Technical Overview

Calculation Principles of Agilent xCELLigence RTCA Software

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Abstract

The Agilent xCELLigence system monitors cellular events in real-time by measuring electrical impedance through microelectrodes at the bottom of each cell culture plate well. The Agilent xCELLigence RTCA software calculates Cell Index (CI) as the relative change in measured impedance to represent cell status. In this technical overview, the mathematics for calculating the related parameters used in RTCA software are described.

All calculations are based on the RTCA software version 2.6, and are not applicable in the same way to RTCA software version 1.1.

Basic terms of statistics

For Cell Index calculations, some basic statistical methods are used. Take a set of data, $y_1, y_2, \ldots, y_n$, as an example. The average represents a measure of the arithmetic mean value of this set of data. The standard deviation (std dev) represents a measure of the spread or dispersion of the set of data. The average and standard deviation of the data set $y_1, y_2, \ldots, y_n$ are calculated as follows:

$$\text{Average } (y_1, y_{2, \ldots,} y_n) = \frac{\sum_{i=l}^{n} y_i}{n}$$

$$\text{Std dev } (y_1, y_{2, \ldots,} y_n) = \sqrt{\frac{\sum_{i=l}^{n} (y_i - \bar{y})^2}{(n-1)}}$$

Cell Index (CI)

In the Agilent xCELLigence RTCA system, the parameter termed Cell Index (CI) is a measure of the relative change in electrical impedance at a certain frequency (f_n). The Cell Index at a given time point t (CI(t)) is calculated as follows:

$$CI(t) = \frac{R(f_n,t) - R(f_n,t_0)}{Z_n}$$

Where:

- f_n is the frequency in which the impedance measurement is carried out.
- $R(f_n,t)$ is the measured impedance at frequency f_n at time point t.
- $R(f_n,t_0)$ is the measured impedance at frequency f_n at time point t_0 (usually t_0 is the time when the background is measured).
- Z_n is the corresponding frequency factor of f_n.

The xCELLigence system measures impedance at three discrete frequencies: f_1 = 10 kHz, f_2 = 25 kHz, and f_3 = 50 kHz. The corresponding frequency factors are Z_1 = 15 Ω, Z_2 = 12 Ω, and Z_3 = 10 Ω, respectively.

Average of Cell Index

The average of the Cell Index ($CI_{Average}$ (t)) is the average of Cell Index in all selected replicate wells 1, 2, ..., n at time point t. The replicate wells are wells with the same cell type, cell number, compound name, and compound concentration as inserted on the **Layout** page. $CI_{Average}(t)$ is calculated as follows:

$$CI_{Average}(t) = \text{Average}(CI_{well\_1}(t), CI_{well\_2}(t), \ldots, CI_{well\_n}(t))$$

Where:

$CI_{well\_1}(t)$, $CI_{well\_2}(t)$, ..., $CI_{well\_n}(t)$, are the Cell Indices of well 1, 2, ..., n at time point t, respectively.

The calculated average of the Cell Index is displayed in a single-color curve on the **Plot** page of the RTCA software.

Standard deviation of Cell Index

The standard deviation of the Cell Index ($CI_{Std\ dev}(t)$) is the standard deviation of Cell Index in all selected replicate wells 1, 2, ..., n at time point t. $CI_{Std\ dev}(t)$ is calculated as follows:

$$CI_{Std\ dev}(t) = \text{Std dev}(CI_{well\_1}(t), CI_{well\_2}(t), \ldots, CI_{well\_n}(t))$$

Where:

$CI_{well\_1}(t)$, $CI_{well\_2}(t)$, ..., $CI_{well\_n}(t)$ are the Cell Indices of wells 1, 2, ..., n at time point t, respectively.

The calculated standard deviation of the Cell Index is shown as error bars added to the corresponding Cell Index average curve on the **Plot** page of the RTCA software.

Normalized Cell Index, baseline Cell Index, and delta Cell Index

Normalized Cell Index

The Cell Index of different wells (1, 2, ..., n) can be selected for normalization at user-specified time points. The normalized Cell Index (NCI(t)) is calculated as the Cell Index at a given time point t (CI(t)) divided by the Cell Index at the selected normalization time point ($CI(t_{normalization})$) as follows:

$$NCI_{well\_i}(t) = \frac{CI_{well\_i}(t)}{CI_{well\_i}(t_{normalization})}$$

with

$CI_{well\_i}(t_{normalization}) \neq 0$

and

i =1, 2, ..., n

Where:

- $NCI_{well\_i}(t)$ is the normalized Cell Index of well i at time point t.
- $CI_{well\_i}(t)$ is the Cell Index of well i at time point t.
- $CI_{well\_i}(t_{normalization})$ is the Cell Index of well i at the normalization time $t_{normalization}$.

Note: In RTCA software, the Cell Index values at the selected normalization time point should be ≥0.001. Otherwise, there will be a warning message displayed, and all normalized Cell Index values of that well will be set to zero, which results in a horizontal Cell Index curve with a value of zero.

By definition, the normalized Cell Index is equal to 1 at the normalization time point, so $NCI(t_{normalization}) = 1$.

The average and standard deviation of the normalized Cell Index for selected wells at time point t are calculated as follows:

$$NCI_{Average}(t) = \text{Average}\ (NCI_{well\_1}(t), NCI_{well\_2}(t), ..., NCI_{well\_n}(t))$$

$$NCI_{Std\ dev}(t) = \text{Std dev}\ (NCI_{well\_1}(t), NCI_{well\_2}(t), ..., NCI_{well\_n}(t))$$

Where:

- $NCI_{Average}(t)$ is the average of the normalized Cell Index of well 1 to n at time point t.
- $NCI_{Std\ dev}(t)$ is the standard deviation of the normalized Cell Index of well 1 to n at time point t.
- $NCI_{well\_1}(t)$, $NCI_{well\_2}(t)$, ..., $NCI_{well\_n}(t)$ are the normalized Cell Indices of wells 1, 2, ..., n at time point t, respectively.

Example 1: Calculation of normalized Cell Index

Two sets of Cell Index data measured at time point 10:06:49 and 10:21:49 are shown in Table 1.

Table 1. Cell Index for an Agilent E-Plate 96 at two time points.

