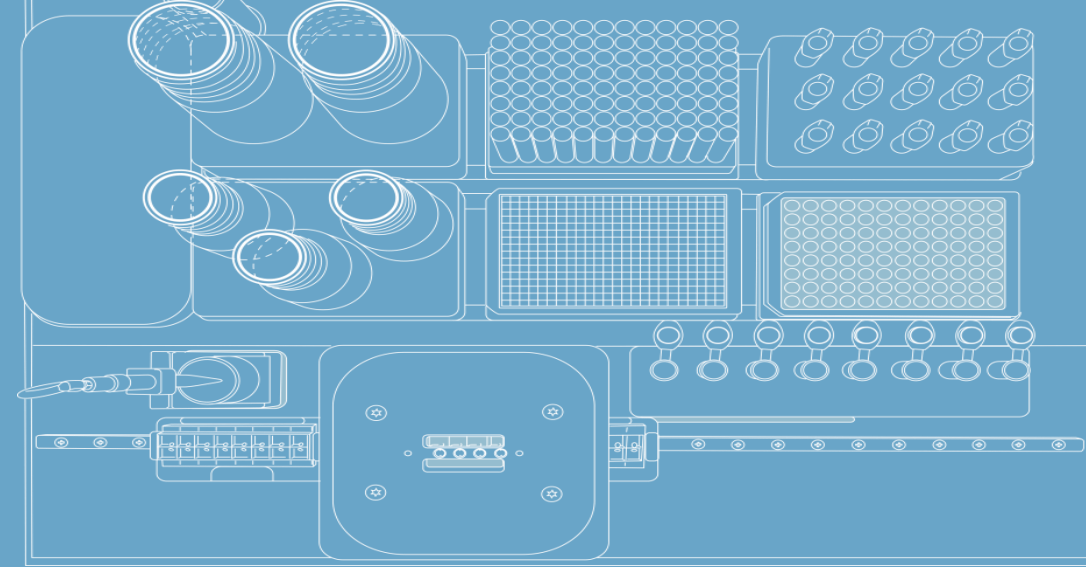


Nanon Technologies

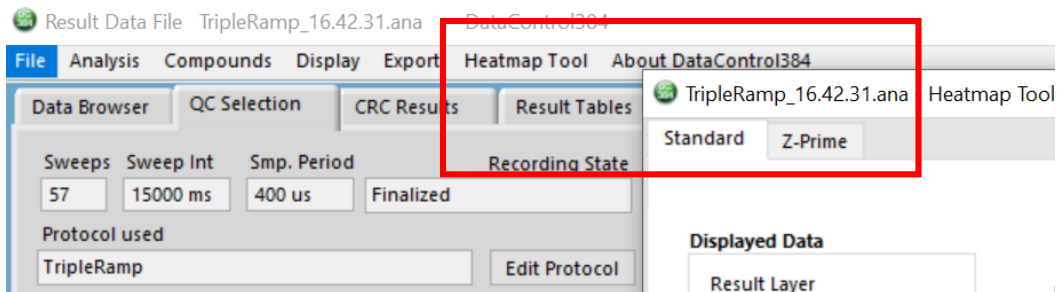
Heatmaps in DataControl

Nanon Technologies



nan]i[on

Heatmap – an expansion of Z'



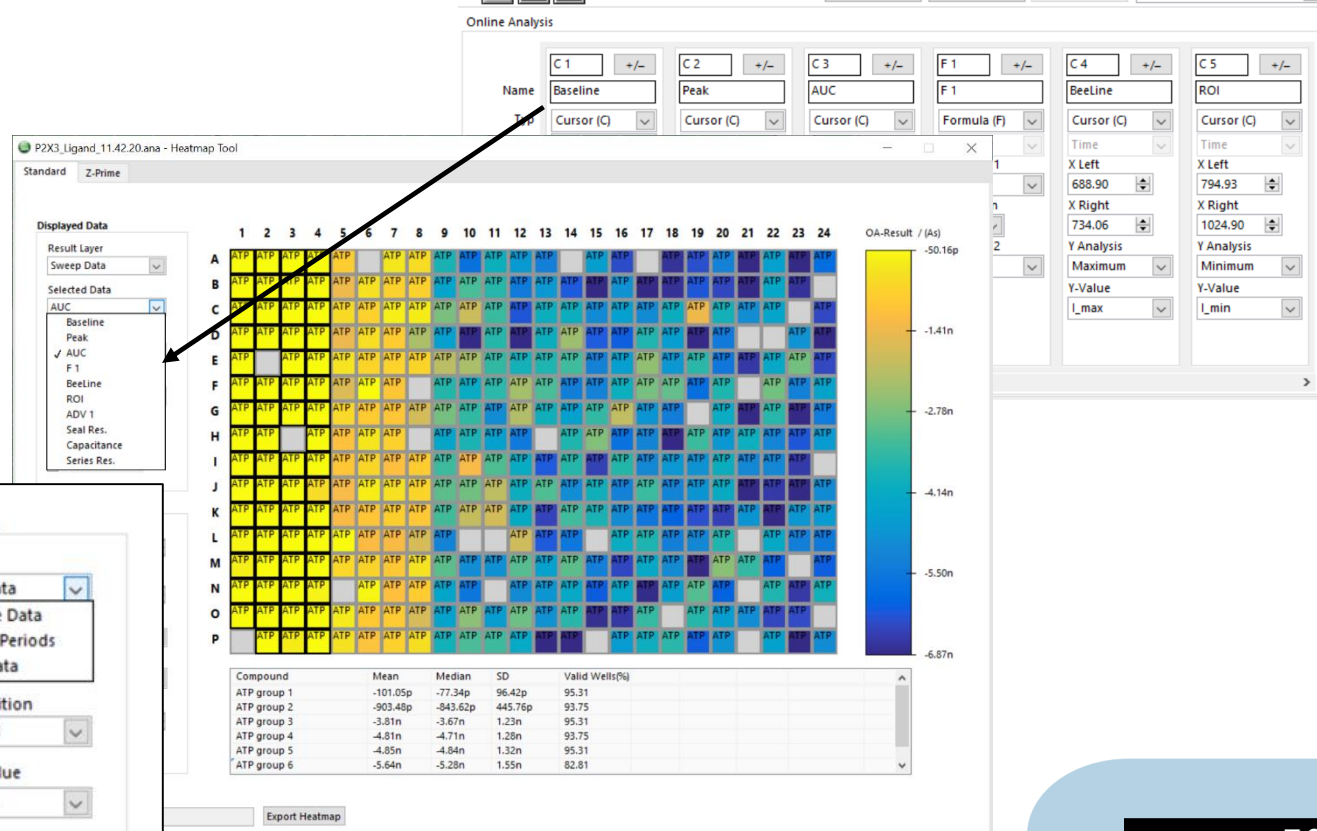
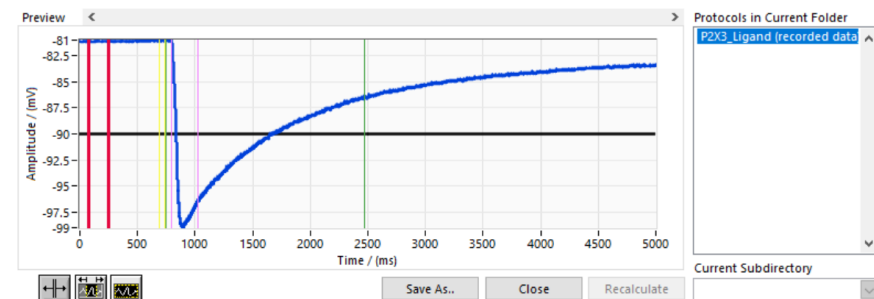
Z' has taken the backseat and the auxiliary tool is now called "Heatmap Tool".

Z' can still be found in there but it is not standard.

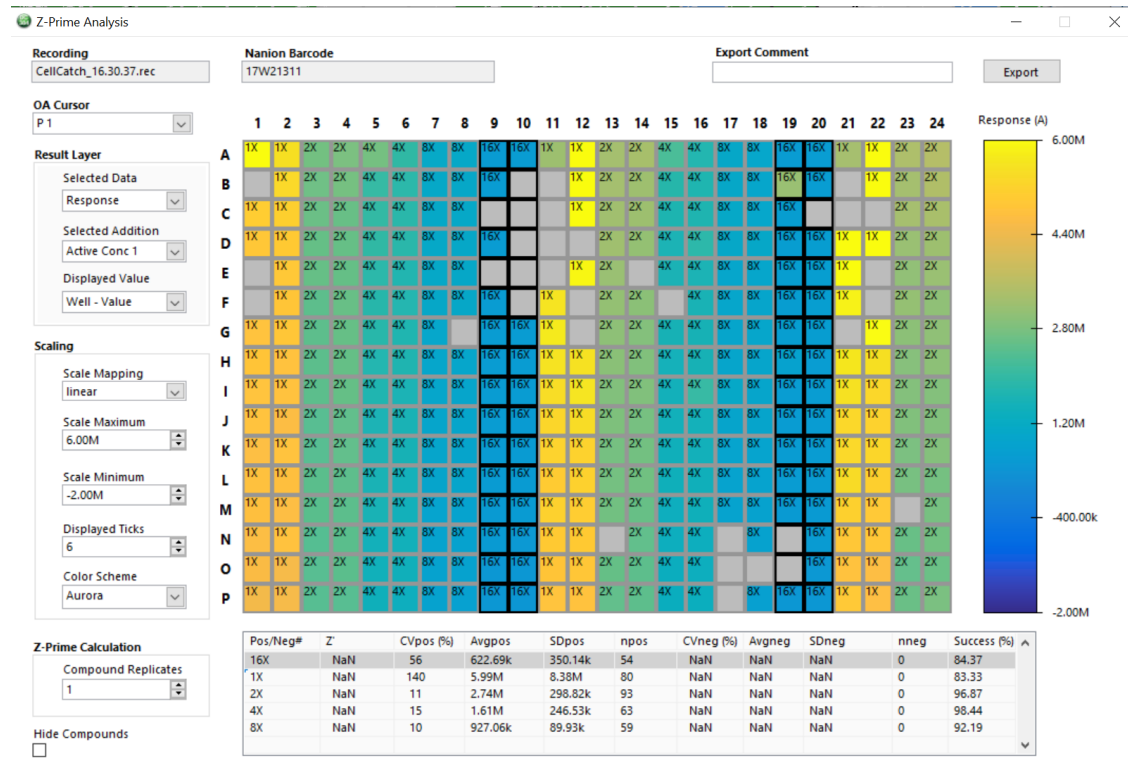
Z' also had heatmaps – but only for cell parameters and only for the Response Data layer.

