Data Analysis with eSight Software

This quick reference guide describes the data analysis features within the xCELLigence RTCA eSight software (310100310).

To analyze the data on a control unit (the computer controlling an instrument), it is suggested to open a new instance of the software. Note that it will automatically open in "Offline mode". This enables the existing window to be used for running a new experiment.

If you would prefer to move the data to another computer for analysis, you will first need to create an archive. For this, select **System > Archive** and follow the prompts to create an archive folder for your experiment(s). Then, copy this archive folder to the second computer (the archive generation can take several minutes to tens of minutes depending on the amount of data).

Image analysis

Image analysis in eSight is performed as a three-step process:

1. View the captured images and create the training images in the Image Browser tab.

2. Define the processing method for the selected training images in the Image Processing tab.

3. Analyze and view the analysis results in the Image Analysis/Data Analysis tabs.

Further details are provided as follows:

Image Browser tab

Select the date and time the image was captured (Figure 1A). Select the well position where the image was captured (Figure 1B). If multiple images were captured in the same well, select the image number (just above the well selection map). The corresponding image will be displayed in the middle of this page. To turn on/off various channels, check/uncheck the checkbox in front of Bright-field, Red, Green, or Blue in the image channel tab (Figure 1C).

The Plate View checkbox (above the well selection map) allows the user to view a montage of all the wells in the plate. Users can also scroll down and quickly view all the captured images in a smaller size to the right of the main window in this page.
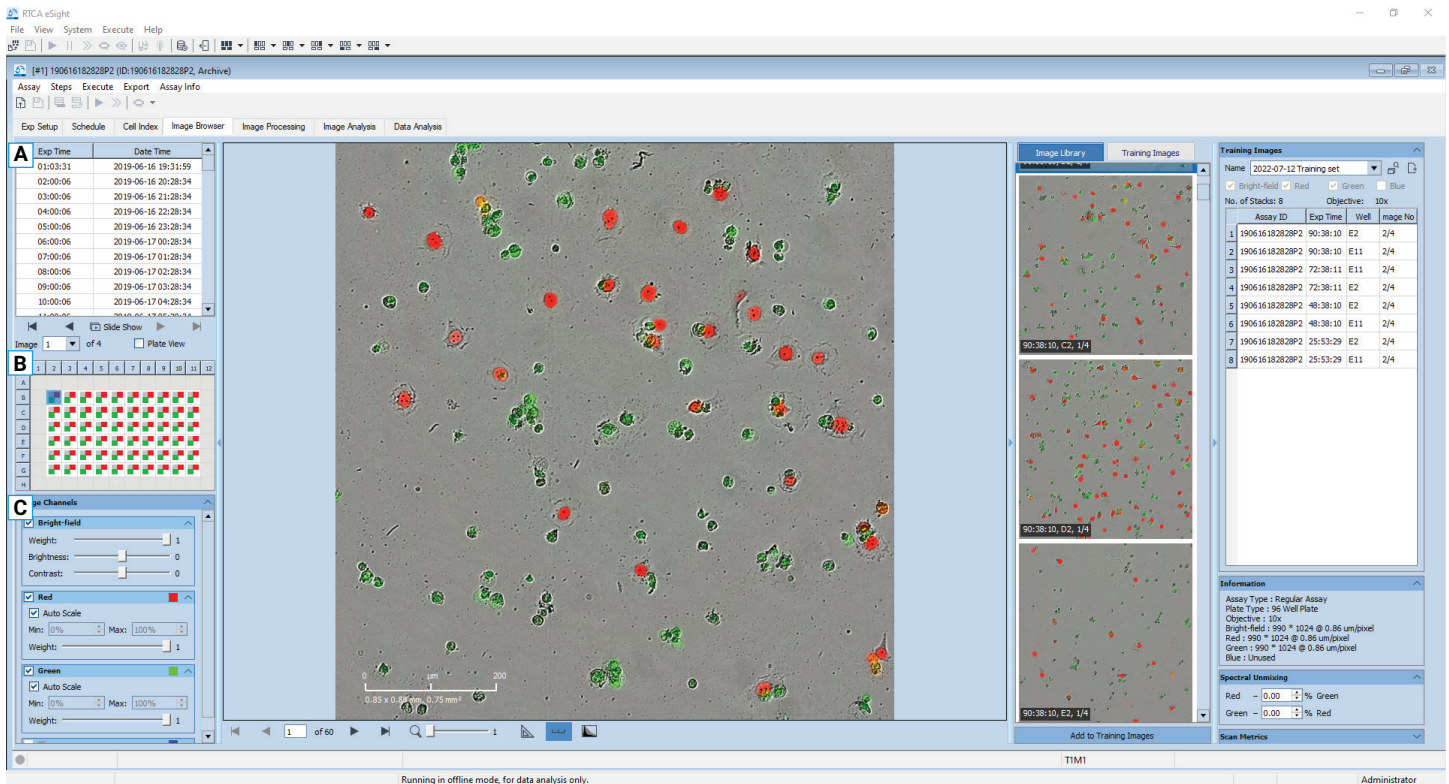
Figure 1. Viewing the captured images in the Image Browser tab.