Cell Index at 10:06:49												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.2774	0.3044	0.2718	0.2656	0.3013	0.2726	0.2215	0.2470	0.2851	0.2443	0.2673	0.2191
B	0.1951	0.2217	0.1634	0.1820	0.2054	0.1996	0.1564	0.1914	0.1672	0.1159	0.1736	0.1097
C	0.1572	0.2065	0.2050	0.2280	0.1897	0.2227	0.1541	0.1986	0.1627	0.1785	0.1794	0.1657
D	0.1732	0.2197	0.2039	0.2295	0.2439	0.2618	0.1824	0.1985	0.2486	0.2197	0.2071	0.1513
E	0.1951	0.1722	0.2366	0.2417	0.2258	0.2056	0.2246	0.2092	0.1965	0.2202	0.1888	0.1834
F	0.2069	0.2235	0.2128	0.1923	0.2226	0.2179	0.2034	0.2283	0.2383	0.1925	0.1982	0.1777
G	0.1699	0.2177	0.1555	0.1572	0.1581	0.2147	0.1797	0.1656	0.1631	0.1252	0.1232	0.1877
H	0.2357	0.2792	0.2683	0.2439	0.2346	0.2307	0.2123	0.2497	0.2490	0.2712	0.2337	0.2607
Cell Index at 10:21:49												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.2819	0.3103	0.2746	0.2734	0.3077	0.2794	0.2239	0.2535	0.2891	0.2512	0.2723	0.2227
B	0.1976	0.2264	0.1673	0.1875	0.2098	0.2029	0.1602	0.1936	0.1713	0.1215	0.1775	0.1143
C	0.1586	0.2098	0.2082	0.2319	0.1926	0.2267	0.1568	0.2023	0.1663	0.1823	0.1816	0.1579
D	0.1751	0.2257	0.2098	0.2330	0.2476	0.2673	0.1891	0.2018	0.2558	0.2257	0.2100	0.1553
E	0.1986	0.1785	0.2405	0.2451	0.2294	0.2092	0.2267	0.2167	0.2012	0.2235	0.1944	0.1857
F	0.2119	0.2273	0.2203	0.1978	0.2286	0.2234	0.2052	0.2333	0.2439	0.1988	0.2010	0.1825
G	0.1751	0.2227	0.1592	0.1622	0.1632	0.2197	0.1827	0.1668	0.1692	0.1308	0.1254	0.1896
H	0.2414	0.2863	0.2736	0.2479	0.2401	0.2369	0.2176	0.2540	0.2539	0.2754	0.2395	0.2647

If 10:06:49 is selected as the normalization time point, the normalized Cell Index at 10:06:49 will all become 1. Here, taking well D4 as an example, the normalized Cell Index of well D4 at time point 10:21:49 is:

$$NCI_{well\_D4}(t_{10:21:49}) = \frac{CI_{well\_D4}(t_{10:21:49})}{CI_{well\_D4}(t_{10:06:49})} = \frac{0.2330}{0.2295} \approx 1.0153$$

The calculated normalized Cell Index values at 10:06:49 and 10:21:49 are shown in Table 2.

Baseline Cell Index

Users can choose one specific well or multiple wells as the baseline wells. The average of the Cell Index of selected baseline well 1 to n is defined as $CI_{baseline\_well}(t)$. The baseline Cell Index (BCI(t)) of one well is calculated by subtracting $CI_{baseline\_well}(t)$ from the Cell Index of that well at the time point t as follows:

$$BCI_{well\_i}(t) = CI_{well\_i}(t) - CI_{baseline\_well}(t)$$

with

$$CI_{baseline\_well}(t) = Average(CI_{baseline\_well\_1}(t), CI_{baseline\_well\_2}(t), ..., CI_{baseline\_well\_n}(t))$$

and

i =1, 2, ..., n

Where:

- $BCI_{well\_i}(t)$ is the baseline Cell Index of well i at time point t.
- $CI_{baseline\_well}(t)$ is the average of the Cell Index of selected baseline well 1 to m at time point t.
- $CI_{baseline\_well\_1}(t)$, $CI_{baseline\_well\_2}(t)$, ..., $CI_{baseline\_well\_n}(t)$ are the Cell Indices of selected baseline wells 1, 2, ..., m, respectively.

Table 2. Calculated normalized Cell Index for an Agilent E-Plate 96 at two time points.

Normalized Cell Index at 10:06:49 (Normalization Time at 10:06:49)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1
Normalized Cell Index at 10:21:49 (Normalization Time at 10:06:49)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.0162	1.0196	1.0103	1.0294	1.0213	1.025	1.0106	1.0266	1.0141	1.0284	1.0186	1.0164
B	1.0125	1.0212	1.0235	1.0302	1.0216	1.0166	1.0239	1.0116	1.0243	1.0487	1.0228	1.0412
C	1.0089	1.016	1.0159	1.0173	1.0153	1.0181	1.0177	1.0184	1.0224	1.0215	1.0123	0.9527
D	1.0111	1.0272	1.0287	1.0153	1.015	1.0211	1.037	1.0166	1.0287	1.0272	1.0144	1.0267
E	1.0182	1.0367	1.0164	1.0142	1.016	1.0176	1.009	1.0354	1.0241	1.0149	1.0301	1.0125
F	1.0243	1.0174	1.0350	1.0285	1.0267	1.0249	1.0089	1.0218	1.0238	1.0326	1.014	1.0270
G	1.0309	1.0227	1.0238	1.0320	1.0322	1.0231	1.0168	1.0074	1.0374	1.0448	1.0177	1.0098
H	1.0239	1.0254	1.0200	1.0165	1.0232	1.0267	1.0249	1.0172	1.0199	1.0152	1.0252	1.0153

The average and standard deviation of the baseline Cell Index for wells 1 to n at time point t are calculated as follows:

$$BCI_{Average}(t) = Average(BCI_{well\_1}(t), BCI_{well\_2}(t), \ldots, BCI_{well\_n}(t))$$

$$BCI_{Std\ dev}(t) = \sqrt{(CI_{Std\ dev}(t))^2 + (CI_{Std\ dev(baseline\_wells)}(t))^2}$$

with

$$CI_{Std\ dev}(t) = Std\ dev(CI_{well\_1}(t), CI_{well\_2}(t), \ldots, CI_{well\_n}(t))$$

$$CI_{Std\ dev\ (baseline\_wells)}(t) = Std\ dev(CI_{baseline\_well\_1}(t), CI_{baseline\_well\_2}(t), \ldots, CI_{baseline\_well\_n}(t))$$

Where:

- $BCI_{Average}(t)$ is the average of the baseline Cell Index of well 1 to n at time point t.
- $BCI_{Std\ dev}(t)$ is the standard deviation of the baseline Cell Index of well 1 to n at time point t.
- $CI_{Std\ dev}(t)$ is the standard deviation of the Cell Index of well 1 to n at time point t.
- $CI_{Std\ dev(basline\_wells)}(t)$ is the standard deviation of the Cell Index of selected baseline well 1 to n at time point t.
- $BCI_{well\_1}(t), BCI_{well\_2}(t), \ldots, BCI_{well\_n}(t)$ are the baseline Cell Indices of well 1, 2, ..., n, respectively, at time point t.
- $(CI_{baseline\_well\_1}(t), CI_{baseline\_well\_2}(t), \ldots, CI_{baseline\_well\_n}(t)$ are the Cell Indices of selected baseline wells 1, 2, ..., n respectively at time point t.

