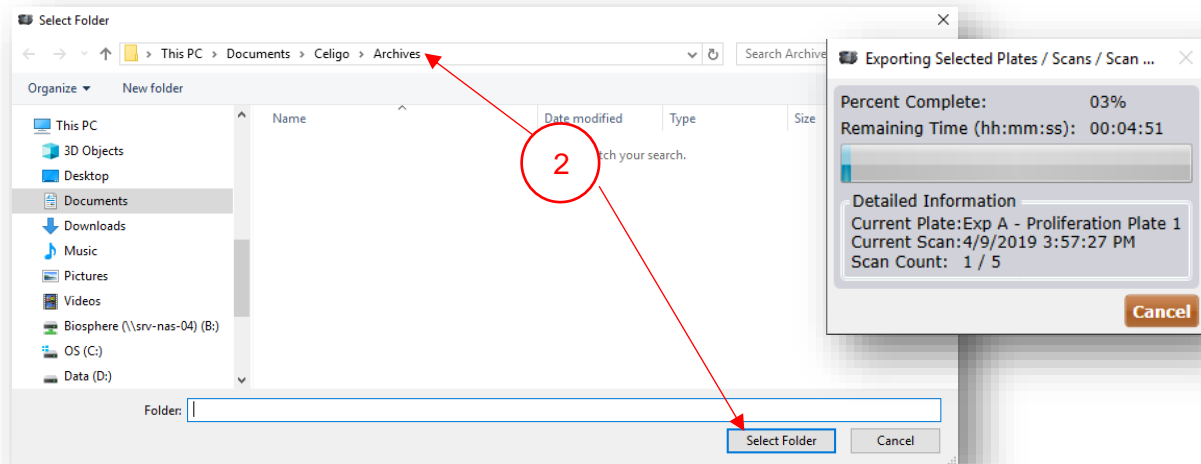
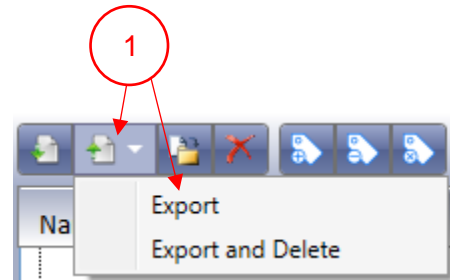


The screenshot shows the 'Data' tab of the software interface. On the left, the 'Search Data' section has 'Exp A - Proliferation' entered in the search field. Below it, a table lists folders with 'Demo' selected. The 'Plate Filters' and 'Scan Filters' sections are visible. On the right, three hierarchical views of data are shown, each with a red circle and number indicating a step: '2' points to the arrow next to the plate ID, '3' points to the arrow next to a scan entry, and '4' points to the check box next to a scan entry. A toolbar at the top right contains icons for search, expand, and other actions, with a red arrow pointing to the right arrow icon.

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**.

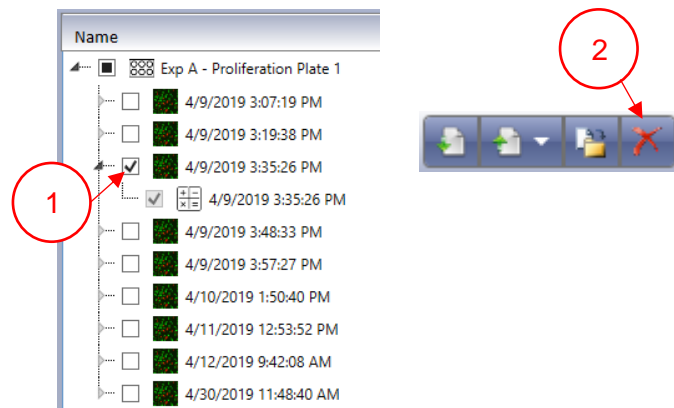
System will present a progress bar for data file export completed.



