

## Corning® Cell Counter: New Application

An improved user interface, multicount cell count features, and live cell size gating

### Introduction

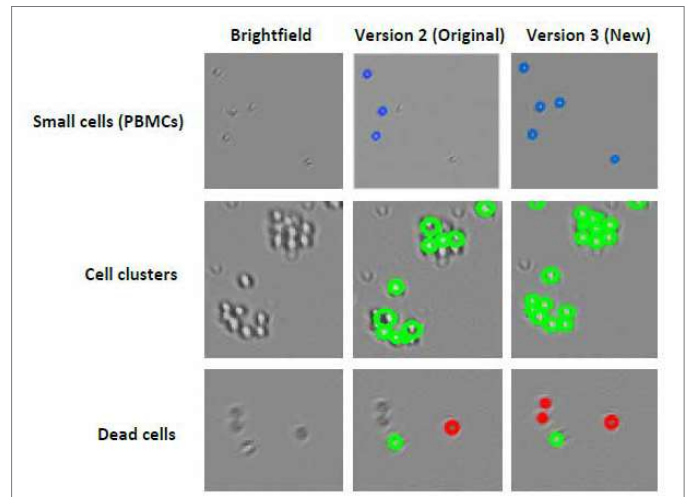
The Corning Cell Counter application includes an array of new features. First of all, a new algorithm, called “Version 3”, has been developed and implemented. This new algorithm has several improvements, including enhanced dead cell detection and improved counting of cells within clusters (Figure 1). Also, the cell size range has been enlarged, enabling counting of cells as small as 4 µm (5 µm for viability detection). Furthermore, you can now perform multiple counts per sample and easily obtain average results (± SD).

In addition, cells will be encircled in blue when “No (1:0)” is selected for the Trypan blue option. Furthermore, cell size gating has been improved which allows for gating of the cell size histogram as well as the processed image. Your results will be adjusted according to this gating. These two features are present in both Version 2 (Original) and Version 3.

In this document we will guide you through all the new elements, demonstrating what is new, and how the results are displayed.

### Start Page

Performing the cell count has remained the same. When you create your experiment, select or create a project, name it, and choose if the sample is diluted with Trypan blue or not. In addition, the user can choose which Algorithm Version will be used (Figure 2A). By default, the dropdown menu at “Algorithm version:” is set to Version 3. For extra clarification, click on “More info” next to the Algorithm version. An explanation of both algorithms will appear to help the user choose the appropriate algorithm (Figure 2B).



**Figure 1. Visualization of the improvements of the Version 3 algorithm.** The columns (from left to right) represent the Brightfield images, the Version 2 (Original) processed image, and the Version 3 processed image, respectively. The rows (from top to bottom) represent samples that contain small cells, cell clusters, or dead cells, respectively.

**2A Start cell count**

Project: Multi\_Count Create new project

Name: Cell Count nr. 2

Algorithm version: Version 3 (New) More info

Sample dilution used  No  Yes

Trypan Blue  No (1:0)  Yes (1:1)

Dilution factor: 1

**2B**

Algorithm version: Version 3 (New) Less info

Algorithm **Version 3** of our Cell count algorithm is now generally available. We recommend that you start using it today.

**Version 3 (New):**

- Counting range: 4 µm to 70 µm.
- Viability range: 5 µm to 70 µm.
- Improved user interface which allows you to perform up to eight counts within one experiment.
- Improved dead cell detection.
- Improved detection of cells within clusters of cells.

**Version 2 (Original):**

- Detect cells ranging between 10 – 70 µm in diameter.

**Figure 2. Cell count algorithm.** (A) Next to choosing a project, naming your count, and selecting the appropriate Trypan blue dilution, you need to select a cell counting Algorithm [Version 2 (Original) or Version 3]. (B) When you click on “More info”, a short explanation will appear describing the improvements of Version 3.

There is an additional feature for pre-diluted samples. When using this option, the cell count concentrations are automatically recalculated for this specific sample. Therefore, the extra calculation step required by the user is eliminated. The user can select this option by clicking “Yes”, which will result in a dropdown menu where the dilution can be adjusted (Figure 3A). The information icon (Figure 3B) briefly explains when and how to use this option.

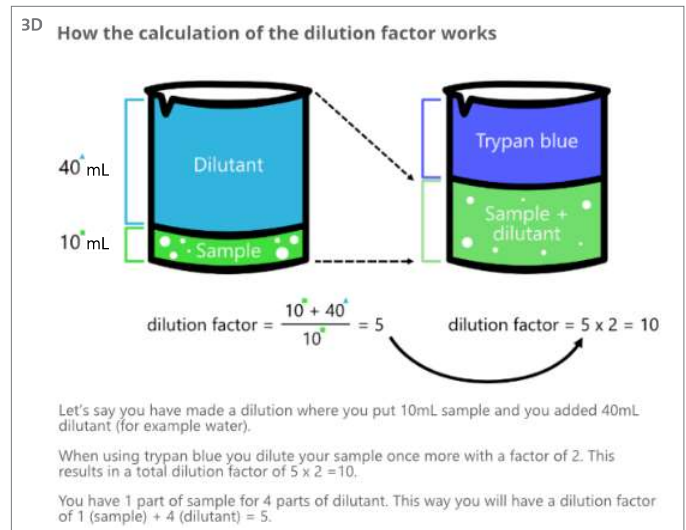
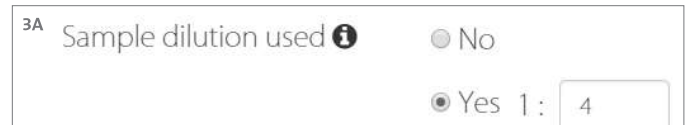
When a diluted sample and/or Trypan blue staining is used, the dilution factor changes. The calculation leading to this dilution factor is displayed at the bottom of the Start Page (Figure 3C). For clarification, the user can click on the “More info” button. This will show a pop-up window (Figure 3D) that explains how the calculation of the dilution factor works.

This example shows a sample dilution of 1:10 with Trypan blue staining.

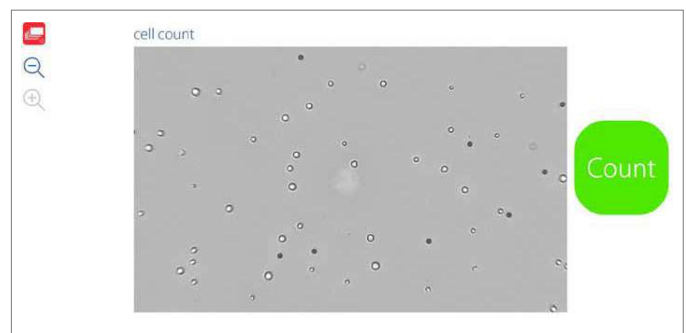
### Version 2 (Original)

#### Live View Page

After choosing Version 2, the screen will display the live image of the sample. Focus your cells in a way that live cells are black with a white center and dead cells are completely black (Figure 4).



