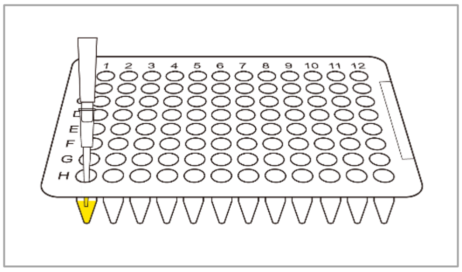
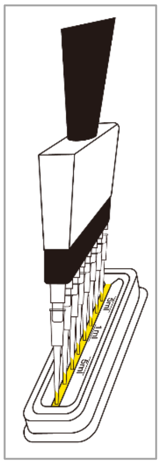
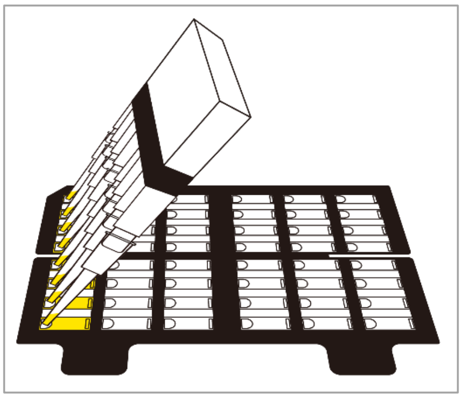
# EVE™ HT FL Quick Guide

## 1. Preparing and loading samples

1) Prepare cell suspensions
   - Measurable ranges: **1 x $10^5$ ~ 1 x $10^7$** cells/mL
2) Add **20 μL of well-mixed samples** into a **mix well plate**.
   - Mix well plates are included in the kit
3) Dispense a sufficient amount of Cell viability reagent (AO/DAPI) into a reservoir
   - Reservoirs are included in the kit.
4) Add **20 μL of Cell viability reagent** to the mix well plate and **mix sample with reagent well**
   - 8-channel multichannel pipette is provided
5) Load **20 μL of mixture** into a **EVE HT FL counting plate**

