Nanion Technologies
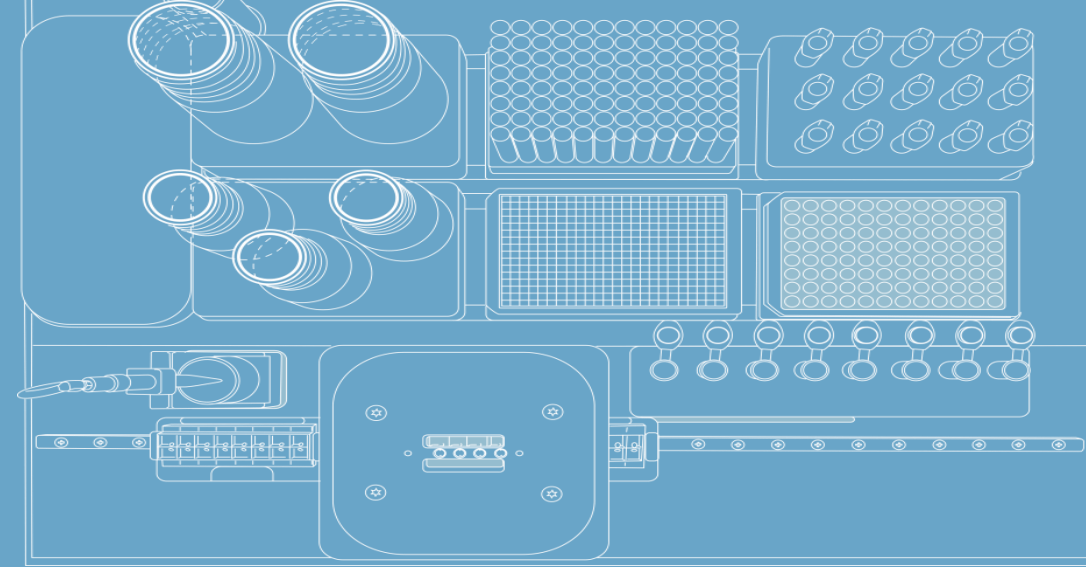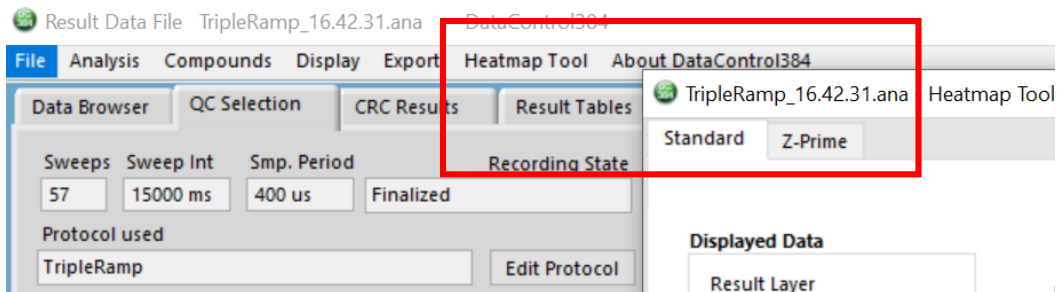
Heatmaps in DataControl

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Heatmap – an expansion of Z'

Z' has taken the backseat and the auxilary tool is now called "Heatmap Tool".
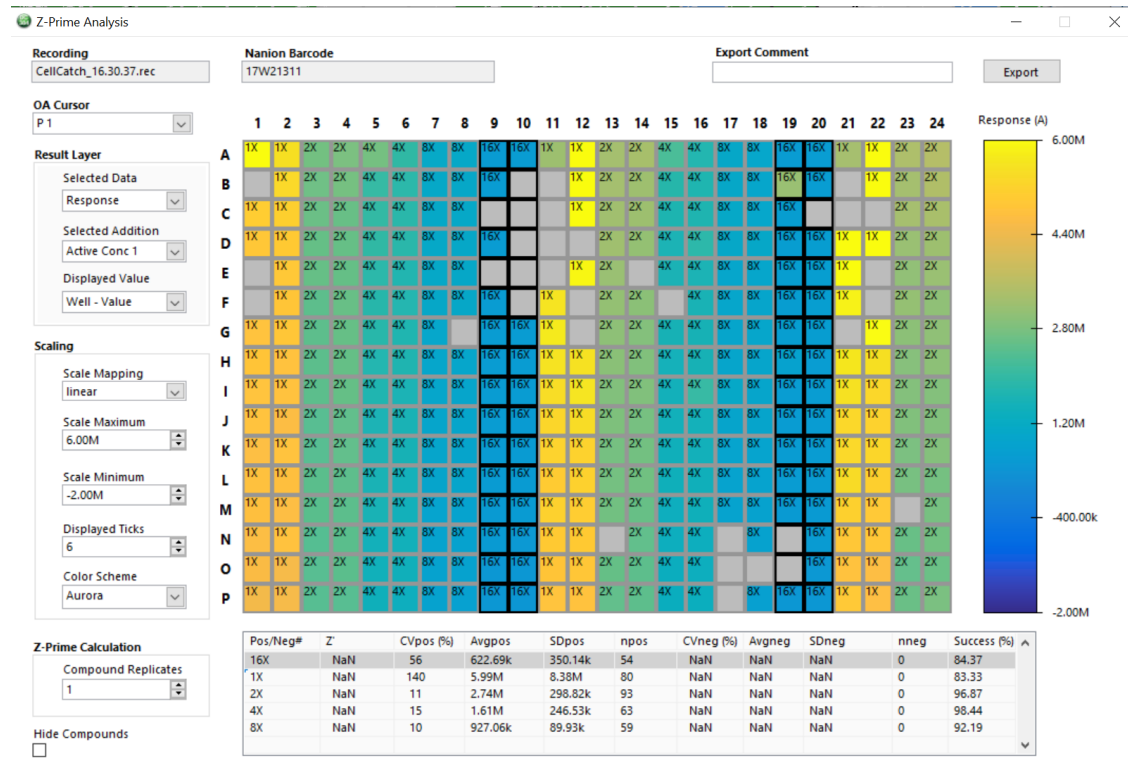
Z' can still be found in there but it is not standard.

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.