**Figure 3. Sample dilution.** (A) The Sample dilution option on the Start page. When selecting “Yes”, you can adjust the dilution. (B) The information icon displays an explanation on when and how to use this new option. (C) The dilution factor is calculated and displayed at the bottom of the Start page. (D) A schematic drawing representing how the dilution factor is calculated.



**Figure 4. Focusing your cells.** Focus your cells correctly before pressing “Count”.

After pressing the “Count” button, you are redirected to the Results page. This is where the first differences can be noticed.

### Results Page

In the Results page the processed image and cell size histogram are displayed. In the old Results page (Figure 5A) these tabs were called “Count” and “Histogram”. In the new Results page (Figure 5B), the two tabs are renamed “Cell count” and “Cell size”. Also, for quick sample recognition, the name of the count is added above the two tabs.

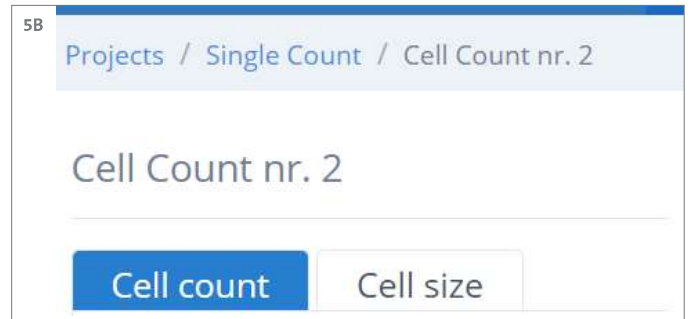
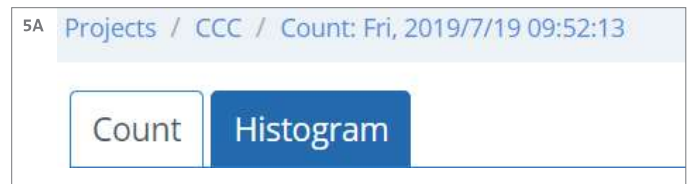


Figure 5. Results page. (A) Old Results page, with the “Count” and “Histogram” tab. (B) New Results page. The two tabs are renamed to “Cell count” and “Cell size”. In addition, the name of the count is added above the tabs, to ensure faster sample recognition.

When the Trypan blue “No (1:0)” option is selected, cells are encircled by blue circles (Figure 6A). The results box displays the gating, cell concentration, and the average cell size. If you go to the “Cell size” tab, the size histogram is also displayed in blue (Figure 6B).

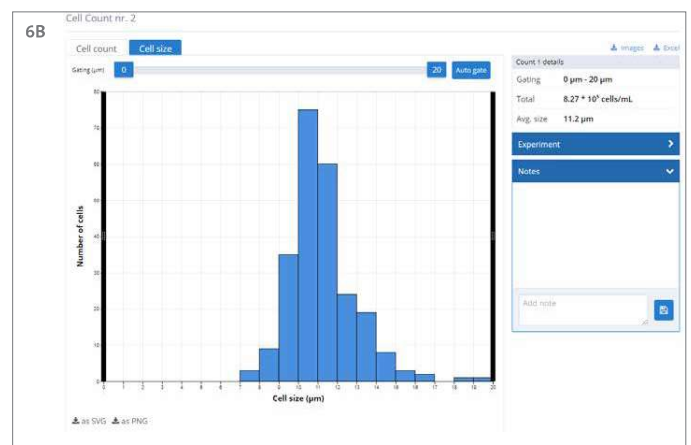
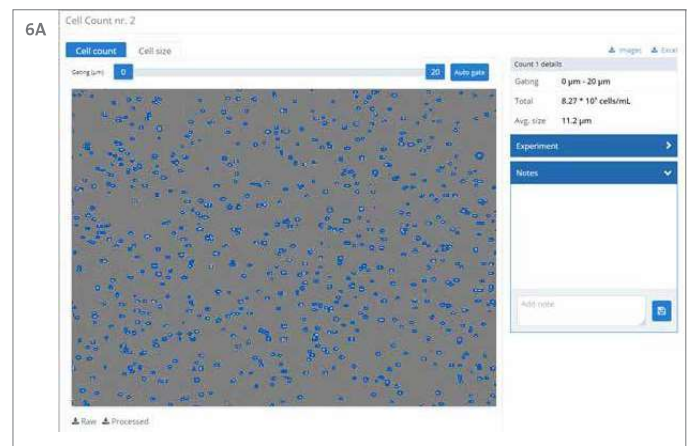


Figure 6. Cell count with the Trypan blue “No (1:0)” option selected. (A) Cells are encircled by blue. The count details are displayed in the result box on the right. (B) The “Cell size” tab shows a blue histogram of the cell size dispersion.

When the option Trypan blue “Yes (1:1)” is selected, the algorithm distinguishes live cells (encircled in green) from dead cells (encircled in red) (Figure 7A). The size histograms of the live and dead cells are displayed in one chart in the “Cell size” tab in green and red, respectively (Figure 7B). If you are only interested in the histogram of either live or dead cells, use the button below the histogram to toggle one on or off. The results box displays the gating, the concentration of live and dead cells, the total cell concentration, the viability percentage, and the average live and dead cell size.

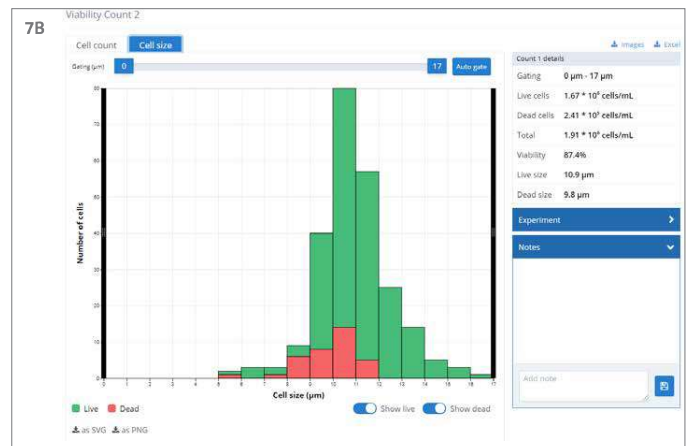
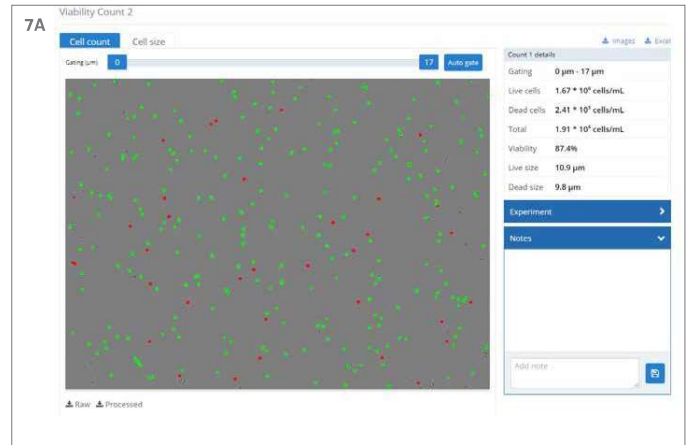
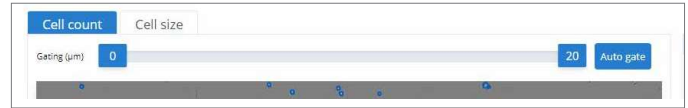


Figure 7. Cell count with the Trypan blue “Yes (1:1)” option selected. (A) Live cells are encircled by green while dead cells are encircled by red. The count details are displayed in the Results box on the right. (B) The size histograms of the live and dead cells are displayed in one chart. You can use the “show live” or “show dead” buttons to display just one of the histograms.

