Trypan Blue Dilution Calculation and Histogram Gating for Corning® Cell Counter

Two new features have been added to the Corning Cell Counter: The first allows you to select the Trypan blue dilution used and corrects the cell concentration(s) for the dilution used; the second is histogram gating. The features will be added automatically.

Trypan Blue Dilution Calculation

Performing a cell count has remained the same; you only need to select whether you have added Trypan blue in a 1:1 dilution to your sample when you are creating your experiment (Figure 1). You then focus on your cells. Live cells have bright centers with dark edges, wheras dead cells are completely black (Figure 2). Focus on your cells and press "Count."
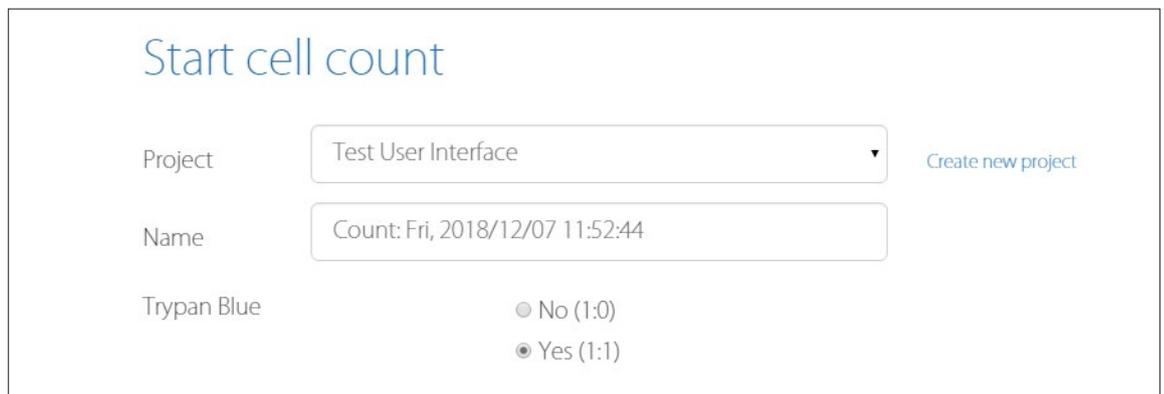

Figure 1. Name your experiment before starting a cell count and then select whether you have added Trypan blue to your cells or not. The default is "Yes (1:1)."
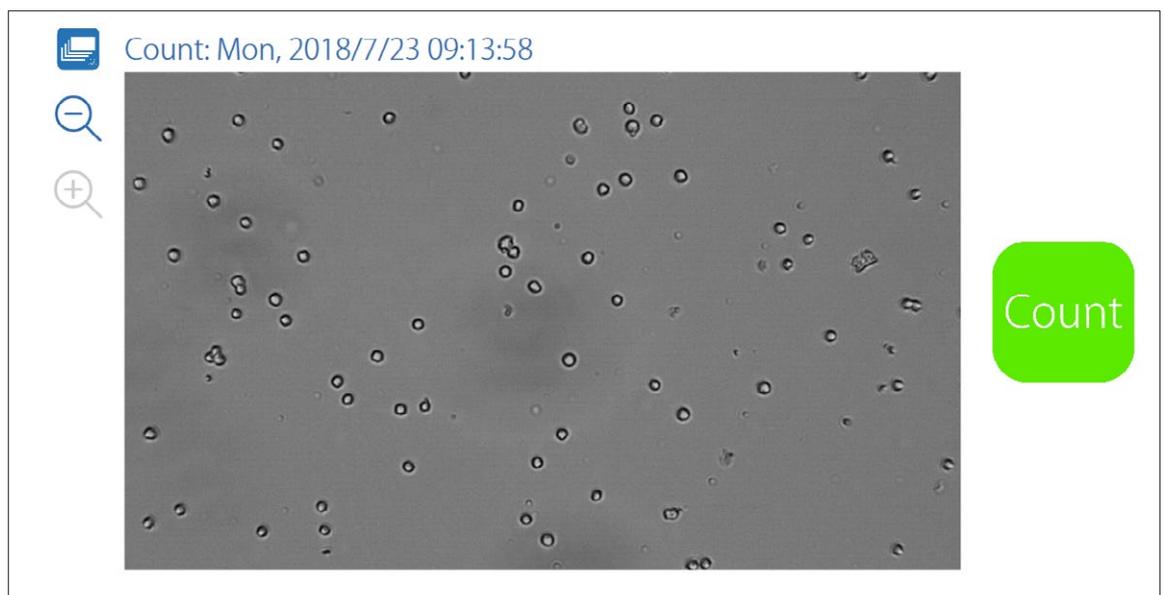

Figure 2. Focus on your cells before pressing "Count."