The imaging processing parameters are optimized using a subset of images called Training Images. Click ⊡ in the Training Images pane (Figure 2A) to create a new training image collection. Users may select one or more fluorescence channel when creating the new training image set (selecting all is recommended). Click **OK** to finish creating the training image set then proceed to populate the set. Adding nine to 15 images is generally recommended–one image each for the no treatment, highest treatment dose, and an intermediate treatment dose (three images per timepoint). Further, it is recommended that several timepoints be chosen (e.g., one image just before treatment addition, and one set of three images as described above) for every 24-hour period post-treatment addition.

To add an image to the collection, right-click on the desired image in either pane and select **Add to '*Training Image Set*'** (Figure 2B). The information for all the images added to the Training Images will be displayed in the Training Image window (Figure 2C).
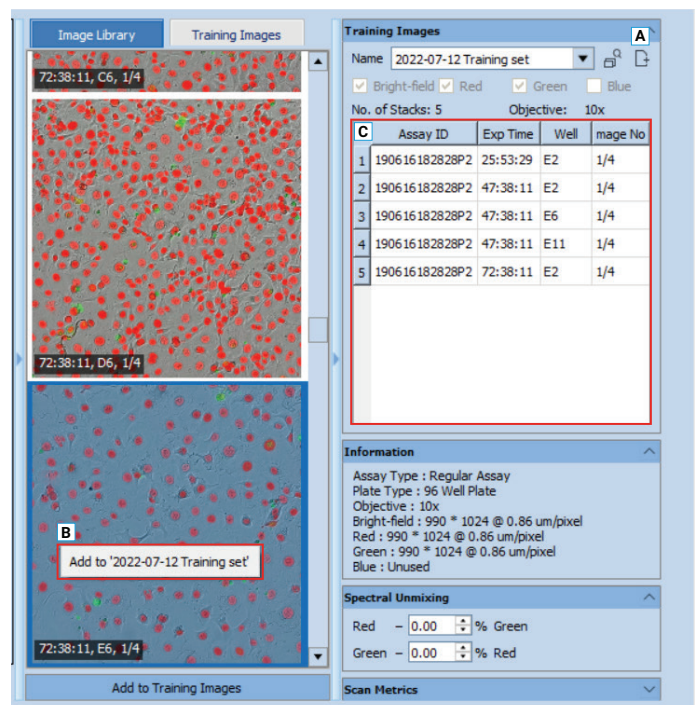

Figure 2. Adding images to the Training Image collection.

Image Processing tab

1. Select the training image set in the Training Images tab drop-down menu (Figure 3A).

2. Create a new Processing Method by clicking [icon] in the left pane. Enter the name and select the channels (Figure 3B). At this stage, it may be beneficial to select only the channel of interest to minimize the processing time (though multiple channels may also be selected).

3. Click **Preview Current** (Figure 3C) to view the segmentation result for the current image, or click **Preview All** to view the default segmentation result for all images. The objects selected will be overlaid by a mask. To adjust the overlay, use the **Weight** slider in the Analysis Masks pane (Figure 3D) or check **Outlines** in the corresponding Mask sub-window below the slider.

4. To zoom into the image, use the slider below it (Figure 3E).

5. Change the segmentation settings in the Processing Method panel (Figure 3F) for one image channel at a time until you are satisfied with the result. For counting live cells, it is typically useful to set a minimum area for excluding debris. Good starting points for optimization are Minimum = 300 μm^2 for selecting complete cells and Minimum = 75 μm^2 for selecting nuclei. Move to the next image channel.