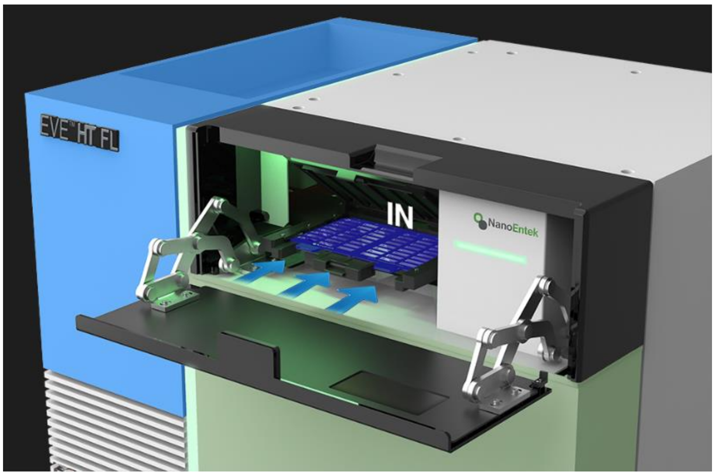






## 2. Insert counting plate

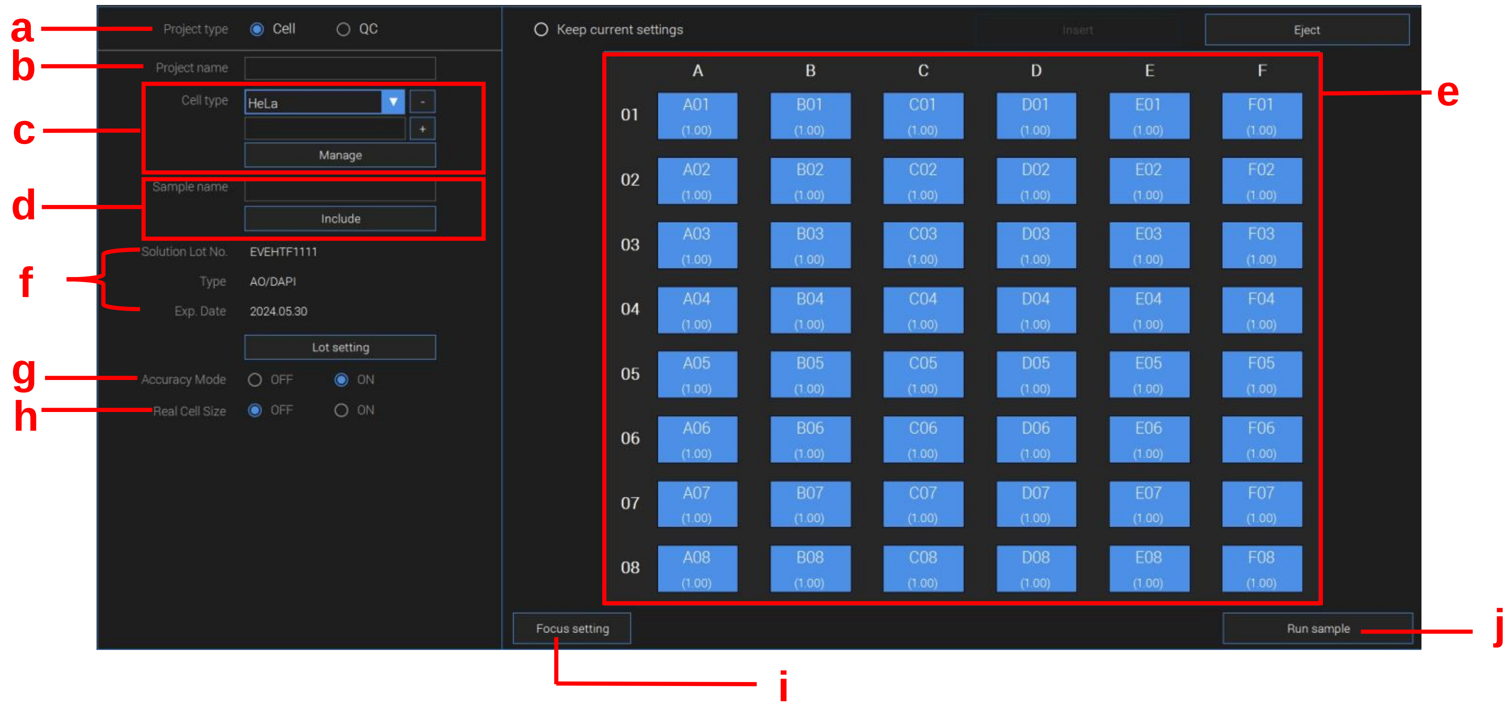
1) Open the EVE HT FL door and open the plate holder
   - Press the button on the plate holder
2) Insert counting plate
3) Close the plate holder and the door.




### ⚠ Caution

- Allow sample to **settle for at least <u>2 minutes</u>** before starting measurements
- Make sure to put the plate all the way in when inserting the plate
- Make sure to properly close the door and the plate holder

## 3. Measure [Project name and defining a plate map]




a. Project type: select "Cell" to run cell samples
   - Select "QC" to run quality control samples

b. Project name: enter project name

c. Cell type: select one of existing cell types or add a new one.
   - Click "Manage" to review and revise the parameters of cell types

d. Define plate map: repeat the following steps for all the wells to be measured
   ① After selecting a cell type, enter the name of sample
   ② Select the wells in the plate map
   ③ Click "Include" button
   For grouping samples, right-click the plate map and select "Include as a Group"

e. Plate map: click individual wells or click-and-drag group of wells to select wells

f. Solution: confirm the information about the solution.
   - Click "Lot setting" to change

