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Quick Reference Guide

Running Experiments on the xCELLigence RTCA eSight

This quick reference guide describes the setup of experiments on the RTCA xCELLigence eSight (380601600) using RTCA eSight software. If the Instrument Status LED is RED, the instrument has failed the self-test, and you should contact Agilent technical support.

A cytolysis experiment is used as an example for illustration purposes.

1. Start the RTCA eSight Software by double-clicking the RTCA eSight Software icon on the desktop and login under a user. Select a specific cradle.
2. **Set up the Exp Notes page.**

   This page records experiment-related information such as Assay information, Target cell and Treatment information. Enter the information in the appropriate fields. When the experiment starts, this information is automatically saved.

   Set up the Cell subpage.

   Step 1: Enter assay name, device (plate) serial number, and comments in the corresponding boxes of the Assay Information panel (Figure 1).

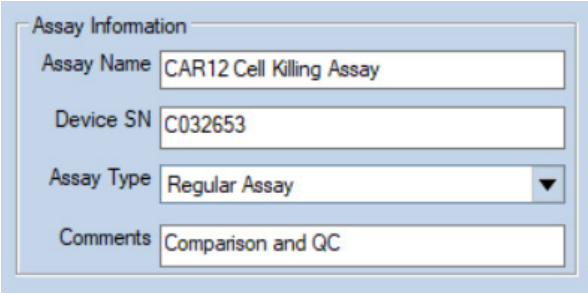

Figure 1. Assay Information panel.

   Step 2: Enter information into the appropriate boxes in the Target Cell panel (Figure 2). Select the well type (i.e., Sample, Positive Ctrl, or Negative Ctrl). Click **Apply** to save the edits and apply all information to the selected wells.

   Note: Only a~z, A~Z, 0~9, and underscore "_" are acceptable, other characters will be ignored.

   An example of a completed Cell subpage is shown in Figure 3.

   Similarly set up the Treatment subpage.

3. **Set up the Schedule page.**

   Click the icon on the plate tool bar (Figure 4).

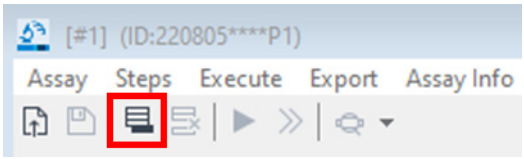

Figure 4. Plate tool bar.

For cradles 1 to 3, the first step will be an impedance-only reading (background/medium measurement). For cradles 4 and 5, it will be image-only (skip to section 9).

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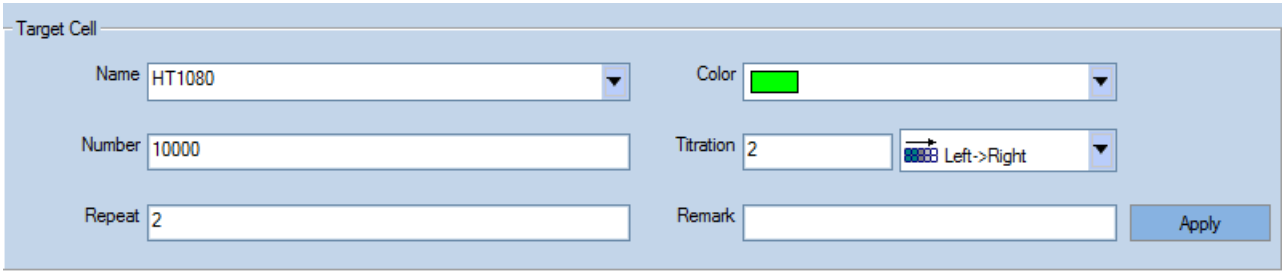


Figure 2. Target Cell information panel.

