

# Rare Cell Analysis Software

## Introduction

The Bio-Rad Rare Cell Analysis Software is a platform used in tandem with the Celselect Slides™ Enumeration Direct Stain Kits and Enumeration Indirect Stain Kits. It is designed to enable analysis of target cell populations using images and is compatible with multiple microscope models. The software allows users to create a custom algorithm to identify target cells with up to six fluorescent channels or five fluorescent channels and brightfield. These settings can be saved for reuse with future datasets or to share among users. Results can also be revisited and augmented using updated analysis algorithms.

The software also has the capability to generate a PDF report that includes information about and images of verified target cells. This report information can then be used for presentations and publications.

## Setup

### System Requirements

Rare Cell Analysis Software is supported on Windows operating system 10 (minimum 1903) and 11 versions.

### Microscopes with Supported Image Analysis Compatibility

Models
Agilent Lionheart
Keyence BZ-X
Leica DMI8
Nikon Eclipse
Olympus VS200
Zeiss Observer

- The software is compatible with a Windows platform that at minimum supports .NET Standard 2.0
- The recommended minimum RAM configuration is 2 GB
- Anti-virus or anti-malware software can be running simultaneously with the software

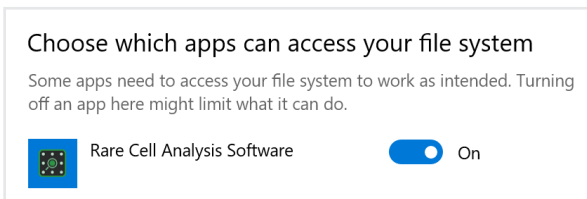
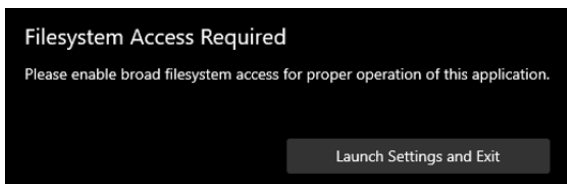
## Installation

### Installation on Windows OS

**Note:** Remove any previous versions, as older versions will interfere with installation.

1. Type “Add or Remove Programs” in the Windows search bar.
2. Click **Add or Remove Programs** and then type Rare Cell Analysis Software under the Apps and Features section.
  - a. If an older version of Rare Cell Analysis Software is not installed, continue to step 3a.
  - b. If an older version of Rare Cell Analysis Software is installed, uninstall the program before continuing to step 3a.
3. Certificate Installation
  - a. Download or obtain the installer file, dependency files, and certificate files.
  - b. Open the directory from the Windows Start menu by searching for the filename or by accessing the folder where the files are stored: .../RareCellAnalysis.UWP\_X.X.X.X\_x64.
  - c. Click the directory to open the location of the .cer file.
  - d. Double click the .cer file and click **Install Certificate**.

- e. Select **Local Machine** as the store location in the Certificate Import Wizard and click **Next**.
  - f. A prompt will appear; click **Yes** to allow the certificate to be installed.
  - g. Select **Place all certificates in the following store**.
  - h. Click the **Browse...** button.
  - i. In the pop-up window, select **Trusted Root Certification Authorities** and click **OK**.
  - j. Click **Next**.
  - k. Click **Finish**. A window notification will appear with: "The import was successful."
4. Install Dependencies
    - a. Install the following dependencies by double clicking the following files:
      - 1) Microsoft.NET.CoreFramework.Debug.2.2.appx
      - 2) Microsoft.NET.CoreRuntime.2.2.appx
 If an internet connection is not available, you will need to install these two dependencies manually:
      - 3) Microsoft.UI.Xaml.2.4
      - 4) Microsoft.VCLibs.x64.14.00
  5. Application Installation
    - a. Double click the .../RareCellAnalysis.UWP\_X.X.X.X\_x64.msixbundle file.
    - b. Click **Install**.
  6. Enable File Access
    - a. By default, this application does not have permission to access the active file system. If this setting is not enabled, the user will be prompted to enable this setting when the software is launched.
  - b. Click **Launch Settings and Exit** to open the Windows File Access Settings menu. To manually open this window, go to Windows Settings > Privacy > File System. Turn on file access for Rare Cell Analysis Software.