On the Results page, you will notice some differences. On the previous results page (Figure 3), you had the option to view the counted image and the size histograms of the live and dead cells separately.

Figure 3. Previous Results page had three tabs to enable viewing of the count or one of the histograms.

In the new Results page (Figure 4), only two tabs are visible above the image:

1. **Count**: Image tab with the live and dead cells encircled by green and red circles.
2. **Histogram**: Histogram tab with histograms of the diameter of all counted live and dead cells.

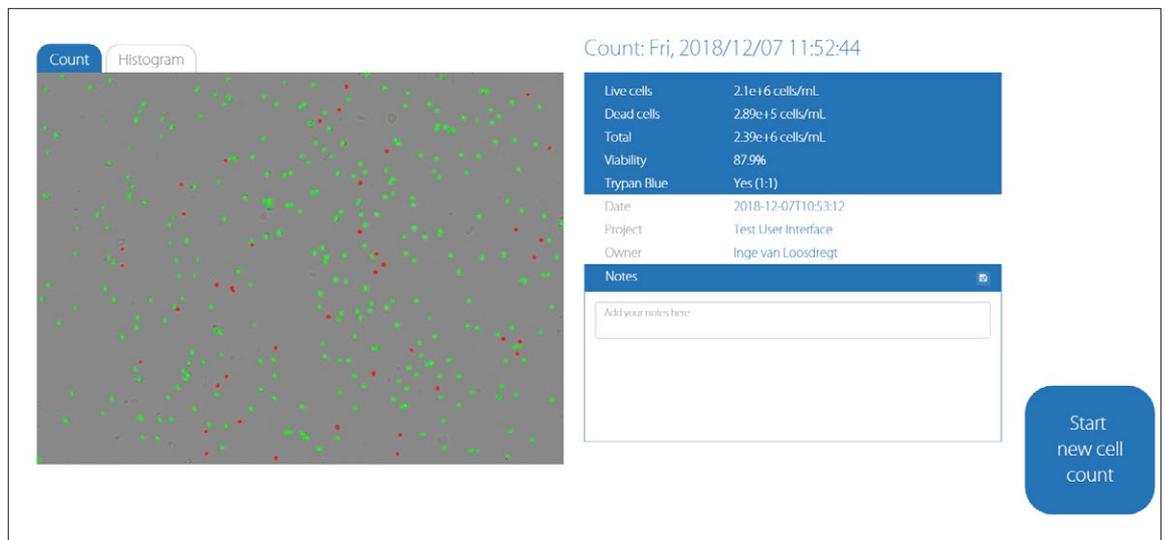By clicking on the "Histogram" tab, you will go to the new histogram panel.

Figure 4. New results page. Click on the "Histogram" tab to view the histogram of the diameter of live and dead cells. The Trypan blue dilution is displayed in the results box.

The Trypan blue dilution that you have selected is displayed in the results box on the right (Figure 4). If you have used Trypan blue, the cell concentration that is displayed is corrected for the 1:1 dilution, so you no longer have to correct the results yourself. If you did not use Trypan blue, the results are not corrected for any dilution, as in the previous CytoSMART Cloud App.

Histogram Gating

In the new "Histogram" tab, the histograms of the live and dead cells are displayed in one chart (Figure 5). The black bars on the left and right side of the chart can be used to gate the histogram. Just click and drag the bars (Figure 6). You will notice that the concentration and viability of your count will adjust according to the position of the gates. These numbers are displayed below the histogram.

NOTE: Concentrations and viability displayed in the blue results box will always display the ungated results.

Figure 5. The size histograms of the live and dead cells are displayed in one chart. The histograms can be gated by moving the black bars on the left and right side of the chart.