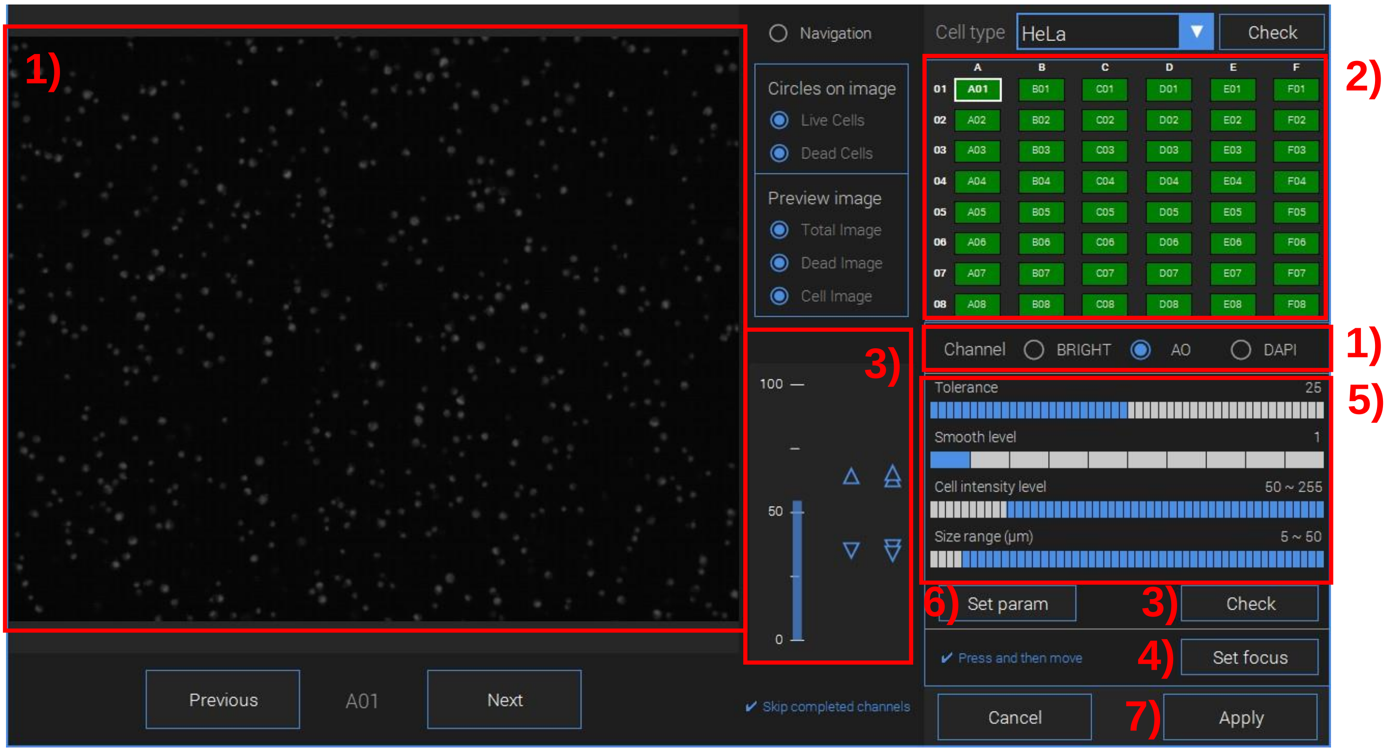
g. Accuracy Mode: choose "OFF" to take one image per well and "ON" to take 4 images per well

h. Real Cell Size: choose "ON" to take BR images which will give cell size estimates

i. Focus setting: see next page for details

j. Run sample : click this button to start measurements

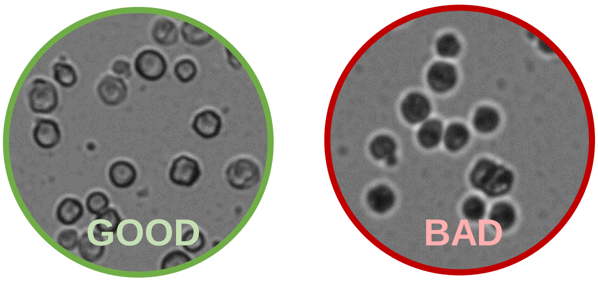
## 3. Measure [Focus setting]



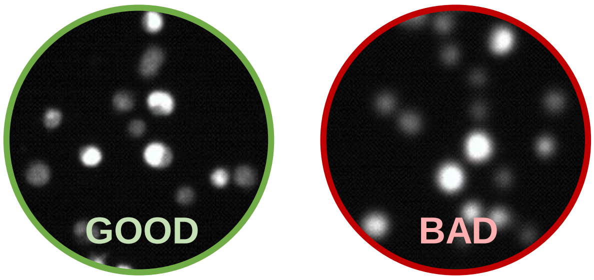

1) Preview
   - Zoom In and Out: put mouse cursor on the preview and turn mouse wheel
   - Select channel: choose between BRIGHT, AO, or DAPI
2) Plate map: wells marked with BLUE indicate the wells of which focuses have not set. When focuses are set, wells will be marked GREEN
   - Only the focus of the first well of each cell types needs to be adjusted
3) Adjust focus: there are multiple ways to adjust focuses
   ① Click single (fine steps) or double (coarse steps) arrows
   ② Put mouse cursor on the preview, hold “ctrl” or “shift” key and turn mouse wheel
   Examples of good and bad focuses can be found on the new page
   ③ Click “Check” to see how cells are counted
4) Save focus: click “Set focus” to save focuses
   - If there are more than 2 cell types, Preview will automatically move to the first well of the next cell type
5) Image analysis parameters: please see next page for details
6) Set param: To save a changed parameter to that cell type, click this button
7) Finish focus setting: after saving focuses of all wells, click “Apply” button

