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Quick Reference Guide

Calculating %Cytolysis with RTCA Pro

This quick reference guide describes the procedure for calculating %Cytolysis from Cell Index data, using RTCA Software PRO v2.0 and newer (5454433001).

Before calculating the cytolysis, the Cell Index must be normalized. Figure 1 shows an example in which the effectors were added at approximately 19 hours. There are some differences even before this time, as shown by the blue arrows. These are due to pipetting differences and edge effect.

Normalizing to the point just before effectors are added corrects this (Figure 2).

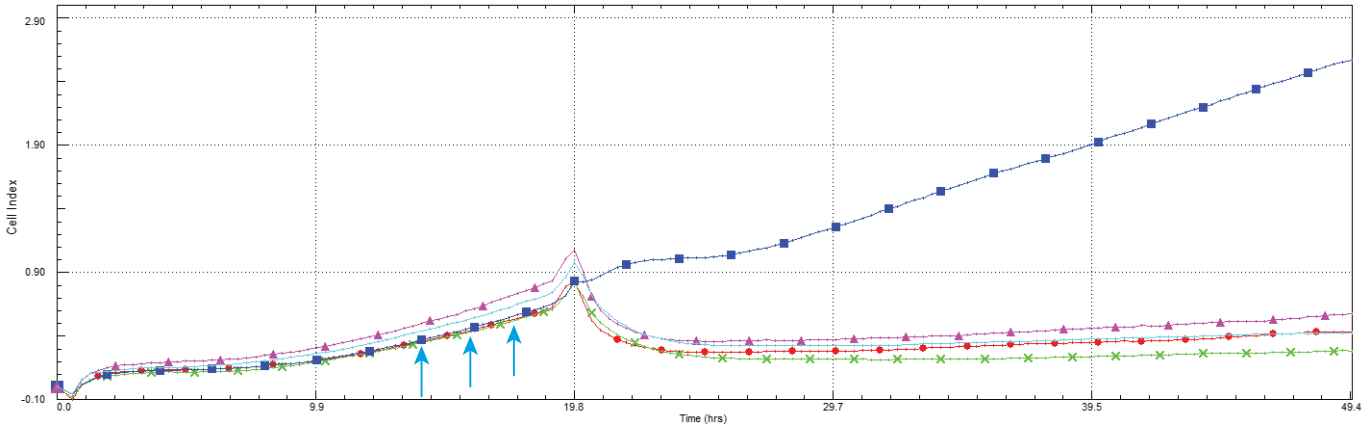

Figure 1. Pre-effector differences in Cell Index.

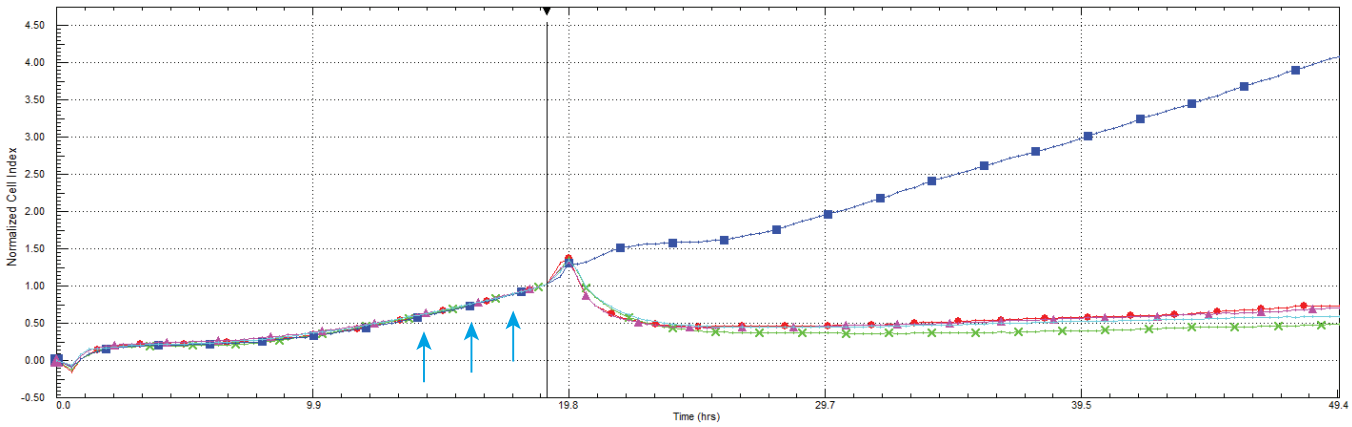

Figure 2. Normalization corrects discrepancies due to experimental error.