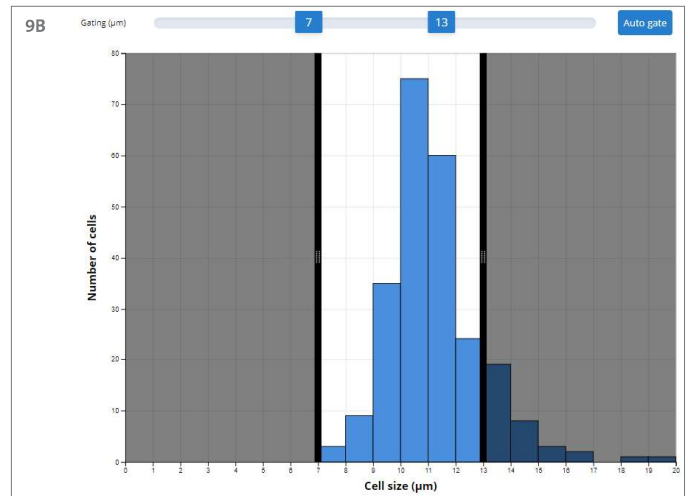
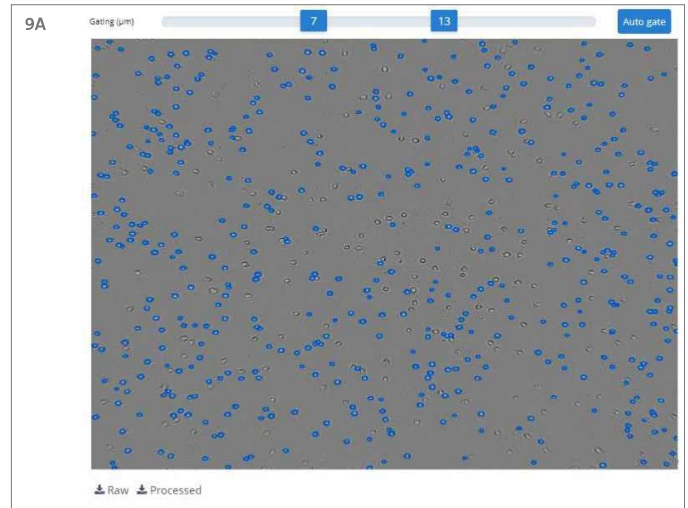
An extra addition is the gating slider above the image (Figure 8). The gating slider is present in the “Cell count” tab as well as in the “Cell size” tab. When moving the blue sliders, you can set the minimum and maximum cell size that you want to include into your count. These values will appear in the blue sliders. When you use the gating slider, the results will change accordingly in the Results box on the right. Cells that are below the minimum or above the maximum size will no longer be encircled in blue (Figure 9A). Furthermore, the histogram will display these excluded regions in grey (Figure 9B). This allows for live gating of your cell count.

An additional feature is the “Auto gate” button, which is located on the right of the gating slider (Figure 8). When this button is selected, the algorithm will recognize the optimal minimum and maximum gating for this specific cell count.

The gating slider and “Auto gate” buttons are available in Version 2 (Original) and Version 3, with both Trypan blue “No (1:0)” and “Yes (1:1)”.



**Figure 8. The gating slider in the new application.** The slider can be used in both the “Cell count” tab as well as in the “Cell size” tab. The results of your count will change according to the gating and are displayed in the Results box on the right. On the right of the gating slider you can find the “Auto gate” button, which recognizes the optimal gating for your specific cell count.

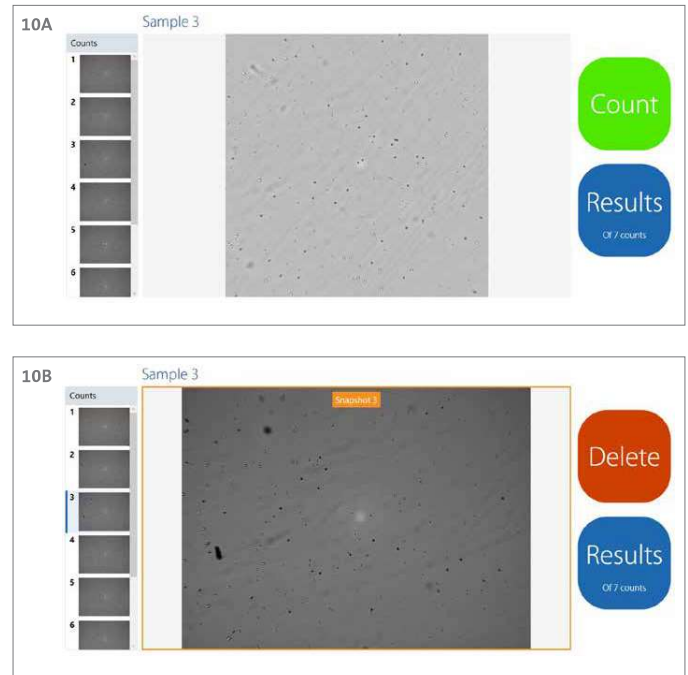


**Figure 9. Example of live gating using the gating slider.** The minimum and maximum are set on 7 and 13  $\mu\text{m}$ , respectively. (A) Live gating in the “Cell count” tab. Cells that are smaller than 7  $\mu\text{m}$  or larger than 13  $\mu\text{m}$  are not encircled in blue. (B) Live gating in the “Cell size” tab. The shaded regions represent the excluded values.

## Version 3

### Live View Page

When Version 3 is selected, it is possible to take multiple counts of one sample. Instead of getting redirected to the Results page after pressing “Count”, you can simply move your sample to a new position and press count again. This can be done up to 8 times. The images of the counts are collected at the left side of the screen (Figure 10A). If you are not satisfied with one of the counts, select that specific count by clicking on the thumbnail on the left and delete it from your count selection (Figure 10B). The “Results” button displays how many counts are included in the Results page.