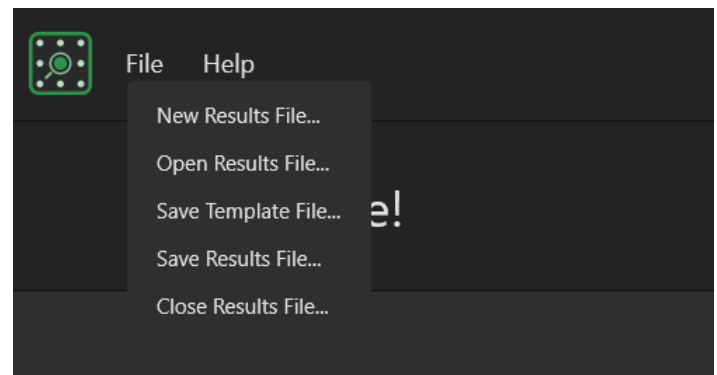


7. End User License Agreement (EULA)
  - a. After reading the EULA, click **Accept**.

### File Menus

Click **File** in the top left corner to access file options at any time. Options include:

- **Save Template File:** Saves selected parameters entered in the Template File Configuration screen
- **Save Results File:** Saves data on selected experiment. Parameters and reference images entered in the Document Information and Template File Configuration screen will be saved with the results. An asterisk at the top of the screen next to the Results File name indicates unsaved results
- **Close Results File:** Exits current experiment and relocates to the Start Menu
- **New Results File:** Begins a new image analysis setup with no saved parameters
- **Open Results File:** Opens existing file that already has analysis parameters set

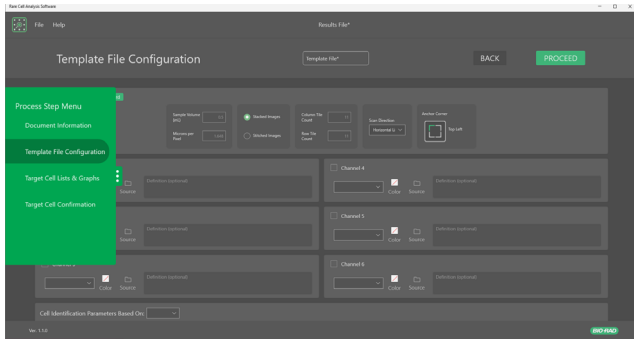


This screen also contains buttons to progress through the two main workflows. Select one of the following buttons based on intended operations:

- **New Results File:** For analyzing new images with an existing template file or from scratch. Selecting this will open the Document Information screen to enter experiment details
- **Open Previous Results File:** For revisiting or adjusting analysis on an experiment analyzed before. Selecting this will take the user to File Explorer, where a previous session may be reopened

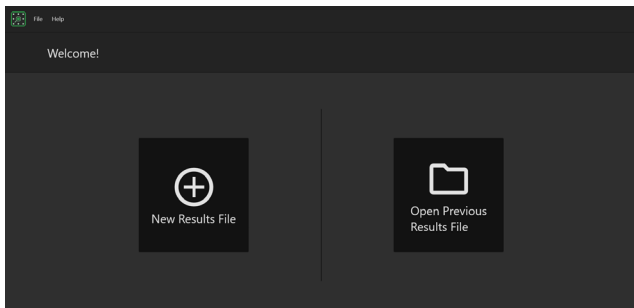
### Process Step Menu (Navigation Menu)

To navigate between analysis parameter screens, select **Proceed** or **Back** in the top menu. To choose specific steps for analysis, access the Process Step Menu by clicking the green tab with three circles on the left side of the screen. Click the page title to return to that step in the analysis process.