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1. To find the correct point to start normalization, look for the point where the curve usually has a small bump or change in direction. (When the plate is removed and effectors are added, the plate temperature changes, and the cells slightly shrink or expand, depending on the cell type. This causes a change in the Cell Index.) Drag a square around that region with your mouse to zoom in (Figure 3).
2. Once you have zoomed in, you can change to Normalized Cell Index (Figure 4A).
3. Drag the black arrowhead (Figure 4B), or use the drop-down menu (Figure 4C) to choose the point just before the bump/direction change.

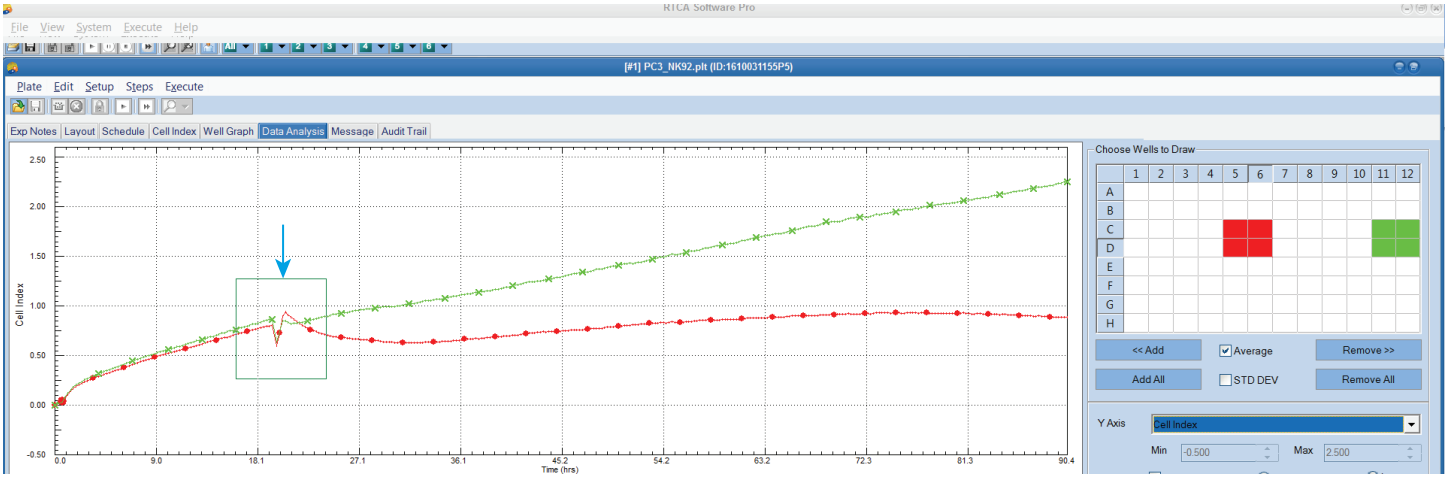

Figure 3. Selecting an area to zoom.