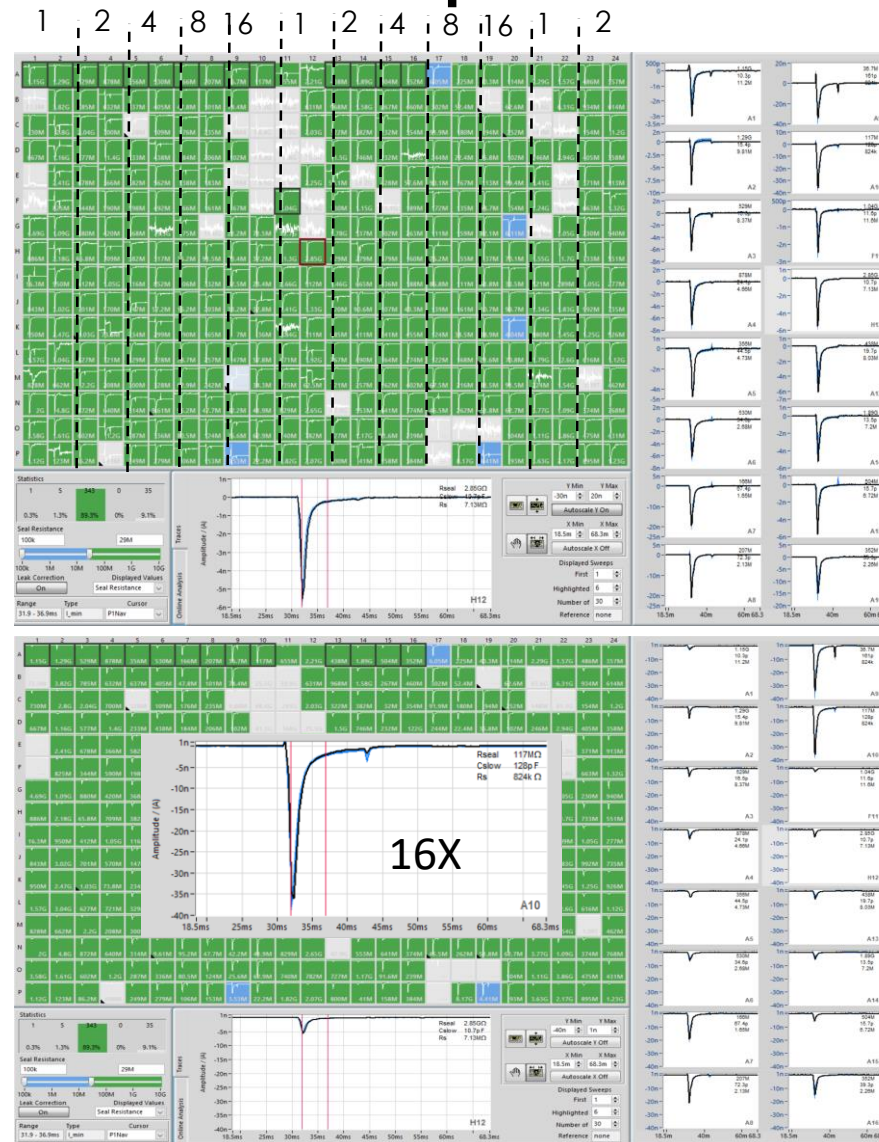Simple statistics on the chosen OA's are easily derived from the groups that has been defined.
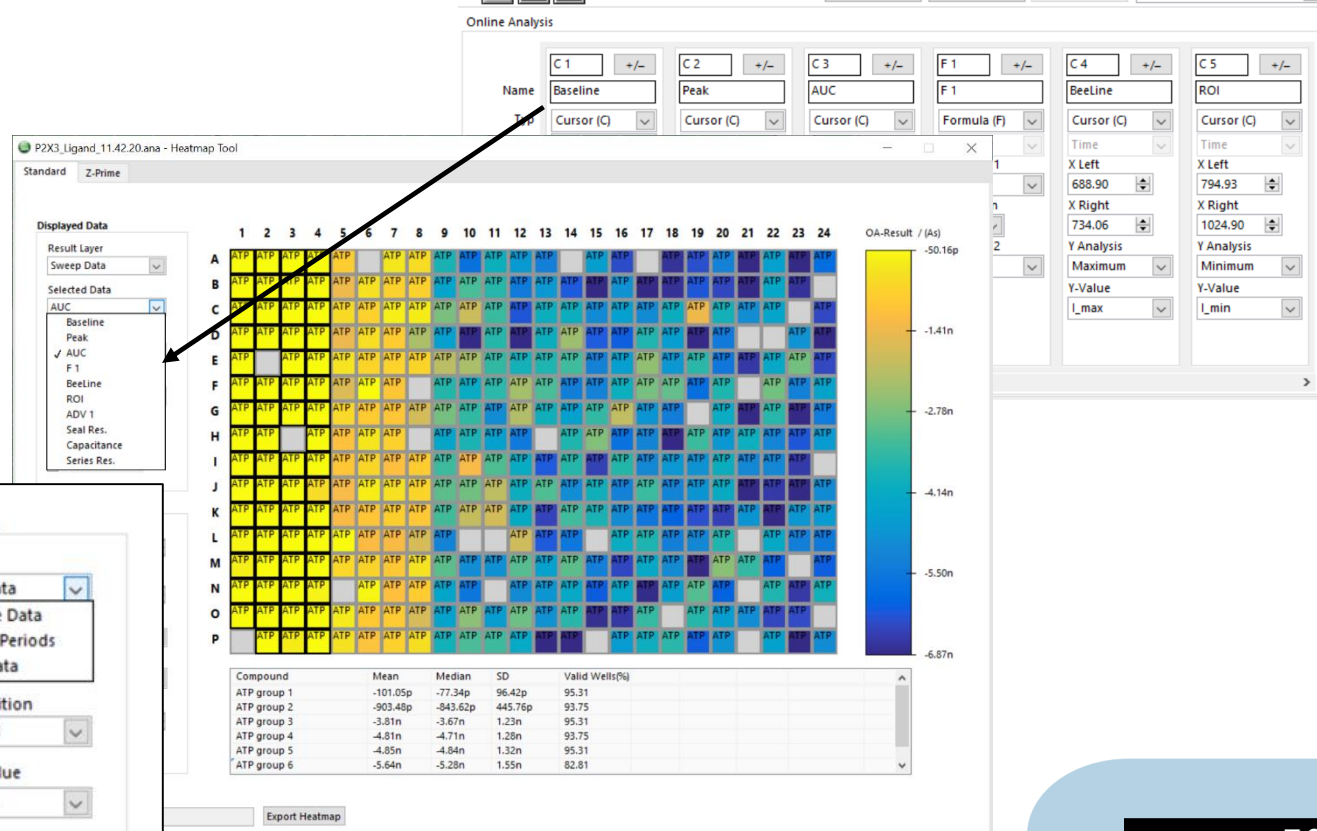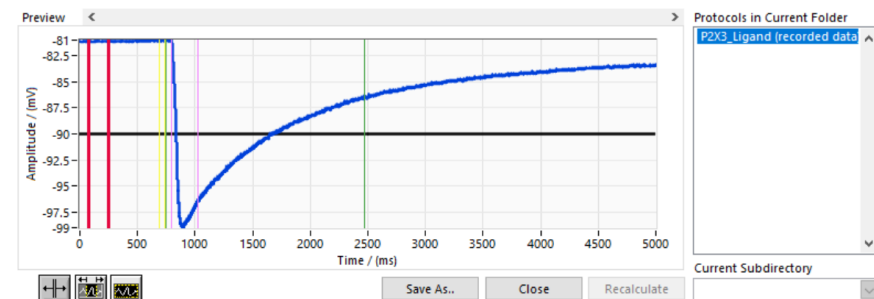
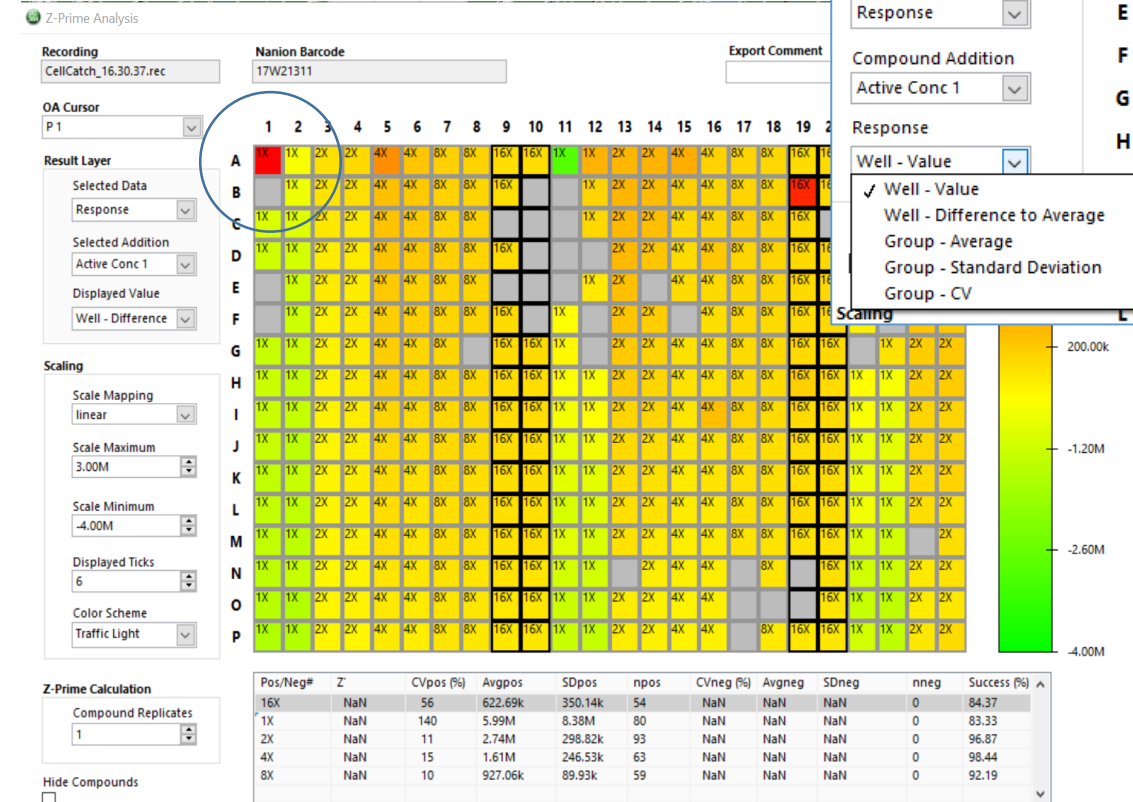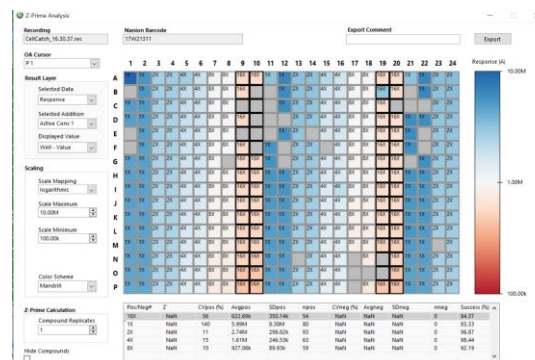# Test data is from a so called "Rainbow plate"

A rainbow plate is develomental 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.