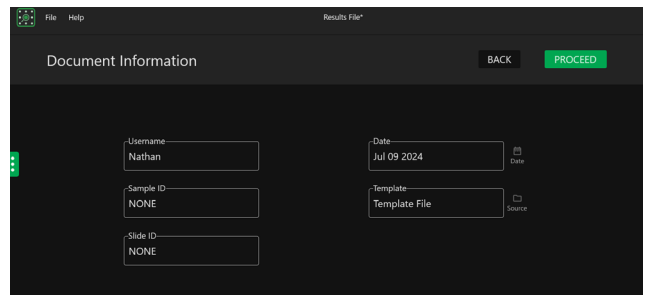
### New Results File

- To begin analyzing a new set of images, open the Rare Cell Analysis Software.
- On the Start menu, click **New Results File**.

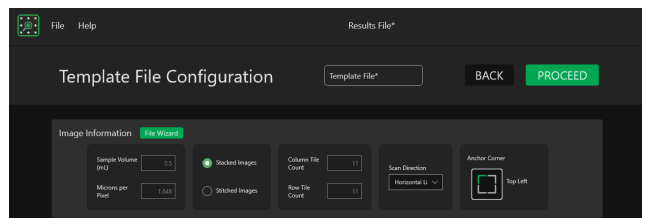


- In Document Information, enter the appropriate information.
  - Username: Open text field that autofills based on the current user directory (required).
  - Sample ID: Open text field that assigns the name of the sample being analyzed (optional).
  - Slide ID: Open text field for the user to enter the serial number of the Celselect Slides being analyzed (optional).
  - Date: Field for user to define a date, normally when an image set was collected or analyzed (required). The default setting will fill in the date on the computer being accessed. Select the calendar icon and select the date by clicking a day from the calendar navigation pane.

- Template: Allows the user to select a general set of established parameters saved as a file locally (optional). If a Template File is not selected, the parameters will need to be entered or optimized by the user. Click the file icon to navigate to a file of choice through a File Explorer window.

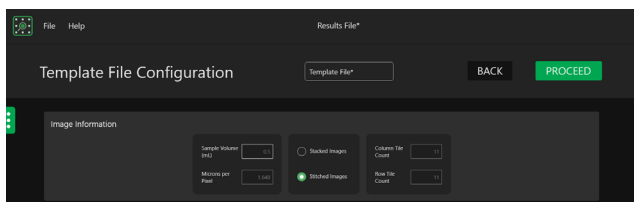


- Click **Proceed** to continue.
- In most cases, the settings for an image can be initiated from a Template file. This file can be loaded in the Document Information Page and may be imported or created by the user. Once these settings are established for a particular microscope, the image information should not need to be recreated. If this is the first Template file for a particular microscope, follow the steps below based on whether the microscope produces a mosaic of images (stacks) or one image for the entire slide (stitched):



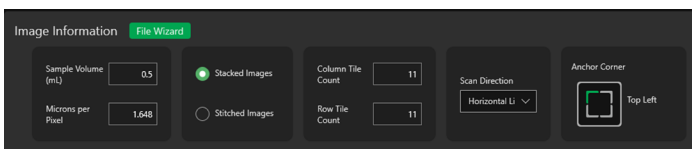
- Stacked Images:
  - Sample Volume: To calculate the concentration of target cells, enter the undiluted volume of sample that was loaded into the Inlet Funnel (mL, range 0.1–99).
  - Microns per Pixel: To calculate multiple metrics about cell size, enter the ratio of microns per pixel (range 0.2–2.0).
  - Tiles per Column: In image stacks, to correctly identify cells on the borders of multiple images, enter the number of columns that make up the entire scanned area (range 1–52).

- 4) Tiles per Row: In image stacks, to correctly identify cells on the borders of multiple images, enter the number of rows that make up the entire scanned area (range 1–52).
- 5) Scan Direction: In image stacks, to correctly identify cells on the borders of multiple images, enter the direction in which the image index increases. It can be left to right row after row (Horizontal Line), it can be left to right for one row and then right to left for the row underneath (Horizontal Zigzag), or it can be the same two fundamental patterns in the vertical direction: top to bottom for every column (Vertical Line) or alternating between top to bottom and bottom to top (Vertical Zigzag).
- 6) Anchor Point: In image stacks, in addition to knowing how many tiles there are and how they're arranged, the software needs to know where the images start. The user must select the corner where the first image of the series is positioned. The options are top left, top right, bottom left, and bottom right.