Now all defined OA's are selectable and also the layers that can be shown has been expanded.

Simple statistics on the chosen OA's are easily derived from the groups that has been defined.

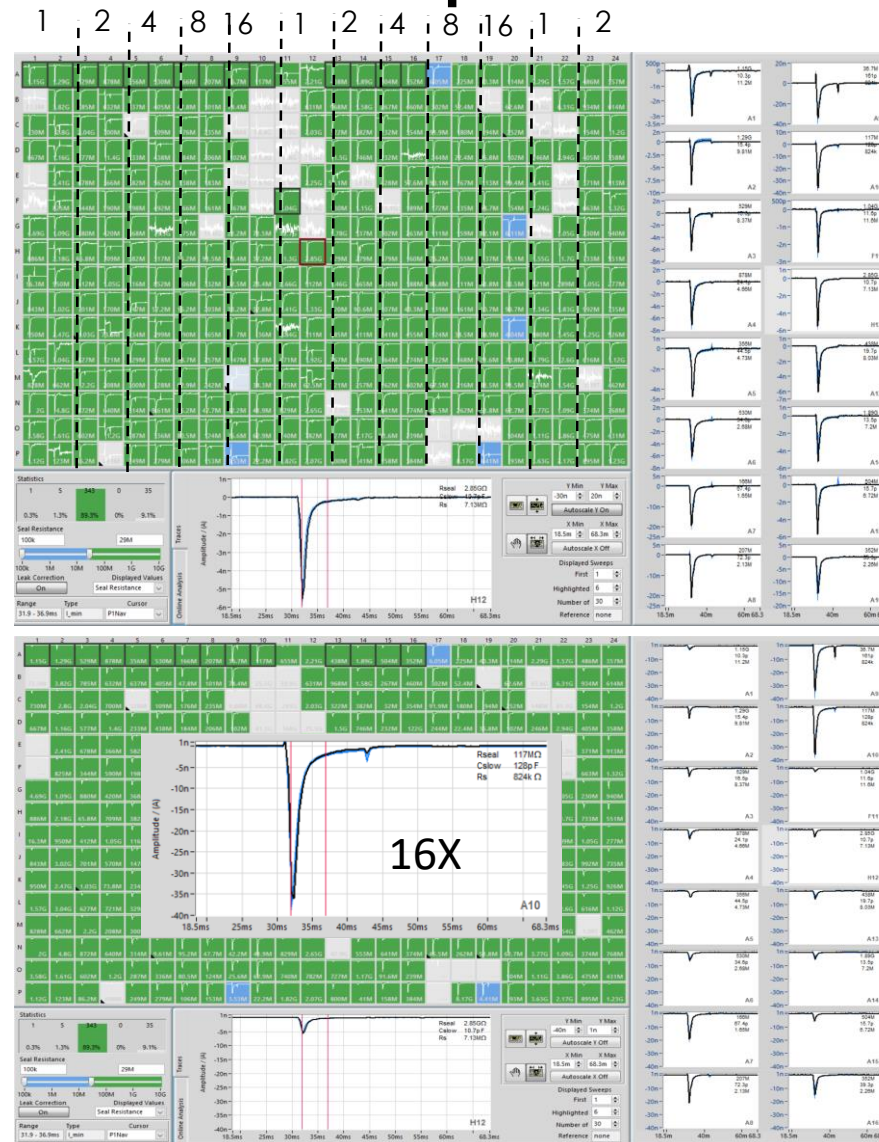


Test data is from a so called "Rainbow plate"

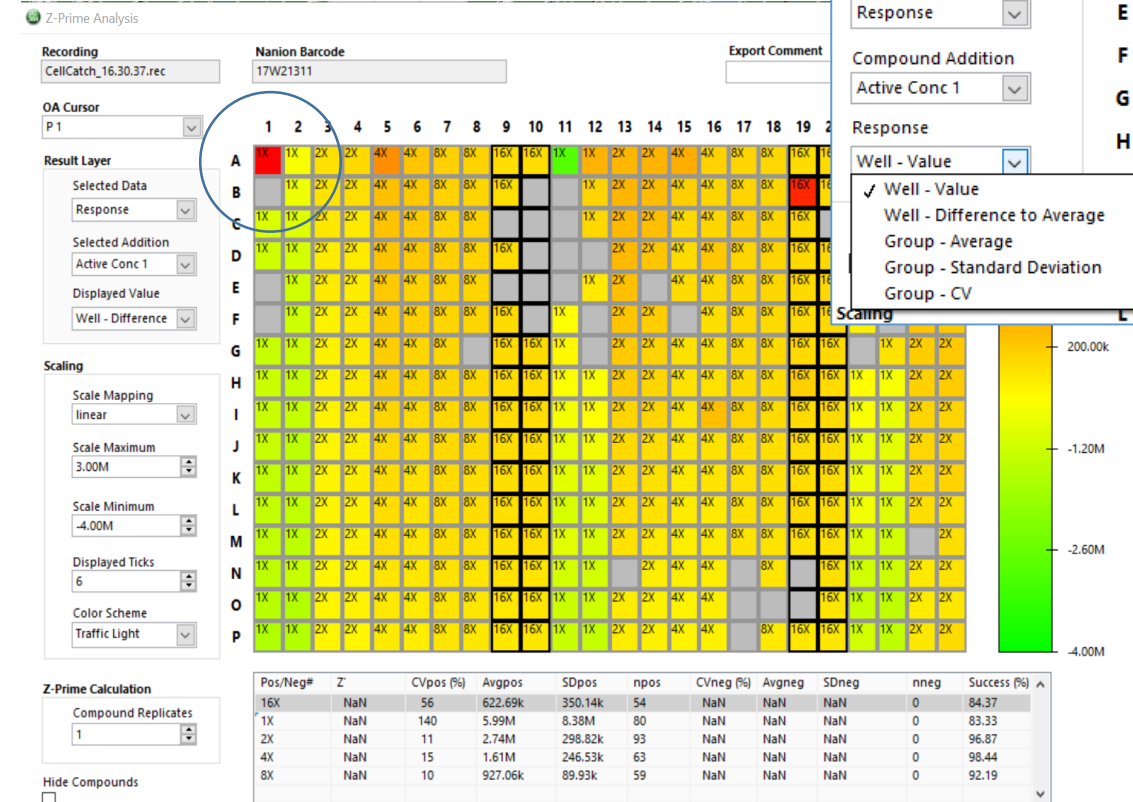
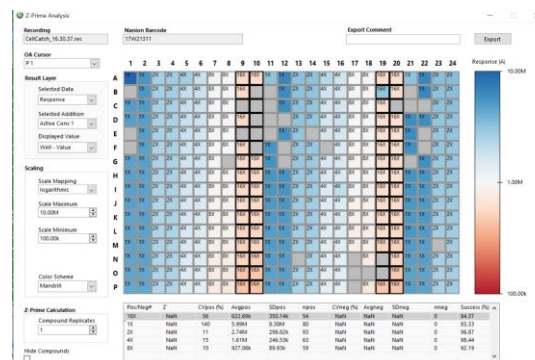
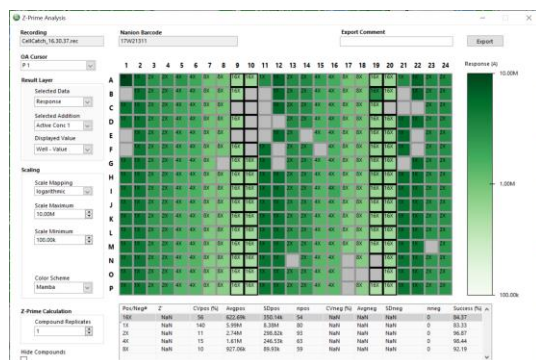
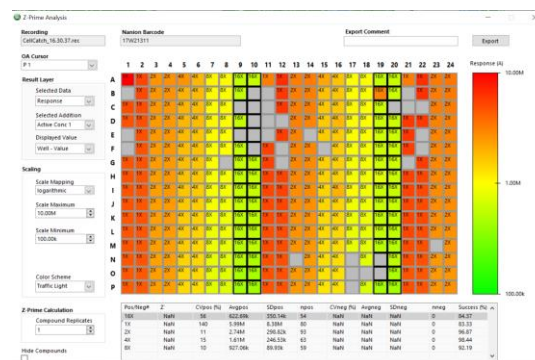
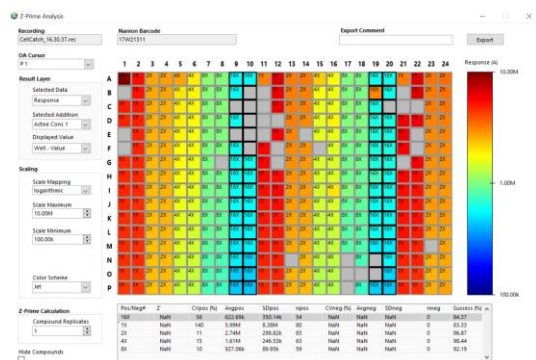


A rainbow plate is developmental plate made in small numbers with different numbers of holes 1,2,4,8 and 16 in the pattern shown above.