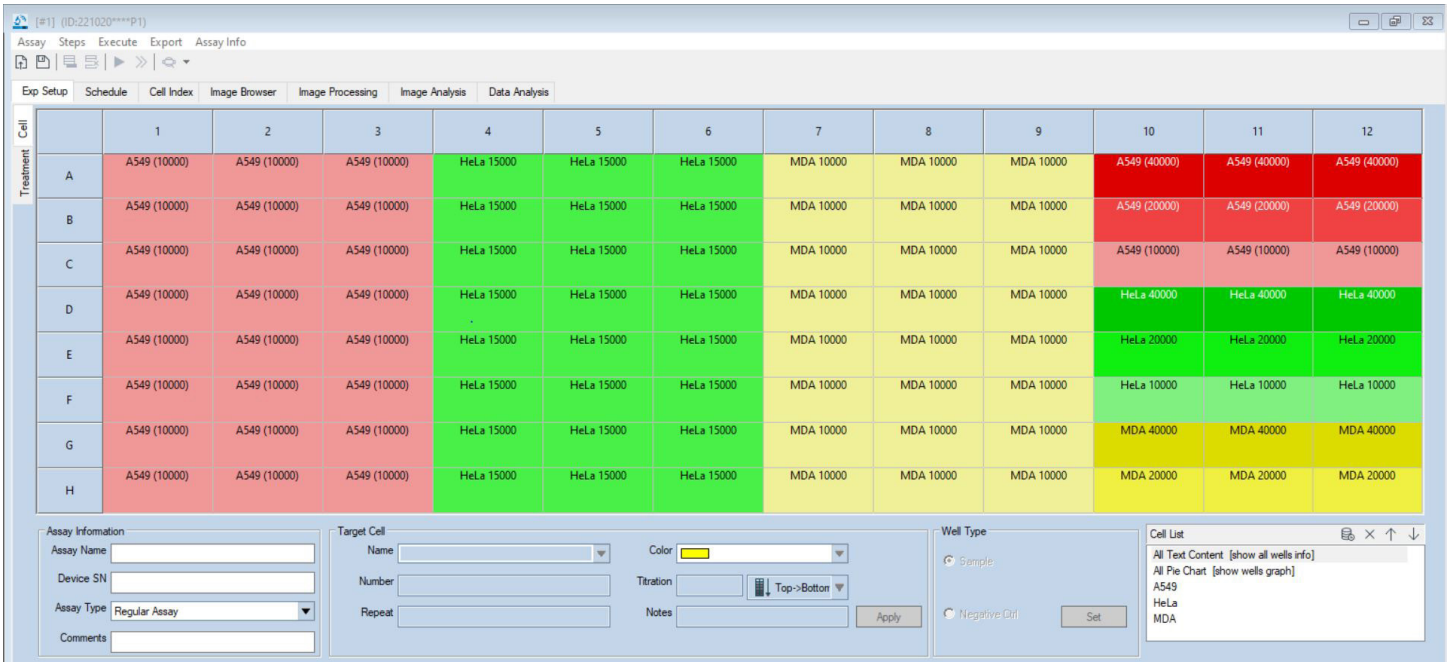


Figure 3. A completed Cell subpage.

4. Remove the Agilent E-Plate View 96 from its packaging and add 50 µL of cell culture media to each of the 96 wells.
   - Use reverse pipetting to minimize the generation of bubbles as these will negatively impact image quality.
   - The use of powder-free safety gloves is recommended.
   - Do not touch the electrical contacts at the bottom of the plate (green strips).
   - **Do not add more than 230 µL of cell culture medium to each well.**

5. Insert the E-Plate View 96 into one of cradles 1 through 3. Lock the plate with the clamp (Figure 5).

Figure 5. Locking an Agilent E-Plate View 96 into place with the locking clamp.

6. The software will perform an automatic scan each time an E-Plate View 96 is inserted into the cradle. The instrument will not scan the wells that are turned off.

   Click **Assay Info > Message** to open the message window. If the measured impedance value is within the expected range (i.e., the connection between the plate and the instrument is good), the message *"Plate Scanned. Connections ok."* will be displayed at the top of this window. If the connection between the plate and the instrument is not made, the message *"Plate Scanned. Please check the connection on position: xx (for example, C11)"* will be displayed in this window. In this case, remove the plate and re-insert the plate into the cradle. If the error persists, clean the pins using the provided brush.

   Start the experiment to take the background measurement. Click **Execute > Start** from the plate window menu or click ▶ from the plate window tool bar to begin the experiment. This step measures the background impedance of cell culture media. After completion of the measurement, *Ready for Next Step* is displayed at the bottom of the software window.

8. Remove the plate from the cradle.

9. Add target cells to the plate.

   – It is recommended to add cell suspension to each well for a total volume of 100 µL. For cell-mediated cytolysis, effectors can be added the next day in 100 µL. For drug testing, a day one total volume of 180 µL and addition of 20 µL of drug at 10x after cell attachment is recommended.

