

Calculating EC₅₀ and IC₅₀ Values using Area under the Curve

A major component to Live Content Imaging is translating phenotypic changes in cultured cells into quantitative biology. The integrated image analysis algorithms within the IncuCyte™ ZOOM software package facilitates the kinetic quantification of cellular processes over time (i.e. proliferation, cell death, invasion/migration, neurite outgrowth, and more) and enables researchers to use alternative strategies to evaluate kinetic pharmacology. Area under the curve (AUC) is an integrated measurement that can be used as a cumulative measurement of a drug effect. EC₅₀ and IC₅₀ values can be quantified using repeated measures to reinforce confidence in the collected biological data. This technical note outlines steps to export data from IncuCyte™ ZOOM into GraphPad Prism® Version 5.00 to calculate AUC and illustrates the process of translating that data into EC₅₀/IC₅₀ calculations. Similar analyses can be performed in other graphing/statistical software programs.

Experimental Overview

To set up a concentration response assay, the experimentalist should choose a range of compound concentrations and replicates that fully encapsulates (including maximal and minimal responses) the biology being tested. An analysis job specific to the assay of interest should be scheduled at the time of assay initiation for real-time data quantification. Once data is collected follow these simple steps (covered in detail below):

- 1) Export the data from the IncuCyte™ ZOOM and paste into a spreadsheet program such as Microsoft® Excel.
- 2) Calculate the AUC for each well in GraphPad Prism®.
- 3) Log transform the concentration values and plot against AUC.
- 4) Perform a non-linear regression to calculate EC₅₀ and IC₅₀ values.

Exporting and Organizing Data

Export data from the IncuCyte ZOOM onto the clipboard and paste into Excel. Arrange the data so that all replicates are next to each other.

1. Select the wells of interest, click “Data Export”.
2. Layout: Show each scan as a single row in one large table (**Screenshot 1**)
 - a. If replicates run vertically (i.e. A5, B5, C5 are all replicates), then select “Column by column”
 - b. If replicates run horizontally (i.e. A5, A6, A7 are all replicates), then select “Row by row”
3. Destination: Clipboard
4. Click Export and paste data into a new sheet in Excel (**Screenshot 2**)





Calculating IC_{50} and EC_{50} Values using Area under the Curve

Screenshot 1: Export Data

Export Metrics

Layout

- ☒ Show each scan as a single row in one large table.
 - ☒ Column by column: A1, B1, C1, ... A2, B2, C2, ...
 - ☐ Row by row: A1, A2, A3, ... B1, B2, B3, ...
 - ☐ Organize the metrics by replicate wells per the Plate Map
 - ☐ Show each scan as its own table. (columns: 1, 2... rows: A, B...)
- ☒ Show row/column labels

Destination

- ☒ Clipboard
- ☐ All scans in one file: C:\Users\Katherine Artymovich\Desktop\File1a
- ☐ Each scan in a separate file:

Other Options

- ☒ Include experiment details in header
- Fill holes in the data with the following characters: (optional)
- ☐ Include ☒ Std Error ☐ Std Deviation
- ☐ Break data down into individual images.

Export

Screenshot 2: Paste Data into Excel

Column by column

	A	B	C	D	E
1	Date Time	Elapsed	A5	B5	C5
2	5/30/2013 17:36	0	509.1151	325.7965	529.533
3	5/30/2013 19:46	2.166667	633.4932	345.7527	561.558
4	5/30/2013 21:46	4.166667	730.9537	380.0959	637.20
5	5/30/2013 23:46	6.166667	758.3355	407.9418	698.002
6	5/31/2013 1:46	8.166667	687.3286	444.1414	762.048
7	5/31/2013 3:46	10.16667	700.7874	468.7386	849.762

Row by row

	A	B	C	D	E
1	Date Time	Elapsed	A5	A6	A7
2	5/30/2013 17:36	0	509.1151	593.1168	488.694
3	5/30/2013 19:46	2.166667	633.4932	697.0746	582.908
4	5/30/2013 21:46	4.166667	730.9537	796.8556	684.54
5	5/30/2013 23:46	6.166667	758.3355	817.2759	735.130
6	5/31/2013 1:46	8.166667	687.3286	759.7278	685.472
7	5/31/2013 3:46	10.16667	700.7874	738.3793	660.87

Calculate Area under the Curve for Each Well

1. Create a new XY Table/Graph in GraphPad Prism® and select “Enter and plot a single Y value for each point” (Screenshot 3).
2. Copy and Paste data from Excel into GraphPad Prism®. Time elapsed should go in the X column.
3. Click “Analyze”, then expand “XY Analyses”, select “Area under the Curve”, and click “OK” (Screenshot 4).

Screenshot 3: Enter Data into GraphPad Prism

New table & graph:

XY

Column

Grouped

Contingency

Survival

Clone from:

Opened project

Recent project

Saved example

Shared example

Free update available

New version available

Sample data

- ☒ Start with an empty data table
- ☐ Use sample data

Choose a graph

Selected graph: **Points & connecting line**

Subcolumns for replicates or error values

X error bar: ☐ Enter X error values to plot horizontal error bars

Y: ☒ Enter and plot a single Y value for each point

☐ Enter 3 replicate values in side-by-side subcolumns, and plot Mean and Error SEM

☐ Enter and plot error values already calculated elsewhere

Enter: Mean, SD, N

Cancel **Create**



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Format Data for Pharmacological Analyses

1. Copy the values from the row of "Total Area" (**Screenshot 4**).
2. Create a new XY Table/Graph and select the # of replicates in your data set (e.g. this example has 4 replicates) (**Screenshot 5**).
3. Right Click on well A:Y1. Select "Paste Special". Select the "Placement" tab. Select "By rows. Place the # of replicate values on each row" (assuming your data is arranged with replicates next to each other). Click "Paste" (**Screenshot 6**).
4. Enter the drug concentrations in the X column for each set of replicates.