This one has a 1X resistance of app 5.5 Mohm.



Different colour schemes – to suit your needs

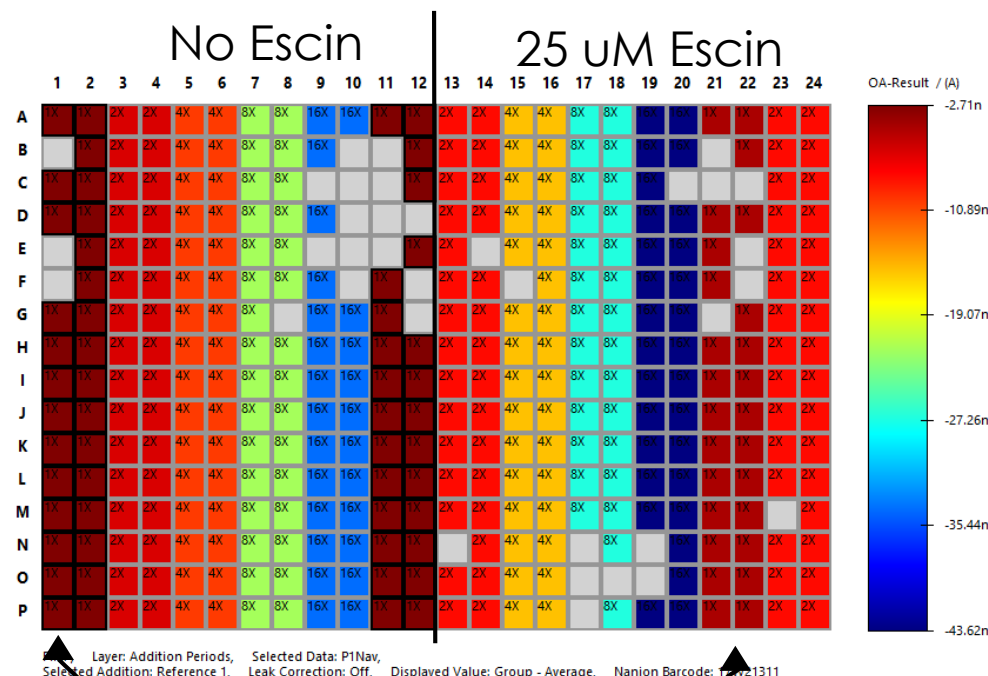
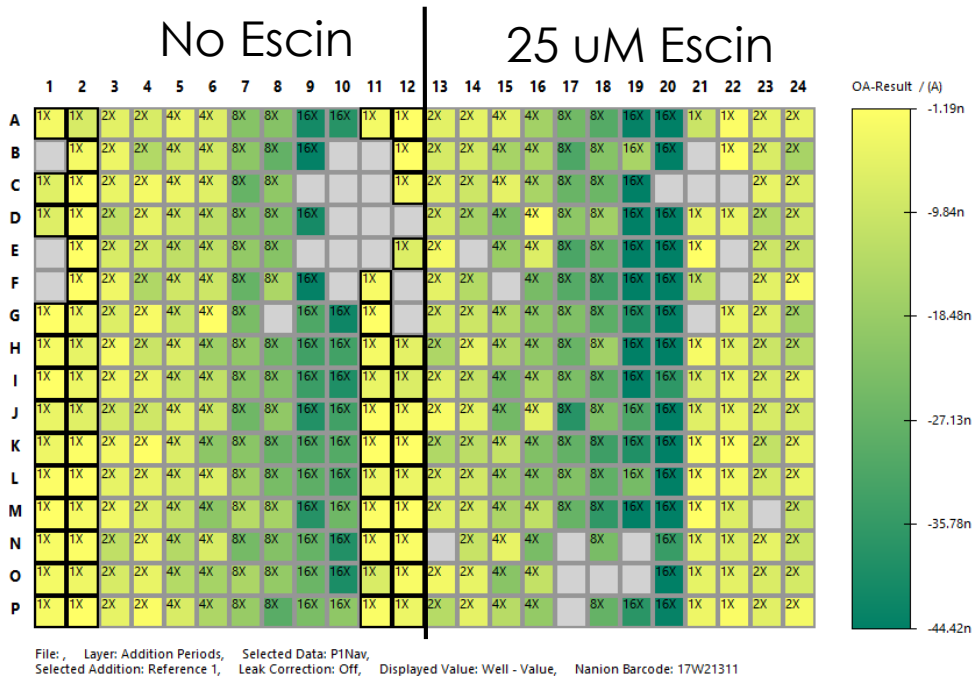


Various different colour schemes to choose from – the scaling and axis's can be configured.

Data is grouped per compound/condition.

Here it is presented as Difference from Mean – to easy detect outliers.

Choose the right colouring



Nav 1.4 current amplitude

Compound	Mean	Median	SD	Valid Wells(%)
1X group 1	-2.71n	-2.51n	2.46n	84.37
1X group 2	-4.38n	-4.46n	2.77n	81.25
2X group 1	-6.26n	-5.19n	3.52n	100.00
2X group 2	-8.17n	-7.64n	3.58n	95.31
4X group 1	-10.14n	-9.33n	4.48n	100.00
4X group 2	-15.52n	-16.13n	6.62n	96.87
8X group 1	-21.68n	-21.69n	3.90n	96.87
8X group 2	-26.97n	-27.17n	5.20n	87.50
16X group 1	-34.14n	-33.47n	6.56n	78.12
16X group 2	-43.62n	-44.89n	8.84n	90.62

Escin effect across multiple hole numbers

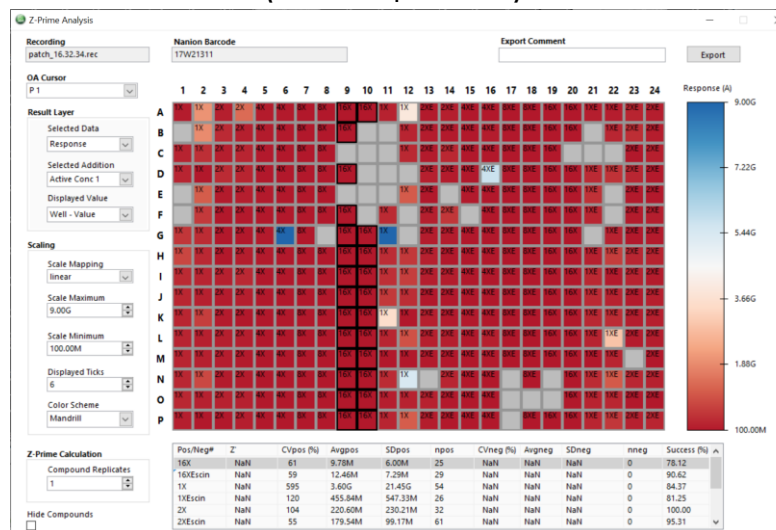
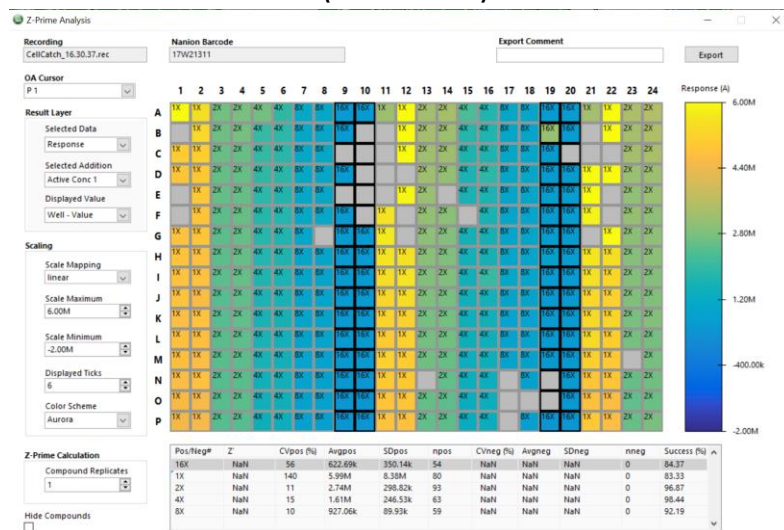
The difference can be seen in the right using "Jet", but it is not so clear with "Summer" on the left.