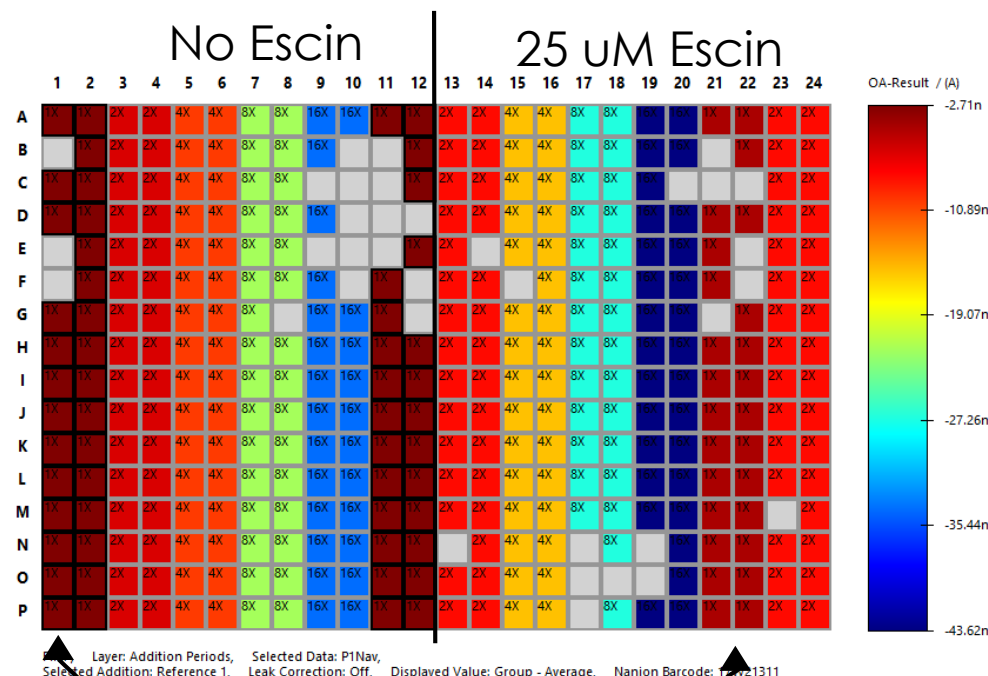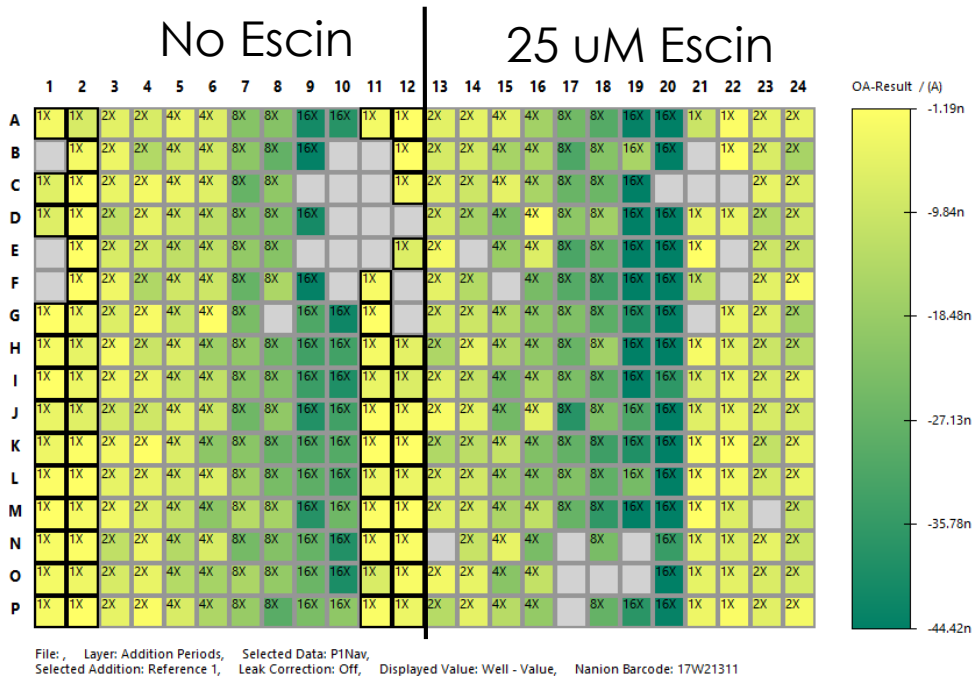# Different colour schemes – to suit your needs
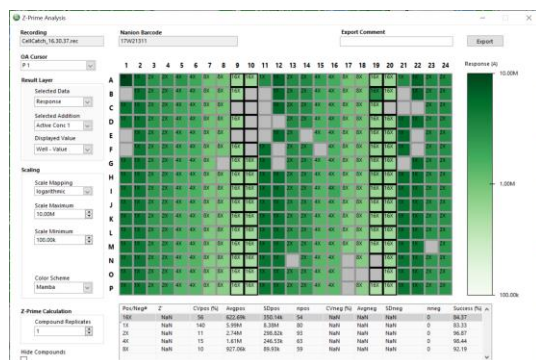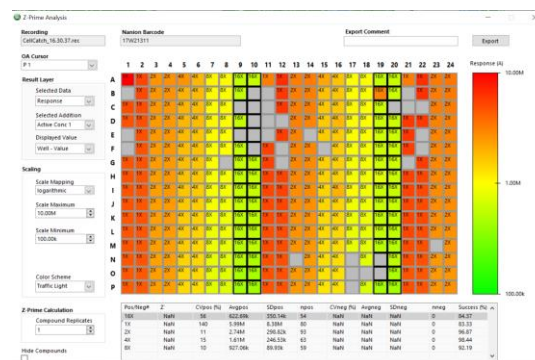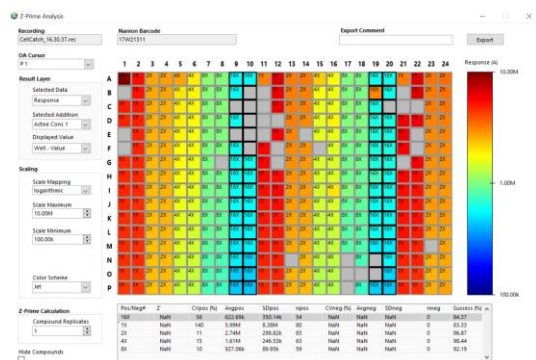
Various different colour schemes to choose from – the scaling and axis's can be configured.

Data is grouped per compound/condition.

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

Choose the right colouring

No Escin   25 uM Escin   No Escin   25 uM Escin

Nav 1.4 current ampitude

| 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 multible hole numbers

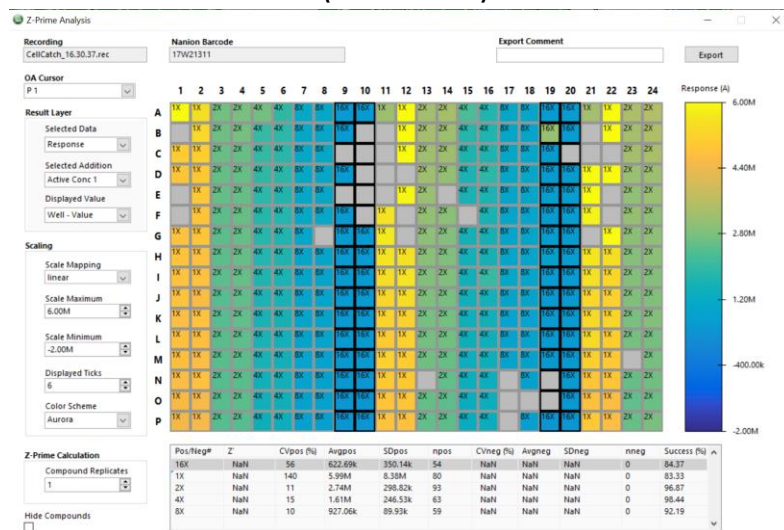The difference can be seen in the right using "Jet", but it is not so clear with "Summer" on the left.