   – **Optional:** To reduce edge effect, leave the plate in the tissue culture hood for 30 minutes at room temperature so that the cells settle to the bottom of the well evenly. https://www.ncbi.nlm.nih.gov/pubmed/14567784

10. Select the Schedule tab. For cradles 1 to 3, add Step 2. For cradles 4 and 5, instead add Step 1 from the Steps menu. The default sweep number is 500 for impedance measurements and 120 for imaging acquisition; the interval is 15 minutes for impedance measurements and 60 minutes for image acquisition. Only the image acquisition schedule will be displayed in cradles 4 and 5.

    – To edit these values, double-click on the appropriate Interval or Sweep value, and enter the desired value. Select the desired interval units (Figure 6).

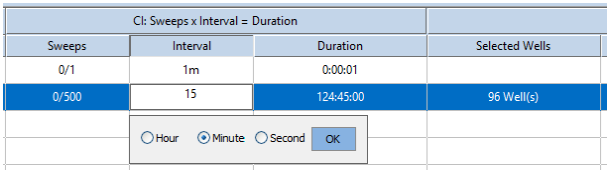


Figure 6. Changing the Interval value in the Schedule tab.

The experiment duration is automatically calculated as the product of sweep number and internal time (e.g., 48 sweeps and 30-minute intervals will result in a 24-hour experiment duration). It is recommended that excess sweeps be programmed, since a step can be stopped before all sweeps are completed.

– Select the number of images to be acquired from each well by clicking the drop-down menu of Image/Well (Figure 7). Note that this cannot be changed once the experiment has been started.

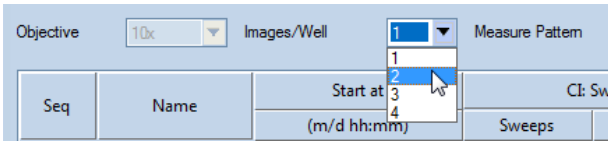


Figure 7. Changing the number of images to be acquired.

– Click **96 Well(s)** in the Selected Wells column to prompt the 96 well map window (Figure 8). Select the desired wells, then select the desired imaging channels (i.e., Bright-field, Red, Green, and Blue). Enter the exposure time for each selected fluorescence channel, then click **Apply**. Users can also click **Remove** to delete the selected wells.

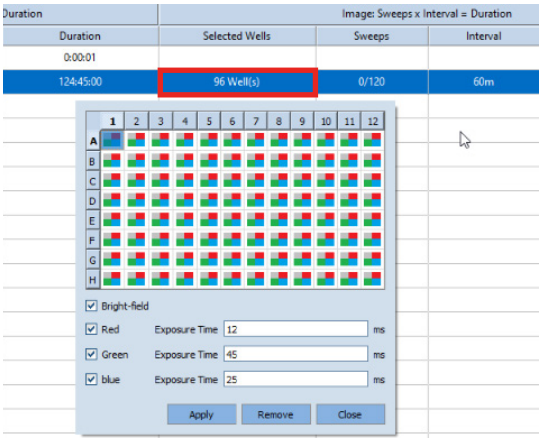


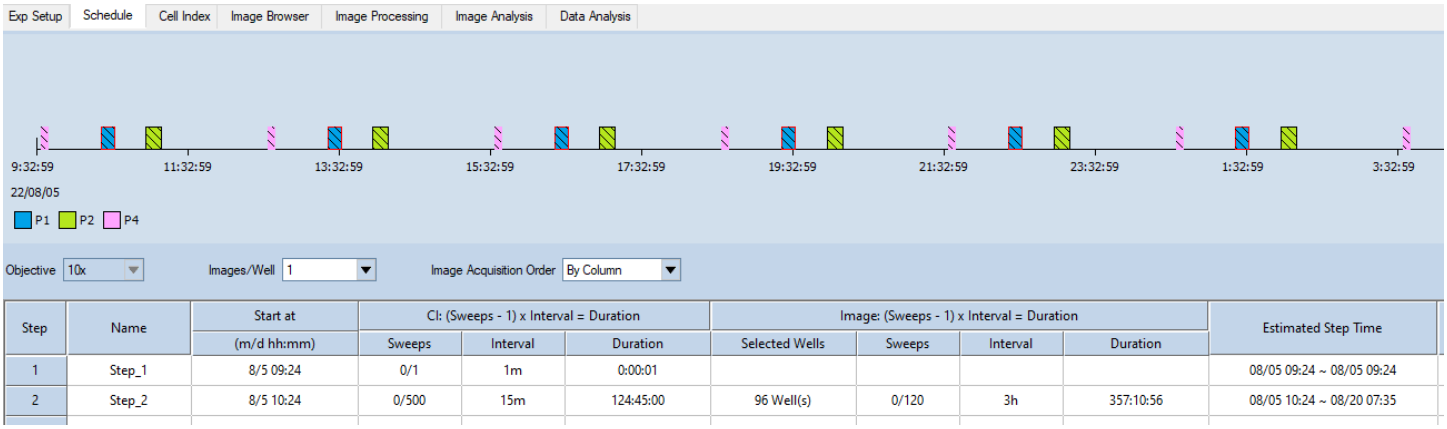
Figure 8. The 96 well map window.