Liniar vs. logarithmic colour scales

Low resistance
(contact)

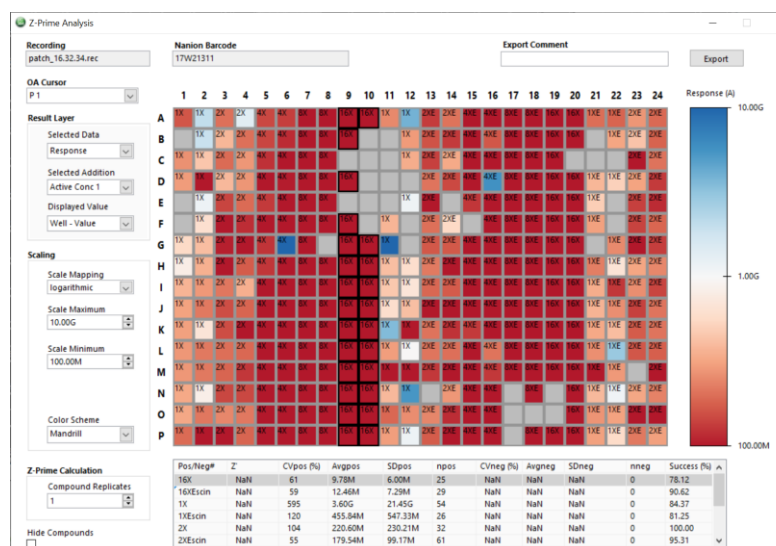
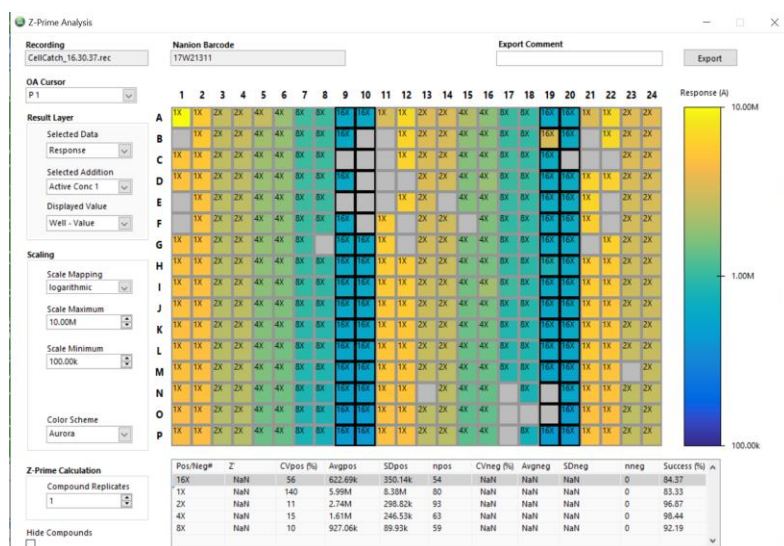
High resistance
(Patch period)

Liniar scale



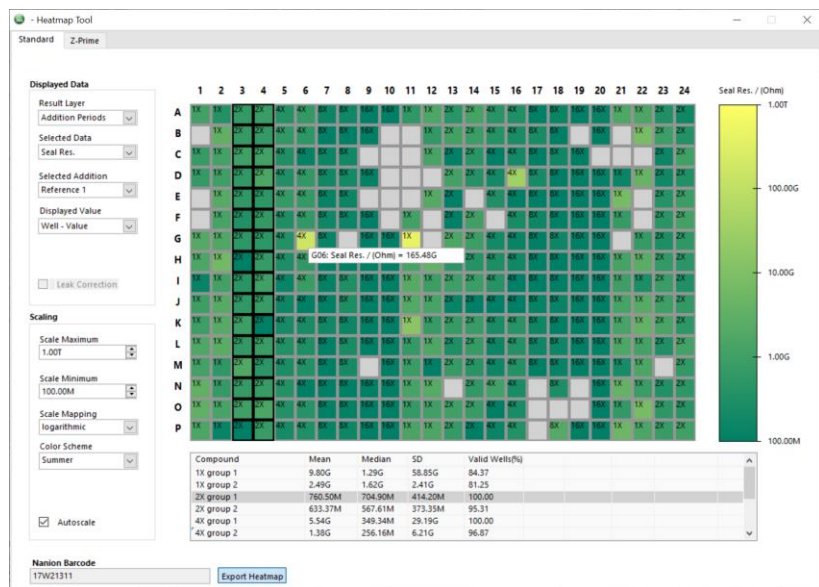
Liniar scales are optimal for low resistances – notice the gradient North/South in the plate.

Log scale



Log scales are optimal for high resistances – notice the difference between number of holes.

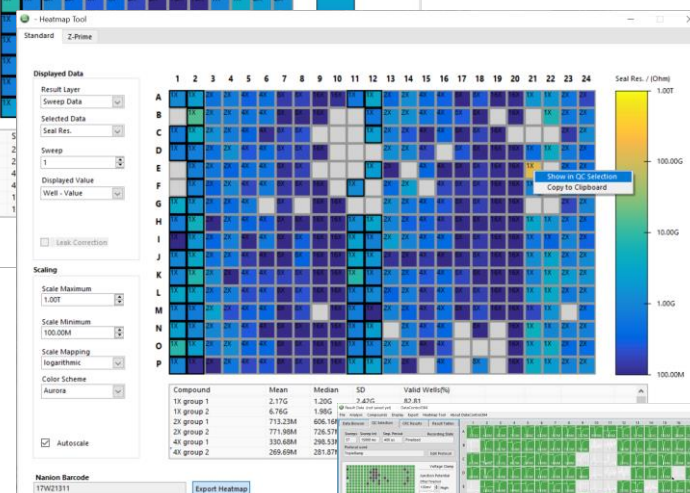
Detection and filtering of outliers



Check for groups with outliers
e.g. Mean \neq Median



- Find outlier by color difference
- Mouse over to see actual numerical value



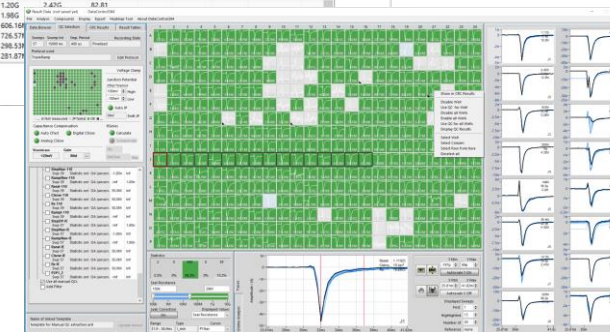
Right click and
jump to QC
selection



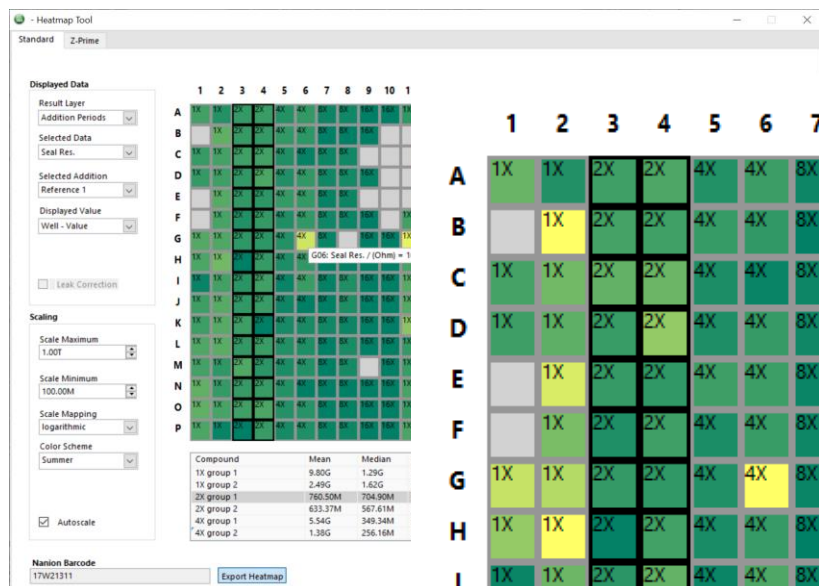
Export map and table
by right clicking

Compound	Mean	Median	SD	Valid Wells(%)
1X group 1	2.17G	1.20G	2.4	80.81
1X group 2	2.21G	1.82G	1.5	81.25
2X group 1	713.23M	606.16M	485	100.00
2X group 2	771.98M	726.57M	485	95.31
4X group 1	330.68M	298.53M	176	100.00
4X group 2	269.69M	281.87M	134	96.87
8X group 1	137.32M	132.99M	78.58M	96.87
8X group 2	149.43M	131.41M	144.62M	87.50
16X group 1	42.48M	36.13M	25.10M	75.00
16X group 2	60.49M	65.69M	32.90M	84.37