Liniar vs. logarithmic colour scales

Low resistance
(contact)
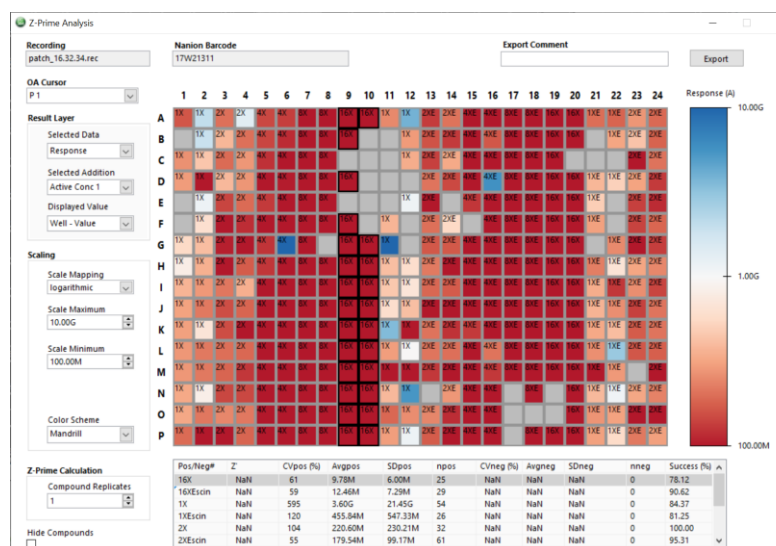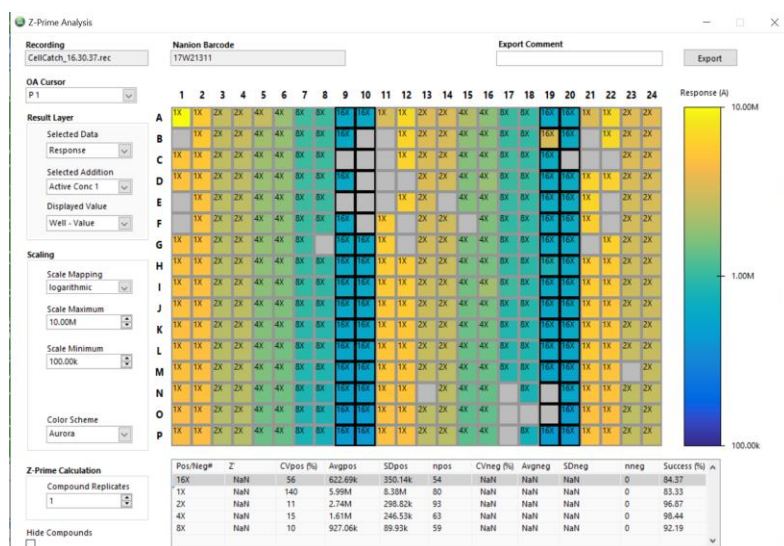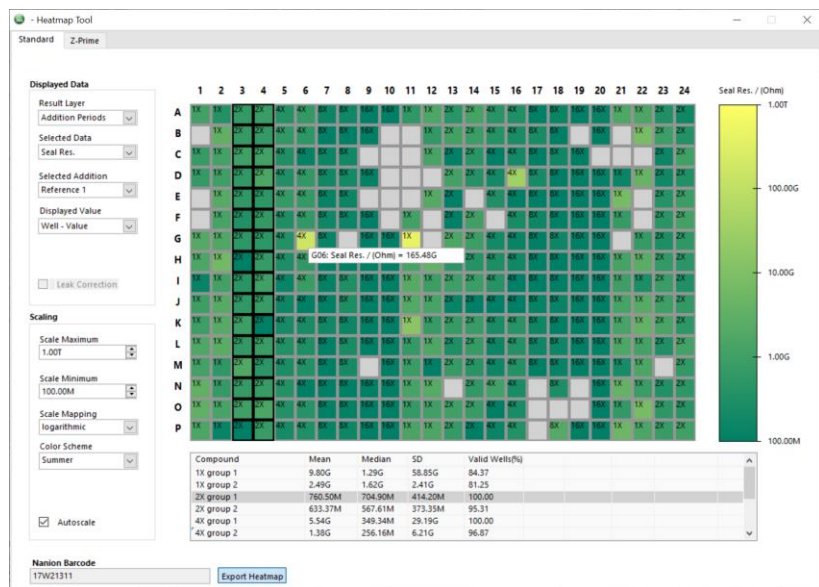
High resistance
(Patch period)

Liniar scale
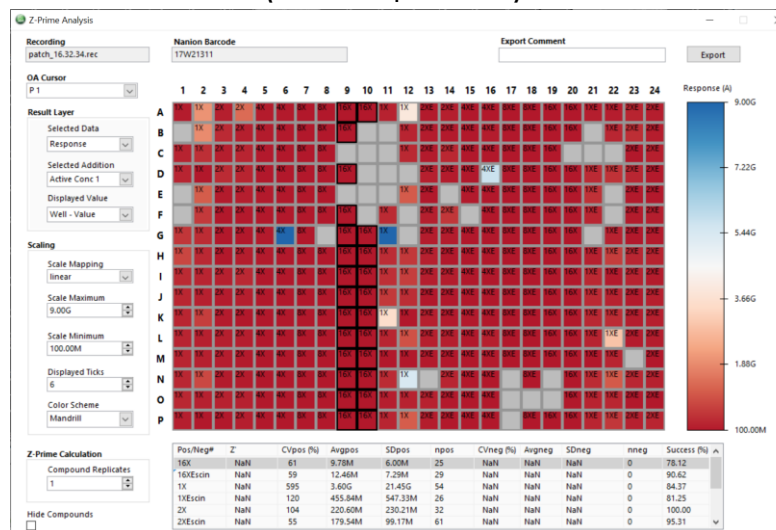
Log scale

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

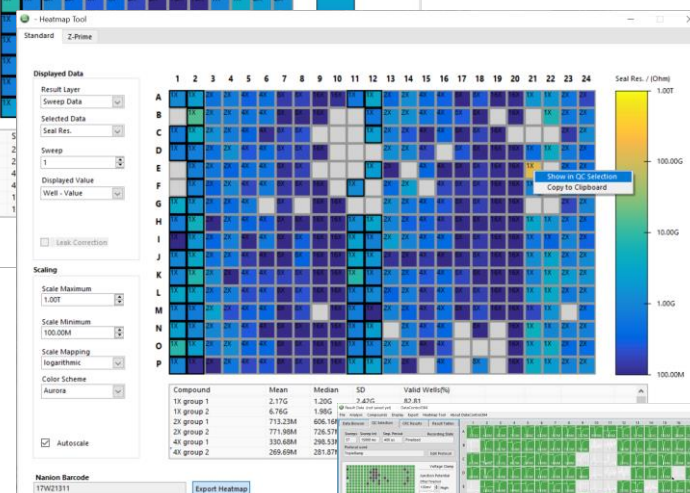Log scales are optimal for high resistances – notice the difference between number of holes.

Detection and filtering of outliers

Check for groups with outliers
e.g. Mean ≠ Median

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

Right click and jump to QC selection

Disable site

Export map and table by right clicking

Compound	Mean	Median	SD
1X group 1	2.17G	1.20G	2.4
1X group 2	2.21G	1.82G	1.5
2X group 1	713.23M	606.16M	485
2X group 2	771.98M	726.57M	486
4X group 1	330.68M	298.53M	176
4X group 2	269.69M	281.87M	134
8X group 1	137.32M	132.99M	78.58M
8X group 2	149.43M	131.41M	144.62M
16X group 1	42.48M	36.13M	25.10M
16X group 2	60.49M	65.69M	32.90M

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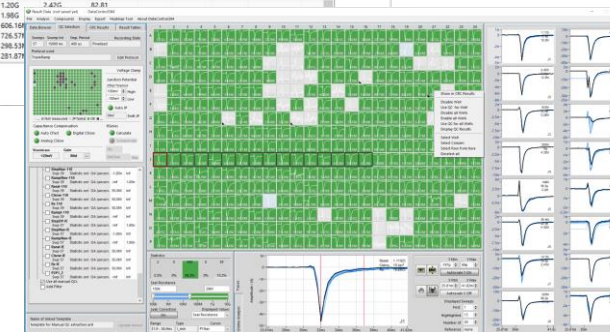
Detection and filtering of outliers