## 3. Measure

[Adjusting focus examples]

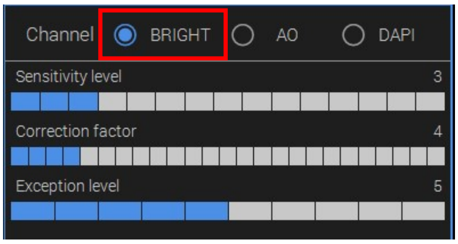



**BRIGHT** : The outline is clear, and the brightness inside the cells is the same as the background.



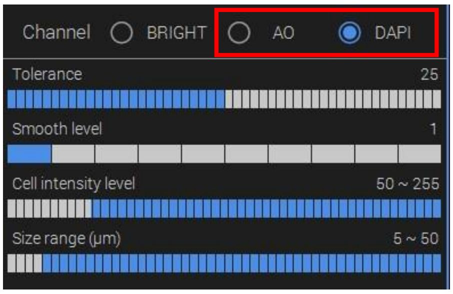

**AO&DAPI** : clear cell outline

[Setting image analysis parameters]




**BRIGHT**

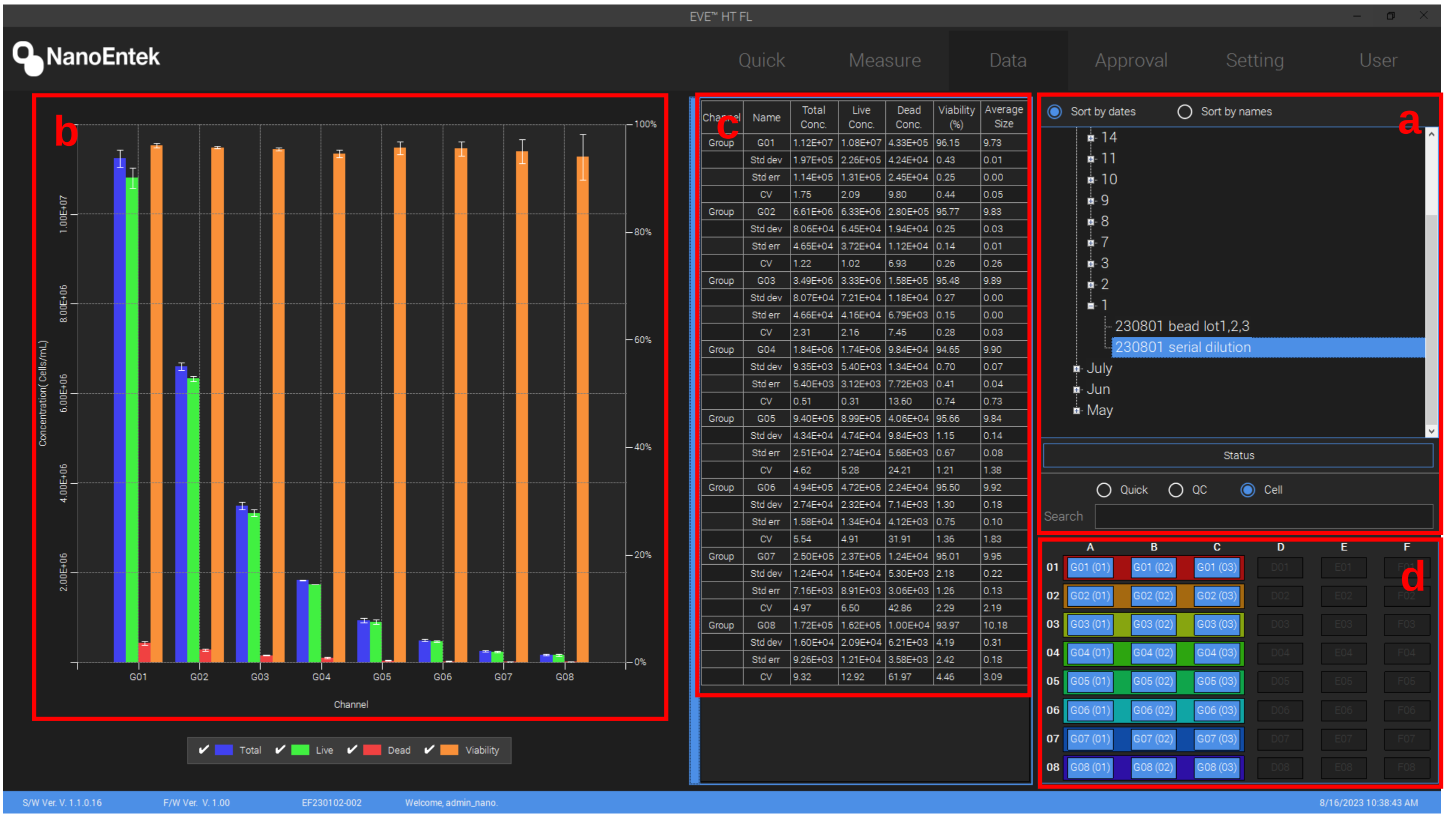
1. **Sensitivity level** : Decreasing this sensitivity parameter will make it easier to prevent debris from counting. (Caution: if the sensitivity level is too low, then some of real cells may not be counted)
2. **Correction factor** : Decreasing this correction factor will make it easier to detect cells which are slightly darker than background. When cells look very transparent, you can decrease this correction factor to pick up vague objects.
3. **Exception level** : Decreasing this exception level will make it easier to detect small objects.