Disable site



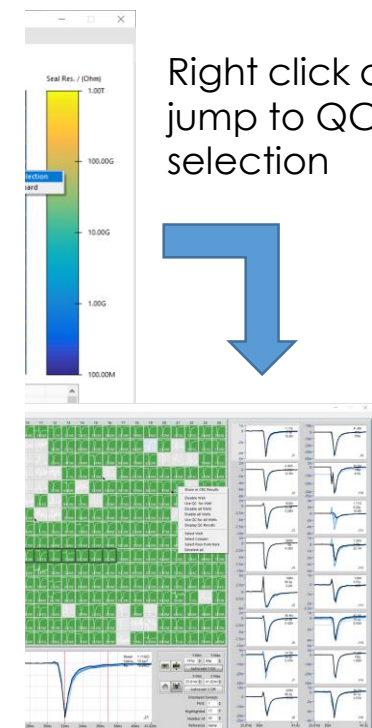
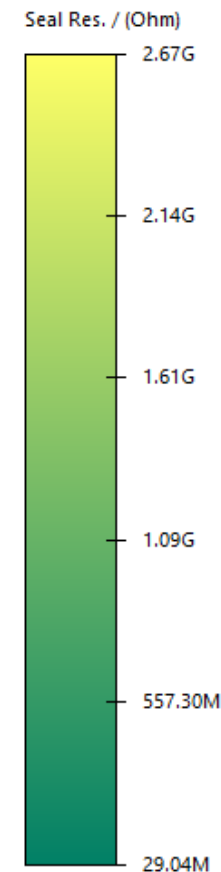
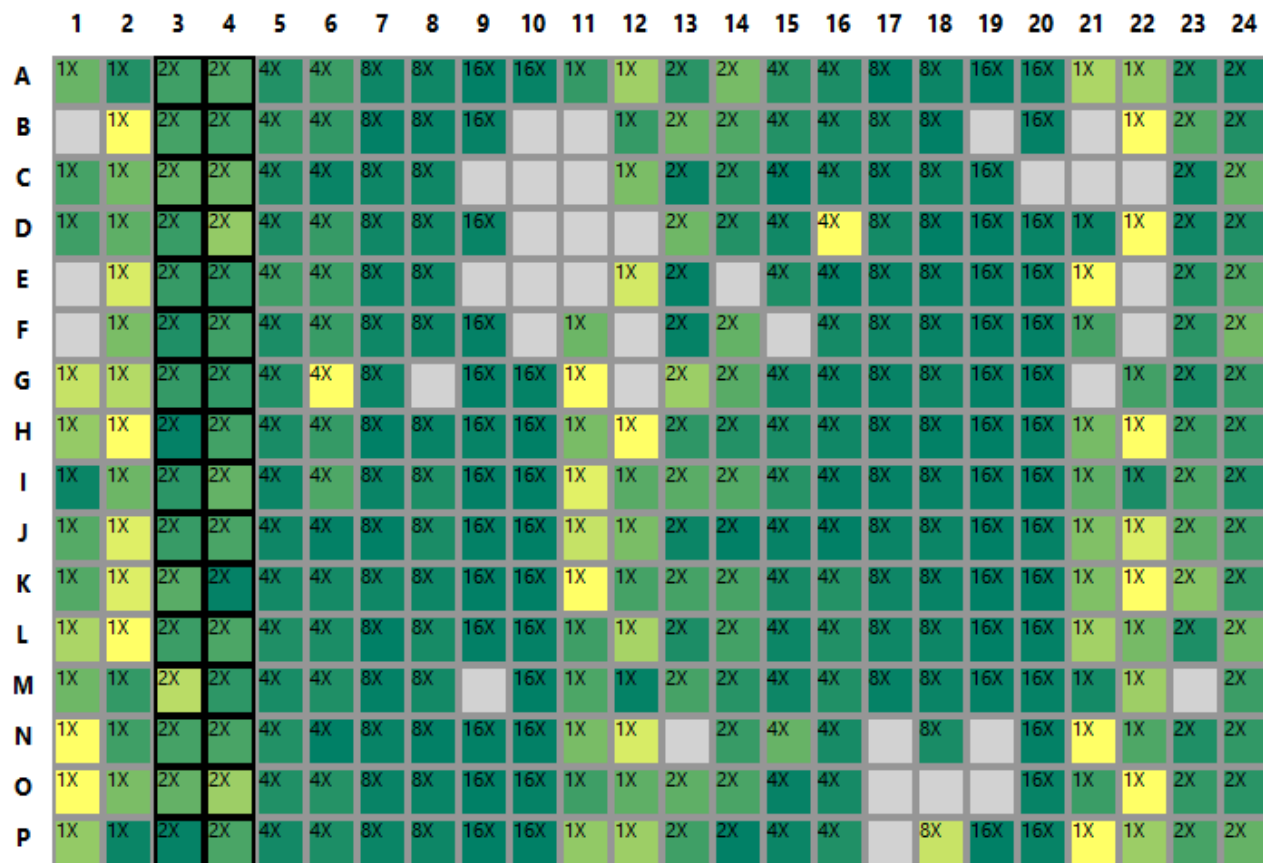
Detection and filtering of outliers



ers



- Find outlier by color difference
- Mouse over to see actual numerical value



Right click and jump to QC selection



Export map and table by right clicking

Compound	Mean
1X group 1	2.17G
1X group 2	2.21G
2X group 1	713.23M
2X group 2	771.98M
4X group 1	330.68M
4X group 2	269.69M
8X group 1	137.32M
8X group 2	149.43M
16X group 1	42.48M
16X group 2	60.49M

File: , Layer: Addition Periods, Selected Data: Seal Res.,
Selected Addition: Reference 1, Leak Correction: Off, Displayed Value: Well - Value, Nanion Barcode: 17W21311

151.41M	144.02M	87.50
36.13M	25.10M	75.00
65.69M	32.90M	84.37

Subgrouping assay conditions in heatmaps using concentrations

Compounds of M1_17W21311 \ M1_17W21311_Rainbow \ TripleRamp_16.42.31.rec

File

Compounds Solutions Cells

Compound ID	Batch	Lot	Comment
1	1X		
2	2X		
3	4X		
4	8X		
5	16X		
6	32X		
7	64X		
8	128X		
9	256X		
10	512X		

Concentration (mol/l)	Comment
1	30.00u
2	10.00u
3	3.000u
4	1.000u
5	300.0n
6	0.000

Cells

14 13 12 11

I J K L

E F G H

A B C D

Compound defined Concentration defined

Solution defined

Cell defined

Position C

Plate Type 384 well plate

Plate ID (Barcode)

Content Type Compounds

Context of Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
B	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
C	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
D	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
E	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
F	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
G	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
H	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
I	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
J	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
K	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
L	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
M	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
N	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
O	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2
P	1	1	2	2	3	3	4	4	5	5	1	1	2	2	3	3	4	4	5	5	1	1	2	2

Concentration of Active Compound on Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
B	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
C	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
D	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
E	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
F	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
G	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
H	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
I	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
J	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
K	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
L	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
M	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
N	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
O	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5
P	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	5	5	5	5	5

Apply Discard Changes Close

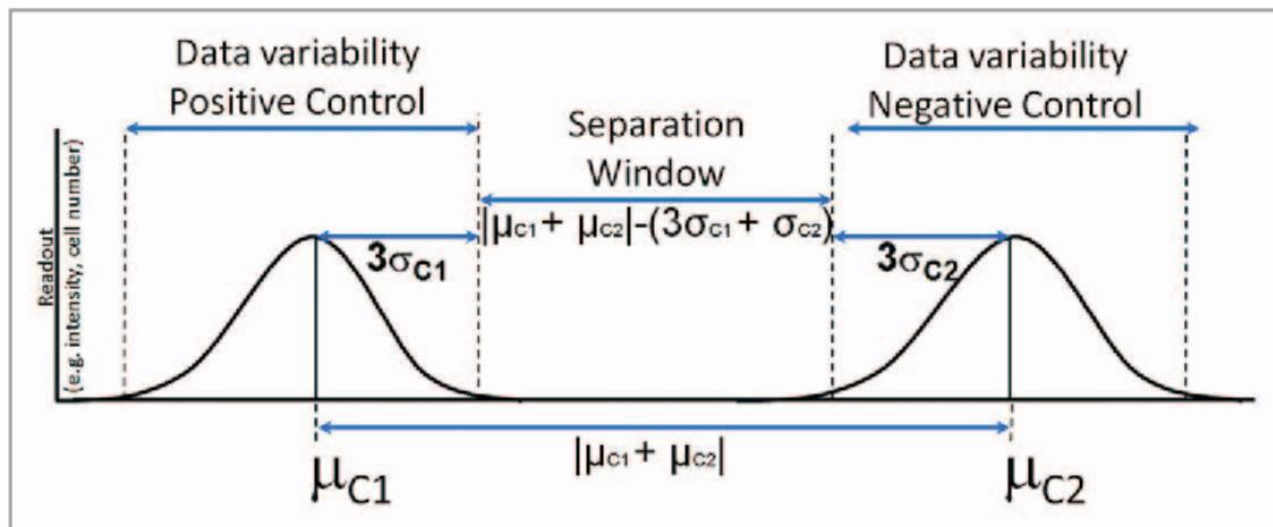
Conditions can be subdivided using different concentrations to define a subset of parameters.

From the previous slide left (No Escin) and right (25 uM Escin) where used.

The compound name is from Compound ID and group 1 and 2 are the concentration groups.

Compound	Mean	Median	SD
1X group 1	-2.71n	-2.51n	2.46n
1X group 2	-4.38n	-4.46n	2.77n
2X group 1	-6.26n	-5.19n	3.52n
2X group 2	-8.17n	-7.64n	3.58n

Z' analysis



Z' is a quality parameter that you can use to evaluate your assay.

It gives you information if there is an unwanted overlap between your negative and positive controls that can result in false positives/negatives.

The implemented function in Datacontrol is:

$$Z' = \frac{(AVG_{max} - 3SD_{max}/\sqrt{n}) - (AVG_{min} + 3SD_{min}/\sqrt{n})}{AVG_{max} - AVG_{min}}$$

Typically n=1, but it is possible to increase the number of repeats in the software, which you can then adapt in your assay if needed.

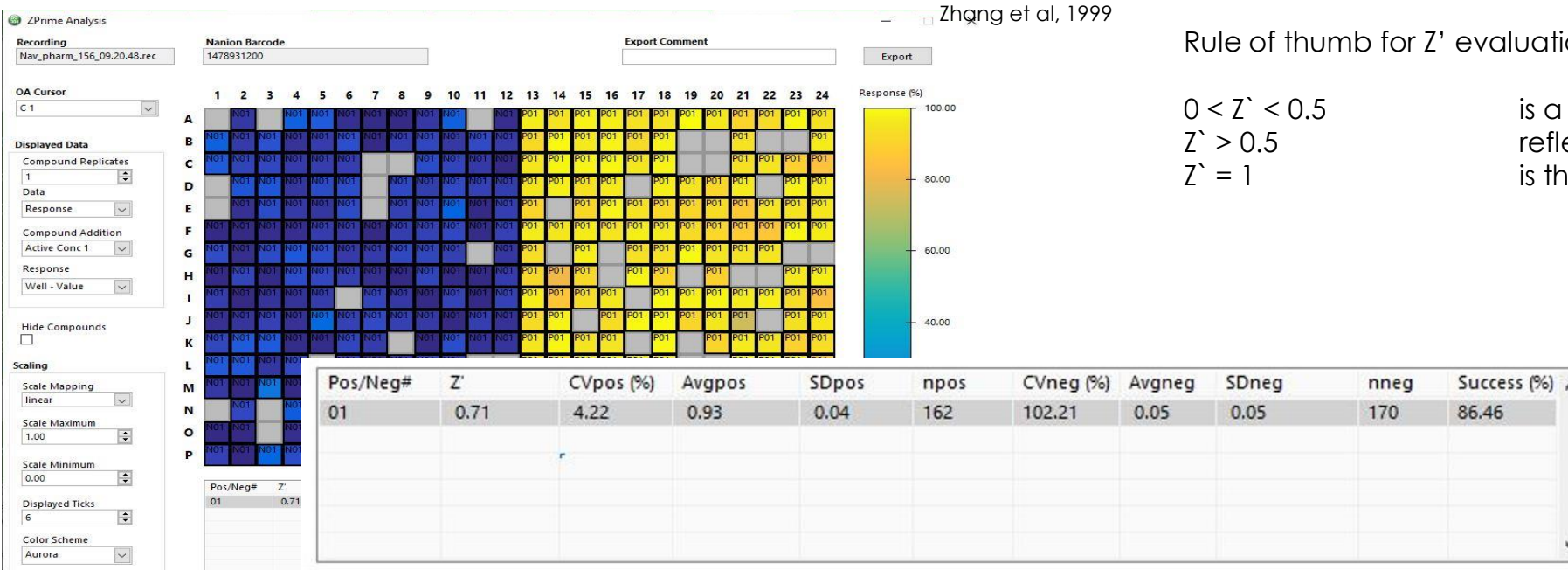
Rule of thumb for Z' evaluation:

$0 < Z' < 0.5$

$Z' > 0.5$

$Z' = 1$

is a workable assay
reflects an excellent assay
is the ideal assay



Z' analysis

The screenshot shows the DataControl384 software interface. The 'Compounds' tab is active, displaying a table with columns: Compound, Batch, Lot, Comment, Concentration (mol/l), and Comment. The 'Cells' tab is also visible, showing a 4x4 grid of wells (A-D, 1-4) with a legend indicating 'Compound defined' (yellow), 'Solution defined' (pink), and 'Cell defined' (green). The 'Pos01' and 'Neg01' wells are highlighted in yellow.

Compound	Batch	Lot	Comment	Concentration (mol/l)	Comment
1 Tetracaine				1 0.000	
2 Pos01				2 600.0u	
3 Neg01					

The analysis reads the fractional response that has been setup in the Analysis settings.

The average and SD are calculated from your controls and you tell the analysis which sites are used as controls by naming them Pos01/Neg01.

You can add more controls by increasing the index number.

The screenshot shows the DataControl384 software interface. The 'Result Data File' window is open, displaying the file path 'TRPA1_Ligand1_10.37.10.ana' and the file name 'DataControl384'. The 'File' menu is open, showing options: File, Analysis, Compounds, Display, Export, Z-Prime, and About DataControl384. The 'Data Browser' tab is selected, showing a list of data files including 'neg1'.

The screenshot shows the 'Analysis Settings' dialog box. The 'General Options' section shows 'Analysis Type' set to 'Blocker (Hill Equation)', 'Normalization' set to 'Reference', and 'Correct Baseline with Control' set to 'No Current'. The 'Data Selection' section shows 'First Parameter / Fit' set to 'C 1', 'Second Parameter / Fit' set to 'C 2', and 'Third Parameter / Fit' set to 'F 1'. The 'OA Point Selection Rule' section shows 'Averaging Algorithm' set to 'Mean', 'Selection Region' set to 'End', and 'Points to Select' set to '3'.

Examples on how to use the tool –

Test if the Z' can be used to determine how many replicates are required for IC₅₀ determination?

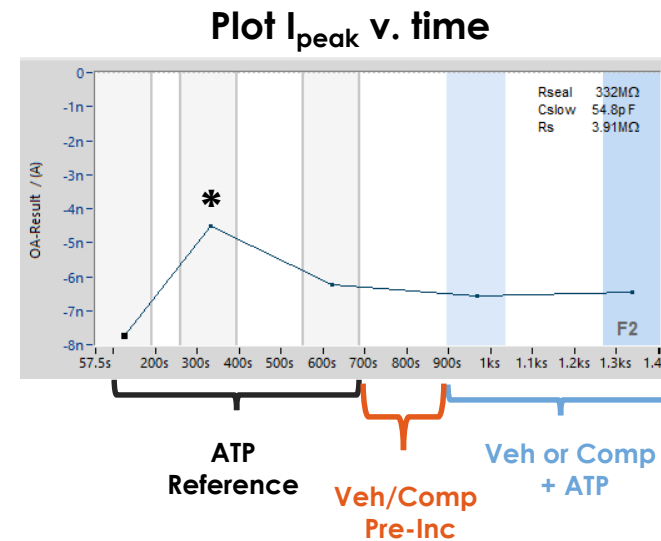
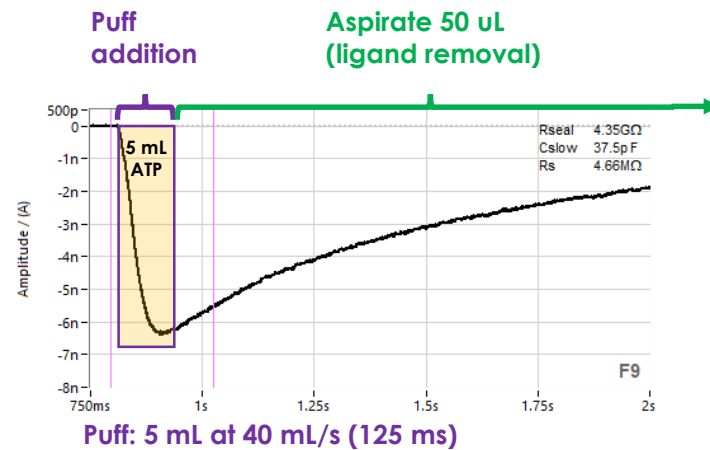
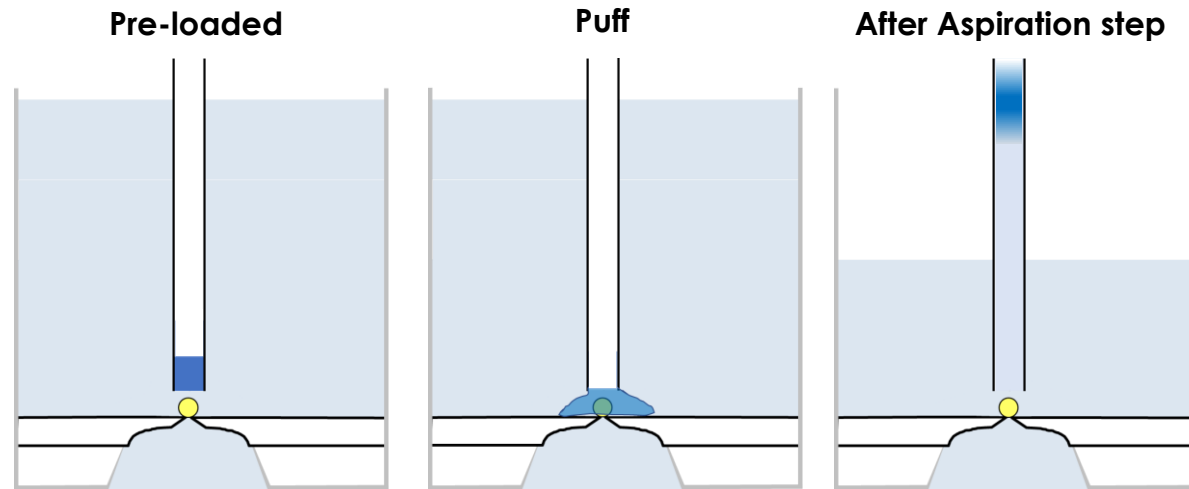
Experimental outline

1. Run a full plate dose response experiment with e.g. 6 concentrations (one full and one no effect).
2. Determine Z' using No and Full effect data and increase Replicates.
3. Use Compound Editor to decrease replicates.
4. Check consistency.

- Use Compound Editor in DataControl to decrease replicates (n=2)

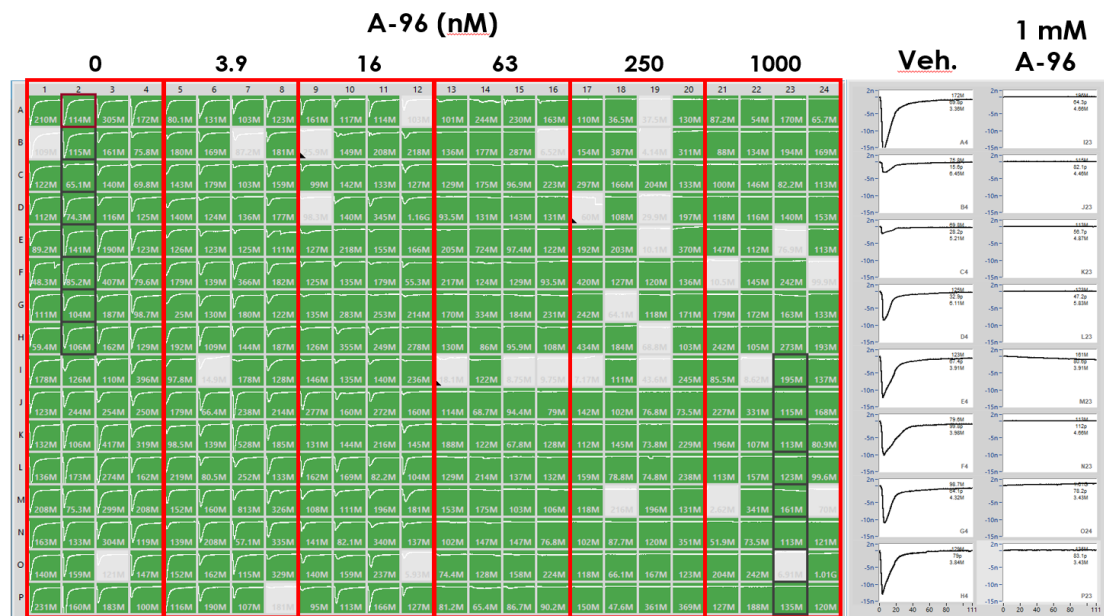
	0				1				3				10				30				100			
Content of Plate																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	3	3	4	4	3	3	4	4	3	3	4	4	3	3	4	4	3	3	4	4	3	3	4	4
B	5	5	6	6	5	5	6	6	5	5	6	6	5	5	6	6	5	5	6	6	5	5	6	6
C	7	7	8	8	7	7	8	8	7	7	8	8	7	7	8	8	7	7	8	8	7	7	8	8
D	9	9	10	10	9	9	10	10	9	9	10	10	9	9	10	10	9	9	10	10	9	9	10	10
E	11	11	12	12	11	11	12	12	11	11	12	12	11	11	12	12	11	11	12	12	11	11	12	12
F	13	13	14	14	13	13	14	14	13	13	14	14	13	13	14	14	13	13	14	14	13	13	14	14
G	15	15	16	16	15	15	16	16	15	15	16	16	15	15	16	16	15	15	16	16	15	15	16	16
H	17	17	18	18	17	17	18	18	17	17	18	18	17	17	18	18	17	17	18	18	17	17	18	18
I	19	19	20	20	19	19	20	20	19	19	20	20	19	19	20	20	19	19	20	20	19	19	20	20
J	21	21	22	22	21	21	22	22	21	21	22	22	21	21	22	22	21	21	22	22	21	21	22	22
K	23	23	24	24	23	23	24	24	23	23	24	24	23	23	24	24	23	23	24	24	23	23	24	24
L	25	25	26	26	25	25	26	26	25	25	26	26	25	25	26	26	25	25	26	26	25	25	26	26
M	27	27	28	28	27	27	28	28	27	27	28	28	27	27	28	28	27	27	28	28	27	27	28	28
N	29	29	30	30	29	29	30	30	29	29	30	30	29	29	30	30	29	29	30	30	29	29	30	30
O	31	31	32	32	31	31	32	32	31	31	32	32	31	31	32	32	31	31	32	32	31	31	32	32
P	33	33	34	34	33	33	34	34	33	33	34	34	33	33	34	34	33	33	34	34	33	33	34	34