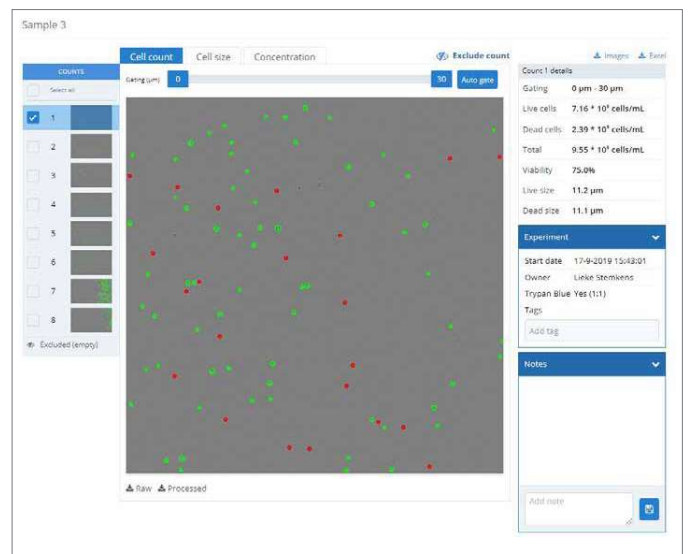


**Figure 10.** Live view interface after selecting algorithm Version 3. (A) The counts that are taken are displayed on the left. (B) To delete count number 3, click on the thumbnail on the left, and use the red “Delete” button to delete this count from your selection.

### Results Page

When you click on the “Results” button, you are redirected to the Results page. By default, count 1 is selected (Figure 11). The Results box on the right contains all details of the selected count: the gating range, live/dead/total cell concentration, viability percentage, and the average live/dead cell size. The “Experiment” box underneath contains general information, such as the start date, the owner of the experiment, and the selected Trypan blue option. The “Notes” box can be used to add your own findings or particularities about a specific count or about the sample.

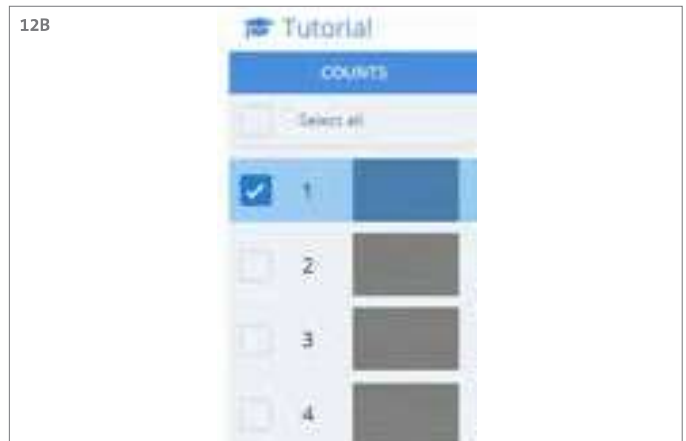
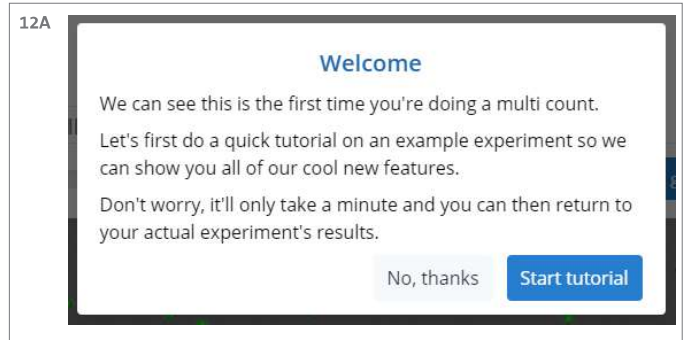
In addition, the gating slider is positioned in the same location as when using Version 2 (Original). When using the gating slider or clicking the “Auto gate” button, the results in the box on the right will change according to the selected gating.



**Figure 11.** Results page after performing multiple counts with the Version 3 algorithm. In the top left the sample name is displayed. Underneath this you can see the tutorial button, the counts taken of this sample, and the dilution calculator. The box on the top right contains details of the count. General information is displayed in the “Experiment” box, while notes can be added in the “Notes” box.

**NOTE:** All the features that are explained in the upcoming pages use examples where Trypan blue is used. Because of this, the graphs and processed images will be displayed in green (live), red (dead), and blue (total) colors. The features that will be discussed are also all applicable when Trypan blue is not selected, but all results (graphs, bars, and circles) will be displayed in blue, and no information about viability is available.

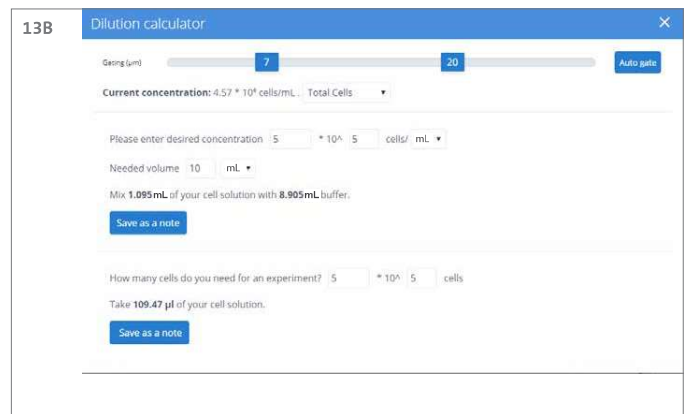
To better familiarize with the new layout and features of Version 3, a tutorial has been developed that will guide users through all the major changes and possibilities using the new application. After selecting Version 3 for the first time, the tutorial will automatically open (Figure 12A). If a user wishes to replay the tutorial, simply click the “Tutorial” button on the results page, which is located above the thumbnails of the counts (Figure 12B).



**Figure 12. Tutorial.** (A) The pop-up text box for the Tutorial, when using Version 3 for the first time. (B) The “Tutorial” button located above the thumbnails of the counts.

Another new feature is the addition of a dilution calculator. This button can be found below the thumbnails of the counts (Figure 13A). This feature calculates what volume of the cell suspension should be mixed with buffer to get a desired concentration in a preferred volume (Figure 13B). It can also determine how much of the cell solution should be used for a certain number of cells per experiment. The calculator takes the gating slider into account and uses these values accordingly. Furthermore, when multiple counts are selected, the calculator uses the average value of the selected counts. Calculations can be saved as notes on the Result page.

**NOTE:** the dilution calculator is available in the cloud for Version 2 (Original) as well as Version 3.



**Figure 13. Dilution calculator.** (A) The “Dilution” button is located below the thumbnails of the counts. (B) The dilution calculator can be used for several purposes. A user can enter the concentration they need in a certain volume using the values of the live/dead/total cell concentrations. It also calculates how much volume of the counted cell suspension is required for a certain number of cells.



There are three tabs that contain information about the selected cell count: "Cell count" (Figure 14A), "Cell size" (Figure 14B), and "Concentration" (Figure 14C). The "Cell count" and "Cell size" tab display results in the same way as they do in Version 2 (Original). The extra "Concentration" tab gives you information about the live/dead/total cell concentration in the form of bar graphs. This gives the user a quick impression of ratios of the live and dead cell concentration in relation to the total cell concentration of the selected count. These bar graphs can be downloaded as SVG or PNG files.