Note: In software version 1.1, the calculation of standard deviation of the baseline Cell Index is different.

Example 2: Calculation of baseline Cell Index

The same sets of Cell Index data measured at time points 10:06:49 and 10:21:49 (see Table 1) are used for the calculation of the baseline Cell Index.

If well A1 is selected as the baseline well, the baseline Cell Index of well D4 at 10:06:49 is:

$$BCI_{well\_D4}(t_{10:06:49}) = CI_{well\_D4}(t_{10:06:49}) - CI_{baseline\_well}(t_{10:06:49}) = 0.2295 - 0.2774 = -0.0479$$

The baseline Cell Index of well D4 at 10:21:49 is:

$$BCI_{well\_D4}(t_{10:21:49}) = CI_{well\_D4}(t_{10:21:49}) - CI_{baseline\_well}(t_{10:21:49}) = 0.2330 - 0.2819 = -0.0489$$

Since well A1 is the baseline well, the baseline Cell Index of well A1 at 10:06:49 and 10:21:49 are zero. The calculated baseline Cell Index at 10:06:49 and 10:21:49 with well A1 selected as the baseline well are shown in Table 3.

Table 3. Calculated baseline Cell Index at two time points with well A1 selected as the baseline well.

Baseline Cell Index at 10:06:49 (Baseline Well – A1)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.0269	-0.0056	-0.0118	0.0239	-0.0049	-0.0559	-0.0305	0.0076	-0.0331	-0.0101	-0.0583
B	-0.0823	-0.0558	-0.1140	-0.0955	-0.0721	-0.0778	-0.1210	-0.0861	-0.1102	-0.1616	-0.1039	-0.1677
C	-0.1202	-0.0710	-0.0725	-0.0495	-0.0877	-0.0548	-0.1233	-0.0788	-0.1148	-0.0990	-0.0981	-0.1117
D	-0.1042	-0.0577	-0.0735	-0.0479	-0.0335	-0.0156	-0.0951	-0.0790	-0.0288	-0.0577	-0.0704	-0.1261
E	-0.0824	-0.1052	-0.0408	-0.0357	-0.0516	-0.0719	-0.0528	-0.0682	-0.0810	-0.0572	-0.0887	-0.0940
F	-0.0706	-0.0540	-0.0646	-0.0851	-0.0548	-0.0595	-0.0740	-0.0491	-0.0392	-0.0850	-0.0792	-0.0997
G	-0.1075	-0.0597	-0.1220	-0.1202	-0.1194	-0.0627	-0.0977	-0.1118	-0.1143	-0.1523	-0.1542	-0.0897
H	-0.0417	0.0018	-0.0092	-0.0335	-0.0428	-0.0467	-0.0651	-0.0278	-0.0284	-0.0062	-0.0438	-0.0168
Baseline Cell Index at 10:21:49 (Baseline Well – A1)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.0284	-0.0073	-0.0085	0.0258	-0.0026	-0.0581	-0.0284	0.0072	-0.0307	-0.0097	-0.0592
B	-0.0844	-0.0556	-0.1147	-0.0945	-0.0722	-0.0790	-0.1218	-0.0884	-0.1106	-0.1604	-0.1044	-0.1677
C	-0.1233	-0.0722	-0.0737	-0.0500	-0.0893	-0.0553	-0.1251	-0.0797	-0.1156	-0.0996	-0.1004	-0.1241
D	-0.1068	-0.0562	-0.0722	-0.0489	-0.0343	-0.0146	-0.0928	-0.0802	-0.0262	-0.0563	-0.0719	-0.1266
E	-0.0833	-0.1034	-0.0415	-0.0368	-0.0525	-0.0728	-0.0553	-0.0653	-0.0807	-0.0584	-0.0875	-0.0962
F	-0.0701	-0.0546	-0.0616	-0.0841	-0.0534	-0.0586	-0.0767	-0.0487	-0.0380	-0.0832	-0.0809	-0.0994
G	-0.1068	-0.0593	-0.1228	-0.1197	-0.1188	-0.0623	-0.0992	-0.1151	-0.1127	-0.1511	-0.1566	-0.0924
H	-0.0406	0.0044	-0.0083	-0.0340	-0.0419	-0.0451	-0.0643	-0.0280	-0.0280	-0.0066	-0.0424	-0.0173

If wells A1 and A2 are selected as the baseline wells, the Cell Index of the baseline wells is the average Cell Index of wells A1 and A2. At 10:06:49 and 10:21:49, the Cell Index of the baseline wells are (respectively):

$$CI_{baseline\_well}(t_{10:06:49}) = \text{Average}(CI_{well\_A1}(t_{10:06:49}), CI_{well\_A2}(t_{10:06:49})) = \frac{(0.3044 + 0.2774)}{2} = 0.2909$$

and

$$CI_{baseline\_well}(t_{10:21:49}) = \text{Average}(CI_{well\_A1}(t_{10:21:49}), CI_{well\_A2}(t_{10:21:49})) = \frac{(0.3103 + 0.2819)}{2} = 0.2961$$

The baseline Cell Index of well D4 at 10:06:49 is:

$$BCI_{well\_D4}(t_{10:06:49}) = CI_{well\_D4}(t_{10:06:49}) - CI_{baseline\_well}(t_{10:06:49}) = 0.2295 - 0.2909 = -0.0614$$

The baseline Cell Index of well D4 at 10:21:49 is:

$$BCI_{well\_D4}(t_{10:21:49}) = CI_{well\_D4}(t_{10:21:49}) - CI_{baseline\_well}(t_{10:21:49}) = 0.2330 - 0.2961 = -0.0631$$

The calculated baseline Cell Index at 10:06:49 and 10:21:49 with wells A1 and A2 selected as the baseline wells are shown in Table 4.