Puff method of ligand application

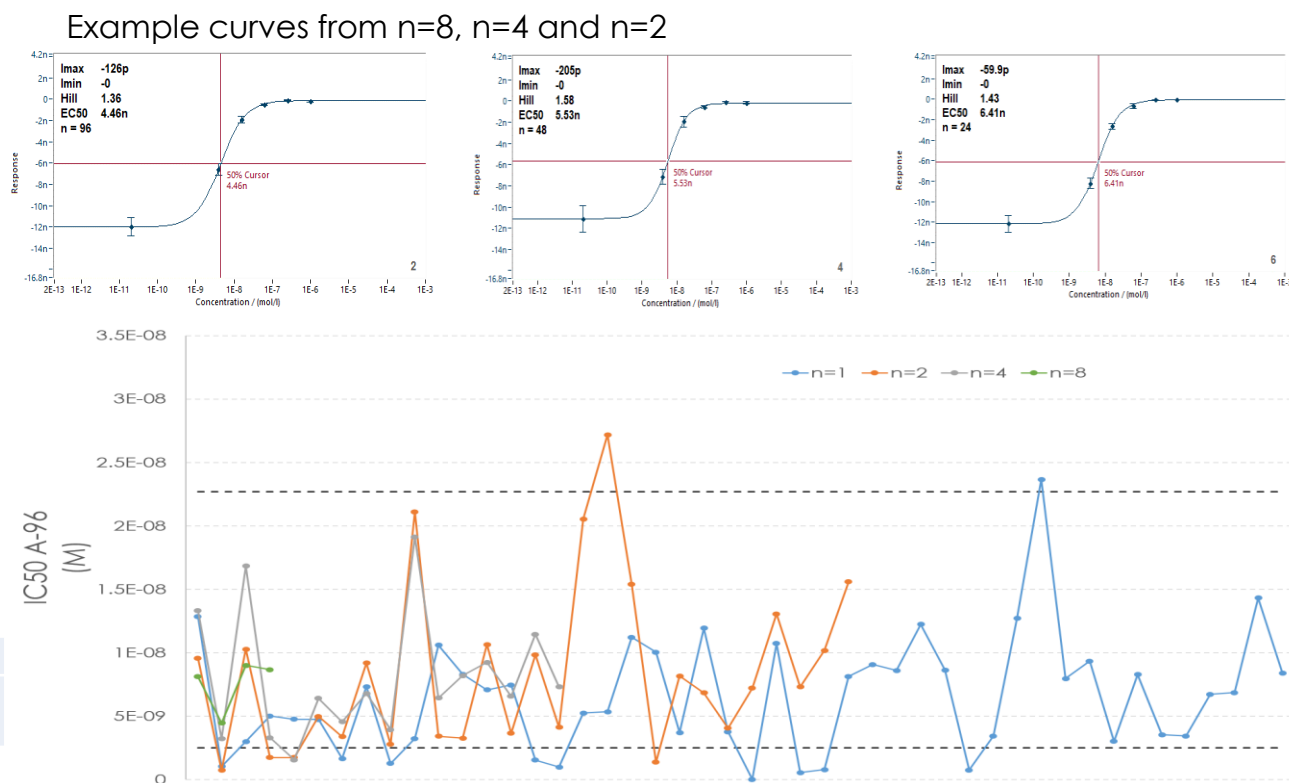
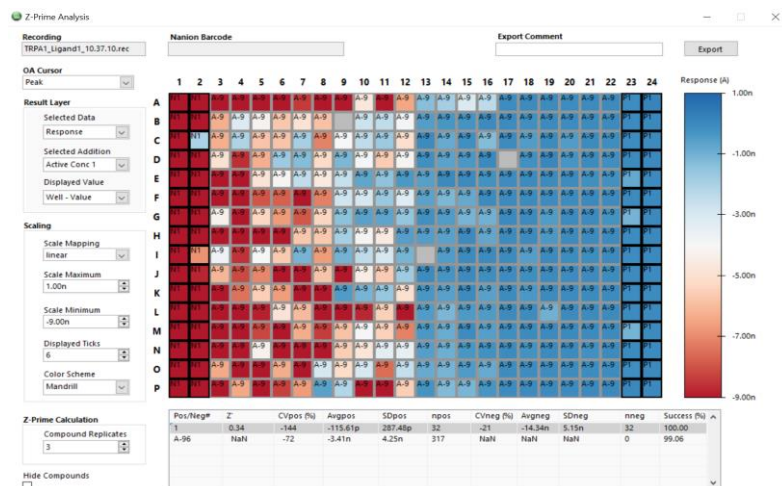


* 2nd Puff gives smaller current due to desensitization; longer inter-puff interval is required for recovery from desensitization.

Z' analysis – TRPa1 EC Ca²⁺ blocker assay



TRPa1 EC Ca ²⁺ assay	Z', n=1	Z', n=2	Z', n=3	Z', n=4	Z', n=5	Z', n=6
	-0.15	0.19	0.34	0.43	0.49	0.53



"One Shot" TRPa1 with extracellular Ca²⁺ antagonist dose response data was analysed with the Z' tool by adding a set of 32 positive and negative controls in each end of the dose response curve.

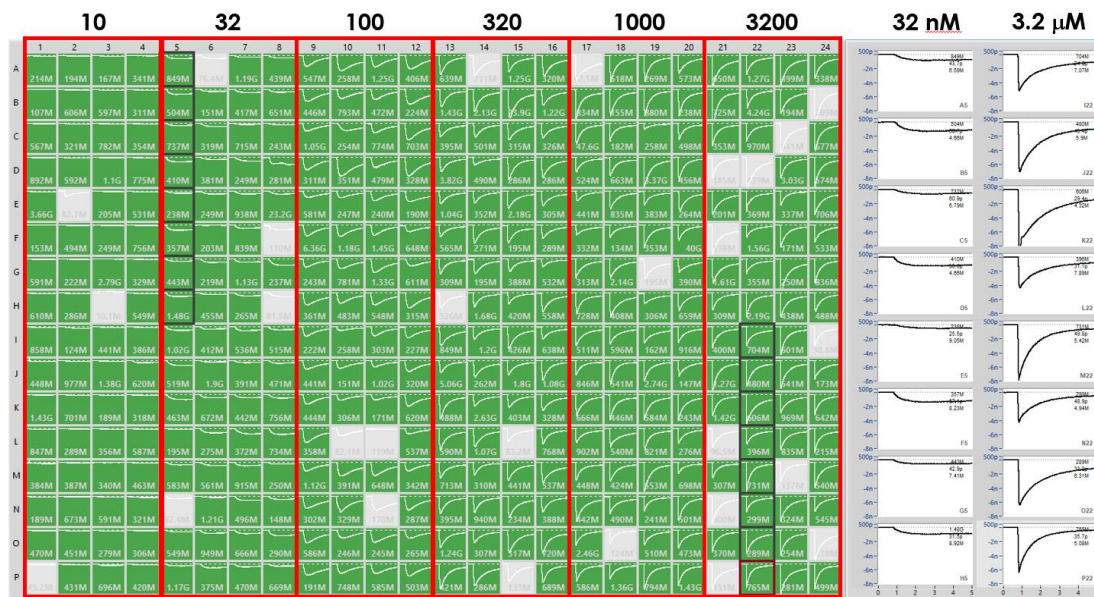
The number of repeats were increased to demonstrate the effect on Z'. It was found that n=4 should be sufficient for running the assay.

The dose response curve was then broken down into groups of 1, 2, 4 and 8 replicates per concentration and the resulting EC50's collected.