Figure 6. Example of gating the histograms. The cells that are in the shaded regions are excluded from the cell count. The cell concentrations and viability corresponding to the gated histograms are displayed underneath the histograms.

If you are only interested in the histogram of either the live or the dead cells, you can deselect the toggle box for one population (Figures 7 and 8). This can be done in both the gated and the ungated histograms.
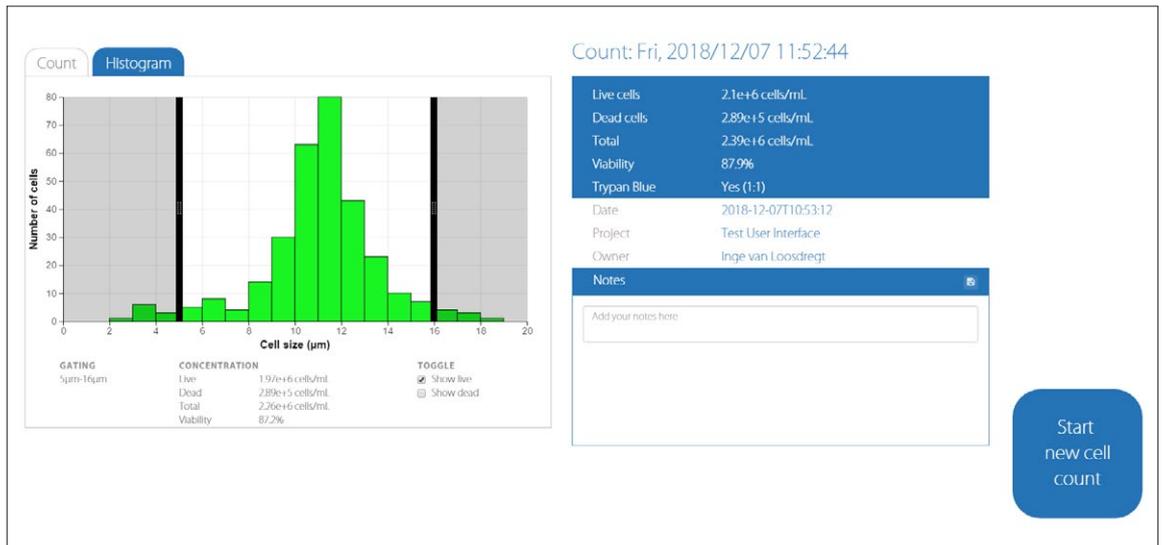

Figure 7. The dead cell population is deselected in the toggle box so that only the histogram of the live cells is displayed.

Figure 8. The live cell population is deselected in the toggle box so that only the histogram of the dead cells is displayed.

The gating of the histograms can also be performed on the CytoSMART Connect Cloud. Go to **https://cloud.cytosmart.com/** and log in using your credentials. Thereafter, open the project and then open the experiment. On the Experiment page, you will notice the same changes as in the CytoSMART Cloud App:

1. Two "Histogram" tabs have changed to one "Histogram" tab (Figures 9 and 10).

2. Your selection of Trypan blue addition [Yes (1:1) or No (1:0)] has been added to the Experiment box. The cell concentrations displayed in the Experiment box are corrected for the Trypan blue dilution that was used (1:1 or 1:0).
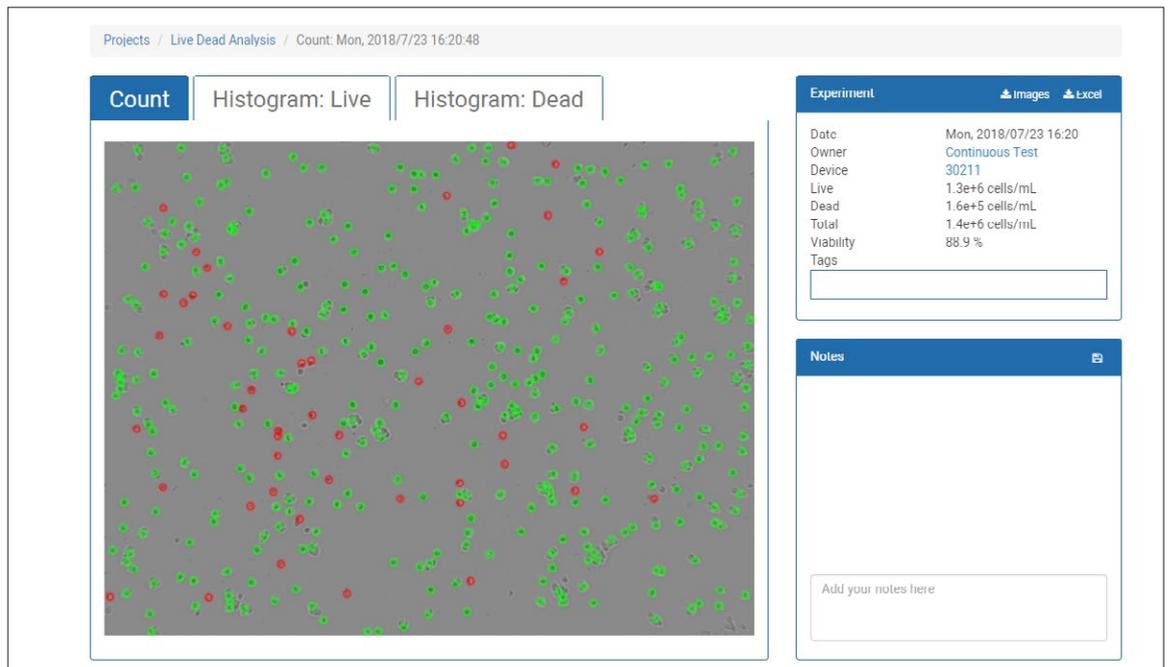

Figure 9. Previous Experiment page on the CytoSMART Connect Cloud had two "Histogram" tabs.
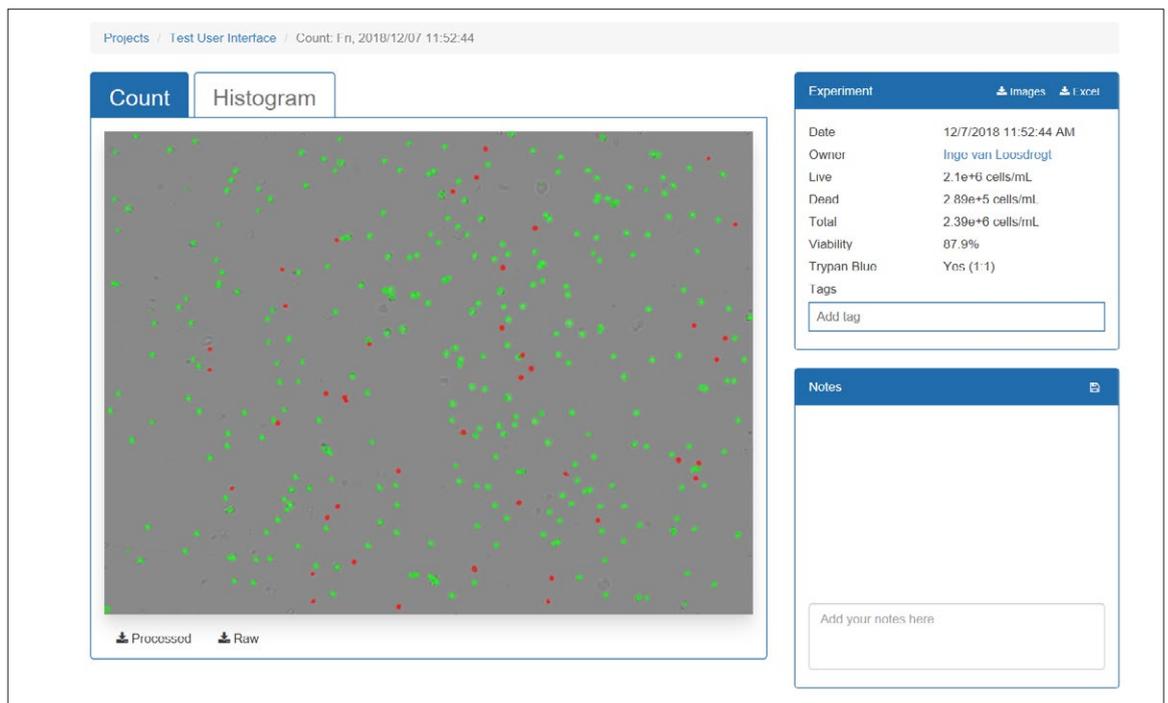

Figure 10. New Experiment page on the CytoSMART Connect Cloud has one "Histogram" tab and the Trypan blue selection in the Experiment box.