- Find outlier by color difference
- Mouse over to see actual numerical value
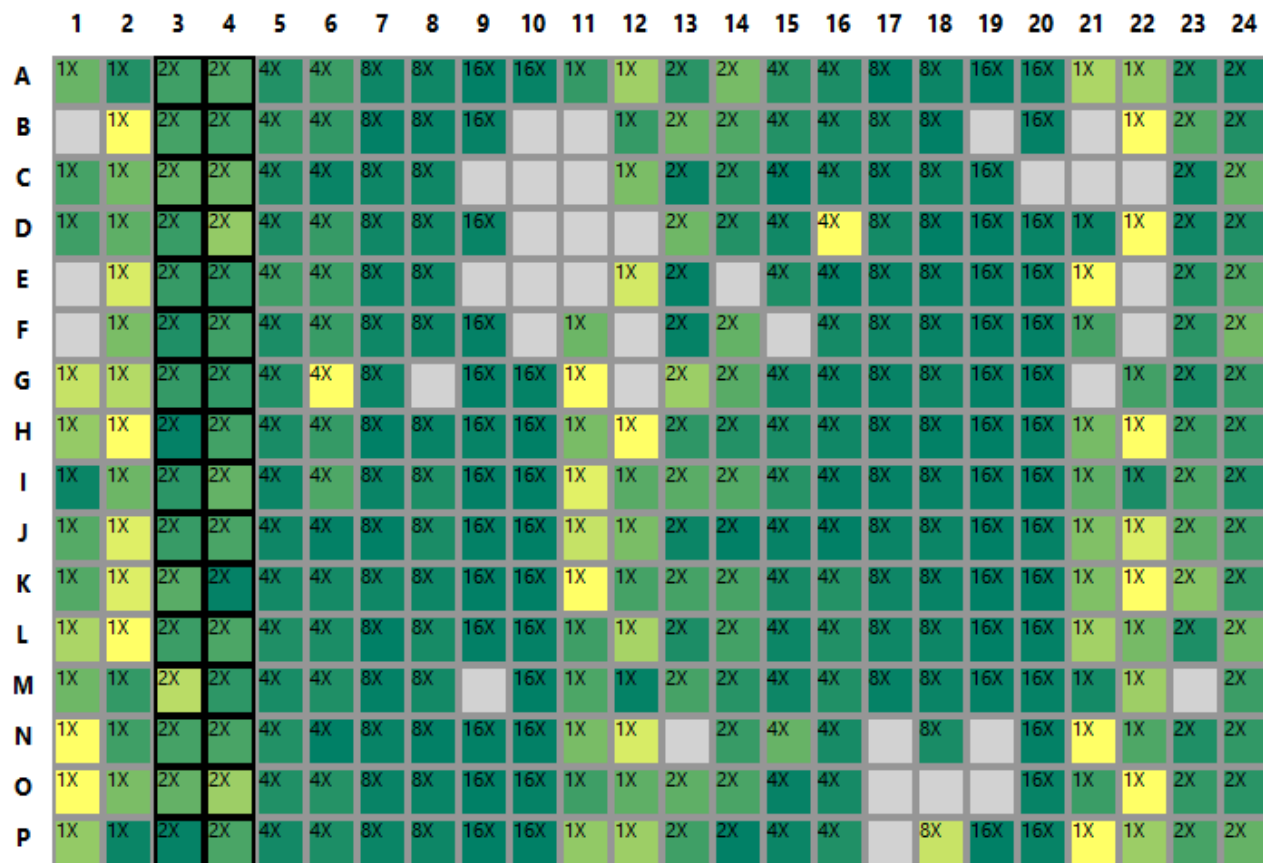
Right click and jump to QC selection

Export map and table by right clicking
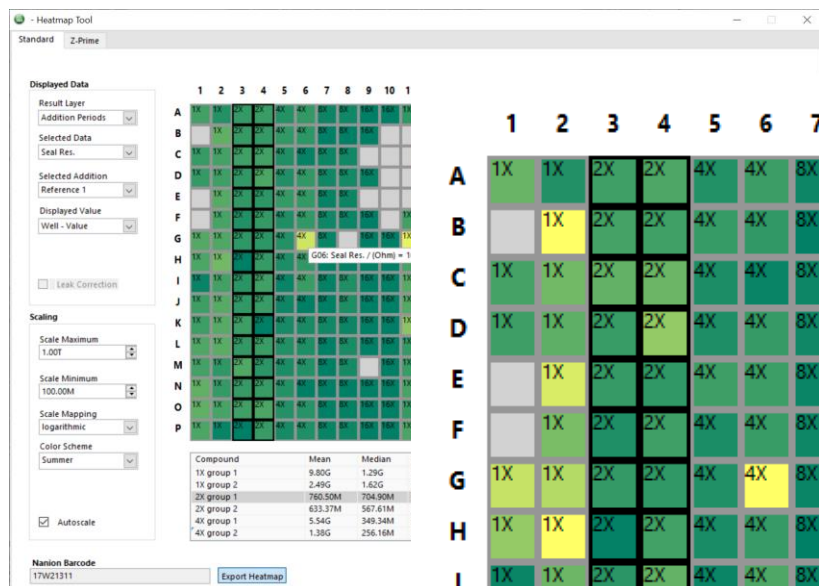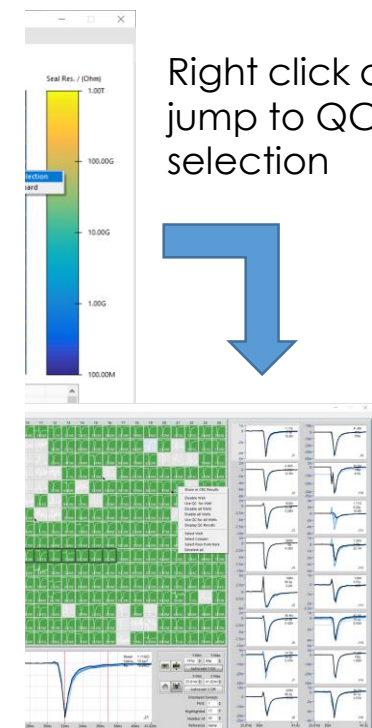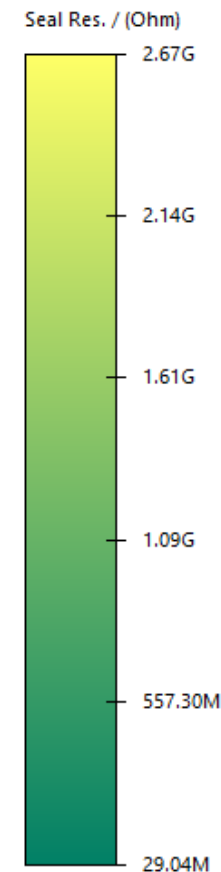
Compound	Mean
1X group 1	2.17G
1X group 2	2.21G
2X group 1	713.23M
2X group 2	771.98M
4X group 1	330.68M
4X group 2	269.69M
8X group 1	137.32M
8X group 2	149.43M
16X group 1	42.48M
16X group 2	60.49M

131.41M	144.02M	87.50	
36.13M	25.10M	75.00	
65.69M	32.90M	84.37	

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

Subgrouping assay conditions in heatmaps using concentrations

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

Zhang et al, 1999

Pos/Neg#	Z'	CVpos (%)	Avgpos	SDpos	npos	CVneg (%)	Avgneg	SDneg	nneg	Success (%)
01	0.71	4.22	0.93	0.04	162	102.21	0.05	0.05	170	86.46
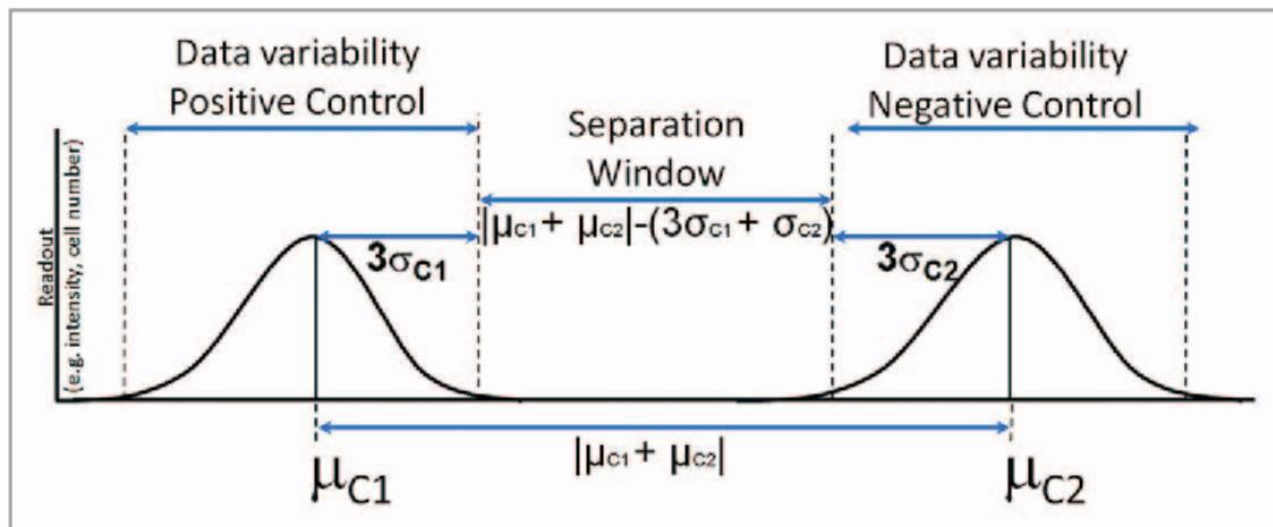
Z' is a quality parameter that you can use to evaluate your assay.