6. When you are satisfied with the segmentation for all the image channels, save the settings and proceed to the next step.
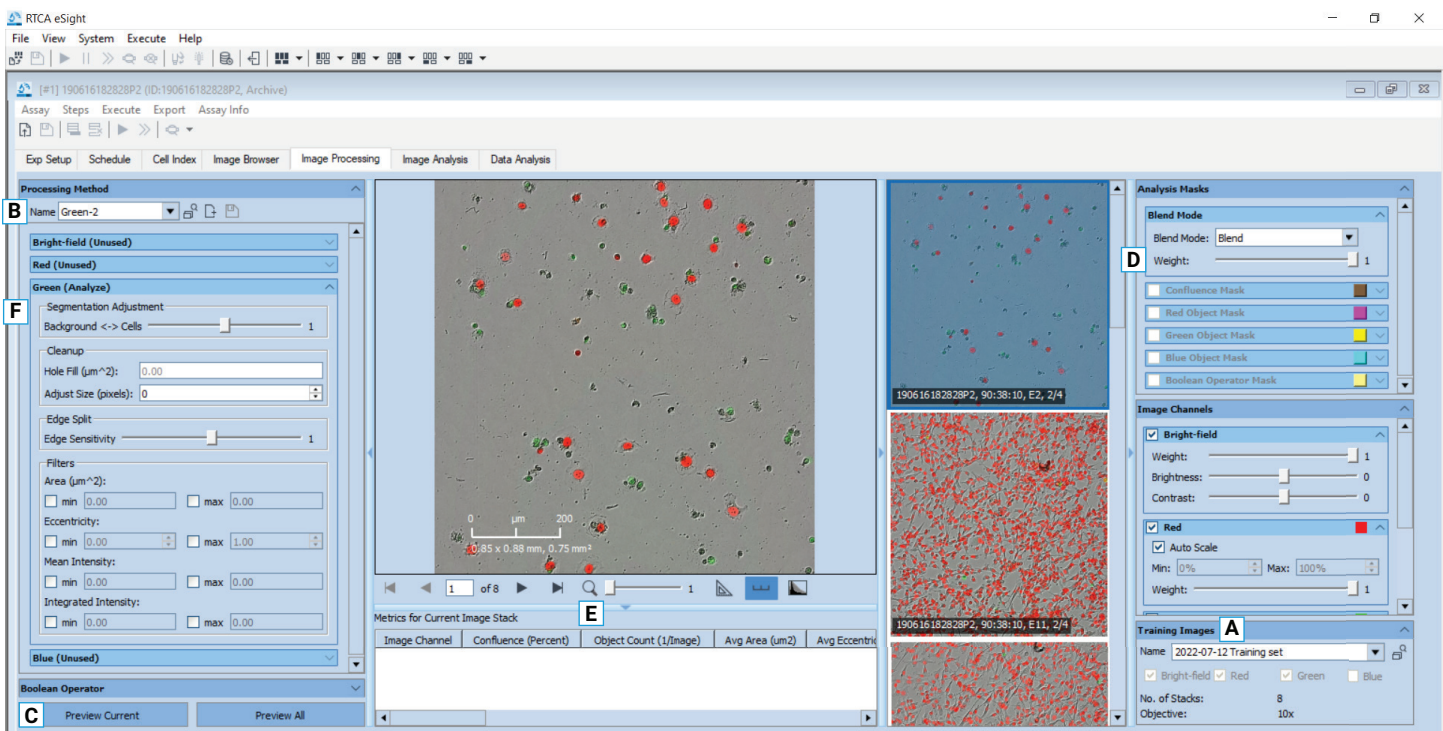