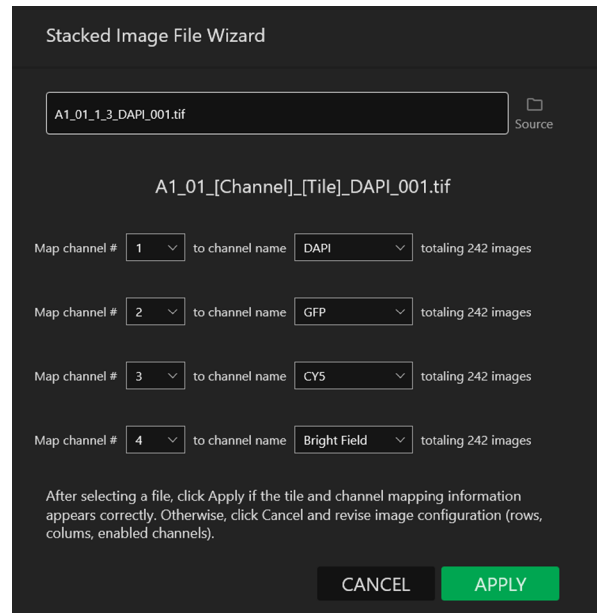


- 7) File Wizard: The File Wizard is used for determining the file pattern name associated with stacked images and can be used to automatically select and map stacked images to channel folders. Note that all of the stacked images should be placed in the same directory. Sub-directories are not included in the search.

After filling out the Image Information and Channel sections of the Template Configuration page, run the File Wizard by clicking the green File Wizard button.



Click the Source button and select one of the image files from your dataset.



Confirm the detected file pattern matches what is expected. For example, A1\_01\_[Channel]\_[Tile]\_DAPI\_001.tif should be displayed for European Visitor datasets with the positions of [Channel] and [Tile] correctly identified.

Select a mapping for the images. By default, the displayed image will map image files with filenames indicating channel 1 to the first enabled channel, channel 2 to the second enabled channel, etc. Both the channel as indicated in the filename and the enabled channel to map to may be changed as necessary toward the expected mapping. Select **Do Not Map** from the dropdown list to not map any images files indicating that channel number.

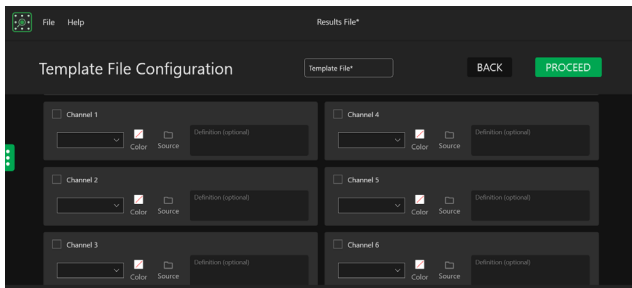
Click **Apply**.

- b. Stitched Images:

- 1) Sample Volume: To calculate the concentration of target cells, enter the undiluted volume of sample that was loaded into the Inlet Funnel (mL, range 0.1–99).
- 2) Microns per Pixel Ratio: To calculate multiple metrics about cell size, enter the ratio of microns to pixels (range 0.2–2.0).
- 3) When using a stitched image, adjust the tile and row count to optimize processing. For best practices, use the same numbers as you would for the stacked images.

6. Scroll down to the Channel configuration.
7. Select the number of channels necessary for the assay by clicking the checkbox next to the channel number. **(Note:** limited to six channel fluorescent markers.)
8. Select the dropdown box to choose the fluorescent marker used for each channel. If a custom marker is being used, select **Custom** and enter the name of the fluorescent label.
9. Click the Source folder to upload the corresponding images for each channel.

**Note:** If using image stacks, all scan files affiliated with the channel must be entered at the same time by selecting the image files while holding the Ctrl and/or Shift keys.