It gives you information if there is an unwanted overlap between your negative and positive controls that can result in false postives/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 addapt in your assay if needed.

Rule of thumb for Z' evaluation:

0 < Z` < 0.5	is a workable assay
Z` > 0.5	reflects an excellent assay
Z` = 1	is the ideal assay

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Z' analysis

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.

Examples on how to use the tool –
Test if the Z' can be used to determine how many replicates are required for IC_{50} determination?

Experimental outline

1. Run a full plate dose reponse 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

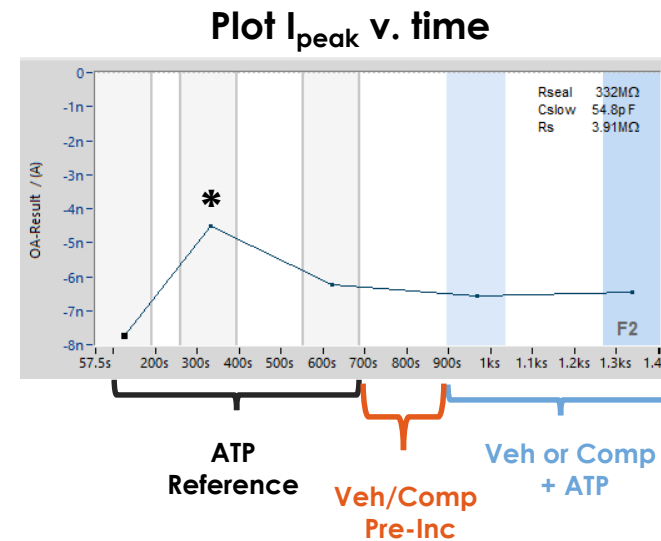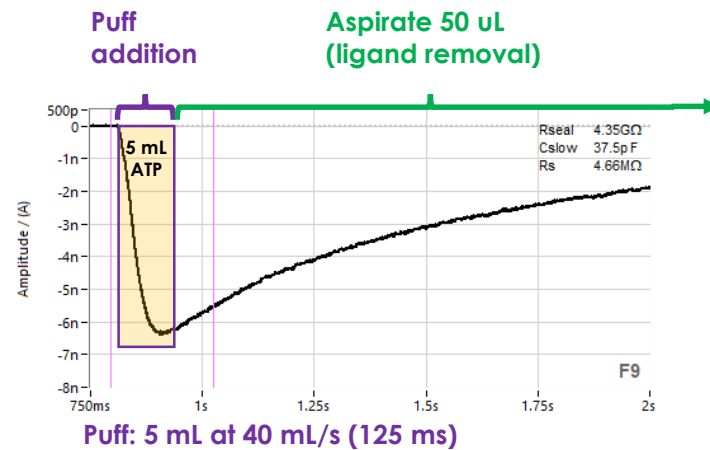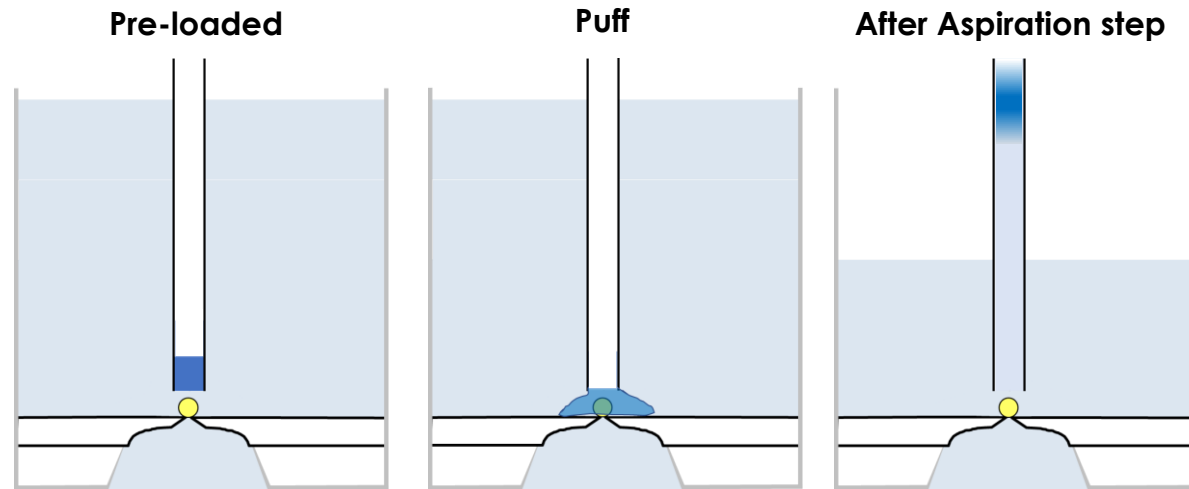|	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	3	3	4	4	3	3	4	4	3	3	4	4	3	3	4	4	3	3	4	4	3	3	4	4
B	5	5	6	6	5	5	6	6	5	5	6	6	5	5	6	6	5	5	6	6	5	5	6	6
C	7	7	8	8	7	7	8	8	7	7	8	8	7	7	8	8	7	7	8	8	7	7	8	8
D	9	9	10	10	9	9	10	10	9	9	10	10	9	9	10	10	9	9	10	10	9	9	10	10
E	11	11	12	12	11	11	12	12	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

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Puff method of ligand application

Pre-loaded

Puff

After Aspiration step

Puff addition

Aspirate 50 uL (ligand removal)

5 mL ATP

Rseal   4.35GΩ
Cslow   37.5p F
Rs   4.66MΩ

F9

Puff: 5 mL at 40 mL/s (125 ms)