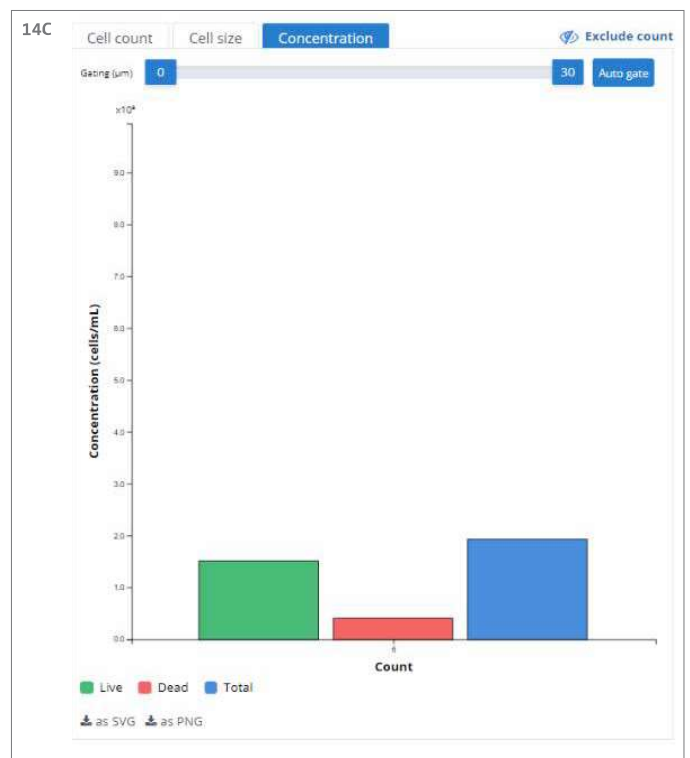
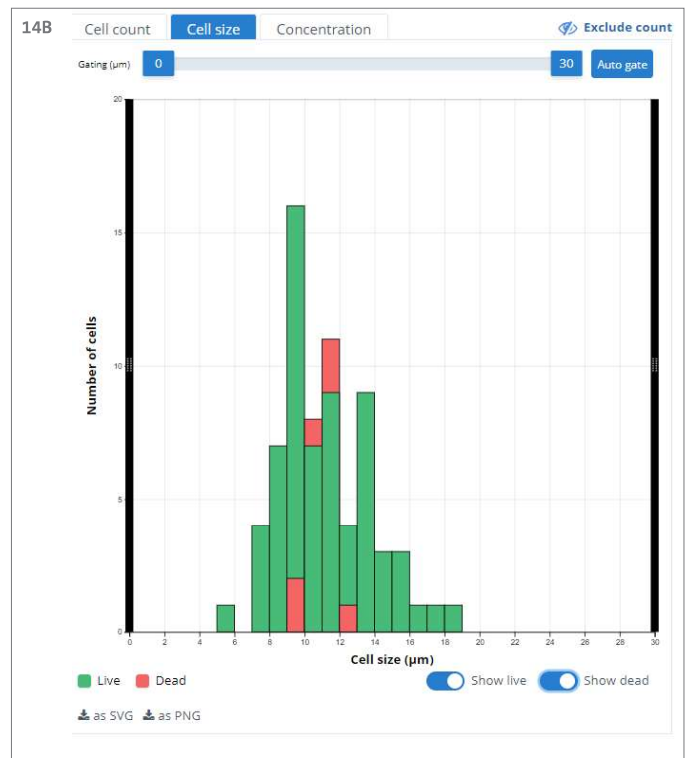
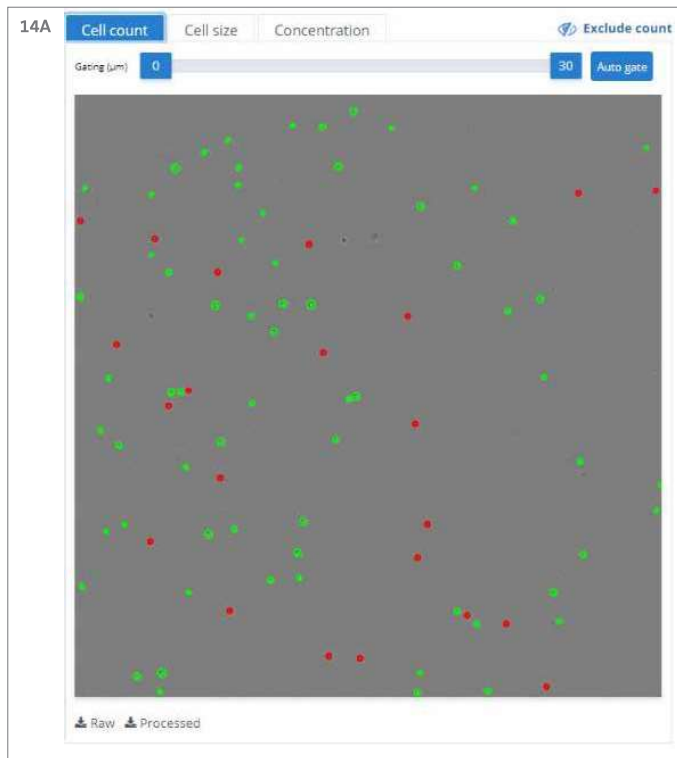


Figure 14. Three tabs that contain information about the selected count. (A) The "Cell count" tab displays the snapshot of the selected count. Live cells are encircled in green while dead cells are encircled in red. (B) The "Cell size" tab includes a dead (red) and live (green) cell size histogram. (C) The "Concentration" tab gives the user a quick impression about the ratios of live (green)/dead (red) cell concentration in relation to the total (blue) cell concentration.

To get an overall average, multiple counts can be selected. This can be done by clicking the boxes next to the thumbnails of the different counts (Figure 15). To select them all, click the “Select all” box above the thumbnails. All three result-tabs will change according to the count selection.