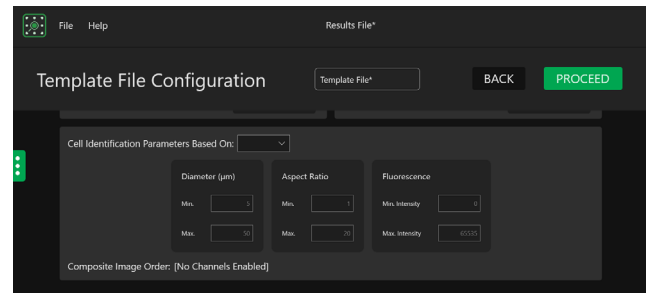
10. An optional text box is available to enter any sample information that may be helpful for reference later. For example, a user may want to associate a specific channel with a protein or other label of interest.

**Total Cell Identification**

The initial analysis begins with identification of all objects that may be cells. This is done by selecting one universal channel and the parameters of interest that will define potential cells.

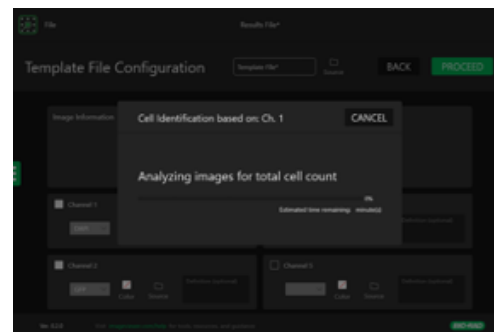
1. Scroll down to the Cell Identification Parameters box, select the desired channel, and enter in the desired values for the following cell identification features:
  - a. **Diameter:** Using the Microns per Pixel Ratio entered in the Image Information section, the software will identify objects based on their outer diameters. For optimal analysis, select a potential range of cell diameters. Enter a value for the range minimum and maximum (maximum range 1–50  $\mu\text{m}$ ).
  - b. **Aspect Ratio:** Using the long and short axis of each object, the software will identify objects based on the aspect ratio of their long-to-short dimensions. For optimal analysis, select a potential range of aspect ratios. Enter a value for the range minimum and maximum (maximum range 1–20).

- c. **Fluorescence Intensity:** Using the intensity of an object in a specific channel, the software will identify objects for further analysis. For optimal analysis, select a potential range of fluorescence levels. Enter a value for the range minimum and maximum (maximum range 1–65535).

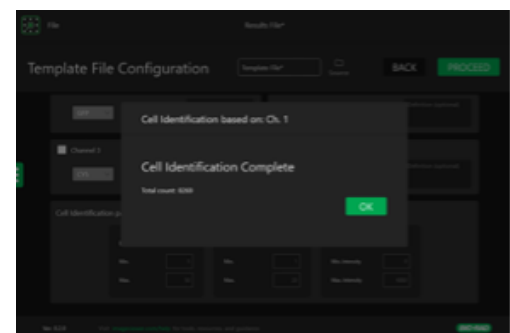


2. Click **Proceed** when ready.
3. A loading screen will appear with the estimated time to analyze images and identify potential cells.

**Note:** The maximum cell count that can be detected is 100,000 potential cells. This prevents the user from using incorrect settings, which would result in a runaway indefinite processing time.



4. After the analysis time has completed, a new screen will appear. Click **OK** to continue.



### Target Cell Identification

In the Target Cell Identification Lists & Graphs page, information can be viewed on the cells identified previously. Additional analysis tools in the Charts menu can be used to narrow search parameters to specific target cell characteristics in one or more of the selected channels.