Please refer to Table 1 for the reference exposure time for select Agilent dyes, which can be used as a starting point for optimization. Users may need to optimize the exposure time for the cell line under study.

Table 1. Reference exposure times.

Fluorescent Channel	Fluorescent Dyes	Reference Exposure Time (ms)
Red	eLenti Red	100 to 200
	All other Agilent red dyes	300 to 400
Green	eLenti Green and all Agilent green dyes	100 to 200
Blue	eLenti Blue	50
	Hoechst and all Agilent blue dyes	50 to 100

For other dyes or tranfected cell lines, determine exposure times by clicking **Assay > Preliminary Experiments**. An example of a completed Schedule tab is shown in Figure 9. Note that the Estimated Step Time is the larger of the Impedance (CI) or Image durations.



Step	Name	Start at	CI: (Sweeps - 1) x Interval = Duration			Image: (Sweeps - 1) x Interval = Duration				Estimated Step Time
		(m/d hh:mm)	Sweeps	Interval	Duration	Selected Wells	Sweeps	Interval	Duration	
1	Step_1	8/5 09:24	0/1	1m	0:00:01					08/05 09:24 ~ 08/05 09:24
2	Step_2	8/5 10:24	0/500	15m	124:45:00	96 Well(s)	0/120	3h	357:10:56	08/05 10:24 ~ 08/20 07:35

Figure 9. Example of a completed Schedule tab.

Schedule conflicts: Since the instrument can run up to five plates simultaneously, it is possible that conflicts may arise between scheduled measurements for plates. In this case, priority is designated based on the time at which the experiment step was set up, with a step set up earlier given higher priority. The measurement for the next plate is performed as soon as the measurement for the preceding plate is completed. If four or five plates are being run, it is strongly recommended to increase the time between images to ≥2 hours.

11. It is recommended that the Start time be set to 1 hour after the plate is inserted in the incubator to allow sufficient time for any condensation on the plate lid to evaporate.

    Cradles 1 to 3: A Device calibration failure is unlikely but may occur in the presence of excessive bubbles in the wells. Examine the wells for the presence of bubbles. If a large number of bubbles are visible, either (A) remove the top from a spray bottle of 70% ethanol/propanol and spray gently over the plate, with the lid open, in a tissue culture hood, or (B) apply vacuum to the plate for a few seconds. Examine the wells again for the presence of bubbles. Once resolved, retry the device calibration.

12. Adding effector cells to the plate.
    1. Click **Execute > Pause** to pause Step 2.
    2. Take out the plate and add effector cells. A volume of 100 µL is recommended.
13. Reinsert the plate with cells and either continue Step 2 or start a new step (Step 3).
14. Plot the Cell Index data on the Cell Index page. Select the desired well(s) in the well map and click **Add**. Charts and data on the charts can easily be exported using a right-click of the mouse.
15. View the captured images and create the training images in the Image Browser tab. Click **Image Browser**. Select the date and time the image was captured. Select the well position where the image was captured. If multiple images were captured in the same well, select the image number. Check the checkbox in front of Bright-field, Red, Green, or Blue in the image channel tab. The corresponding image will be displayed in the middle of this page with all the defined settings. Users can also view smaller versions of captured images to the right of the main display window.

    The processing method for the selected training images is developed in the Image Processing tab. The analysis is performed and viewed on the Image Analysis page. Please see the eSight Analysis Quick Guide for details.

Please refer to the xCELLigence RTCA eSight Software Guide for further details.

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