Plot I_{peak} v. time

Rseal   332MΩ
Cslow   54.8p F
Rs   3.91MΩ
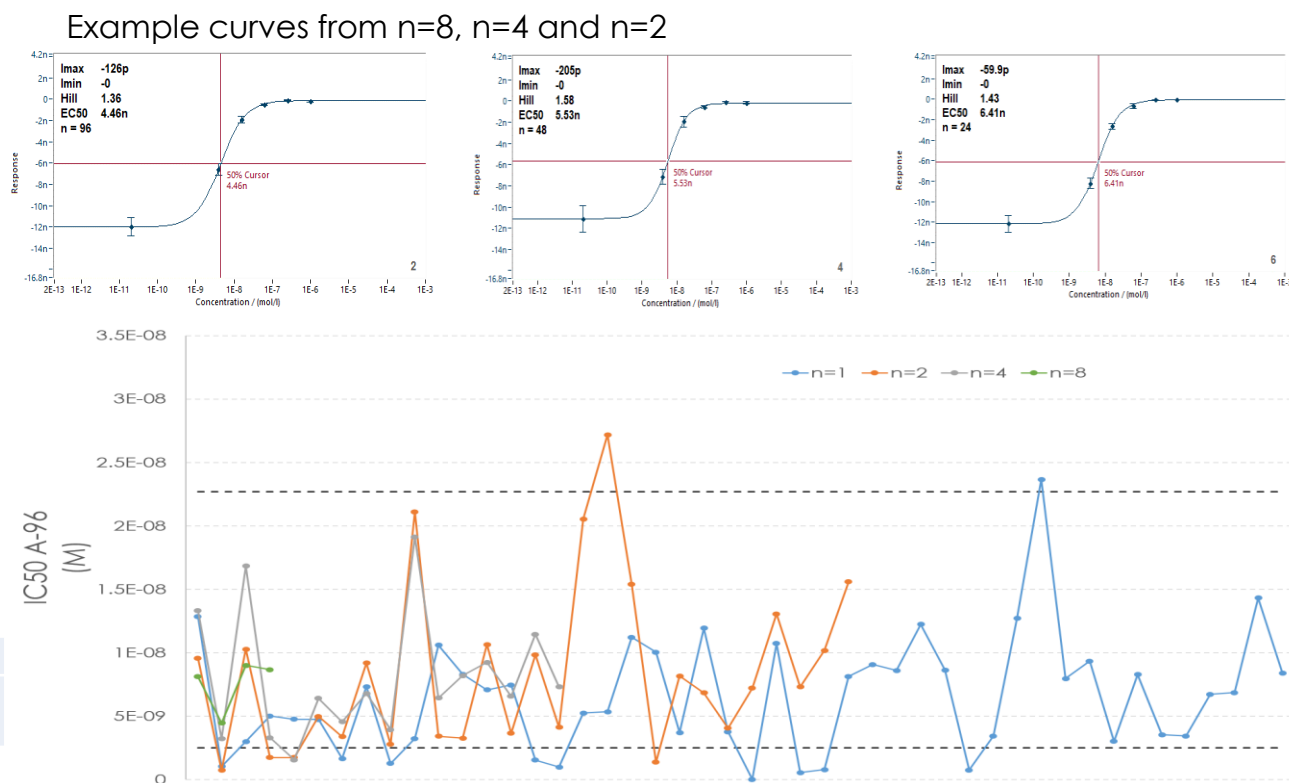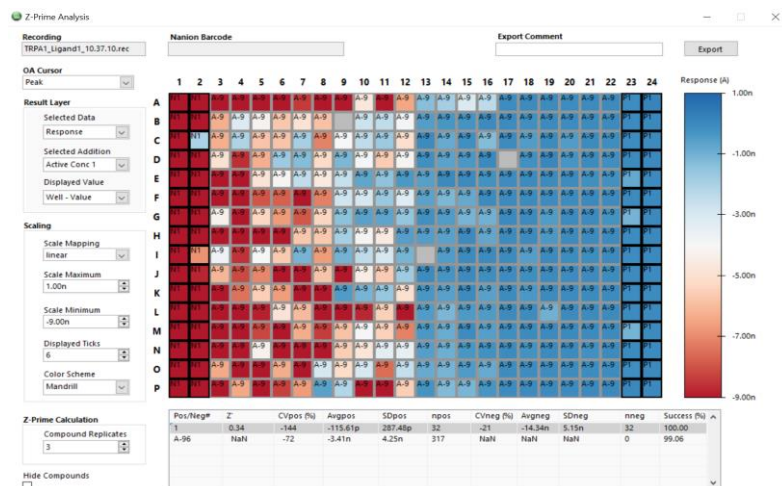
F2

ATP Reference

Veh/Comp Pre-Inc

Veh or Comp + ATP

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

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Z' analysis – TRPa1 EC Ca^{2+} blocker assay

Example curves from n=8, n=4 and n=2
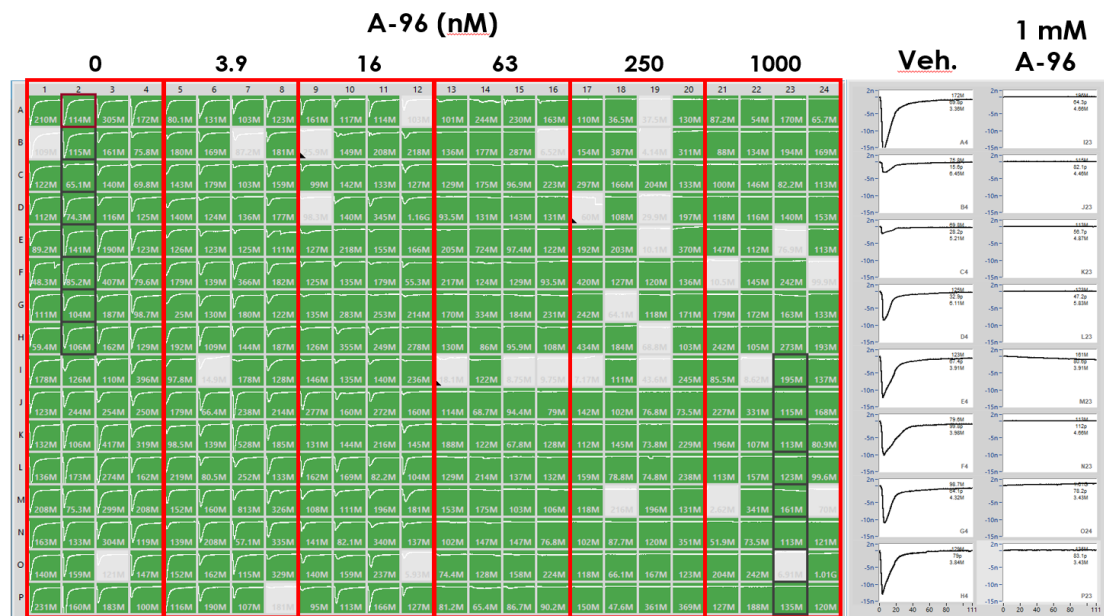
	Z', n=1	Z', n=2	Z', n=3	Z', n=4	Z', n=5	Z', n=6
TRPa1 EC Ca^{2+} assay	-0.15	0.19	0.34	0.43	0.49	0.53

"One Shot" TRPa1 with extracellular Ca^{2+} antagonist dose reponse data was analysed with the Z' tool by adding a set of 32 positive and negative controls in each end if the dose response curve.
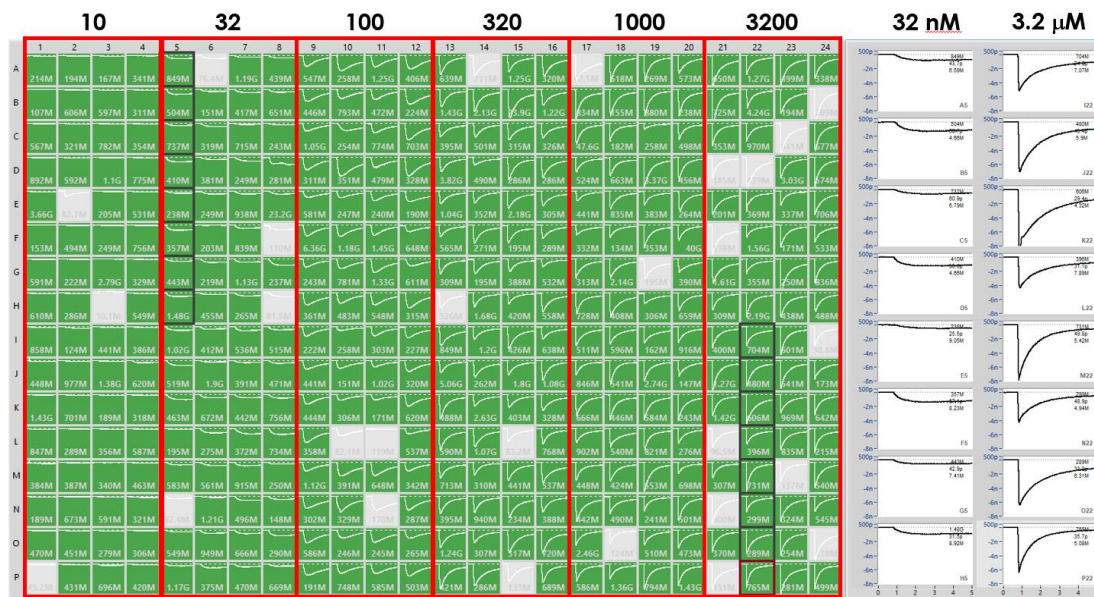
The number of repeats were increased to demostrate the effect on Z'. It was found that n=4 should be sufficient for running the assay.