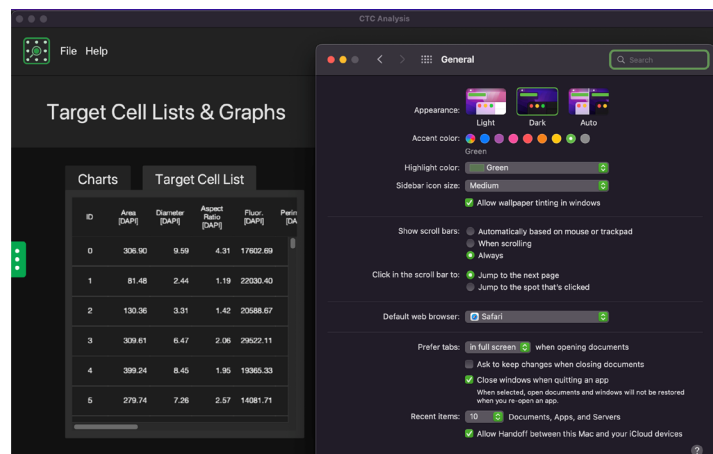
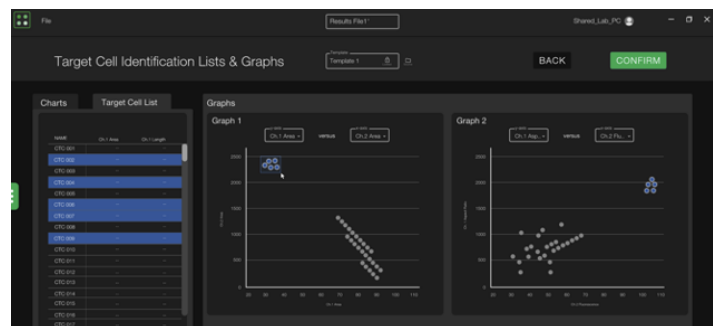
- Click the Charts tab to bring up options for:
  - Area: Using the Microns per Pixel Ratio entered in the Image Information section, the software will identify objects based on their areas. The range may have a minimum, a maximum, both, or neither (maximum range 1–99999  $\mu\text{m}^2$ ).
  - Diameter: Using the Microns per Pixel Ratio entered in the Image Information section, the software will identify objects based on their outer diameters. For optimal analysis, select a potential range of cell diameters. The range may have a minimum, a maximum, both, or neither (maximum range 1–50  $\mu\text{m}$ ).
  - Aspect Ratio: Using the long and short axes of each object, the software will identify objects based on the aspect ratio of their long-to-short dimensions. For optimal analysis, select a potential range of aspect ratios. The range may have a minimum, a maximum, both, or neither (maximum range 1–20).
  - Fluorescence Intensity: Using the intensity of an object in a specific channel, the software will identify objects for further analysis. For optimal analysis, select a potential range of fluorescence levels. The range may have a minimum, a maximum, both, or neither (maximum range 0–65535).
  - Perimeter: Using the Microns per Pixel Ratio entered in the Image Information section, the software will identify objects based on their outer perimeters. For optimal analysis, select a potential range of cell perimeters. The range may have a minimum, a maximum, both, or neither (maximum range 1–99999  $\mu\text{m}$ ).

**Note:** Fluorescence settings are unavailable for brightfield.

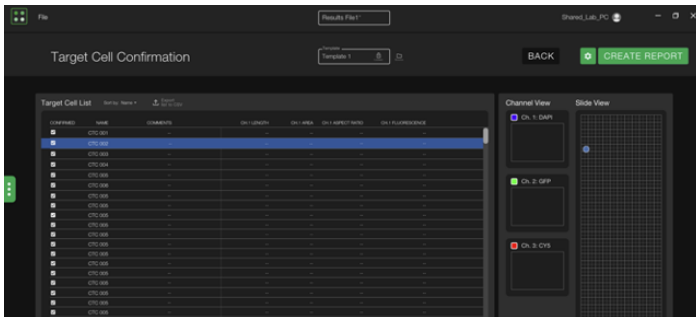
- Click **Refresh Filtering** next to the Graphs heading to update the targeted cell selection in the Identified Target Cell Count.
- In the Graphs tab click the dropdown menu to select which axes will be shown for the target cells, given the ranges of the parameters set earlier.



- In the Target Cell List tab, select the single cell or group of cells by clicking the row to view the fluorescence and morphological data. Selected cells will be highlighted in the Charts tab and in Graphs. Cells can also be selected on the graph, and their corresponding rows in the table will be highlighted.