As with the CytoSMART Cloud App, click on the "Histogram" tab to view the histograms of the live and dead cells (Figure 11), and gate the histograms. The gating can be done by clicking and sliding the black bars on the left or right side of the chart (Figure 12). The histogram can be downloaded as a .SVG or .PNG file by clicking one of the buttons on the bottom right below the histogram. The histogram will be downloaded in the current state. If you have gated the histogram, a gated histogram is downloaded.
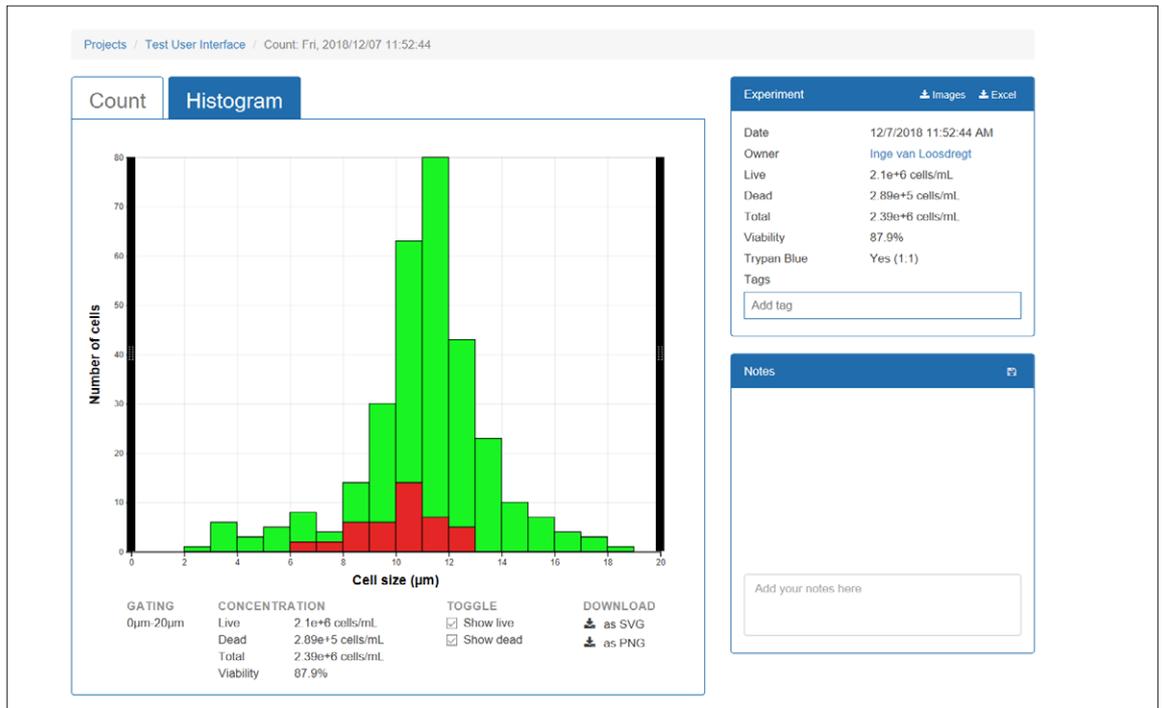