Baseline normalized Cell Index

Baseline Cell Index values can be combined with the normalized Cell Index for data analysis. Baseline normalized Cell Index calculations can be performed by choosing a time point for normalization and a well or several wells as the baseline wells. The calculated baseline normalized Cell Index will not be impacted by whether the user selects the delta Cell Index or baseline Cell Index to be calculated first in the software. The baseline normalized Cell Index is calculated as follows:

$$BNCI_{well\_i}(t) = NCI_{well\_i}(t) - \text{Average}\left(NCI_{baseline\_well\_1}(t), NCI_{baseline\_well\_2}(t), \ldots, NCI_{baseline\_well\_n}(t)\right)$$

with

$i = 1, 2, \ldots, n.$

Where:

- $BNCI_{well\_i}(t)$ is the baseline normalized Cell Index of well i at time point t.
- $NCI_{well\_i}(t)$ is the normalized Cell Index of well i at time point t.
- $NCI_{baseline\_well\_1}(t), NCI_{baseline\_well\_2}(t), \ldots, NCI_{baseline\_well\_n}(t)$ are the normalized Cell Indices of selected baseline well 1, 2, ..., n at time point t.

Table 4. Calculated baseline Cell Index at two time points with wells A1 and A2 as the baseline wells.

Baseline Cell Index at 10:06:49 (Baseline Wells – A1 and A2)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.0135	0.0135	-0.0191	-0.0253	0.0104	-0.0183	-0.0694	-0.0439	-0.0058	-0.0466	-0.0236	-0.0718
B	-0.0958	-0.0692	-0.1275	-0.1089	-0.0855	-0.0913	-0.1345	-0.0995	-0.1237	-0.1750	-0.1173	-0.1812
C	-0.1337	-0.0844	-0.0859	-0.0629	-0.1012	-0.0682	-0.1368	-0.0923	-0.1282	-0.1124	-0.1115	-0.1252
D	-0.1177	-0.0712	-0.0870	-0.0614	-0.0470	-0.0291	-0.1085	-0.0924	-0.0423	-0.0712	-0.0838	-0.1396
E	-0.0958	-0.1187	-0.0543	-0.0492	-0.0651	-0.0853	-0.0663	-0.0817	-0.0944	-0.0707	-0.1021	-0.1075
F	-0.0840	-0.0674	-0.0781	-0.0986	-0.0683	-0.0730	-0.0875	-0.0626	-0.0526	-0.0984	-0.0927	-0.1132
G	-0.1210	-0.0732	-0.1354	-0.1337	-0.1328	-0.0762	-0.1112	-0.1253	-0.1278	-0.1657	-0.1677	-0.1032
H	-0.0552	-0.0117	-0.0226	-0.0470	-0.0563	-0.0602	-0.0786	-0.0412	-0.0419	-0.0197	-0.0572	-0.0302
Baseline Cell Index at 10:21:49 (Baseline Wells – A1 and A2)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.0142	0.0142	-0.0215	-0.0227	0.0116	-0.0167	-0.0722	-0.0426	-0.007	-0.0449	-0.0238	-0.0734
B	-0.0985	-0.0697	-0.1288	-0.1086	-0.0863	-0.0932	-0.1359	-0.1025	-0.1248	-0.1746	-0.1186	-0.1818
C	-0.1375	-0.0863	-0.0879	-0.0642	-0.1035	-0.0694	-0.1393	-0.0938	-0.1298	-0.1138	-0.1145	-0.1382
D	-0.1210	-0.0704	-0.0863	-0.0631	-0.0485	-0.0288	-0.1070	-0.0943	-0.0403	-0.0704	-0.0861	-0.1408
E	-0.0975	-0.1176	-0.0556	-0.0510	-0.0667	-0.0869	-0.0694	-0.0794	-0.0949	-0.0726	-0.1017	-0.1104
F	-0.0842	-0.0688	-0.0758	-0.0983	-0.0675	-0.0727	-0.0909	-0.0628	-0.0522	-0.0973	-0.0951	-0.1136
G	-0.1210	-0.0734	-0.1369	-0.1339	-0.1329	-0.0764	-0.1134	-0.1293	-0.1269	-0.1653	-0.1707	-0.1065
H	-0.0547	-0.0098	-0.0225	-0.0482	-0.056	-0.0592	-0.0785	-0.0421	-0.0422	-0.0207	-0.0566	-0.0314

Delta Cell Index

On rare occasions when negative Cell Index values are recorded and cause problems for further analysis, the **delta Cell Index function** can be used to make all Cell Index values positive by adding a constant to the Cell Index of each well. This operation corresponds to shifting the Cell Index curve along the Y-axis, so that the entire curve is above zero.

To calculate the delta Cell Index, the Cell Index values of the selected wells at a reference time point (delta time, t_{delta}) are set to a constant (delta constant) with a default value of one. It is also possible for the user to change this constant to a different value. The difference between this chosen constant and the original Cell Index is called delta value and is calculated as follows:

$$\text{Delta value}_{well\_i} = \text{delta constant} - CI_{well\_i}(t_{delta})$$

with

$i = 1, 2, ..., n$

At a given time point t, the delta Cell Index (DCI(t)) is calculated as the sum of the Cell Index at time point t and the delta value as follows:

$$DCI_{well\_i}(t) = CI_{well\_i}(t) + \text{delta value}_{well\_i} = CI_{well\_i}(t) - CI_{well\_i}(t_{delta}) + \text{delta constant}$$

Where:

- $DCI_{well\_i}(t)$ is the delta Cell Index of well i at time point t.
- $CI_{well\_i}(t)$ is the Cell Index of well i at time point t.
- $CI_{well\_i}(t_{delta})$ is the Cell Index of well i at delta time t_{delta}.

Therefore, the delta Cell Index at delta time t_{delta} is equal to the selected delta constant.

$$\begin{aligned} DCI_{well\_i}(t_{delta}) &= \text{delta value}_{well\_i} + CI_{well\_i}(t_{delta}) \\ &= (\text{delta constant} - CI_{well\_i}(t_{delta})) + CI_{well\_i}(t_{delta}) \\ &= \text{delta constant} \end{aligned}$$

The average and standard deviation of the delta Cell Index at time point t are calculated as follows:

$$DCI_{Average}(t) = \text{Average}(DCI_{well\_1}(t), DCI_{well\_2}(t), ..., DCI_{well\_n}(t))$$

$$DCI_{Std\ dev}(t) = \text{Std dev}(DCI_{well\_1}(t), DCI_{well\_2}(t), ..., DCI_{well\_n}(t))$$

Where:

- $DCI_{Average}(t)$ is the average of the delta Cell Index of selected well 1 to n at time point t.
- $DCI_{Std\ dev}(t)$ is the standard deviation of the delta Cell Index of selected well 1 to n at time point t.
- $DCI_{well\_1}(t)$, $DCI_{well\_2}(t)$, ..., $DCI_{well\_n}(t)$ are the delta Cell Indices of well 1, 2, ..., n at time point t.

Example 3: Calculation of delta Cell Index

The same sets of Cell Index data measured at time points 10:06:49 and 10:21:49 (see Table 1) are used for the calculation of delta Cell Index.