Delete Scans and Results

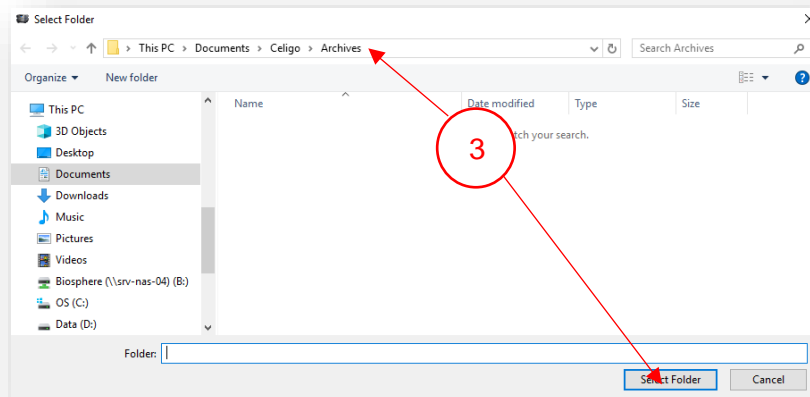
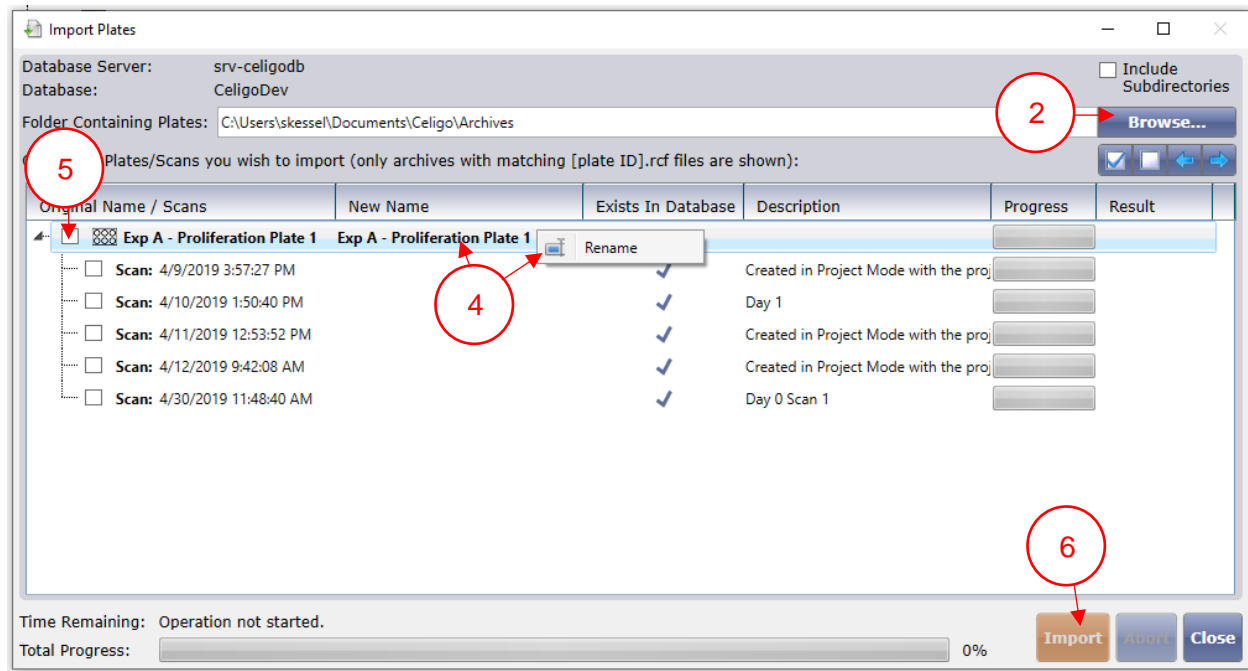
1. Click **Check Box** on Scan(s) and/or Scan Result(s) to be deleted
2. Click on **Delete** icon (red X)

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



Import Scans and Results

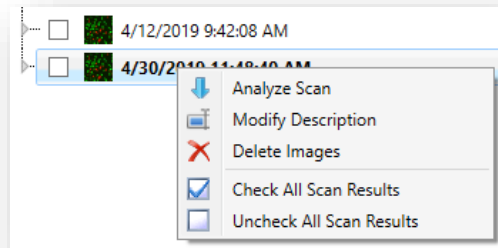
1. Click on the **Import** icon above the data file display area.
2. Click on the **Browse...** button.
3. Select the **Folder** where the data is located. Click **Select Folder**.
4. (Optional) Right-click on the **Plate ID name** in the New Name column, click **Rename** and enter the new name. Click 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

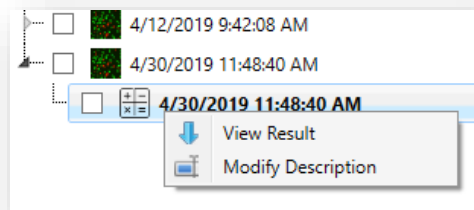
Load a Scan for Analysis

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.