Figure 11. Click on the "Histogram" tab on the Experiment page to view the histograms of the diameter of the live and dead cells.
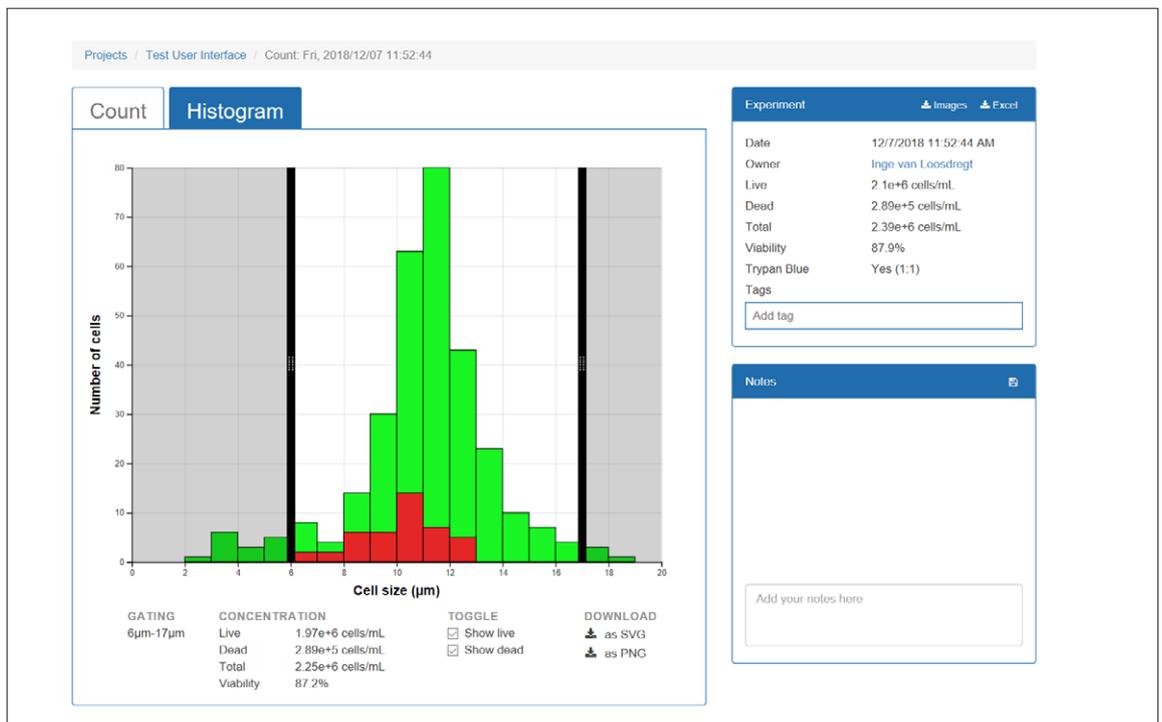

Figure 12. Click and slide the black bars on the left and right of the chart to gate the histograms. The cells in the shaded regions are excluded from the count. The cell concentration and viability corresponding to the gated histograms are displayed underneath the histograms.

Same as in the CytoSMART Cloud App, you can deselect one of the populations in the CytoSMART Connect Cloud environment (Figures 13 and 14). This can be done in both the gated and the ungated histograms. If you would like to download the histogram of only the live or dead cells, click on one of the download buttons on the bottom right while one of the histograms is deselected.
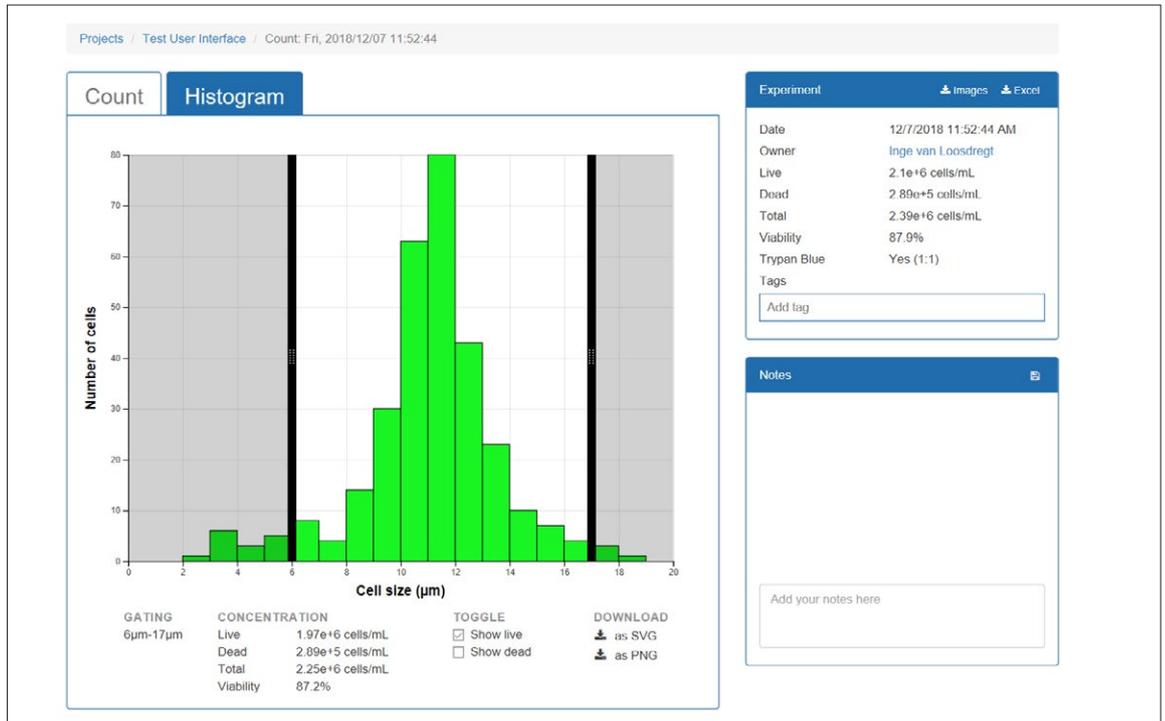