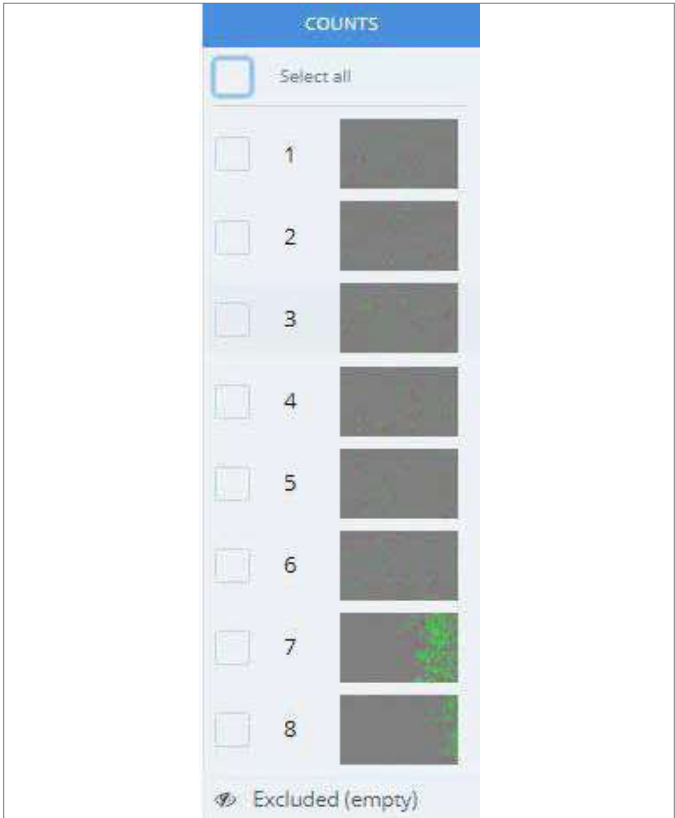

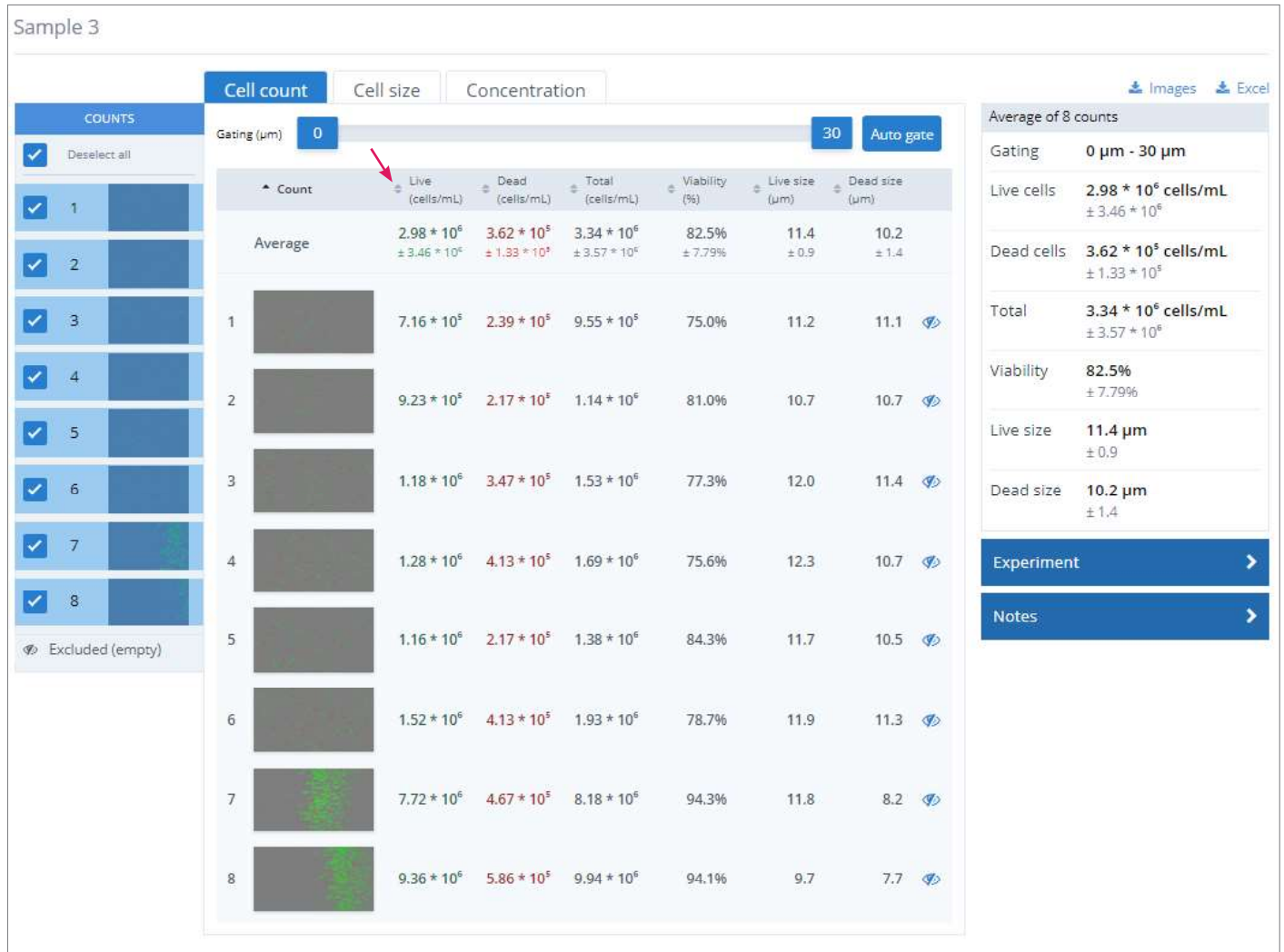


Figure 15. An overview of the counts is presented by displaying the thumbnails of every count in the box on the left. Separate counts can be selected by clicking the boxes next to the count number. To select them all, simply click the “Select all” box on the top.

The Cell count tab gives an overview of all selected counts. It displays the count number with its corresponding image and values. The icon  (Figure 16, red arrow) next to the result parameters can be used to sort the counts. For instance, when you click on this icon next to the “Total (cells/mL)” column, the counts will be sorted from low to high total concentration. If you click on the icon again, the counts will be sorted from high to low total concentration. This helps in identifying any outliers. To exclude counts, you can click on the eye icon (Figure 16, green arrow), positioned on the right side in the overview. The box on the right displays the average of all the selected counts.

When using the gating slider or the auto gate option, the values in the overview and in the Results box will change accordingly.



**Figure 16. Cell count tab using Version 3 algorithm.** In this example, 8 samples were counted. The counts can be (de)selected in the box on the right or by clicking the eye icon indicated with the green arrow. The overview displays the live, dead, and total number of cells/mL per count. Also, the viability and the live and dead cell size are shown. These values can be sorted by clicking on the icon indicated with the red arrow. The average of all the selected counts is displayed in the Results box on the right. The “Cell count” tab gives the user the opportunity to identify outliers and to visually compare the data of the counts.

Whenever counts are excluded, they are displayed in the “Excluded” box on the left (Figure 17, red box). These counts are no longer considered in the Overview page and the Results box. To include these counts again, click on the eye icon on the left side of the excluded count.