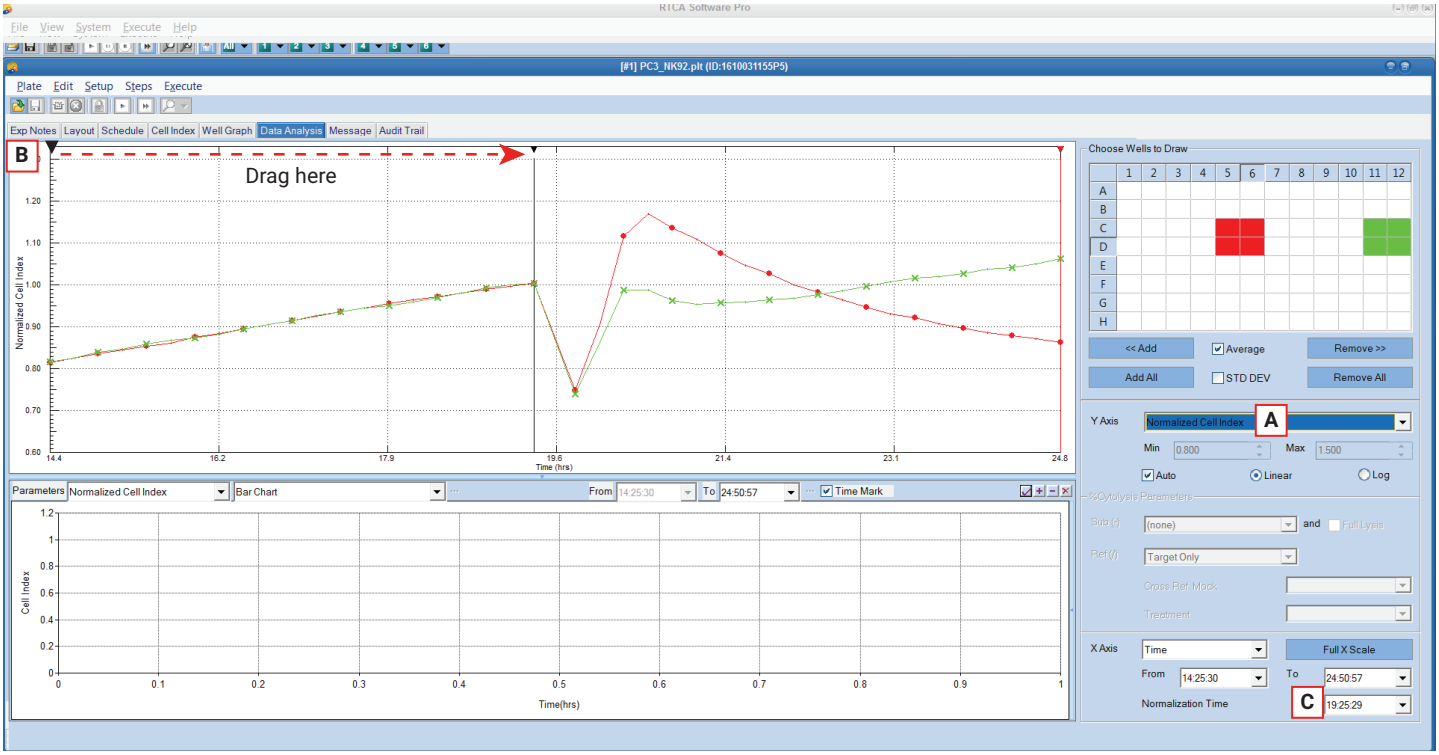

Figure 4. (A) Select "Normalized Cell Index" from the Y Axis drop-down menu. (B) Drag the black arrowhead, or (C) select a time point at which to start normalization.

Setting the normalization time

The software will attempt to find a break in the sequence of readings and mark it in a different color (Figure 5). The time point highlighted is the time when the plate is returned to the instrument after adding the treatment. Select the time *just above* the highlighted time, which corresponds to the last time when all the conditions are theoretically identical (that is, just before the treatments are added).

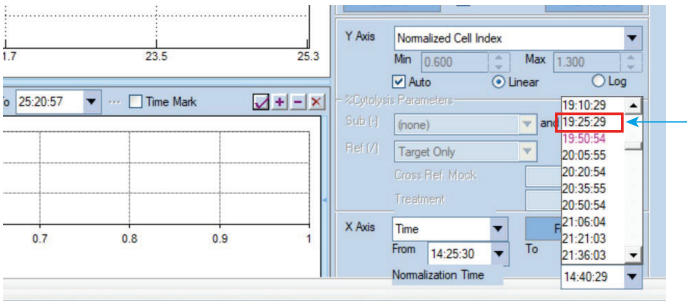

Figure 5. Select the time point just above the highlighted one.