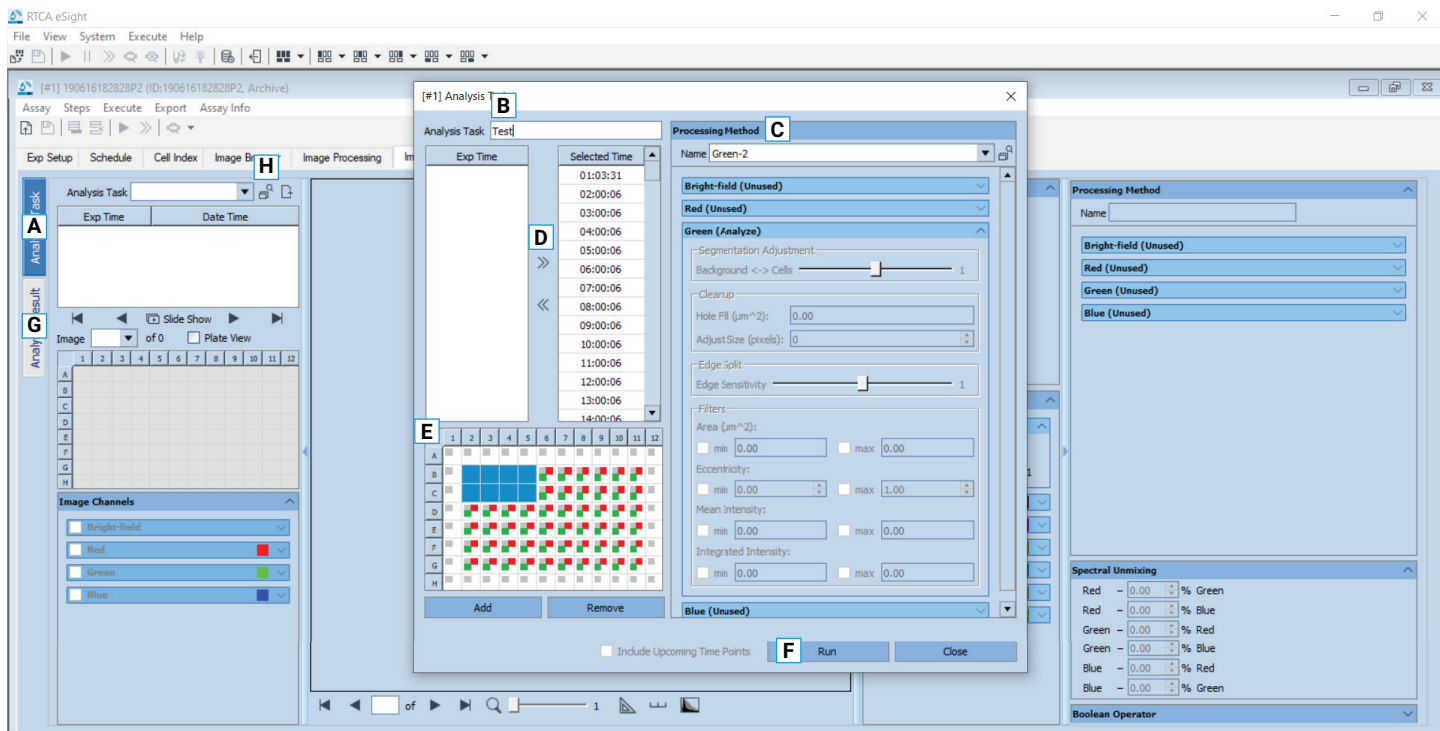**Figure 3.** The Image Processing tab.

Image Analysis tab

Figure 4. The Image Analysis tab.

Ensure that the Analysis Task sub-tab is selected (Figure 4A).

1. Click ⬇ to open the Analysis Task window. Enter the analysis task name (Figure 4B).

2. Select the processing method from the drop-down menu (Figure 4C).

3. Select the time points to be analyzed from the Exp Time pane and click ≫ to add them to the image pool (Figure 4D).

4. Select the well(s) to be analyzed within the plate schematic and click **Add** (Figure 4E).

5. Click **Run** to start the analysis (Figure 4F).

6. When the analysis is complete, the results can be viewed in this tab, or combined with Cell Index and analyzed further on the Data Analysis tab. To plot selected metrics (e.g., Confluence or Count) on this tab, click the **Analysis Result** sub-tab (Figure 4G). To view a previously run task, click 🔍 and select the desired analysis task (Figure 4H). Click **Open**.

7. Select the desired Metrics Category and Metric (e.g., Confluence or Count) to be plotted in the region below the well selection map.

Finally, select the well(s) in the well map and click **Add**. The selected metrics will be ploted in the plot window.

Note 1: Some researchers have reported the total fluorescence intensity for dead cell markers in the literature.

Note 2: Dead cells fragment over time. Depending on the cell type, it may be possible to select early apoptotic cells by using a size filter.

Data analysis

To analyze the acquired impedance and image data in combination, open the Data Analysis tab (Figure 5) from the RTCA eSight window.

1. Select the desired Analysis task (Figure 5A).

2. Select the imaging channel of interest (Brightfield/Red/Green/Blue) (Figure 5B).

3. Select the parameter to be plotted (Y-axis) (Figure 5C).

4. Highlight the desired well(s) in the well map (Figure 5D).

5. Click ✓ to plot the corresponding data. Click **+** or **−** to add or delete additional wells (Figure 5E).

6. Additional parameters can be plotted in the lower plot window: Slope, Doubling Time ("Y Value" in the Parameters drop-down menu), IC_{50} ("DRC" in the Bar Chart drop-down menu), etc. (Figure 5F) Select the time point desired using the To drop-down (Figure 5G).

7. Select the wells and use ✓ or **+** and **−**. Use ↓ to plot the same wells as selected in the upper chart (Figure 5H).

To export the data, right-click on either graph, or use **Assay > Export Experiment Info**. Please refer to the xCELLigence RTCA eSight Software Guide for further details.

Figure 5. Example of a completed Data Analysis tab.

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