If 10:06:49 is chosen as the delta time and the delta constant is set to 1, the delta Cell Index for all wells at 10:06:49 becomes 1. Here, taking well D4 as an example, the delta value for well D4 is:

$$\text{Delta value}_{well\_D4} = \text{delta constant} - CI_{well\_D4}(t_{delta}) = 1 - CI_{well\_D4}(t_{10:06:49}) = 1 - 0.2295 = 0.7705$$

The delta Cell Index for well D4 at 10:21:49 is:

$$DCI_{well\_D4}(t_{10:21:49}) = \text{delta value}_{well\_D4} + CI_{well\_D4}(t_{10:21:49}) = 0.7705 + 0.2330 = 1.0035$$

The calculated delta Cell Index at 10:06:49 and 10:21:49, with 10:06:49 chosen as the delta time, is shown in Table 5.

Table 5. Calculated delta Cell Index for an Agilent E-Plate 96 at two time points.

Delta Cell Index at 10:06:49 (Delta Time at 10:06:49)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1
Delta Cell Index at 10:21:49 (Delta Time at 10:06:49)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.0045	1.0060	1.0028	1.0078	1.0064	1.0068	1.0024	1.0066	1.004	1.0069	1.005	1.0036
B	1.0024	1.0047	1.0038	1.0055	1.0044	1.0033	1.0037	1.0022	1.0041	1.0056	1.004	1.0045
C	1.0014	1.0033	1.0033	1.0039	1.0029	1.004	1.0027	1.0037	1.0036	1.0038	1.0022	0.9922
D	1.0019	1.0060	1.0058	1.0035	1.0037	1.0055	1.0067	1.0033	1.0071	1.0060	1.003	1.0040
E	1.0035	1.0063	1.0039	1.0034	1.0036	1.0036	1.0021	1.0074	1.0047	1.0033	1.0057	1.0023
F	1.0050	1.0039	1.0075	1.0055	1.0059	1.0054	1.0018	1.0050	1.0057	1.0063	1.0028	1.0048
G	1.0052	1.0049	1.0037	1.0050	1.0051	1.0050	1.0030	1.0012	1.0061	1.0056	1.0022	1.0018
H	1.0056	1.0071	1.0054	1.0040	1.0054	1.0062	1.0053	1.0043	1.0049	1.0041	1.0059	1.0040

Baseline delta Cell Index

Baseline Cell Index values can also be combined with the delta Cell Index for data analysis. A baseline delta Cell Index calculation can be performed by choosing one well or several wells as the baseline wells and choosing delta Cell Index in the **Plot** page of the software. The calculated baseline delta Cell Index will not be impacted by whether the user selects the delta Cell Index or baseline Cell Index to be calculated first in the software. The baseline delta Cell Index is calculated as follows:

$$BDCI_{well\_i}(t) = DCI_{well\_i}(t) - Average(DCI_{baseline\_well\_1}(t), DCI_{baseline\_well\_2}(t), \ldots, DCI_{baseline\_well\_n}(t))$$

with

$i = 1, 2, \ldots, n.$

Where:

- $BDCI_{well\_i}(t)$ is the baseline delta Cell Index of well 1 to n at time point t.
- $DCI_{well\_i}(t)$ is the delta Cell Index of well 1 to n at time point t.
- $DCI_{baseline\_well\_1}(t)$, $DCI_{baseline\_well\_2}(t)$, …, $DCI_{baseline\_well\_n}(t)$ are the delta Cell Indices of selected baseline wells 1, 2, …, n at time point t.

Slope calculation

The slope describes the steepness, incline, gradient, and changing rate of a Cell Index curve within a given time period. The slope parameter can be used for evaluation of cell proliferation, cell adhesion, cytotoxicity, receptor activation, and other cell-based assays.

For a single Cell Index curve, a corresponding set of measured points for Cell Index with n in the selected time period is:

$(t_1, CI_1), (t_2, CI_2), \ldots, (t_n, CI_n)$

where,
$t_1, t_2, \ldots, t_n$ are the time points from 1 to n.

$CI_1, CI_2, \ldots, CI_n$ are the Cell Index at time points 1 to n, respectively.

A linear fitting of the defined Cell Index curve of $(t_1, CI_1), (t_2, CI_2), \ldots, (t_n, CI_n)$ is:

$CI = a + b \cdot t$

Where:
a is the intercept and b is the slope of the linear fitting line, respectively. a and b can be calculated as follows:

$$a = \left(\frac{\sum_{i=1}^{n} CI_i}{n} \right) - b \cdot \left(\frac{\sum_{i=1}^{n} t_i}{n} \right)$$

$$b = \frac{n \sum_{i=1}^{n} (t_i CI_i) - \left(\sum_{i=1}^{n} t_i \right) \cdot \left(\sum_{i=1}^{n} CI_i \right)}{n \sum_{i=1}^{n} (t_i)^2 - \left(\sum_{i=1}^{n} t_i \right)^2}$$

The standard deviation of slope b is calculated as follows:

$$\text{Std dev}(b) = \sqrt{\frac{S_{yy} - S^2_{xy}/S_{xx}}{(n-2)\, S_{xx}}}$$

where

$$S_{xy} = \sum_{i=1}^{n} \left(t_i - \frac{\sum_{i=1}^{n} t_i}{n} \right) \cdot \left(CI_i - \frac{\sum_{i=1}^{n} CI_i}{n} \right)$$

$$S_{xx} = \sum_{i=1}^{n} \left(t_i - \frac{\sum_{i=1}^{n} t_i}{n} \right)^2$$

and

$$S_{yy} = \sum_{i=1}^{n} \left(CI_i - \frac{\sum_{i=1}^{n} CI_i}{n} \right)^2$$

To calculate slope b and the standard deviation of slope b for an averaged Cell Index curve of multiple selected wells, the average Cell Index is calculated first.

Example 4: Calculation of slope b and standard deviation of slope b of a defined Cell Index curve

Here the Cell Index curve of well H2 during the time period 30:34:02 to 36:03:24 is used to calculate slope b and the standard deviation. The Cell Indices of well H2 at different time points used for the calculation are shown in the table.

Time Point	Cell Index of Well H2
30:34:02	1.0560
30:49:01	1.0675
31:03:57	1.0803
31:18:58	1.0935
31:33:57	1.1094
31:48:54	1.1241
32:03:48	1.1411
32:18:47	1.1588
32:33:46	1.1758
32:48:44	1.1965
33:03:43	1.2120
33:18:42	1.2307
33:33:41	1.2489
33:48:39	1.2663
34:03:38	1.2850
34:18:37	1.3023
34:33:33	1.3210
34:48:32	1.3424
35:03:31	1.3612
35:18:30	1.3804
35:33:26	1.4083
35:48:25	1.4309
36:03:24	1.4528

Results

See Figure 1.