1. Click **Full X Scale** to again visualize the complete plot (Figure 6).

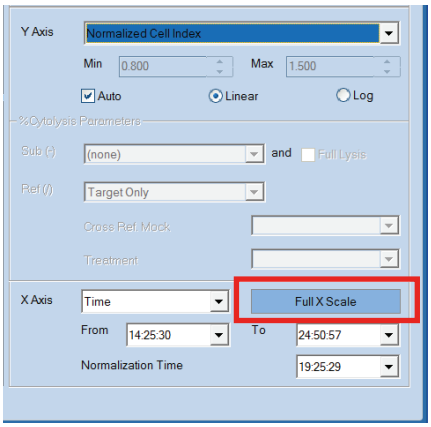

Figure 6. The **Full X Scale** button.

2. From the Y Axis drop-down menu, select **%Cytolysis** (Figure 7).

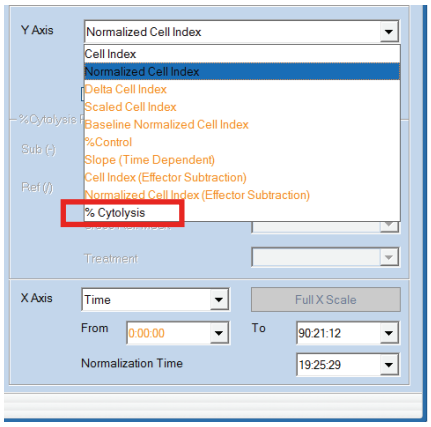

Figure 7. Select **% Cytolysis** from the Y Axis drop-down menu.

As a quick check, the wells used in the calculation as the No Effectors (i.e. No Cytolysis) value are marked with "t" (Figure 8). If the incorrect wells are being used, correct the values in the Layout tab.

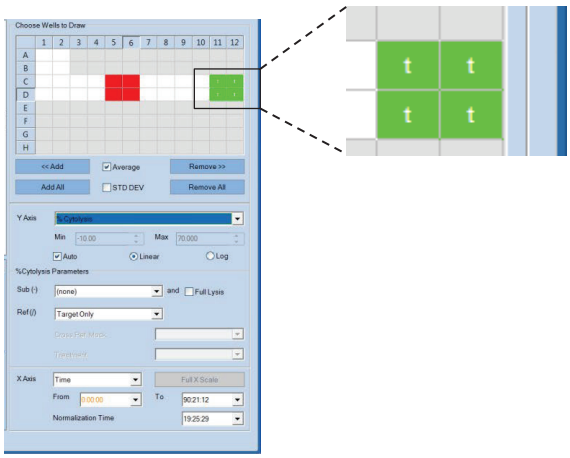

Figure 8. "No Effector" cells are marked with a "t".

Specific cytolysis

If you have mock effector controls in the experiment, and wish to calculate specific cytolysis relative to these, choose "Target + Mock" as the reference from the Reference (Ref(/)) drop-down menu. The corresponding wells are marked with "tm" (Figure 9).

Bar charts

To create bar charts of cytolysis at specific times, choose the time using the drop-down on the lower chart menu (Figure 10A). Click the check button (Figure 10B) twice to refresh the chart window and plot the data, or "+" to add the data to the current chart (Figure 10C).

Exporting data

To export either graph either as an image file, or the data from the graph, right-click on it and choose the desired option.

Alternately, click **Plate > Export Experiment Info**.

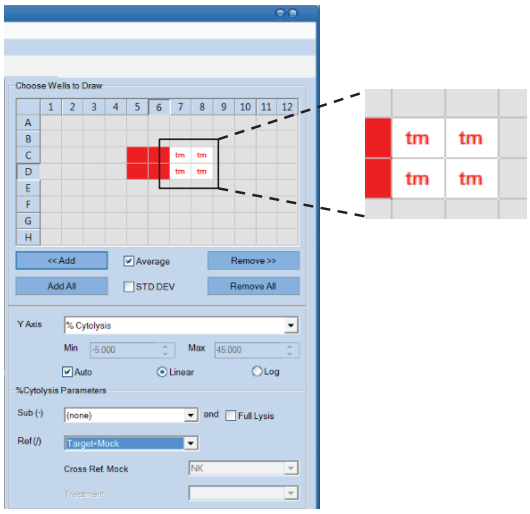

Figure 9. The Ref (/) drop-down menu, with **Target+Mock** selected. The corresponding wells are marked with "tm".