The dose resonse 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
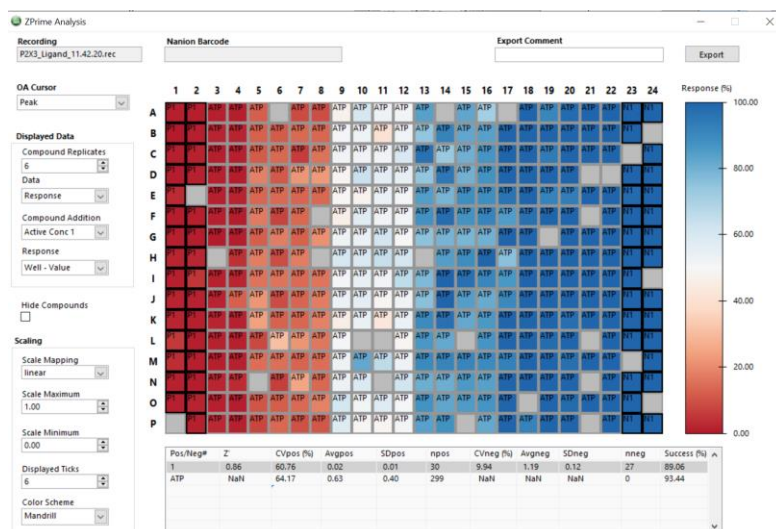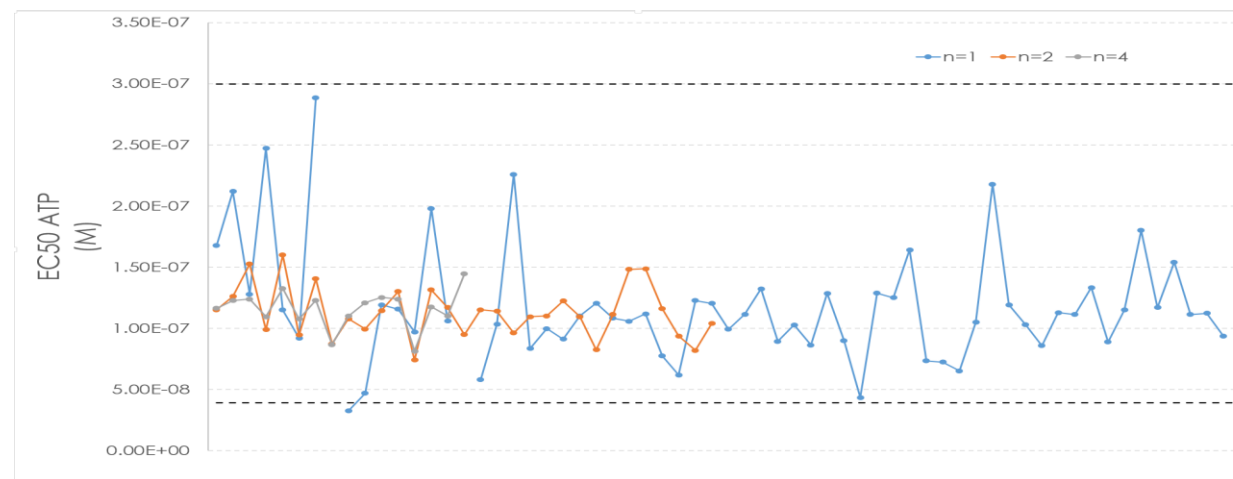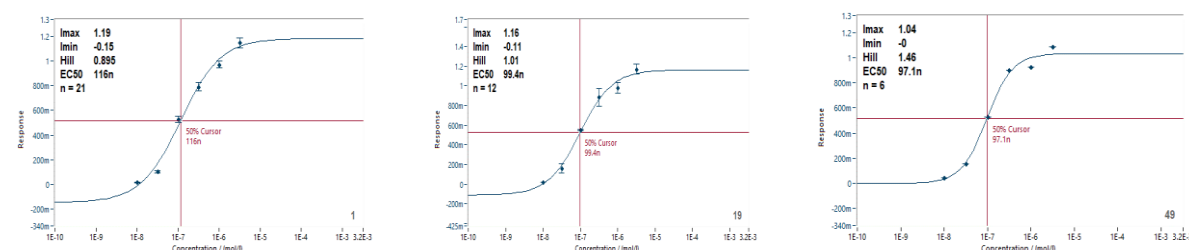
Z' analysis – P2X3 ATP EC50

ATP (nM)

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

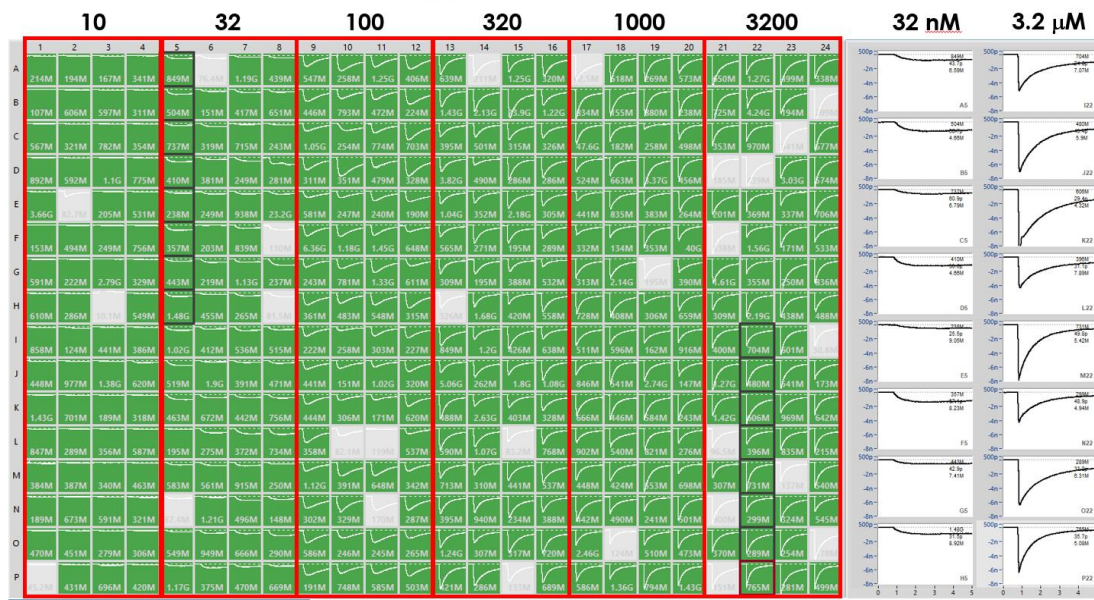|	Z', n=1	Z', n=2	Z', n=3	Z', n=4	Z', n=5	Z', n=6
P2X3 EC50 assay	0.67	0.76	0.81	0.83	0.85	0.86

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

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

The dose resonse 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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Z' analysis – P2X3 ATP EC50

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

Points missing!

	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

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

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

The dose resonse 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.
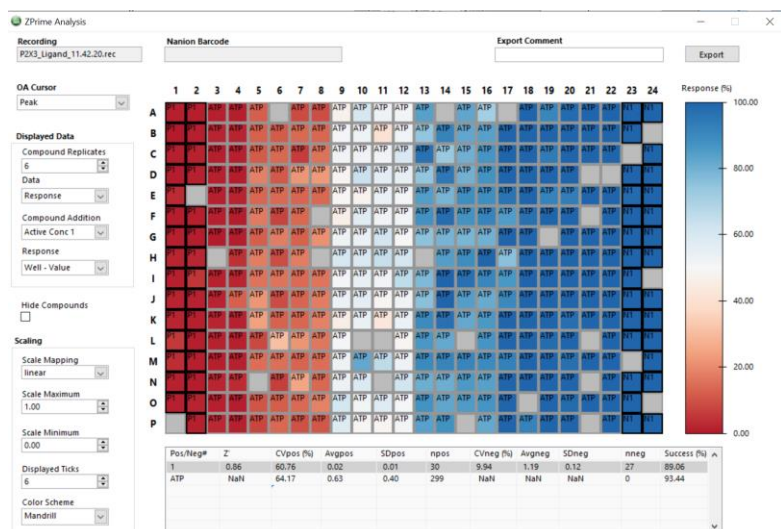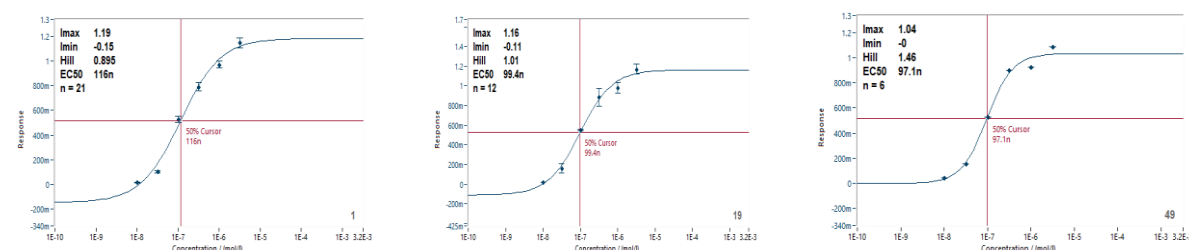
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