Doubling time calculation

The doubling time is the period of time required for a given quantity to double in size or value, assuming that the quantity undergoes exponential growth. The doubling time in RTCA software is used to describe the time required for the Cell Index value to double (with positive slope b within the selected time period) or to halve (with negative slope b within the selected time period). The doubling time parameter is used for cell proliferation and other cell-based assays.

Note: The Cell Index doubling time is not equal to the time when the cell number doubles, because Cell Index values are a combination of the measure of cell viability, cell number, cell morphology, and the degree of cell adhesion.

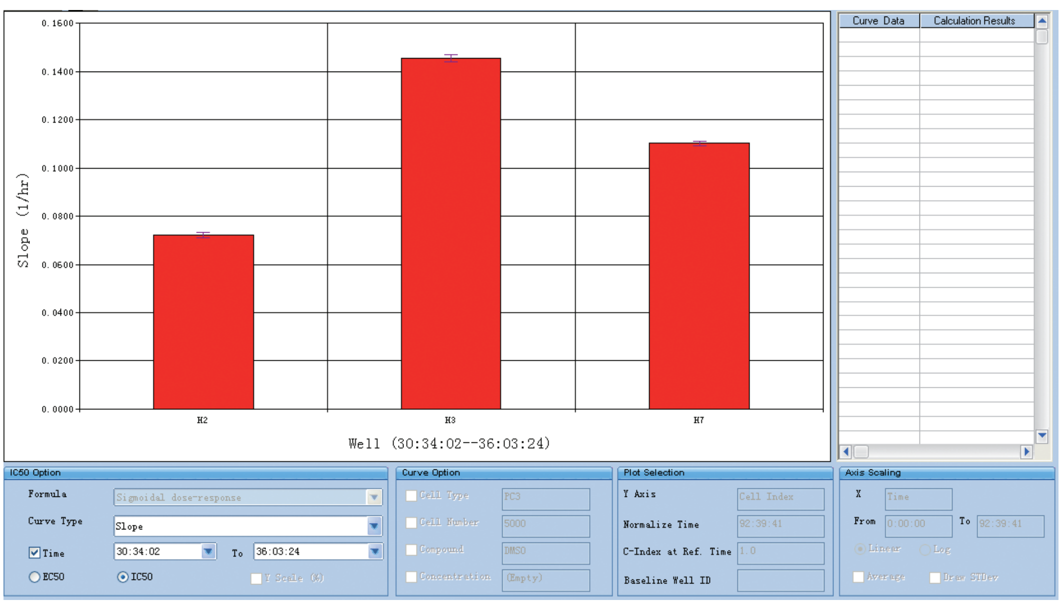
For a single Cell Index curve, a corresponding set of measured points for Cell Index with n in the selected time period is:

$(t_1, CI_1), (t_2, CI_2), \ldots, (t_n, CI_n)$

Where:

$t_1, t_2, \ldots, t_n$ are the time points 1 to n.

$CI_1, CI_2, \ldots, CI_n$ are the Cell Indices at time points 1 to n, respectively.



Formula: Cell Index = a + bt

Well	Slope b	Standard deviation of slope b
H2	7.25E-02	1.06E-03

Figure 1. Calculation of slope and standard deviation of a Cell Index curve.

An exponential fitting of the defined Cell Index curve of $(t_1, CI_1), (t_2, CI_2), ..., (t_n, CI_n)$ is:

$$CI_i = CI_1 \cdot 2^{\frac{t_i - t_1}{DT}}$$

with

$i = 1, 2, ..., n$

Where:

– DT is the doubling time.

– CI_i is the Cell Index at time point i.

The above equation can be transformed into a linear equation and become:

$$\log(CI_i) = \log(CI_1) + \frac{\log(2)}{DT} \cdot (t_i - t_1)$$

The slope of this linear equation is:

$$\text{Slope} = \frac{\log(2)}{DT}$$

The slope can therefore be calculated using the formula in *Section 4 – Slope calculation from the data series* $(t_1, \log(CI_1)), (t_2, \log(CI_2)), ..., (t_1, \log(CI_n))$. To determine doubling time for a set of Cell Index data $(t_1, CI_1), (t_2, CI_2), ..., (t_n, CI_n)$, we would first convert the data into its logarithmic form $(t_1, \log(CI_1)), (t_2, \log(CI_2)), ..., (t_1, \log(CI_n))$ and perform a linear regression analysis to determine the slope of the logarithmic data and the standard deviation of the slope. From such a slope, the doubling time of the original data set is calculated:

$$DT = \frac{\log(2)}{\text{Slope}}$$

$$\text{Std dev}(DT) = \text{Max}(|DT - DT_1|, |DT - DT_2|)$$

where

$$DT_1 = \frac{\log(2)}{\text{Slope} + \text{std dev(slope)}}$$

$$DT_2 = \frac{\log(2)}{\text{Slope} - \text{std dev(slope)}}$$

Std dev(slope) is the standard deviation of the calculated slope.

Example 5: Calculation of doubling time of a defined Cell Index curve

The same Cell Index curve of well H2 during the time period 30:34:02 to 36:03:24 (see Example 4) is used to calculate the doubling time and standard deviation of doubling time.

The results are:

Formula: $\text{Cell Index} = A \times 2^{(t/\text{CI doubling time})} = CI_{t_i} \times 2^{\frac{t_i - t_1}{DT}}$

Chosen time interval:
30:34:02 – 36:03:24 (30.560 – 36.057 hours)

Well	Doubling Time (hours)	Standard Deviation of Doubling Time
H2	1.18E+01	7.41E-02

EC_{50}/IC_{50} calculation

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. The compound in question can be a drug candidate. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process, or component of a process, such as an enzyme, cell, cell receptor or micro-organism, by half. It is therefore the half maximal inhibitory concentration (IC) of a substance (IC_{50}). The IC_{50} is commonly used as a measure of antagonist drug potency in pharmacological research. The IC_{50} is sometimes converted to the pIC_{50} scale, or $-\log(IC_{50})$, where higher values indicate exponentially greater potency.

The time-dependent IC_{50}/EC_{50} describes how the IC_{50}/EC_{50} changes during a given time period following the compound treatment. To calculate the time-dependent IC_{50}/EC_{50}, the RTCA software automatically extracts 20 time points within the chosen time period, and calculates the IC_{50}/EC_{50} values at each point. If there are less than 20 time points in the chosen period, the IC_{50} values for all points will be calculated. The calculated time-dependent IC_{50}/EC_{50} values can also be plotted against the sampled time points.