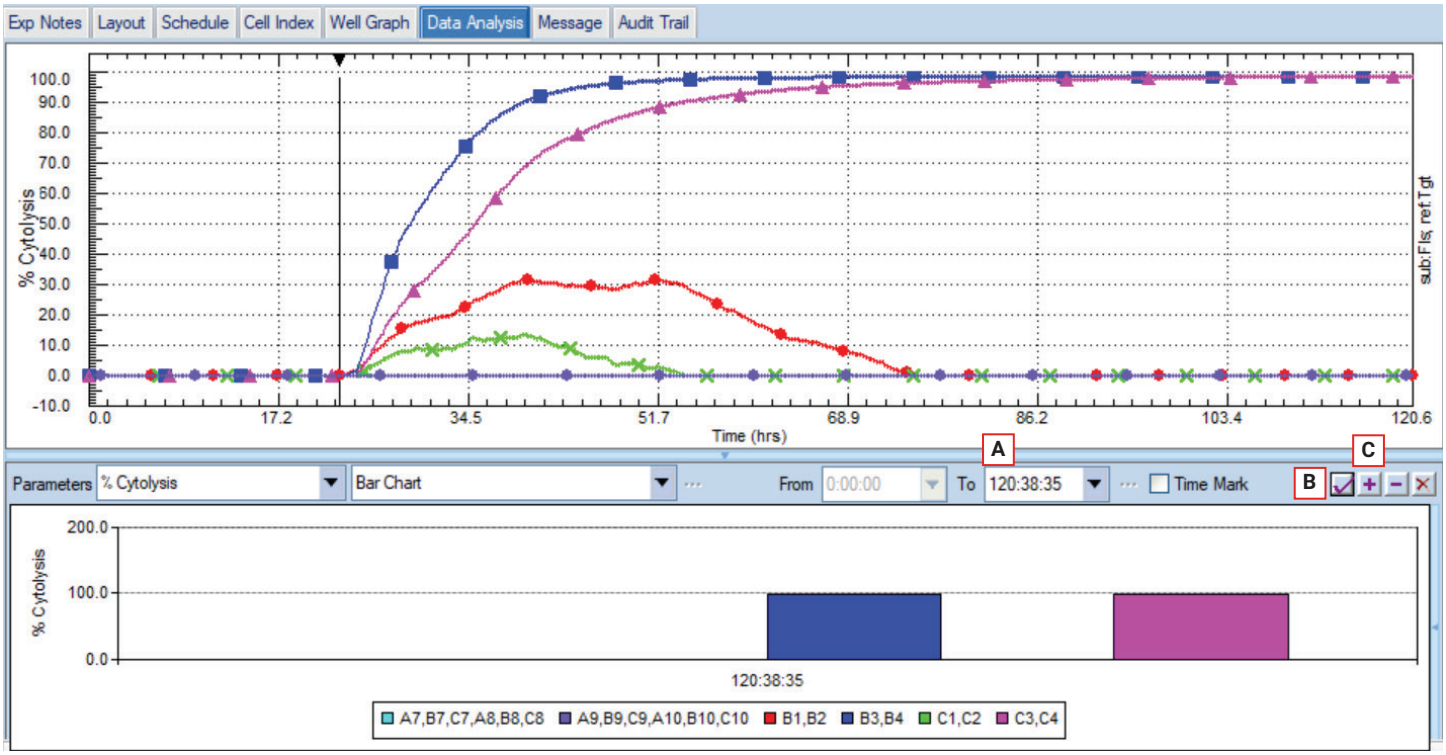

Figure 10. (A) Drop-down menu for selecting a time frame from which to create a bar graph. (B) **Check** button. (C) **Add** button for adding data to the current chart.

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RA44707.3849537037

Printed in the USA, July 19, 2022
5994-4903EN

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