Screenshot 4: Calculate AUC for Each Well

1	Baseline	0.0	0.0	0.0	0.0
2	Total Area	30529	31841	28510	28489
3	Total Peak Area	30529	31841	28510	28489

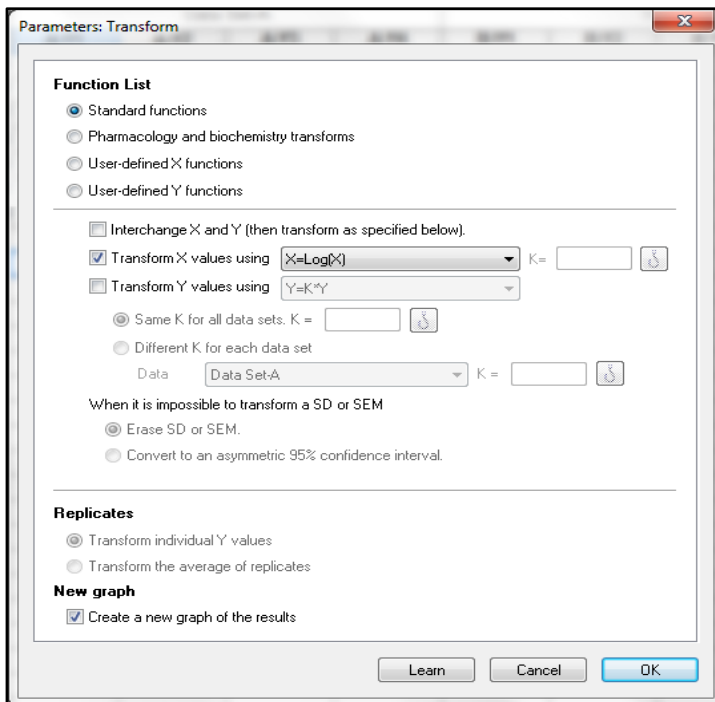
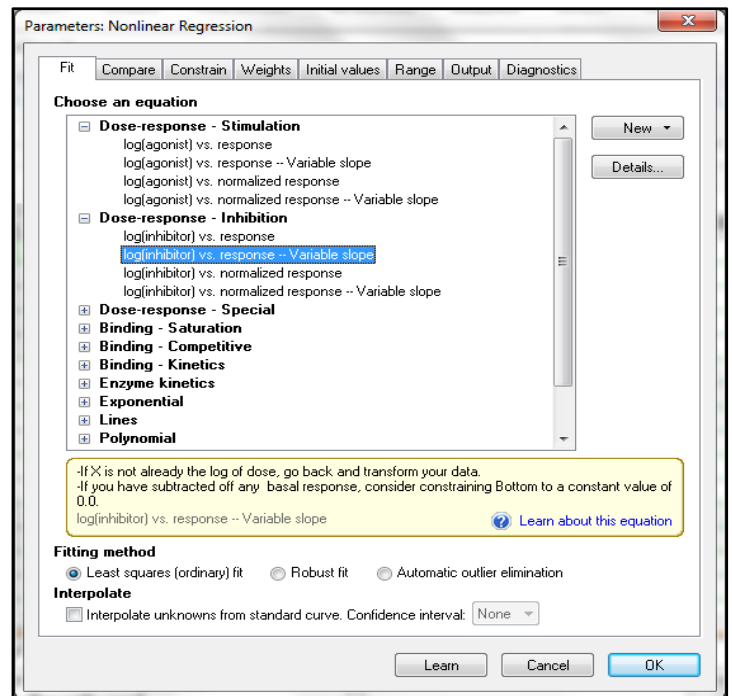
Screenshot 5: Create New Graph for Pharmacology

Screenshot 6: Paste AUC Values in Replicates

		X	A			
		[Cmpd] M	Nuclear Counts/mm ²			
		X	A:Y1	A:Y2	A:Y3	A:Y4
1	Title	0.000003	30529	31841	28510	28489
2	Title	0.000001	26970	26901	34564	28491
3	Title	3.333330e-007	54576	65035	45648	47173
4	Title	1.111110e-007	81709	83111	66462	69568

**Transform Data and Perform Nonlinear Regression**

1. Click “Analyze” and “Transform”. Select “Transform X values using $X=\text{Log}(X)$ ” and click “OK” (**Screenshot 7**).
2. Click “Analyze”, expand “XY analyses”, select “Nonlinear regression [curve fit]”, and click “OK”.
3. To calculate EC_{50} , expand “Dose-response – Stimulation”, select “log(agonist) vs. response – variable slope”, and click “OK”.
4. To calculate IC_{50} , expand “Dose-response – Inhibition”, select “log(inhibitor) vs. response – variable slope”, and click “OK” (**Screenshot 8**).
5. View final data output – Graph is viewable under “Graphs” (**Screenshot 9**).

Screenshot 7: Transform Data**Screenshot 8: Calculate IC50 Value****Screenshot 9: Final Data Output**