Depending on specific applications, a user can choose to use either EC_{50} or IC_{50} for calculations. The formula for the IC_{50} calculation is described below. An identical formula applies for the EC_{50} calculation as well.

The sigmoidal dose-response can be modeled by three- and four-parameter logistic equations as follows:

Three-parameter logistic dose-response model:

$$CI(3PL)_i = CI_{Low} + \frac{CI_{High} - CI_{Low}}{1 + \frac{IC_{50}}{C_i}}$$

Four-parameter logistic dose-response model:

$$CI(4PL)_i = CI_{Low} + \frac{CI_{High} - CI_{Low}}{1 + \left(\frac{IC_{50}}{C_i}\right)^{Hill\text{-}slope}}$$

with

i =1, 2, ..., n

Where:

- CI_{Low} is the modeled Cell Index value when compound concentration approaches zero.
- CI_{High} is the modeled Cell Index value when compound concentration attains the highest value.
- C_i is compound concentrations.
- Hill-slope is the hill coefficient or slope factor of the dose-response curve.

The Levenberg-Marquardt algorithm is used to fit the concentration Cell Index data, (C_1, CI_1), (C_2, CI_2), ..., (C_n, CI_n), to the three- or four-parameter logistic dose-response model to derive the IC_{50}/EC_{50} values. The best fit is reached when the error function converges to a minimum value:

$$S = \sum_{i=1}^{n} (CI_i - CI(3PL/4PL)_i)^2$$

The parameter square R (R^2) is used to determine how well the dose-response model fits the experimental data. Square R is calculated as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (CI_i - CI(3PL/4PL)_i)^2}{\sum_{i=1}^{n} (CI_i - average(CI_i))^2}$$

Square R is a value between 0 and 1. The higher the square R value is, the better the model fits the data.

The dose-response curve (DRC) defines the relation between measured Cell Index (Y-axis) and compound concentrations (X-axis). The parameters used to derive DRC can be Cell Index at a time point, maximum Cell Index in a time period, minimum Cell Index in a time period, the difference of Cell Index (maximum CI minus minimum CI) in a time period, or the area under curve in a time period.

Note: We do not recommend using the SD function for EC_{50}/IC_{50} calculations when using DRC ((max-min) CI in a time period versus concentrations) and DRC (area under curve in a time period versus concentrations).

Example 6: Calculation of IC_{50}/EC_{50}

Figure 2 shows the calculation of IC_{50}/EC_{50} using DRC derived by Cell Index at a time point (68:17:32) versus concentrations.

Formula:

Sigmoidal dose-response = bottom + (top – bottom)/(1 + $10^{(\log_{10}(EC_{50}) - X)}$)

$$= CI_{Low} + \frac{CI_{High} - CI_{Low}}{1 + \frac{IC_{50}}{C_i}}$$

X is the logarithmic concentration of the compound, $X = \log_{10}(C_i)$.

Bottom (CI_{Low}) = 5.95E+00

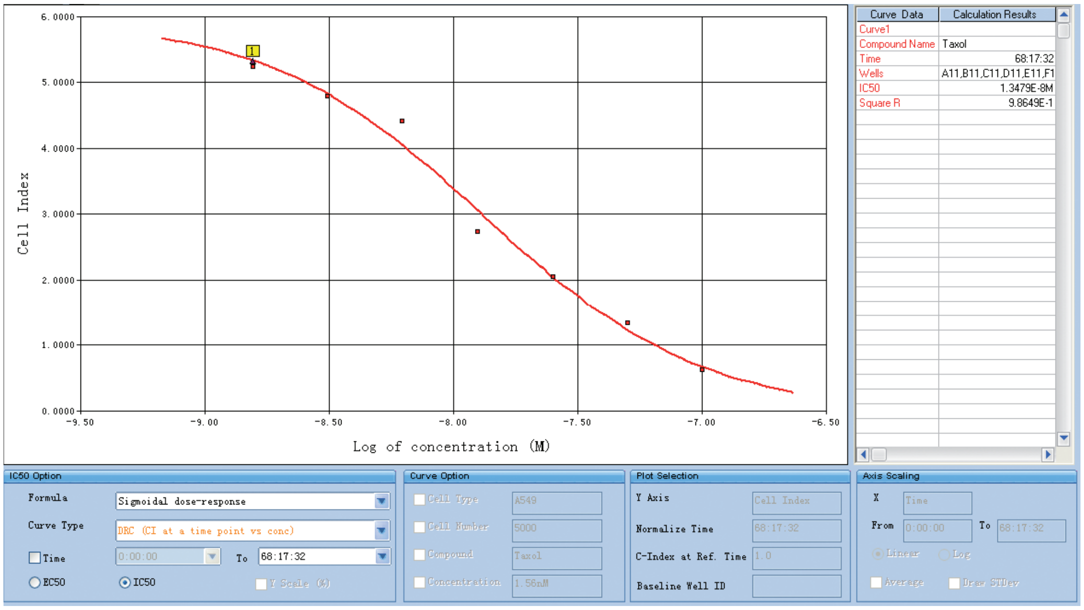
Top (CI_{High}) = −4.56E-02

IC_{50}/EC_{50} = 1.35E-08 (M)

Square R = 9.86E-01

By using this formula at the chosen time point of 68:17:32 (68.292 hours), we can defer the results in the table and calculate a dose-response curve (Cell Index at time point 68:17:32 versus concentrations).

Well ID	Concentration (M)	Log Concentration (M)	Cell Index
A11	1.00E-07	-7.0000	0.6313
B11	5.00E-08	-7.3010	1.3385
C11	2.50E-08	-7.6021	2.0546
D11	1.30E-08	-7.9031	2.7356
E11	6.30E-09	-8.2041	4.4166
F11	3.10E-09	-8.5045	4.7990
G11	1.60E-09	-8.8069	5.2386



Curve 1: DRC (CI at a time point versus concentration)

Y-axis type: Cell Index

Figure 2. Calculation of IC_{50}/EC_{50} using DRC derived by Cell Index at a time point (68:17:32) versus concentrations.

Example 7: Calculation of IC_{50}/EC_{50}

The second example shows the calculation of IC_{50}/EC_{50} using DRC derived from the area under curve in a time period versus concentrations.

Figure 3 shows the normalized Cell Index curve from 18:52:01 to 34:10:15. It displays dose-dependent responses of staurosporine on HeLa cells (2,000 cells/well). The normalized time is 18:52:01.