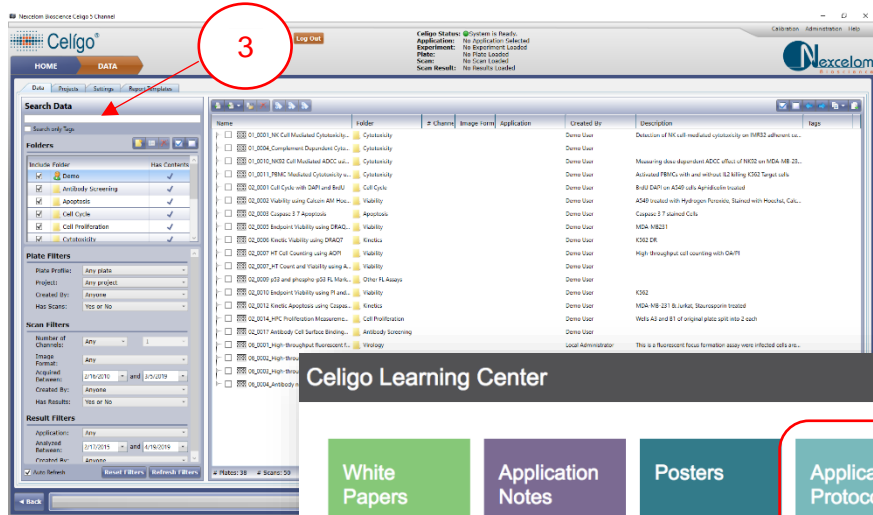


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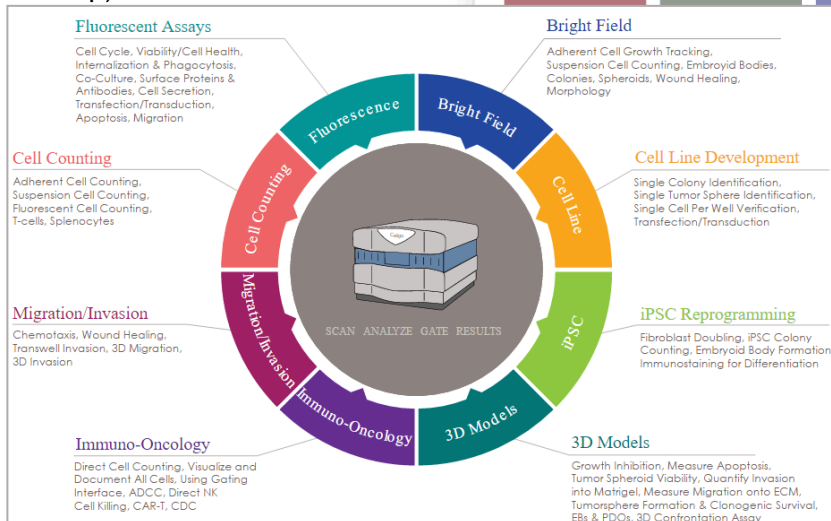
Chapter 11: Demo Data Scans for Review

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).



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Chapter 12: Additional Resources

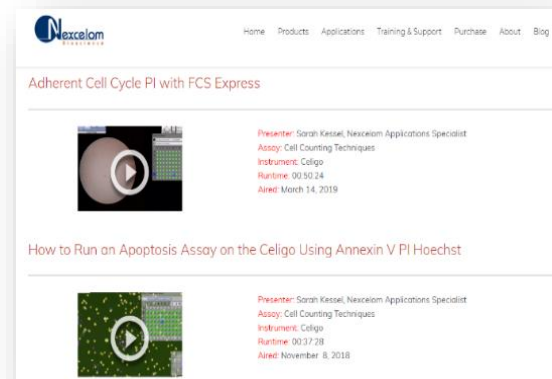
Celigo Learning Center:

(short cut on Celigo computer desktop)



Celigo Training Videos and Webinars:

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



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