It was found that 5 curves fell out of the 3 fold band @ n=2, 1 @ n=4 and 0 @ n=8

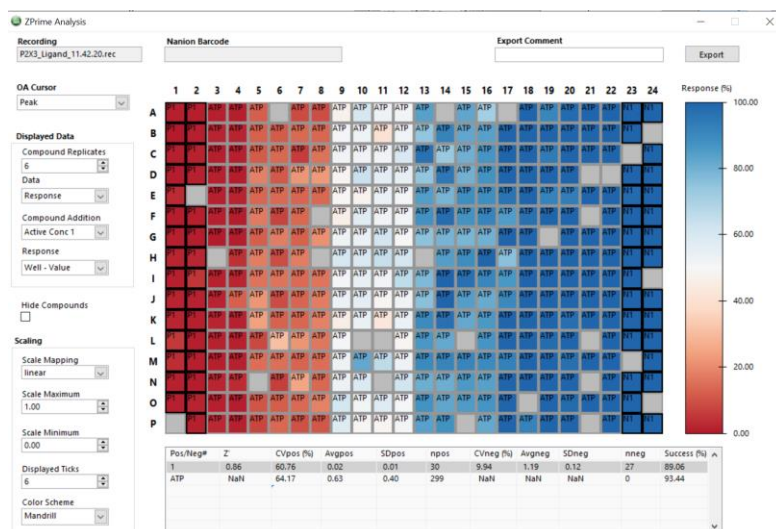
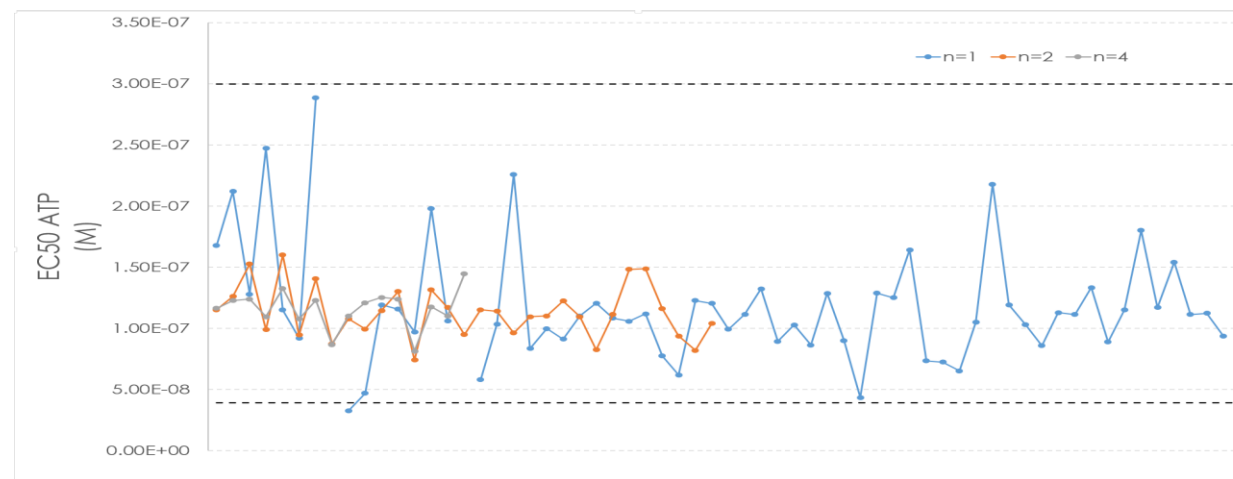
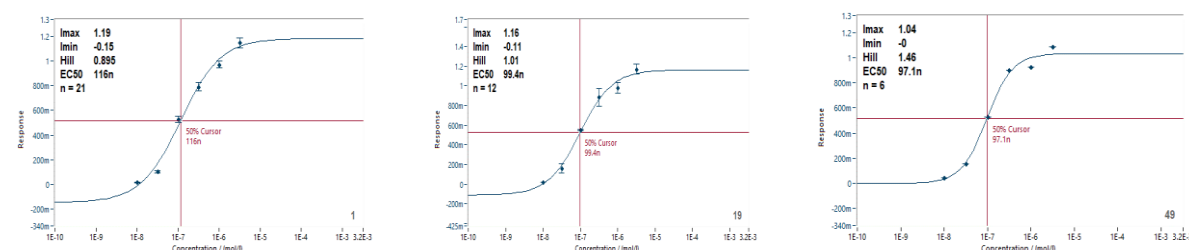
Z' analysis – P2X3 ATP EC50

ATP (nM)



	Z', n=1	Z', n=2	Z', n=3	Z', n=4	Z', n=5	Z', n=6
P2X3 EC50 assay	0.67	0.76	0.81	0.83	0.85	0.86

Example curves from n=4, n=2 and n=1



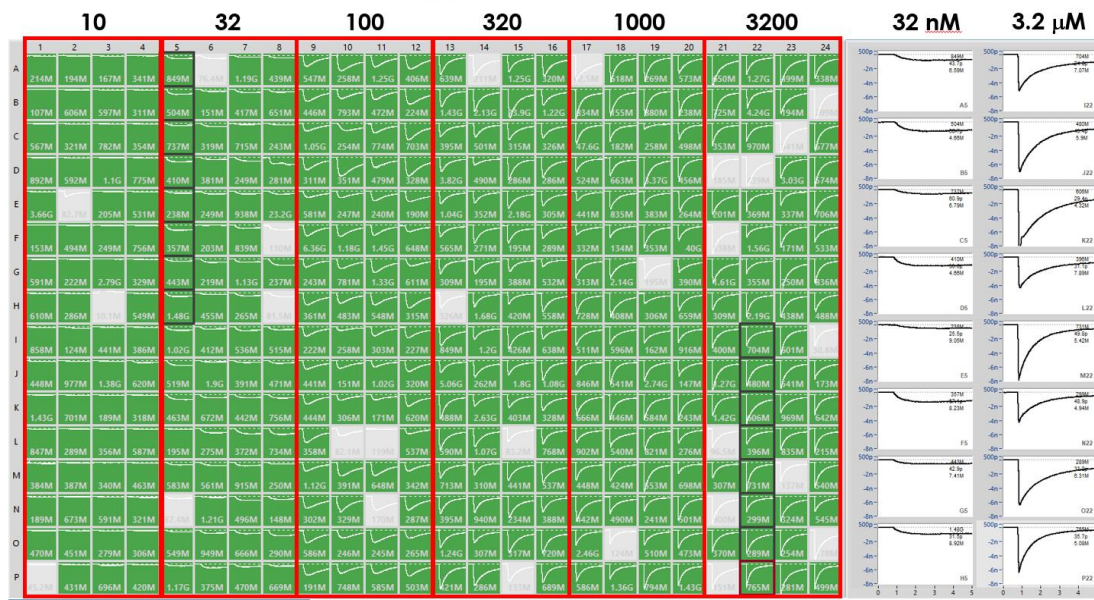
P2X3 ATP dose response data was analysed with the Z' tool by adding a set of 32 positive and negative controls in each end of the dose response curve.

The number of repeats were increased to demonstrate the effect on Z'. It was found that n=1 should be sufficient.

The dose response curve was then broken down into groups of 1, 2 and 4 replicates per concentration and the resulting EC50's collected. It was found that all curves fell within a 3 fold band.

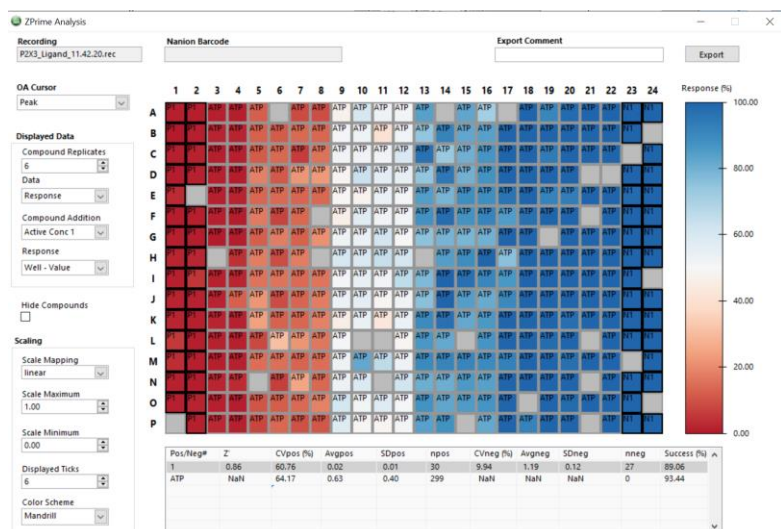
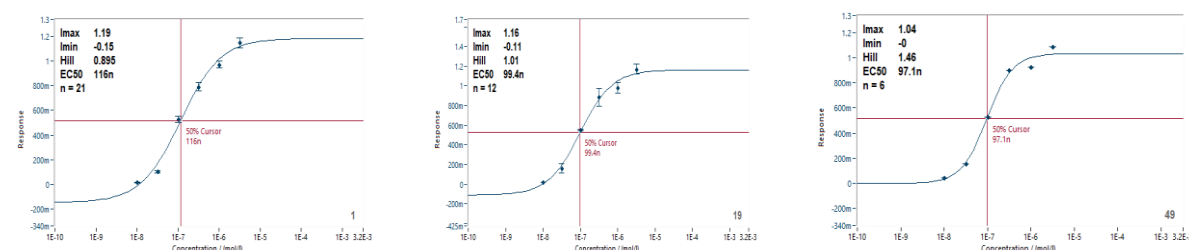
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Example curves from n=4, n=2 and n=1



P2X3 ATP dose response data was analysed with the Z' tool by adding a set of 32 positive and negative controls in each end of the dose response curve.

The number of repeats were increased to demonstrate the effect on Z'. It was found that n=1 should be sufficient.

The dose response curve was then broken down into groups of 1, 2 and 4 replicates per concentration and the resulting EC50's collected. It was found that all curves fell within a 3 fold band.