**AO&DAPI** (Set parameters for each channel)

1. **Tolerance** : Tolerance defines the size of a circular window in which smoothing would be applied. Decreasing tolerance will increase a chance to count clustered cells as individual cells. Increasing tolerance will increase a chance to count clustered cells as a big single cell. Because nucleus in dividing cells before cytokinesis look similar to two cells close to each other, they may be counted as two cells with a low tolerance level.
2. **Smooth level** : Smooth level will control smoothness of images. When cells are big and intracellular organelles are so distinct that organelles are counted as individual cells, you can increase smooth level to smooth out such distinctive textures and prevent organelles to be counted as cells.
3. **Cell intensity level** : Cell intensity level works similar to intensity gating. When cells are very dim or too bright, you can change the range of nuclear intensities to be counted as cells. Here, you can choose both minimum and maximum values.
4. **Size range** : Size range refers to the range of fluorescent cell sizes. This allows you to determine the size range and exclude small debris from the count.

## 4. Data




Data tab allows users to review, edit, and export results including cell counts, viabilities, and images.

a. Data list: Data are listed based on the types of experiments (Quick Count, QC or Cell). In each type, data are lists in chronological order.
- Group : To re-edit group setting, Right-click a data and select Group.
- Export : Right-click a data entry to select a data to export the results
- Rename or Delete : Right-click a data entry to select a data to rename or delete

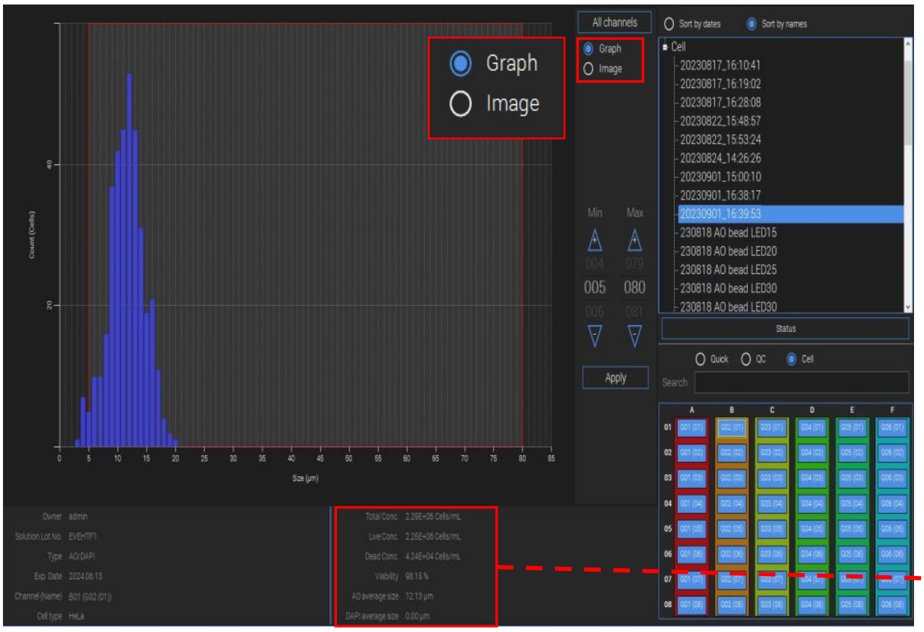
Click a data entry to review the results

b. Bar graph: shows the results in a bar graph
- Left Y-axis is for Total, Live, or Dead cell concentrations [cells/mL]
- Right Y-axis is for viability [%]