5. Clicking the gear in the Graphs section opens additional options.
  - a. Copy Graph to Clipboard: Copies the graph as an image for use in presentations and publications.
  - b. Open in Expanded Window: Enlarges the selected graph in a separate window for additional manipulation.
  - c. Edit Graph Axes: Allows the user to alter the range of the parameters shown on the graph without changing the filter parameters.
  - d. Add New Graph: Creates an additional graph in the same workspace. Up to four graphs can occupy the workspace at one time. These all showcase the same selected data but different axes can be showcased in each separate graph.
  - e. Remove Graph: Deletes the currently selected graph.
6. After the parameter ranges are finalized, click **Proceed** to advance to the Target Cell Confirmation page.
7. In the Target Cell Confirmation page, after reviewing the cell data, users can select or deselect individual cells or groups of cells that do not match desired cell identification features. Information about each potential cell is listed, as well images of the cell in all channels selected and the cell's location translated onto a representative grid of the stitched image or stack montage. Click the checkbox next to the target cell to confirm or cancel the item.



8. This table of cell data can be exported into a .csv file for additional statistical analysis or other forms of manipulation in third-party software.

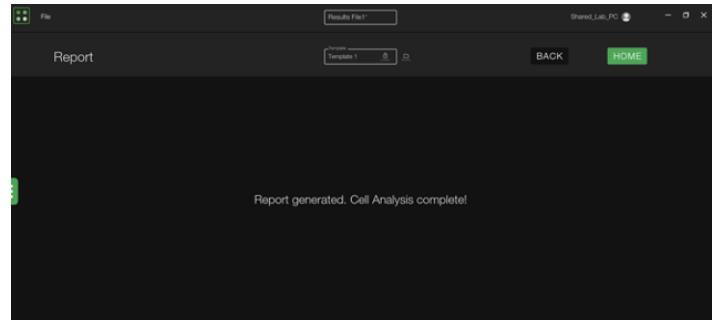
### Generate Report

When analysis is completed, a PDF report of the selected target cells can be generated.

Click the gear toolbox to open Report File Settings to set up:

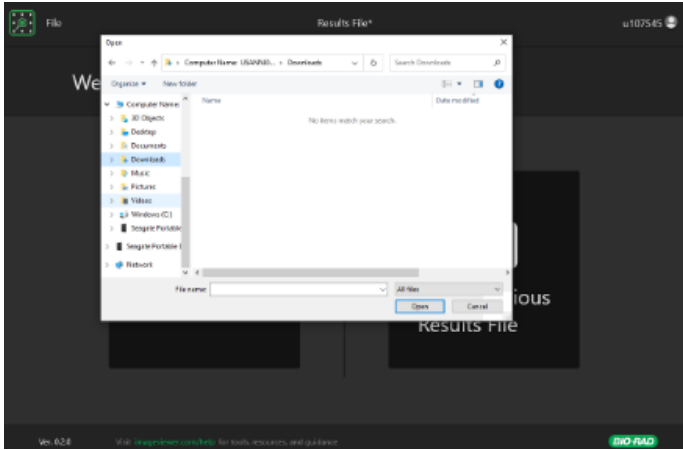
1. Location: The folder where the report will be saved.
2. Report Logo: A user-defined image that will be in the header of the report.
3. Header Configuration: Contains fields in the report that will be specific to the user, including the company/lab name, its website, and a potential report title.

After the PDF report is complete, a confirmation message will appear. Click **Home** to return to the Welcome page. Click **Save Results File** to save all the settings and image associations (just the thumbnails, not the entire files) as a single file. This can be done at any time while setting parameters. In addition, saving the settings by clicking **Save Template File** will allow the same metrics to be used to analyze other datasets.



### Open Previous Results File Option

1. In the Welcome page, click **Open Previous Results File** to open past experiments to edit.



2. Locate the desired file and click **Open**.
3. Users will be transferred to the Document Information page.
4. Click **Proceed** or use the Navigation bar to be transferred to the Template File Configuration page to make any necessary changes to the image source, color, channel, or cell identification parameters. After adjusting these parameters, the settings may be saved as a Template File or the modified findings can be saved as a Results File.
5. Click **Proceed** or use the Navigation bar to be transferred to the Target Cell Identification Lists & Graphs page to review data or generate a PDF report.

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