Figure 13. The dead cell population is deselected so only the histogram of the live cells is displayed.
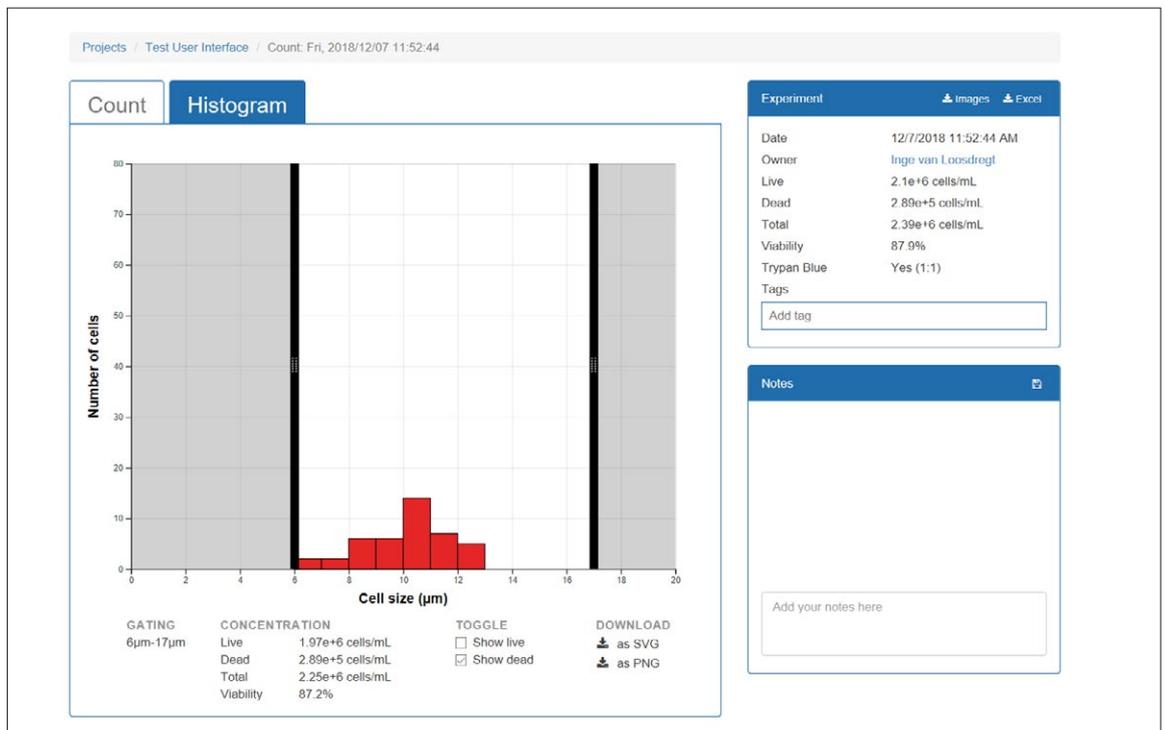

Figure 14. The live cell population is deselected so only the histogram of the dead cells is displayed.

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