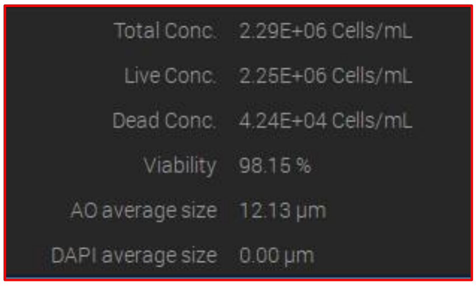
c. Table: shows the results in a table

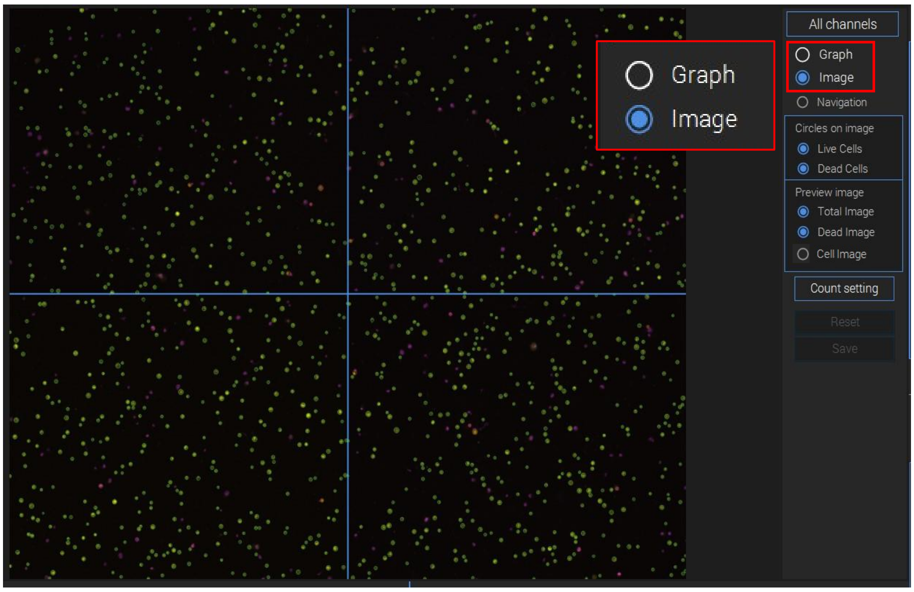
d. Plate map: shows the plate map of the results
- Review images: If you select individual wells, you can check the results, graphs, and images for each well. Please see the next page for details

## 4. Data [Individual well review]

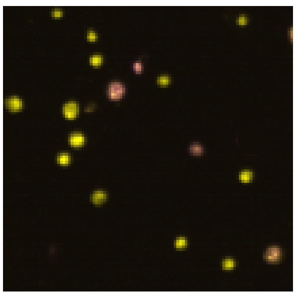



When one well is selected, cell counts (Total, Live and Dead cell concentrations), viability, and average cell size will be shown below cell size histogram. Users can change minimum or maximum cell sizes to be included in the results.

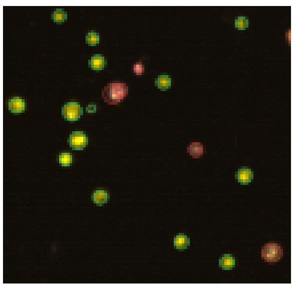


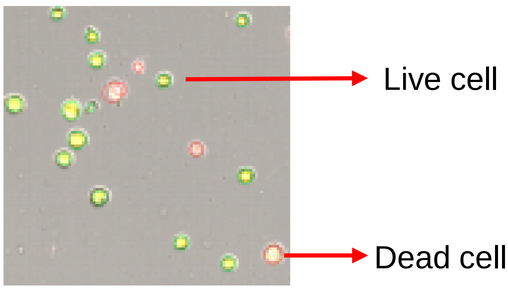

By selecting 'Image', users can review raw images. If 'Accuracy mode' has been selected, 4 images will be displayed. If 'Real cell size' has been selected, Bright channel images will also be shown.



Total, Dead Image



Live, Dead cells
Circle on




Circle on
Cell Image on