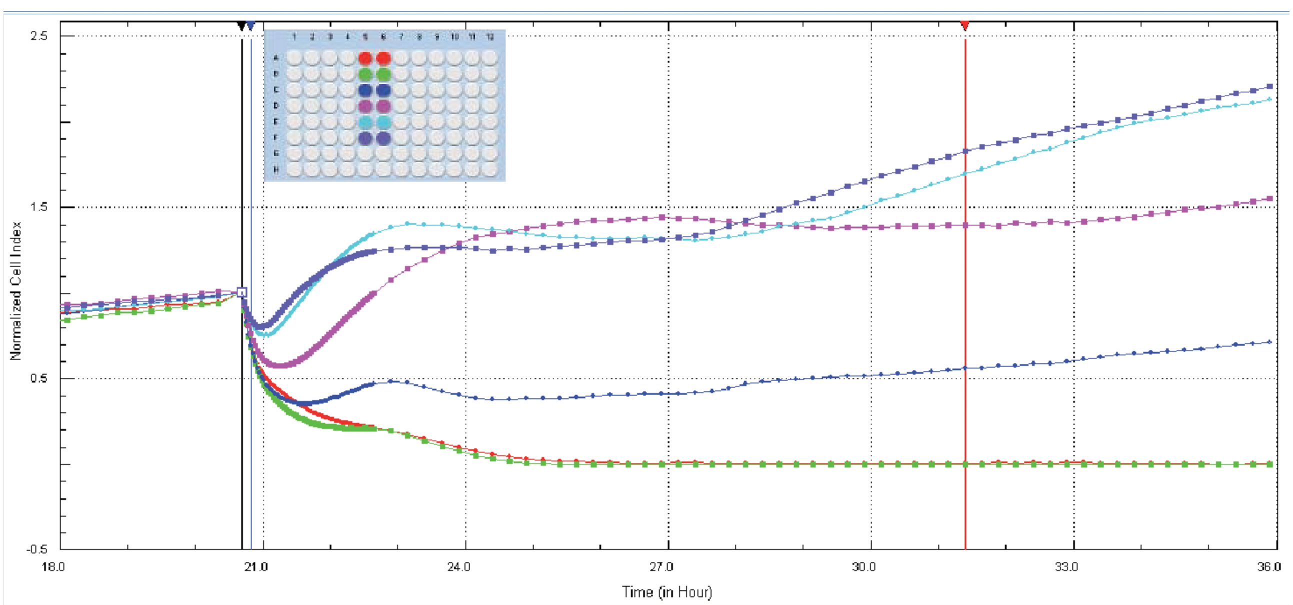


Figure 3. Calculation of the IC_{50}/EC_{50} using DRC derived by Cell Index at a time point (68:17:32) versus concentrations.

Take the time period of 20:50:10 to 31:25:15 and use the DRC (area under curve in a time period versus concentrations) to calculate the IC_{50}/EC_{50} of the previous dataset. The results are shown in Figure 4.

Formula:

Sigmoidal dose-response = bottom + (top – bottom)/(1 + $10^{(\log_{10}(EC_{50}) - X)}$)

$$= CI_{Low} + \frac{CI_{High} - CI_{Low}}{1 + \frac{IC_{50}}{C_i}}$$

X is the logarithmic concentration of the compound, $X = \log_{10}(C_i)$.

Bottom (CI_{Low}) = 1.6274e+001

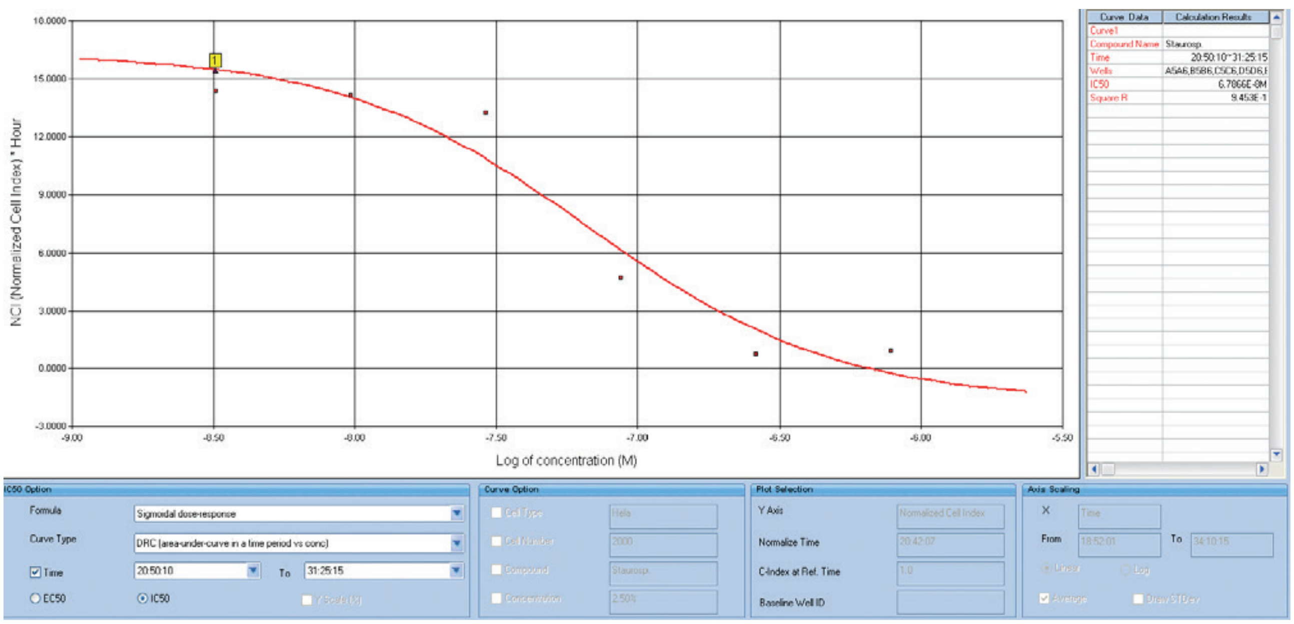
Top (CI_{High}) = –1.7378e+000

IC_{50}/EC_{50} = 6.7866E-008 (M)

Square R = 9.4530e-001

By using this formula at the chosen time interval of 20:50:10.0 to 31:25:15.0 (20.836 to 31.421 hours), we can defer the results and produce a DRC curve.

Well ID	Concentration (M)	Log Concentration (M)	Normalized Cell Index × Hour
A5A6	7.8e-007	-6.1073	0.9165
B5B6	2.6e-007	-6.5845	0.7350
C5C6	8.7e-008	-7.0616	4.7181
D5D6	2.9e-008	-7.5387	13.2894
E5E6	9.6e-009	-8.0159	14.1982
F5F6	3.2e-009	-8.4935	14.3835



Curve 1: DRC (area under curve in a time period versus concentration)

Y-axis type: Normalized Cell Index hour

Figure 4. Area under curve in a time period versus concentrations.

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