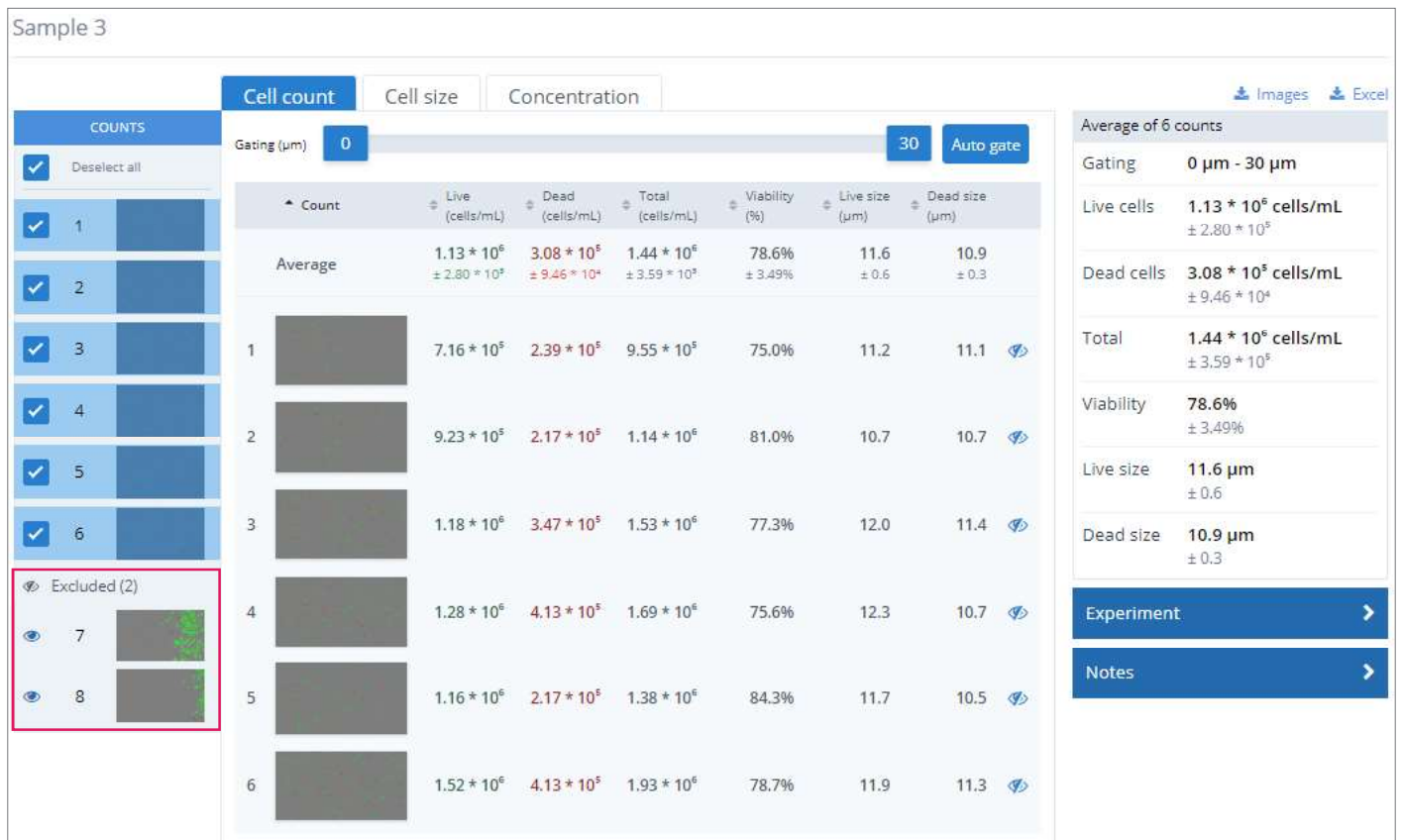
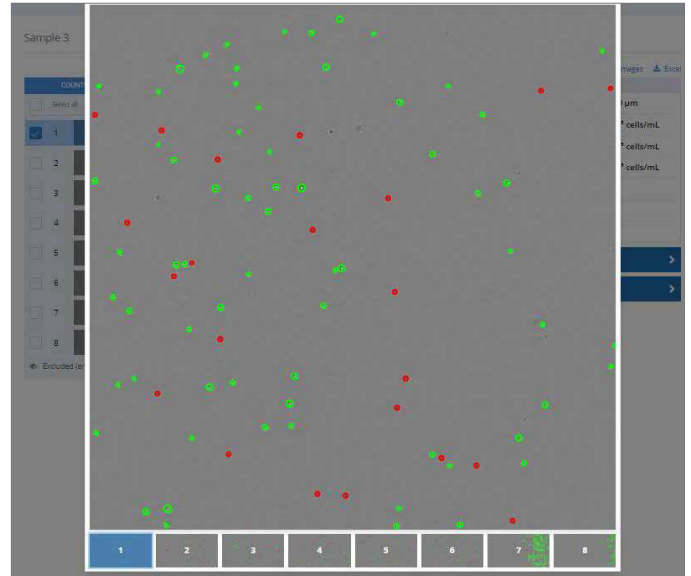


Figure 17. “Cell count” tab when counts are excluded. The excluded counts are displayed in the “Excluded” box on the left. The result is that these counts are not included into the overview page. The average results in the overview and in the Results box on the right change according to the exclusions. To include these counts again, click on the eye icon on the left of the excluded count

The image of a count will be enlarged when the user clicks on the image in the “Cell count” tab. All the other counts are displayed underneath the enlarged image, sorted by their count number (Figure 18). When you click on one of the other counts, the respective image will be displayed. Processed images will change according to the selected gating



**Figure 18. Enlarging a process image.** The user can click on a count to get the enlarged processed image. The other counts are displayed beneath this image and can be selected by clicking on them.

The "Cell size" tab provides information about the cell size ratios. It shows the average histogram of live and dead cell sizes of the selected counts (Figure 19).

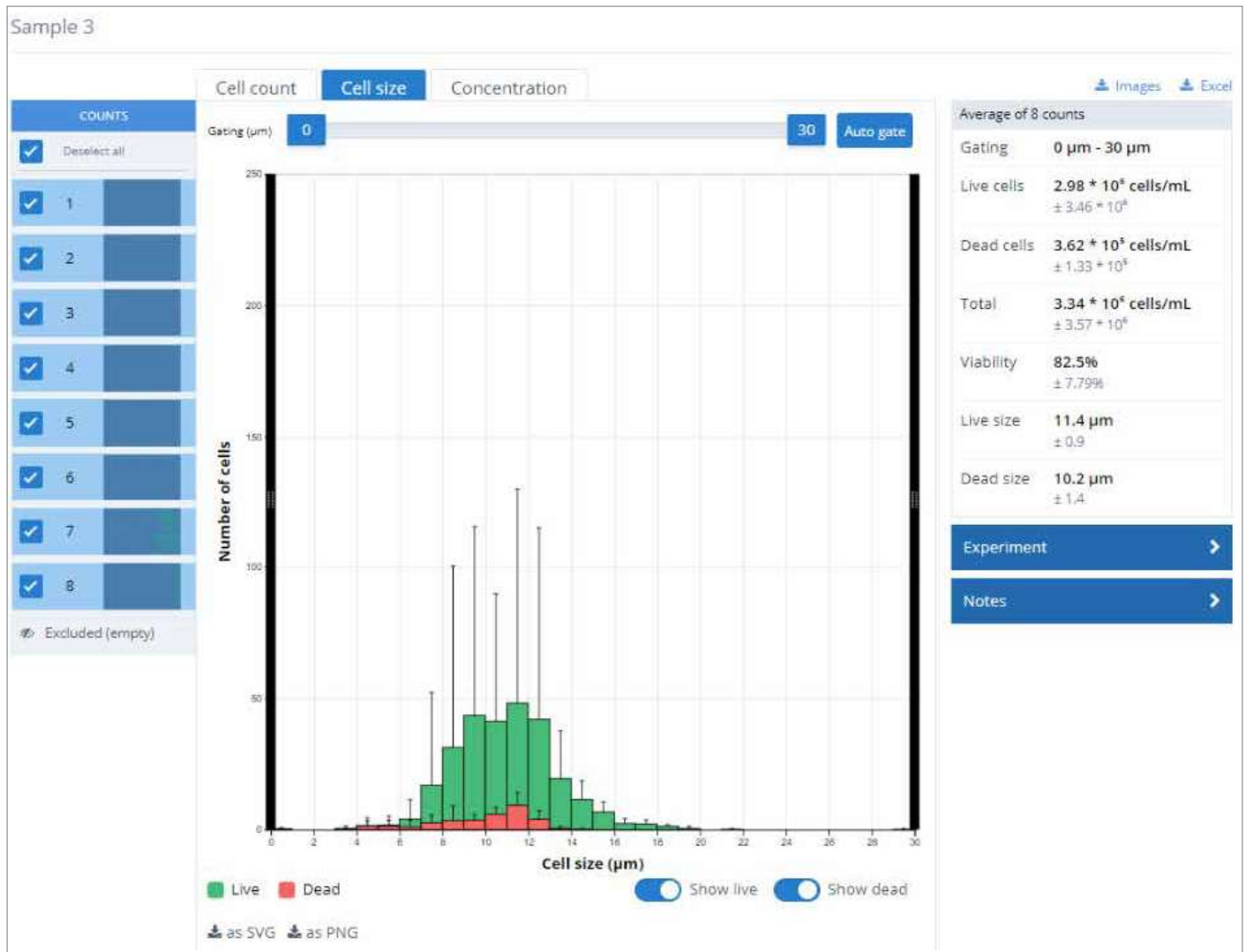


Figure 19. "Cell size" tab after performing multiple counts with the Version 3 algorithm. (A) All 8 counts are included in the live/dead cell size histogram. (B) Count numbers 7 and 8 are excluded. Their data is no longer incorporated in the live/dead cell size histogram.

The “Concentration tab” displays a bar graph of the live, dead, and total cell concentration of the selected counts. Which provides a quick overview of your count concentrations (Figure 20). The average and standard deviation are displayed in the grey shaded area on the left of the graph.

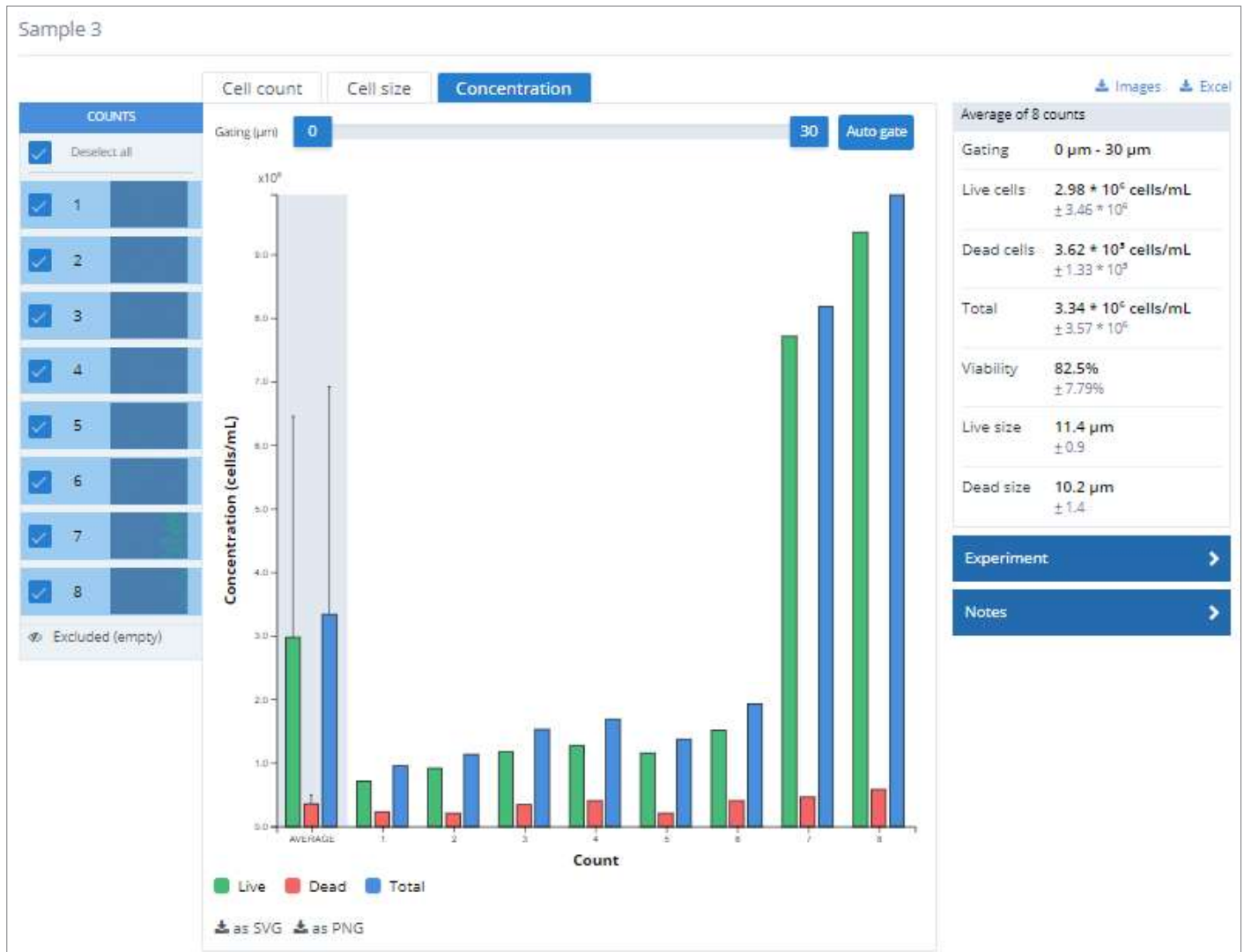


Figure 20. “Concentration tab” after performing multiple counts with the Version 3 algorithm. The live, dead, and total cell concentration are displayed for every count in the form of a bar graph, along with on the left in the shaded grey area is the average (+SD) of the selected counts.

For more specific information on claims, visit the Certificates page at [www.corning.com/lifesciences](http://www.corning.com/lifesciences).

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